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Stem Cells in Clinical Practice and Tissue Engineering

Edited by Rakesh Sharma

STEM CELLS IN CLINICAL PRACTICE AND TISSUE ENGINEERING

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Contents

Preface

Dedicated to my demised father and Innovations and Solutions AMET University Global

Every individual faces many injuries and recoveries over a period of time. In fact, healing occurs due to a proliferation of stem cells capable of restoring the injured tissue. These stem cells contain regenerative potential with enormous impact on clinical applications. The regenerative potential may arise from stem cell self-renewal, multipotency, and paracrine functions (restoration). The paracrine secretion of growth factors or cytokines from retained stem cells leads to endogenous progenitor cells capable of biotransforming in the desired tissue cells (rejuvenation).

Stem cells are progenitor cells originating from a small number of natural cells from tissues. These progenitor cells may be cultured in desired media conditions to grow and differentiate to transform in different tissues of desired organs with improved physiological functions by tissue engineering, pronounced "**The-i-su En-ge-ni-ea-ring.**" The art of differentiation and tissue regeneration and putting cells at endogenous injury sites is called transplantation, pronounced "**Tren-s-phlen-te-san.**" These stem cells may be categorized into four types: embryonic, induced pluripotent, mesenchymal, and allogenic cells.

The last decade has seen the growing popularity of stem cell treatment and tissue engineering applications in regenerative medicine as a lifeline to patients suffering from cancer and incurable diseases. However, a number of public health concerns are still obstructing this lucrative treatment in clinical centers and hospitals due to ethical and political preferences. In spite of this, the clinical needs of engineered, induced pluripotent stem cells have extended to successful organ transplantation and tissue reconstruction for organ repair. Recent estimations indicate that stem cell transplantation for organ repair may be useful in 80% of stem cell clinical centers around the globe to treat diseases, including stem cell experimental research that surprisingly has created great public concern. However, globally, different federal and government agencies have shown their concern over the safe and proper use of stem cells in privately run stem cell clinics or health laboratories as "basic right to get benefit of existing art" available as unfounded words from mouth as projected in introductory chapter. Stem cells are used as an autograft means of self-renewing undifferentiated clonigenic transplantation in organ repair or regeneration to bring tissue functionality back to normal for the long-term survival of patients with permanent endogenous organ damage.

Some cells differentiate into pluripotent cells. Induced pluripotent cells have a unique capacity to differentiate into desired cell types that may be grown into specific organ types. Stem cells are successfully grown for the regeneration and reconstruction of neural, skin,

platelet, cardiac, maxillofacial, kidney, bone cartilage, adipose tissue, eye, hair, abdominal, and gastrointestinal tissue regenerative medicine. Further, new prospects for transplantation are continuously being discovered in almost every part of the human body.

This book is an attempt to compile and highlight the need for basic information on stem cells, differentiation behavior in cultures, experimental data on host–pluripotent cell interaction, and investigative and conclusive evaluation of successful endogenous organ or tissue repair with post-transplantation management issues in a timely fashion. The knowledge of stem cells, transplantation methods, evaluation, monitoring, and post-transplantation management will certainly enrich the scope of stem cell therapy among clinicians, scientists, entrepreneurs, academic politicians, physicians, and health authorities with global acceptance of an "enriched fountain of youth" in future years as a boon to humankind. With this goal, the book is divided into four sections comprising 15 chapters.

Section 1 introduces the purpose of stem cell therapy and tissue engineering.

Chapter 1 introduces readers to the origin of stem cells and types, purpose, and regulation of stem cell therapy with clinical applications in current medical practice around the globe. A panoramic account of legal permissions is presented on stem cell research with limited use of stem cells in regenerative medicine, restoration, rejuvenation, or tissue reconstruction to treat certain diseases at presently available clinical centers in Europe, the United States, Asia, and other countries. The objectives of successful pluripotent stem cell therapy, ethical and regulatory oversight, and its public concern highlight the present paradigm shift from the practice of unestablished stem cell treatments to extensive research on stem cell and tissue engineering products used in their preclinical and clinical trials within permitted regulatory limits before use in medical practice. The scope of regenerative medicine is reviewed with the emerging role of stem cell therapy at different clinical centers under the stringent supervision of federal authorities.

Section 2 focuses on mechanisms of stem cell differentiation and tissue engineering.

Chapter 2 presents the current view of hematopoiesis and beyond. The stem cells originating from embryos and migrating to bone marrow are shown to develop hematopoietic environments participating in interactions such as stem cell survival, self-renewal, and differentiation to hematopoiesis, and regulate the proliferation, differentiation, and mobilization of stem cells. The authors highlight the trafficking of stem cells in various tissues and organs for regeneration, and hematopoietic speedy recovery in diseases such as leukemia and aplastic anemia after chemotherapy for hematopoietic reconstruction after stem cell transplantation.

Chapter 3 witnesses the aging factor of stem cells that contributes to stem cell-based therapy, as well as in tissue engineering procedures. Different mechanisms of aging are described such as molecular chromatin-based oxidative stress, mitochondrial biogenesis and mitochondrial DNA mutation, nuclear damage, telomere shortening, epigenetic changes and gene expression dysregulation, microRNA changes, RNA splicing, proteostasis, cell polarity changes, nutrient sensing, niche deterioration, stem cell exhaustion, and senescence in the adult stem cell aging processes responsible for self-renewal and differentiation essential during rejuvenation. These stem cell aging mechanisms will answer various queries related to current cell-based therapies and design future counter aging longevity procedures.

Chapter 4 describes the monitoring of chimerism levels in post-hematopoietic stem cell transplantation (HSCT) for immune system reconstitution. The chimerism levels are specific

conditioning regimens depending upon disease type. The predictive role of chimerism analysis is a choice in monitoring the early risks of graft versus host disease (GvHD) development, minimal residual disease (MRD), graft failure or rejection, and disease relapse. Chimerism analysis is the preferred method for monitoring the outcome of HSCT.

Section 3 focuses on the current practice of stem cells in medical practice.

Chapter 5 describes optimal delivery strategy for stem cell therapy in patients with ischemic heart disease. The administration of stem cells to the heart is described using three preferred approaches by intracoronary, intramyocardial, and epicardial injection for optimal delivery. Advantages and disadvantages of optimal injection delivery methods and limitations of NOGA clinical systems are analyzed in the light of advances in the last 5 years, with future prospects. The authors believe that a combined intracoronary artery and intracoronary sinus injection approach could reduce washout and increase adhesion to the necrosed area with high clinical success using stem cell therapy administrated for ischemic heart disease.

Chapter 6 introduces *in vivo* evaluation of cardiovascular remodeling and dynamic monitoring for successful repair of myocardial viability by molecular noninvasive imaging methods. Several methods, including cardiovascular 900 MHz or 21 tesla magnetic resonance microimaging, bioluminescence or radionuclide gene reporter methods, may be useful in differentiating stem cells. Ultra-high magnetic field cardiovascular magnetic resonance has possible preclinical prospects for *in vivo* noninvasive molecular imaging or restorative monitoring of the reporters of rejuvenating stem cell genes to evaluate the success of transplantation and cardiac repair. Smart magnetic resonance spectroscopy imaging sequences with improved magnetic resonance imaging-sensitive, specific stem cell differentiation may detect rejuvenation by targeting energy metabolites, myocardial viability, and vital physiochemical molecules. The author believes that with the help of stem cell imaging and monitoring, transplantation of stem cells will eventually be optimized for the effective long-lasting therapy of myocardial infarction and heart failure.

Chapter 7 describes a single center study on the **impact of the donor** killer cell immunoglobulin-like receptor **(KIR) genotype on the clinical outcome of hematopoietic stem cell unrelated transplants. The authors proposed a** "KIR B-content score" based on the number of centromere and telomeric Group B KIR haplotype gene-content motifs to evaluate clinical outcome when patients with acute myeloid leukemia received a hematopoietic stem cell transplant from unrelated Group B KIR haplotype donors. The authors reported a significant role of KIR genotypes, natural killer cells, and HSCT in acute myeloid leukemia to enhance overall survival, relapse, and incidence of acute GvHD for longer disease-free survival.

Chapter 8 describes hematopoietic stem cell transplantation for acute lymphoblastic leukemia with an account of novel therapies. The authors have reviewed various modalities of stem cell therapies in different types of acute lymphoblastic leukemias among children and adults with closely related issues of graft versus tumor effect, MRD, and conditioning therapies such as haploidentical stem cell transplantation and allogenic stem cell transplantation in T-cell Ph^+ leukemia types. The authors comment on relapse before and after stem cell transplantation therapies, including immunotherapies by tyrosine kinase inhibitors imatinib, dasatinib, nilotinib, ponatinib, nelarabine, and blimatumomab; however, integration of other therapeutic interventions before and after transplantation will further improve the outcome of patients.

Chapter 9 describes post-transplantation management strategies for hematologic malignancies treated with bone marrow transplantation. The most effective and safest drug maintenance therapies are best suited to the post-transplantation management of multiple myeloma, chronic myeloid leukemia, Philadelphia chromosome positive acute lymphoblastic leukemia, acute myeloid leukemia, Hodgkin lymphoma, and non-Hodgkin lymphoma diseases. The authors reviewed the beneficial effects of maintenance therapy by thalidomide, lenalidomide, and bortezomib to enhance patient survival, relapse, and longevity.

Chapter 10 reviews the new horizons of regenerative medicine in organ repair with stringent pathological evaluation of clinical use or its success. The latest evidence based on pathological evaluation is presented as an indicator of tissue reconstruction success in urethral defects, retinal and corneal damage, orthopedic injury, bronchomalacia, skin transplantation in bullosa, and amniotic cell grafting. However, investigative studies are suggested on the mechanisms of endogenous injury and interactions at organ or tissue cell interface with activated endogenous progenitor cell populations to explain how progenitor cells behave with cells of the immune system.

Section 4 focuses on tissue engineering mechanisms and stem cell-based product manufacturing.

Chapter 11 describes human-induced pluripotent stem cell-derived engineered cardiac tissues in cardiac disease remodeling and regeneration. The authors describe different available technologies, including cell sheet technology, embedded cardiac biomaterials, spongy polymeric scaffolds, and decellularized tissues to regenerate cardiac tissue. The authors highlight a method of human pluripotent stem cell maturation in cardiac-transplanted sites and evaluate electromechanical properties and mechanical load conditions in stem cell matrix conditions with a detailed account of the use of engineered cells in drug testing for post-transplantation, disease modeling, and establishing its mechanism of cardiorejuvenation. A properly designed manufacturing process and stem cell behavior during maturation may likely expand the scalability and reduce the cost of generating these novel engineered *in vitro* myocardial tissues.

Chapter 12 proposes an improved manufacturing process of immune cells as advanced therapy medicinal products for current clinical needs of stem cell treatment at clinical centers. Currently available different products and bioreactor systems are described such as T-lymphocyte cells, natural killer cells, apheresis, tumor infiltrating lymphocytes, chimeric antigen receptors—T cells, and mesenchymal stem cells in reported clinical trials with a detailed account of withdrawn products from global markets. To maintain the high-quality medical practice of natural killer cells and mesenchymal stem cells, the authors emphasize a detailed characterization of *ex vivo* immune cell receptor subtypes and ligands on the cell surface as a highly recommended protocol in expanded immune cell population with information of pattern and amount of secreted effector molecules over time, with influences from *in vivo* components on them.

Chapter 13 presents a survey of tissue engineering in oral and maxillofacial complexes with an emphasis on the advantages and clinical applications to restore, maintain, and stabilize facial tissue functions. The authors describe the principles of oral and facial tissue engineering with the introduction of mandibular, bone, oral skin, oral mucosa, temporomandibular joint disc, condyle, fibrocartilage, and salivary gland defects with limited success of different transplantation procedures using maxillary sinus augmentation and dorsal augmentation in

rhinoplasty as effective oral and facial procedures. The authors carefully conclude the Food and Drug Administration denial of engineered products, chances of oncogenesis, and difficulties of compatible transplantable scaffolds.

Chapter 14 describes human adipose-derived stem cells for tissue engineering applications with current challenges and future perspectives. Different 3D scaffolds made of gelatin–alginate–polyacrylamide and collagen–sericin are proposed. The authors introduce human adipose-derived stem cell products, their source, properties, and different adipogenic, osteogenic, chondrogenic, neurogenic, retinal, corneal, cardiac, and hepatic differentiation potentials in current clinical practice and research trials with a note on risks and future perspectives. Controlled use of these cells could become a very powerful tool to increase the quality of life in patients with different tissue defects and are the ultimate advance in regenerative medicine.

Chapter 15 reviews the current status of tissue engineering for *in vivo* and *ex vivo* skin replacement methods and manufactured skin scaffold biomaterials. The authors introduce skin structure, skin substitutes, dermal stem cells, *in vivo* autologous skin transfer, skin tissue alternatives with *in vitro* dermal stem cell experimental application models, prospects of skin gene therapy, and chitosan, hyaluronic acid, collagen, silk, fibrin glue, and artificial polymeric scaffold biomaterials with vast future scenarios of pluripotent stem cell reprogramming within scaffolds.

The editor presents this volume as a textbook-cum-ready reckoner guide book in a lucid, effective, simple, and user-friendly manner for novice researchers, teachers, stem cell scientists, and medical experts in regenerative medicine clinical practice. For interested readers, physicians, researchers, and health care authorities, available resources on stem cell treatments and the legal status of clinical practice are included as an introductory chapter.

Books, monograms

1. The Clinical Practice of Stem-Cell Transplantation. Volume 1, 1st Edition. 1998. Editors: Barrett J and Treleaven J, Isis Medical Media. ISBN: 9781899066704, 1899066705
2. Stem Cell Transplantation: A Clinical Trial Textbook. 1st Edition. 2000. Editors: Buchel PC and Kapustay PM, Oncology Nursing Society Publishers. ISBN-10: 1890504157, ISBN-13: 978-1890504151.

Articles

1. Santostefano KE, Hamazaki T, Biel NM, Jin S, Umezawa A, Terada N. A practical guide to induced pluripotent stem cell research using patient samples. Laboratory Investigation (2015), 95, 4–13.
http://www.nature.com/articles/labinvest2014104.pdf
2. The past, present and future of stem cell clinical trials for ALS.
www.sciencedirect.com/science/article/pii/S0014488614000739
3. Heart disease and stem cells publications. Stem Cell Institute, Panama.
https://www.cellmedicine.com/heart-disease-and-stem-cells/

Stem cell clinical research

1. Donnelly EM, Lamanna J, Boulis NM. Stem cell therapy for the spinal cord. Stem Cell Research & Therapy (2012), 3, 24. https://stemcellres.biomedcentral.com/articles/10.1186/scrt115
2. Beato Coelho MB, Cabral JMS, Karp JM. Intraoperative stem cell therapy. Annual Review of Biomedical Engineering (2012), 14, 325–349.
3. Yu D, Silva GA. Stem cell sources and therapeutic approaches for central nervous system and neural retinal disorders. Neurosurgery Focus (2008), 24(3–4), E11.
4. Hayashi T, Onoe H. Neuroimaging for optimization of stem cell therapy in Parkinson's disease. Expert Opinion on Biological Therapy (2013), 13(12), 1631–1638.
5. http://www.jci.org/articles/view/40543

US Patents on stem cell therapy 2007-2018. Patents can be downloaded by clicking the first column:

US patent	Title of patent
20179610430	Cell spraying device, method and sprayed cell suspension
20130259843	Skeletal muscle regeneration using mesenchymal system cells US 9757419 B2
20160296637	Method for improving survival after radiation exposure using mesenchymal stem cells as vehicles for extracellular superoxide dismutase delivery
20160287642	Methods of treating or preventing a lung disorder
20160228537	Reverse vaccination therapy of multiple sclerosis
20160206660	Composition of stem cells having highly expressed FAS ligand
20160199414	Use of mesenchymal stem cells for the treatment of oral inflammation
20160184366	Compositions comprising stem cells expressing mesenchymal and neuronal markers and uses to treat neurological disease
20160184364	Management of osteoarthritis using pooled allogeneic mesenchymal stem cells
20160161483	Method of distinguishing mesenchymal cells
20160158292	Method and apparatus for recovery of umbilical cord tissue derived regenerative cells and uses thereof
20160130556	Enhanced differentiation of mesenchymal stem cells
20160123980	Multicolor flow cytometry method for identifying population of cells, in particular mesenchymal stem cells
20160106781	High telomerase activity bone marrow mesenchymal stem cells, methods of producing the same and pharmaceuticals and treatment methods based thereon
20160082042	Use of mesenchymal stem cell-educated macrophages to treat and prevent graft versus host disease and radiation-induced injury
20160060319	Development of protein-based biotherapeutics that induced osteogenesis for bone healing therapy: cell-permeable BMP2 and BMP7 recombinant proteins (CP-BMP2 & CP-BMP7), polynucleotides encoding the same and pro-osteogenic compositions comprising the same

US patent	Title of patent
20160051729	Reparative cell isolation and delivery
20160022739	Closed system separation of adherent bone marrow stem cells for regenerative medicine applications
20160010113	Compositions and methods for engineering cells
20160008401	Protection of the vascular endothelium from immunologically mediated cytotoxic reactions with human CD34-negative progenitor cells
20160000835	Human uterine cervical stem cells population and uses thereof
20160000835	Human uterine cervical stem cells population and uses thereof
20150320833	Ossification-inducing compositions and methods of use thereof
20150265677	Recruitment of mesenchymal stem cells using controlled release systems
20150238532	Methods to isolate human mesenchymal stem cells
20150231244	Regenerative cell and adipose-derived stem cell processing system and method
20150216911	Multipotent prenatal stem cells
20150216908	Quadri-positive stromal cells (QPSC) population for superior cell protection and immunomodulation
20150216908	Methods for generating mesenchymal stem cells which secrete neurotropic factors
20150175971	Multipotent cells having mesenchymal and endothelial lineage potential
20150166959	Immortalized mesenchymal stem cell from adipose tissue
20150157666	Methods, systems and compositions for cell-derived/vesicle-based microRNA delivery
20150139963	Isolated populations of renal stem cells and methods of isolating and using same
20150037882	Scalable process for therapeutic cell concentration and residual clearance
20140341870	Biocomposite for regeneration of injured tissue and organ, a kit for making the biocomposite, and a method of treating injuries
20140322356	CTC biomarker assay to combat breast cancer brain metastasis
20140227339	Regenerative tissue grafts and methods of making sames
20140220597	Neuregulin-1-based prognosis and therapeutic stratification of colorectal cancer
20140220053	Microvesicles isolated from mesenchymal stem cells for use as immunosuppressive agents
20140193473	Materials and methods for controlling vasculogenesis from endothelial colony forming cells
20140178422	Primary mesenchymal stem cells as a vaccine platform
20140017787	Mesenchymal stem cells and related therapies
20130345289	Adult stem cells, molecular signatures, and applications in the evaluation, diagnosis, and therapy of mammalian conditions
20130338092	Compounds and methods for targeting leukemic stem cells

US patent	Title of patent
20130330300	High telomerase activity bone marrow mesenchymal stem cells, methods of producing the same and pharmaceuticals and treatment methods based thereon
20130251690	Stem cell differentiation using keratin biomaterials
20130251689	Keratin compositions for the treatment of bone deficiency or injury
20130251670	Treatment of macular edema utilizing stem cell and conditioned media thereof
20130190729	Derivation of hematopoietic cells from adult mesenchymal stem cells
20130149245	Novel peptides and uses thereof
20130101561	Closed system separation of adherent bone marrow stem cells for regenerative medicine applications
20130101561	Closed system separation of adherent bone marrow stem cells for regenerative medicine applications
20130040304	Compositions and methods for engineering cells
20130034525	Beta islets-like cells derived from whole bone marrow
20130031645	Method for hepatic differentiation of definitive endoderm cells
20130012921	System and methods for preparation of adipose-derived stem cells
20120294836	High yield method and apparatus for volume reduction and washing of therapeutic cells using tangential flow filtration
20120276066	Peptide linked cell matrix materials for stem cells and methods of using the same
20120270826	Adult stem cells, molecular signatures, and applications in the evaluation, diagnosis, and therapy of mammalian conditions
20120269787	Systemic, allogenic stem cell therapies for the treatment of diseases in equines
20120269785	Systemic, allogenic stem cell therapies for the treatment of diseases in felines
20120224053	Method and apparatus for quantitative microimaging
20120219632	Methods for monitoring cellular states and for immortalizing mesenchymal stem cell
20120201786	Methods for use of stem cells and stem cell factors in the treatment of skin conditions
20120128636	Gingiva derived stem cell and its application in immunomodulation and reconstruction
20120100117	Method for chondrogenic differentiation of pluripotent or multipotent stem cells using WNT6
20120094304	Method of preparing human lung tissue stem cells and inducing differentiation into human alveolar epithelial cells
20120087983	Orthopedic application of encapsulated stem cells
20120087901	Engineered mesenchymal stem cells and methods of using same to treat tumors
20120052049	Systemic, allogenic stem cell therapies for treatment of diseases in animals

US patent	Title of patent
20120027860	Encapsulated adipose-derived stem cells, methods for preparation and therapeutic use
20110318414	Regenerative tissue grafts and methods of making the same
20110311984	Composition for diagnosing Parkinson's disease containing adipose tissue-derived mesenchymal stromal cell
20110311495	Isolated populations of renal stem cells and methods of isolating and using same
20110287534	Automated filling of flexible cryogenic storage bags with therapeutic cells
20110263001	Compositions and methods for engineering cells
20110256058	Novel peptides and uses thereof
20110229970	Dual-chamber perfusion bioreactor for orthopedic tissue interfaces and method of use
20110217363	Two-step targeted tumor therapy with prodrug encapsulated in nanocarrier
20110195054	Preparation and use of stromal cells for treatment of cardiac diseases
20110142805	Method of renal repair and regeneration and the treatment of diabetic nephropathy
20110104100	Compositions and methods of stem cell therapy of autism
20110014701	Protection of progenitor cells and regulation of their differentiation
20100278790	Mesenchymal stem cells, compositions and methods for treatment of cardiac tissue damage
20100222877	Tissue engineered human pulmonary valves with cyclic pressure bioreactor accelerated seeding strategies and methods
20100062038	Markers, antibodies and recombinant scFvs for mesenchymal stem cell sub-populations and osteoclasts
20100003674	Adult stem cells, molecular signatures, and applications in the evaluation, diagnosis, and therapy of mammalian
20090214485	Stem cell therapy for the treatment of diabetic retinopathy and diabetic optic neuropathy
20090214484	Stem cell therapy for the treatment of CNS disorders
20080081370	Directed differentiation of human embryonic stem cells into mesenchymal stem cells
20080075699	Method for isolating and/or identifying mesenchymal stem cells (MSC)
20070128722	Human mesenchymal stem cells and culturing methods thereof

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Section 1

Stem Cell Therapy and Tissue Engineering

Chapter 1

Introductory Chapter: Stem Cells and Tissue Engineering in Medical Practice

Rakesh Sharma

Additional information is available at the end of the chapter

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1. Introduction

Stem cell transplant research and tissue engineering, in present time, have emerged as a legalized and regulated stem cell treatment option globally, but, scientifically, their success is unestablished. Novel stem cell-based therapies have evolved as innovative and routine clinical solutions by commercial companies and hospitals across the world. Such rampant spread of stem cell clinics throughout UK, US, Europe, and Asia reflect the public encouragement of benefits to incurable diseases. However, ever growing stem cell therapy developments need constant dogwatch and careful policy making by government regulatory bodies for prompt action in case of any untoward public concern. Therefore, researchers and physicians must keep themselves abreast of current knowledge on stem cells, tissue engineering devices in treatment and its safe legal limits. With this aim, stem cell scientific developments, treatment options, and legal scenario are introduced here to beginner or actively involved scientists and physicians. Introduction to stem cell therapy will provide basic information to beginner researchers and practice physicians on engineered stem cell research concepts and present stem cell therapy federal regulations in different North American, European, and Asian countries. FDA, CDC, EU, ICMR government policies in different countries includes information on the current legal position, ethical policies, regulatory oversight, and relevant laws.

The word "eais-te-am" cell refers to the progenitor cell or human body's master cell means first original embryonic cell with rejuvenating and restorative capability of regenerating any body tissue cell(s) typically called as "Fountain of Youth." Stem cells can divide and develop

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different cell types during early life and help in repair the body by replenishing the damaged cells in disease, wear, and injury.

Stem cell therapy uses mainly human pluripotent stem cells to restore functions of tissues or organs, to maintain or repair the damaged human tissues or organs caused by trauma, genetic disease, or metabolic disease. The stem cell engineered products are at large available and paradigm shift shows a much greater investment in novel stem cell scaffolds, designing new matrices, grafts to treat chronic and incurable diseases.

Four major considerations for successful stem cell therapy and research are:

- Why stem cell clinics need regulation and legalization?
- Purpose and global regulatory norms for stem cell research.
- Reliable stem cell treatment and tissue engineering products.
- Concerns on stem cell treatment regulations and role of government approvals.

Stem cell types and need of human embryogenic cell research and tissue engineering are introduced in Section 2. Purpose and global regulatory norms for stem cell research are summarized in Section 3. Present status of stem cell therapy and clinical practice limitations or alternatives is reviewed with government guidelines in Section 4. Global scenario of stem cell clinical centers to treat different diseases and human organs are tabulated in Section 5. Major introduction is how government regulatory authorities define policies, frame guidelines, and keep watch public concerns and clinical practices at treatment centers globally.

2. Introduction to stem cell

Stem cells are progenitor cells. There are three types of stem cells: adult stem cells (from umbilical cord blood), human embryogenic cells (from embryo from fertilized eggs), and induced-pluripotent cells (by reprograming adult stem cells to differentiate into specific tissue cells). These stem cells share common properties: (1) survive long periods to make more stem cells; (2) up-specialized but capable to develop into specific cells; (3) develop to do specific work in the body.

2.1. What are human embryonic stem cells?

Human embryonic stem cells were first isolated and cultured in year 1998 to confirm their unique capabilities. They can transform into any human tissues up to 200 different cell types found in the body. Under the right conditions, they behave as evergreen, everlasting, and able to multiply indefinitely to form immortal cell line. This amazing capacity of embryonic stem cells to give rise to any type of tissue has intensified the search for adult stem cells to assume paracrine functions [1]. Stem cells have plasticity, means they circulate throughout the body and reside wherever needed to promote regeneration of local tissue.

2.2. Why human embryonic stem cells are in active research?

Stem cell research offers great hope to repair serious life-threatening diseases. The first clinical trial took place in the United States for spinal cord injury repair [2]. The first European study was reported in the United Kingdom for blindness repair [3].

From biologist's standpoint, embryonic stem cell research offers as a tool to understand the tissue maintenance and repair in health, how disease develops, and its possible treatment. The molecular basis of embryonic stem cells growing in three-dimensional culture environments has explored the molecular control of gut development and associated organs to understand the genetic control of fragile-X syndrome. Other example is Parkinson's disease, currently untreatable and life threat.

2.3. What is origin of embryonic stem cell lines?

All human embryonic stem cell lines originate from a 4- to 5-day-old blastocyst. A blastocyst is a hollow ball of around 100 cells. Blastocyst is a left over egg from *in vitro* fertilization (IVF). Some blastocysts are implanted into the woman's uterus, while the rest are stored in a deep-freezer. After implantation, couple decides what to do with remaining blastocysts. They can continue to store blastocysts for research. Only these donated blastocysts are the source of human embryonic stem cell lines.

2.4. New embryonic stem cell line for each research project?

For research, cells are harvested from one 4- to 5-day-old blastocyst. Blastocyst cell multiplies in the laboratory to create a "cell line" able to produce an infinite number of embryonic stem cells. All these cells have same genetic make-up. Many cell lines are kept in not-for-profit stem cell banks. Banks supply these stem cells for research all over the world. Existing cell lines are also exchanged at no cost between laboratories in the context of research programs, under tight legal controls.

3. Purpose and global regulatory norms for stem cell research

3.1. What position do member states take on human embryonic stem cell research?

Different countries have different legislative provisions among different states on human embryonic stem cell research.

In the United States of America, 26 states have active stem cell research legislation policy, while other states have loose policy or no legislative rules for stem cell research and treatment. So far, no state has permitted any stem cell product for medical treatment as valid. FDA only approved cord blood-derived hematopoietic progenitor cells (blood forming stem cells) for certain indications including certain blood cancers and some inherited metabolic and immune system disorders. Last year, a bill HB 810 passed by Texas Governance Springer has taken first

lead to legalize stem cell treatment as "Right to Have Trial" as unproven therapies at their own risk and cost in its report (https://legiscan.com/TX/text/HB810/2017). Another bill HB 661 permits chronic ill persons to try early stage approved clinical trial. New bill HB 3236 permits companies to charge patients for unproven therapies. Earlier, Obama Governance declared policy for operating 570 stem cell treatment clinics across country including Beverly Hills, CA, New York, San Antonio, Los Angeles, Austin, Texas, Phoenix, and Scottsdale, AZ [1].

All 18 countries in the European Union (EU) have stem cell research legislative policy involving human embryonic stem cells, three countries prohibit it (Poland, Latvia, and Slovakia) and the rest have no specific legislation [2, 3]. The EU has no competence to harmonize the legal situation in Member States. Legislation on cell therapy is based on three directives: (1) directive 2003/63/EC defines cell therapy products as clinical products; (2) directive 2001/20/EC emphasizes clinical trials mandatory for all cell therapy products; (3) directive 2004/23/EC establishes standard quality, donation safety, harvesting, tests, processing, preservation, storage, distribution of human tissues and cells. In year 2008, regulation 1394/2007/EC on Advanced Therapy Medicinal Products (ATMP) includes gene therapy medicinal products, somatic cell therapy products, and tissue engineered products (by manipulation, change in physiological or structural functions for therapeutic use) under Committee for Advanced Therapies (CAT) to provide opinion on safety, quality, efficacy of ATMPs acceptable as stem cell-based medical product by marketing authorization [4].

3.2. What are regulations and the policy toward the use of human embryonic stem cells and stem cell products for research and clinical use?

In view of the different legal situations and practices in US, EU member States and Asia, both US and EU have own clear ethical and legal framework on human embryonic stem cell research funded from respective budget. Major regulatory consideration in policy is on batch consistency, product stability, safety, efficacy, and quality of stem cell-based tissue engineered products through pre-clinical, clinical, and marketing authorization.

In US, FDA and CDC government bodies have laid down stringent guidelines on the use of stem cell treatment. Food and Drug Authority (FDA) keeps dog watch over the performance, standard, and any public concern related with stem cell treatment abuse, defective quality, options of tissue engineering for right purpose. In case of non-compliance, irregularities, illicit, and unlawful gain, FDA warns stem cell center, and may take precautionary or prohibitive action. Major stem cell clinics are opened for certain organ diseases to recover them. Notable examples are bone and joint disease, erectile dysfunction, neck and back pain, oral and maxillofacial surgery, tendons, and arthritis [5–10]. For stem cell therapy, FDA approves stem cell clinic for transplantation purpose. FDA defines and regulates different stem cell-based therapies and different stem cell products for safe use.

Code of Federal Regulation 21 CFR defined sections for use of cell therapy products: IND regulations (21 CFR 312), Biologics regulations (21 CFR 600), and cGMP (21 CFR 211), autologous cells, tissues, cell- or tissue-based products HCT/Ps (CFR, Part 1271) in year 2006. Public Health Service Act (PHSA) refers as "361 products" and "351 products." Food and Drug Administration (FDA) codified 361 cells and tissue products for therapeutic use under Good

Tissue Practice (GTP) with guidelines how biologic drug and device regulations apply to cellular and genetic therapies [11]. FDA developed a regulatory framework in three areas: (1) preventing use of contaminated cells or tissues; (2) preventing the cell and tissue contamination by adequate processing; and (3) clinical safety of all cells and tissues. All these areas of framework control both cell and tissue-based products as mass produced drugs [12]. However, several agencies, like American Red Cross, American Society of Clinical Oncology, and Society of Assisted Reproductive Techniques, clarified the role of FDA limited to allogeneic tissue transplants to control spread of communicable diseases means the stem cell transplantations are medical procedures. FDA division "Center for Biologics Evaluation and Research (CBER)" regulates cell-based therapy, and already approved several ApliGraf®, Carticel®, and Epicel® products. The manipulated autologous cells for somatic cell therapy need approval as investigational new drug (IND). However, ATMP minimally manipulated, labeled, or advertised for homologous use, not combined with drug or device, do not require FDA approval. Of course, FDA has wide regulatory coverage including isolation of stem cell rich fractions for orthopedic use, breast augmentation, and 5-day blastocyst transfer as equivalent drug mass production. In general, regulation applies to cells and tissues (HCT/Ps) used for implementation, transplantation, infusion, and transfer into human recipient.

Two regulatory guides for industries were released by FDA. In the year 2007, "Guidance for industry: Regulation of HCT/Ps-Small Entity Compliance Guide" and other in the year 2009, "Guidance for industry on Current Good Tissue Practice and Additional Requirements for Manufacturers of HCT/Ps" [13]. Clinical trials using mesenchymal stem cells fall in the IND category. In FDA regulation policy, physicians may administer stem cell-based products in patients by two ways: (1) compassionate use or expanded access to investigational drugs and biological products without interfering conduct of clinical investigations; (2) off-label prescription of FDA approved stem cell products at full discretion of physician. A new draft 21 CFR 1271 15(b) guideline for industry "Same Surgical Procedure Exception" states three criteria advised to physicians: (1) autologous use or remove HCT/Ps from individual and implant them into same individual; (2) implant the HCT/Ps within same surgical procedure; (3) HCT/Ps must remain in their original form (rinsing, cleaning, sizing, shaping, and manufacturing is permitted). All guidelines, 21 CFR 1271(a), 14(b), and 15(b) exceptions, prohibit the claim of "practice of medicine" of 361 products without FDA compliance. To date, FDA has not approved any stem cell medical product in market place. Moreover, physicians claim of performing innovative surgical procedures (as practice of medicine art not directly regulated by FDA) falling under regulatory exception mentioned in 21CFR 1271 Section 361 Public Health Service (PHS) Act for human cell-tissue-based products (HCT/Ps) in practice of medicine without spreading communicable disease [14]. In contrast, Section 351 of PHS Act defines the premarketing review and FDA approval of drugs, biological products or medical devices.

In the year 2014, two new draft guidelines amended 21CFR 1271.10 and 21CFR 1271.20 regarding Section 361 enacted autologous HCT/Ps only if they are "minimally manipulated" for advertisement or labeling purpose (using of water, crystalloids, sterilizer, or storage preservative) without any clinical safety concern. For example, lipoaspirate SVF for adipose derived stem cell treatments (by autologous HCT/Ps) of Parkinson disease and multiple sclerosis fail to comply 21 CFR 1271.10 for homologous use because processing HCT/Ps breaks down and eliminates

structural cushion and support components so altered original relevant reconstruction, repair, or replacement characteristics of stem cells. It puts them in drug, device, and biological product 361 category requiring premarketing FDA approval. However, combination standards (minimum manipulation and homologous use) allow drugs, device, biological products as exempted investigational new drug or device for premarketing approval with assurance of conducting premarketing trial with safety and efficacy. Companies can advertise FDA-cleared investigational new drugs or device exemptions to gain profits from sale in compliance with federal regulations without known safety and efficacy of products. For interested readers, "minimal manipulation" means "processing that does not alter the original relevant characteristics of tissues related to tissue utility for reconstruction, repair, or replacement." In the year 2014, third draft regulation "HCT/Ps from Adipose Tissue: Regulatory Considerations" states that processing to isolate nonadipose tissue (without subsequent cell culture or expansion) is more than minimal manipulation. Stem cell business centers and clinics may operate sale of unproven and unlicensed cell-based interventions without FDA compliance using three said guidelines. Now, it requires considerable FDA effort to design final regulatory draft.

The current framework in EU was adopted in year 2007 and subsequently renewed as HORIZON 2020 for the duration (2014–2020) to frame the EU's new research and innovation program "triple lock system."

- First and foremost, national legislation is respected—EU projects must follow the laws of the country in which research is carried out;
- In addition, all projects must be scientifically validated by peer review and must undergo rigorous ethical review;
- Finally, EU funds may not be used for derivation of new stem cell lines, or for research that destroys embryos (blastocysts)—including for the procurement of stem cells.

The program operates on a bottom-up basis. European Commission does not publish calls for proposals specifically for research using human embryonic stem cells. Scientists propose the methods and materials for a particular study. EU research allows fair comparison of different stem cell types to find the best cell source for a particular research or clinical application.

Regulatory framework in Asia has no well-defined regulation and policy on stem cell-based products and clinical use. It amounts the risk to patients of physical harm and high financial exploitation. Currently, clinics and pharma companies do not follow clinical trials under regulatory framework or have no national guidelines to follow. Some guides were released as guidelines to clinical trials shown in **Table 1**.

3.2.1. Ethical guidelines for biomedical research in India on human subjects: section V

Stem cell research and therapy (by Indian Council of Medical Research, ICMR 2006) defines the clinical grade stem cells for clinical trial approved by institution committee for Stem Cell Research and Therapy (IC-SCRT) at multinational companies or from abroad. Collaboration is approved by hierarchy of National Apex Committee and Institutional Committee for Stem Cell Research and Therapy, Institutional Ethics Committee, Drug Controller general of India, Health Ministry

Country	Current legal position on embryonic cell lines	Ethical/regulatory oversight	Reference
Austrıa	Banned. imported cell lines permissible	Austrian Bioethics Commission	[24, 25]
	(Fortpflanzungsmedizingesetz) 2004	2009 opinion	
Bulgarıa	Permitted IVF treatment.	Bulgarian Centre for Bioethics	
	Bulgarian Health Act SG83/19,2003.	Central Ethics Commission (CEC)	[26]
Czech Republıc	**Reproductive** cloning is banned	Czech R&D Council	[15]
Fınland	Permitted IVF embryos, somatic	Medical Research Act 2001	[27]
	Cell nuclear transfer	Finnish National Advisory Board 1991	
France	**Permitted research**	Agence de la Biomédicine	[20, 28]
		Fr National Committee of Ethics	
Germany	Permitted research	Embryonenschutzgesetz) 1991	[22]
	2002 Stem Cell Act	Central Ethics Commission (ZES)	[29–31]
Greece	Permitted IVF embryos	Hellenic National Bioethics Commission	[32]
	Law 3305/2005	National Transplantation Organization	
Ireland	No specific legislation	Irish Council of Bioethics (ICB), 2002	
		Assisted Human Reproduction Report	[33]
Italy	Permitted imported stem cell lines	Law 40	
		Comitato Nazionale per la Bioetica)	[34]
Lıthuanıa	Prohibited any embryo research	Law on Ethics of Biomedical Research	
		Lithuanian Bioethics Committee (LBC)	[35]
Portugal	Permitted cell lines from IVF embryos	Law 32/2006 medically assisted procreation	[36]
Spaın	Regulatory framework embryonic stem cells	Law 14/2007 for embryo theranostics	[37, 38]
Sweden	Embryonic cells from IVF/SCNT	Act on Genetic Integrity 2005	[39]
UK	**HEFA** regulated stem cell research only	Fertilisation and Embryology Act 2001	[40]
USA, Canada	Stem cell clinics for limited use	21CFR 1271 sections	[41]

Table 1. Present rules, restrictions, and regulatory mandate enforced in European and Western world.

Screening Committee, and funding agency. Investigators should assure that stem cell lines are in accordance with appropriate Material Transfer Agreement based on country's guidelines on Good Medical Practices. ICMR, Department of Biotechnology laid down "Guidelines for Stem Cell Research and Therapy" in the year 2007 on mechanism of review and monitoring research

and therapy at national level and institution level. Central Drug Standards Control Organization (CDSCO) defined guidelines on new biological/biotechnology products. However, regulation of stem cell products as drugs does not exist for clinical trials. As a result, national committee is proposed as "Cell Biology Based Therapeutic Drug Evaluation Committee" by ICMR to approve the therapeutic products of human gene manipulation, xerotransplant technology, and stem cells in market since year 2011. However, till date no registration requirement technical guidelines of human stem cell-based products are formulated.

3.2.2. International unanimous opinion

International Society of Stem Cell Research (ISSCR) and Hinxton group have published "guidelines on clinical translation of stem cell" to emphasize: (1) quality controlled stem cells with known characteristics; (2) *a priori* information of delivery efficacy, safety of stem cells in animal model; (3) peer reviewed clinical protocols in pre-clinical research; (4) awareness of tumorigenic risk without evidence of clinical benefit evidences at the time of voluntary informed recipient consent to perform clinical trial [15]. In spite of all, ISSCR recommendations remain as undefined code of professional conduct to assure safety due to no harmony between laboratory-based research and use of approved stem cell-based products with policy differences in different continents [16]. Legislation must regulate scientific progress from lab to clinic in public interest. Public must have confidence in clinical benefits. The public interests may be protected by guidelines for: (1) stem cell-based product is safe, pure, potent for general practice GTP, GMP, and GCP requirements; (2) pre-clinical evidence available on proof-of-principle and safety in animal models; (3) new non-invasive biodistribution monitoring by markers and tumors for clinical trials; (4) preference to patient safety by risk-based approach in granting regulatory approval with conditional marketing authorization.

3.3. How much have the US and EU spent on human embryonic stem cell research?

In the years 2007–2017, the EU has funded 27 collaborative health research projects involving the use of human embryonic stem cells with an EU contribution of about €157 million. Human embryonic stem cell research projects represent approximately one-third of health projects on all forms of stem cells.

In addition, the European Research Council has funded 10 projects for an EU financial contribution of about €19 million, and there have been 24 Marie Skłodowska-Curie actions involving human embryonic stem cell research worth €23 million.

4. Stem cell treatment and tissue engineering products

4.1. Stem cell therapy: a success or a myth

Stem cell treatment is a new option of organ transplantation or tissue and cell transplantation. Stem cells in culture behave differently from the tissue cells behave inside body. As a result, the progenitor cells and embryonic cell behavior entirely depends on media conditions, physiology of cultured cells, environment of breeding, action of added growth factors, vital molecules, additives,

antibiotics, bioactive proteins, colony stimulating factors, cell division regulatory factors, gene regulation, signal molecules, enzymes, hormones, and energy available to intracellular metabolism. All these factors function and act in unison for stem cell treatment to become success in regenerative medicine otherwise stem cells do not grow in the desired manner due to one or the other deficiency and stem cell treatment becomes a myth [16, 17].

Any product derived from stem cells or containing stem cells is referred as "stem cell-based product" (SCBP), including tissue engineering biomaterials in cell- and tissue-based therapy. Around the world, autologous stem cell clinics or hospitals are spreading in China, India, Mexico, Panama, Ukraine, European Union member states to perform stem cell facelifts, sport orthopedics, breast augmentation, treatment of muscle dystrophy, Alzheimer's disease, Parkinson's disease, and multiple sclerosis. In US alone, development of tissue engineering methods have shown significant progress in success of stem cells as therapeutic tools of regenerative medicine in different states of Ohio, Kansas, Minnesota, Wisconsin, and Mayo. These clinics and centers maintain *in vitro* stem cells in cultures that can be transplanted by fixing them at desired site in Matrigel®. With time, stem cells grow in the desired pre-programed manner and regenerate the defective part of the tissue or the organ [18]. Stem cell treatment centers charge prospective patients privately for simple stem cell therapy by bone marrow or peripheral blood liposuction, enzyme digestion, ultrasonic cavitation to prepare stromal vascular fraction (mixture of fibroblasts, endothelial progenitor cells, pericytes, mesenchymal stroma cells, and adipocytes) as therapeutic injection given to said patients or industries manufacture commercial stem cell therapy products without any product regulatory information to patients.

For clinical and commercial use, regulatory challenges are safety testing, *in vitro* functional assays, potency assays, pre-clinical, or clinical trials. The safety testing includes assays for microbial, fungal, endotoxin, mycoplasma, viral contamination, karyotyping testing, and enriched cell population. *In vitro* functional and potency assays act a surrogate measure of clinical effectiveness and validity to meet standards and control. In support, pre-clinical trial on experimental toxicity animal models such as immuno-compromised or tumorigenic animals, *in vitro* manipulation, administration route, and clinical trials with complete safety and sound ethics are necessary [19]. These characteristics establish the potential of these cells for tissue repair after injury or disease so called "stem cell therapies" as stem cell medicinal products made out of minimal manipulation of any target cell type destined for clinical application to improve defective function in the body. Presently, human embryonic stem cells (hESCs) are used in 13% of cell therapy procedures; fetal stem cells in 2%, umbilical cord stem cells in 10%, and adult stem cells in 75% of treatments [20]. Any use of such cell-based medicines is subject to authorization and controls, including their manufacture.

4.2. Clinical development for first-in-man study plan

Study design should demonstrate safety endpoints, efficacy, and its action for proposed clinical trial of new investigational medicinal product (EMEA/CHMP/SWP/28367/07). Safety endpoints may be defined on theoretical basis or any toxicity endpoint. The efficacy assessment should be related to pharmacodynamic effect of ATMP. A safe and minimal effective treatment dose should be identified. The presence of stem cells intended at desired location should be investigated by selected differentiation biomarkers to facilitate *in vivo* monitoring the stem cells during the time of administration in patients and their follow-up *in vivo* effect to establish long-term efficacy.

4.3. Are there alternatives to embryonic stem cells?

Embryonic stem cells have peculiar properties and functions uncommon in other natural cell types. Induced-pluripotent stem cell discovery as an alternative was awarded Nobel Prize in the year 2012 confirming many similar properties common with embryonic stem cells [21]. These cells are in use since then for drug development and screening new medicines. It is believed that drug development will be up to the clinical standard for therapeutic purposes in the future. In recent years, development of induced-pluripotent stem cells has opened new vista of stem cell restoration, repair, rejuvenation, and treatment research as adjuvant therapy.

There are various types of "tissue-specific" or "adult" stem cells. These cells are useful in specific applications. They make the limited number of cells found in the tissue from which they were isolated. So, they are limited in their potential as a clinical application of research. The expansion of adult stem cells in culture may be the answer, but extensive cultures of human adult cells may change their intrinsic properties *in vivo*, rendering them unfit for restoring injured or diseased tissue in patients.

5. Concerns on stem cell treatment regulations

There are concerns raised in the media about uncertain differentiation and matched neotissue functions after stem cell therapy treatment. The unconfirmed outcome of new techniques offers new possibilities of successful treatment in patients with difficult or untreatable conditions. Stem cell therapy techniques have benefits and risks. Specific rules were introduced in the European Union (EU) in 2007 [22] to ensure appropriate authorization, supervision, and control of cell therapy medicines to reduce and manage the risks.

Recent media reports highlight the need of public authorities' attention to enforce their legal responsibilities in favor of patients taking restricted or limited treatment in compliance with relevant quality standards, material authenticity, treatment protocols, and supervised patient follow-up measures. The protection of patients is the core rule. Safety and efficacy of stem cell transplant products rule the quality and engineered tissue manufacturing of these products that are set out in good-manufacturing-practice (GMP) requirements. These are globally recognized standards for quality assurance in the production and control of stem cell products. Security and control of medicines derived from stem cell manipulation is tightly controlled by the FDA in US and EU [23].

Present time, manufacturers avoid compliance with quality standards. Inappropriate unapproved treatment definition or reclassification without mandate of competent authorities for control of stem cell products, may expose patients to cross-contaminated cell preparations, and result in short- and long-term risks to individual patients.

5.1. How is stem cell treatment clinics regulated in different countries?

In a very short span of 10 years, over 600 stem cell clinics were opened with unproven claims and unapproved treatment definition in the name of some benefits to individual diseases. Competent authorities in different countries have laid down ethical or regulatory policies.

Globally, the present major focus is on stem cell therapy in finding new options of incurable diseases with following objectives:

- to promote advances in the treatment of infertility
- to increase knowledge about the causes of congenital disease
- to increase knowledge about the causes of miscarriages
- to develop more effective techniques of contraception
- to develop methods for detecting the presence of gene or chromosome abnormalities
- to increase knowledge about the development of embryos
- to increase knowledge about serious disease
- to enable any such knowledge to be applied in developing treatments for serious disease.

Licensed research is permitted on embryos created *in vitro* for its limited use in fertility treatment research within 14 days of harvesting cells. Human Reproductive Cloning Act (2001) does not permit cell nuclear replacement, or any other technique, to create a child or human reproductive cloning. The Human Tissue Act 2004 [42] regulates the use of human biological materials.

5.1.1. Ethical and regulatory oversight

The regulatory Human Tissue Authority (HTA), the HFEA and the Medicines and Healthcare products Regulatory Agency (MHRA), Gene Therapy Advisory Committee (GTAC) are research ethics bodies examine and issue reports on ethical issues relating to stem cell research [43].

5.2. Major concerns of stem cell therapy and loopholes in stem cell research

Major concerns are:

- **Where do the embryos come from to create stem cell lines for clinical use?**

All the human embryonic stem cell lines currently in use come from 4- to 5-day-old embryos left over from *in vitro* fertilization (IVF) procedures. In IVF, researchers mix a man's sperm and a woman's eggs together in a lab dish. Some of those eggs get fertilized. At about 5 days, the egg divides to become a hollow ball of roughly 100 cells called a blastocyst. These early embryos (blastocyst) are implanted into the woman's uterus to develop pregnancy.

For research, unused blastocysts are stored in the IVF clinic freezer for following use in future:

- Continue paying to store the embryos in freezer
- Defrosting the embryos, which destroys them, so, they are kept in freezer
- Donation of the embryos for supervised adoption
- Choice to donate the frozen embryos for research. These donated embryos are the main source of human embryonic stem cell lines.

Some embryonic experimental stem cell lines also come from embryos carrying harmful genetic mutations like cystic fibrosis or Tay Sachs disease. These are discovered by genetic testing prior to implantation. People who donate leftover embryos for research go through an extensive consent process. Under national and international regulations, no human embryonic stem cell lines can be created without explicit consent from the donor and without stringent regulatory protocols.

- **Do embryonic stem cell lines come from aborted fetuses?**

No. Embryonic stem cells only come from 4- to 5-day-old blastocysts or younger embryos otherwise it is bad criminal clinical practice.

- **Does creating embryonic stem cell lines destroy the embryo?**

In most cases, Yes. The hollow blastocyst—source of embryonic stem cells—contains a cluster of 20–30 cells called the "inner cell mass." These are the cells that become embryonic stem cells in a lab dish. The process of extracting these cells destroys the embryo. There is a second method that creates embryonic stem cell lines without destroying the embryo. Instead, scientists take a single cell from a very early stage IVF embryo and can use only one cell to develop a new line. The process of removing one cell from an early stage embryo has been done for many years as a way of testing the embryo for genetic predisposition to diseases such as Tay Sachs. This process is called "preimplantation genetic testing."

5.3. Alternatives of embryonic stem cells

New alternatives are emerging to replace controversial embryonic stem cells. Notably, adult stem cells, pluripotent cells are promising sources.

- **Are adult stem cells as good—or better—than embryonic stem cells?**

Adult stem cells unlike embryonic stem cells can grow only to follow certain cell paths. The adult stem cells do not grow indefinitely in the lab, unlike embryonic stem cells, and they are not as flexible in the types of diseases they can treat. To establish the claims, large trials with both adult and embryonic stem cells are needed to know the value of adult stem cells.

- **Do iPS cells eliminate the need to use embryos in stem cell research?**

Induced-pluripotent stem cells, or iPS cells, represent another type of cells that could be used for stem cell research. The iPS cells are adult skin cells. They can be genetically "reprogramed" to appear like embryonic stem cells. The technology to generate human iPS cells was pioneered by Shinya Yamanaka in 2007 [44].

- Stem cell research may not lead to human cloning because significant regulatory and advisory body has restrictions on reproductive cloning throughout world.

5.4. Scope of stem cell therapy: what clinics offer benefits from stem cell therapy?

Stem cell therapy, in some clinics, is making claims of healing based on new investigational personalized trials. Some clinical conditions are claimed to have limited benefit from stem cell therapies in recent years are mentioned in **Table 2**.

Disease/Problems	Benefits claimed	Stem Cell Clinics posted on Internet webpages*
Knee and Hip problems Disc degeneration Ligament tears	Partial ossification Partial ligament repair	https://www.villarbajwa.com/**stem-cell-therapy**-hip.shtml https://www.wellmark.com/Provider/MedpoliciesAndAuthorizations/MedicalPolicies/policies/Stem_Cell_Ortho.aspx http://www.observer.com/2016/06/**stem-cells**-andrews-institute/ https://www.regenexx.com/the-regenexx-procedures/knee-surgery-alternative/ https://articles.mercola.com/sites/articles/archive/2017/06/04/stem-cell-therapy-repair-regenerate-body.aspx
Blood transfusion	Increased counts	https://www.cancer.net/navigating-cancer-care/how-cancer-treated/bone-marrowstem-cell-transplantation/what-stem-cell-transplant-bone-marrow-transplant
Back arthritis/ spine disease	Partial ossification	http://www.jfas.org/article/S1067-2516(16)00027-2/fulltext https://www.ncbi.nlm.nih.gov/pubmed/17598490 https://stemcellres.biomedcentral.com/articles/10.1186/scrt81
Pain Treatment	Improved ECM	https://www.stemcellcenters.com/conditions/orthopedic-pain-management/ https://www.stemcellcenters.com/conditions/neuropathy-pain/
Neck arthritis	Partial ligament repair	https://www.stemcellfusion.com/post/stem-cell-therapy-for-neck-arthritis/ https://r3stemcell.com/conditions/shoulder-and-elbow-arthritis/
Shoulder, elbow, and hand problems	-do-	http://sportssurgerychicago.com/alternative-shoulder-replacement-stem-cell-therapy-westchester-oakbrook-hinsdale-il/ www.newyorkhandsurgery.com/services.html
Anti-aging and skin rejuvenation	Better skin looks	http://www.startstemcells.com/anti-aging-treatment-naturally.html
Lupus Erythromatosus		https://www.regen-center.com/lupus.html
Asthma	Better physiological	http://stemcellrevolution.com/currently-studying/pulmonary/asthma/
COPD	improvement	http://www.startstemcells.com/COPD-treatment.html
Crohn's Disease	Abdominal pain relief	http://www.startstemcells.com/crohns-disease-treatment.html
Erectile Dysfunction	Better eraction	https://dasilvainstitute.com/stem-cell-therapy-erectile-dysfunction/
Fibromyalgia	Softer muscle, joints	http://renovationstemcellinstitute.com/stem-cell-treatment-fibromyalgia
Kidney Disease	Improved KFT	https://www.kidneycancer.org/get-information/therapies-for-advanced-kidney-cancer/ www.neural**stem**.com/**cell**-therapy-for-sci https://lozierinstitute.org/written-testimony-of-david-a-prentice-ph-d-in-support-of-sb-334/ www.**medical**newstoday.com/articles/264892.php https://www.stemcellmexico.org/kidney-disease-treatments

Disease/Problems	Benefits claimed	Stem Cell Clinics posted on Internet webpages*
Multiple Sclerosis	Loss in lesions	https://www.placidway.com/package/3477
Parkinson's Disease	Better motor function	http://alsworldwide.org/research-and-trials/category/stem-cells
		http://www.healthyhabitswellness.net/**stem-cell**-regenerative/
		http://www.nymag.com/daily/intelligencer/2015/06/gordie-howe-protocol-stem-cells.html
Osteoarthritis	Slow bone loss	**https://www.hindawi.com/journals/bmri/2014/951512/.pdf**
Osteoporosis	Osteogenesis	https://www.macquariestemcells.com/stem-cell-treatment-for-arthritis/
Psoriatic Arthritis, Neck Arthritis	Ossification	www.drlox.com/
	-do-	https://**treating**pain.com/**treatment**/regenexx-**stem-cell-therapy**
Rheumatoid Arthritis	-do-	www.greensidevet**practice**.co.uk/**stem-cell-therapy**/
Back arthritis and spine disease	Neural recovery	http://ocsportsmed.com/wp-content/uploads/2015/07/trifold.pdf
	Bone repair	http://www.cjonline.com/news/2016-12-03/topeka-physician-adds-adult-derived-stem-cell-treatment-practice
Pain Treatment		https://www.paindoctor.com/Pain_Treatments
Oral Maxillofacial Densistry		http://www.whatisstemcelltherapy.com/Regenerative-Medicine/Regenerative-Medical-Technology
TMJ		https://www.cryo-cell.com/cord-blood-treating-diseases
		www.faim.org/why-cant-we-use-our-own-**stem-cells**
Ulcerative Colitis	Colon repair	https://www.cirm.ca.gov/
		https://www.thenewatlantis.com/publications/appendix-b-the-promise-of-stem-cell-therapies
		http://www.allelebiotech.com/cell-therapy/cell-banking/
Hair loss (in both men and women)	Hair growth	https://www.bioinformant.com/product/stem-cell-fact-sheet/
		https://www.bioinformant.com/product/guide-accelerated-regulatory-pathways/
		http://www.orangecountyhairrestoration.org/stem-cell-therapy-hair-loss-treatment.html
Regenerative medicine	Better wound repir	https://books.google.co.in/books?isbn=111997139X

Table 2. Several stem cell therapy clinics claim the benefits are shown on internet websites* (right click active).

For investigational treatment, consumers need to discuss with doctor to know the potential risks and benefits out of SCBP-based treatment with clear information of mandatory EU or FDA or country approval and regulation for clinical trial study before giving consent to participate in study. Consumers often do not know about SCBP product safety and efficacy outside EU and USA. Consumers should be aware of regulatory authority guidelines and safety, efficacy regulations (risk/benefit evaluation) covering SCBPs in countries before taking decision of treatment in those countries. Safety concerns of SCBPs mainly are: possible cell migration from site of administration to differentiate into inappropriate cell types at unexpected tissue sites, excessive new cell growth and tumor, or cancer development.

In nutshell, there is a mixed claim of stem cell therapy success, because of unfounded theory, trials, misguided treatments, and no clinical established research to justify the therapy

and treatment while federal authorities are plagued by false promises of medical experts under influence of stem cell and tissue engineering product manufacturers. Investigators and researchers have to lot of homework and hard efforts to solve this problem.

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References

[1] Ratajczak MZ, Kucia M, Jadczyk T, et al. Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: Can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? Leukemia. 2012;**26**(6):1166-1173

[2] European Commission. Community Research and Development Information Service. Available from: http://cordis.europa.eu/home_en.html

[3] European Commission. International Cooperation and Development. Available from: http://ec.europa.eu/europeaid/work/funding/beneficiaries/index.cfm?lang=en&mode=SM&type

[4] EUR-LEX. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:en:PDF

[5] European Medicines Agency. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/ general/general_content_000295.jsp

[6] Reproductive Medicine Act Amendment 2004. FmedGNov 2004 [Fortpflanzungsmedizingesetz geändert wird (Fortpflanzungsmedizingesetz-Novelle 2004 – FMedGNov 2004)]. http://www.ris.bka.gv.at/Dokument.wxe?Abfrage=BgblAuth&Dokumentnummer=BGBLA_2004_I_163

[7] Research on human embryonic stem research. Opinion of the Austrian Bioethics Commission. 16 March 2009. Bunderkanzleramt Österriech. Austrian Bioethics Commission. http://www.bka.gv.at/site/3575/default.aspx

[8] Ethical aspects of the development and use of assistive technologies. Opinion of the Austrian Bioethics Commission. 13 July 2009. Bunderkanzleramt Österriech. Austrian Bioethics Commission. http://www.bka.gv.at/DocView.axd?CobId=39411

[9] Collecting stem cells from umbilical cord blood. Ruling of the Bioethics Commission. 16 March 2009. Bunderkanzleramt Österriech. Austrian Bioethics

[10] The Health Act (Promulgated in the State Gazette, Issue No. 70/10 August 2004, entered into force on 1 January 2005). Available from: http://solicitorbulgaria.com/index.php/bulgarian-health-act-part-2

[11] Law on the Transplantation of Organs, Tissues and Cells (Promulgated in the State Gazette, issue No. 83/19 September 2003, entered into force on 1 January 2004) [Закон за Трансплантация на Органи, Тъкани и Клетки (обнародван в ДВ, бр. 83 от 19-ти септември 2003г., в сила от 1-ви януари 2004г.)]. Available from: http://bgtransplant.bg/iat/docs/Law%20on%20transplantation.doc; http://www.mh.government.bg/Articles.aspx?categoryid=2997&lang=bg-BG&pageid=380 (Word download) [Accessed 10 October 2011]

[12] Regulation on the terms and conditions of activity of the central ethics commission under the Council of Ministers in accordance with the Law of Medicinal Products in Human Medicine (adopted 28 September 2007, SG 81/0 October 2007). Available from: http://lex.bg/en/laws/ldoc/2135566887

[13] Bulgarian Centre for Bioethics. Available from: http://www.bio-ethics.net/en/content/mission

[14] Act on Research on Human Embryonic Stem Cells and Related Activities and on Amendment to Some Related Acts [Zákon o výzkumu na lidských embryonálních kmenových buňkách a souvisejících činností a na Změně některých souvisejících zákonů]. Available from: http://aplikace.msmt.cz/PDF/JJHumanstemcells.pdf

[15] Statute of Bioethics Commission. Bioethical Commission of the Czech Research and Development Council. Urad vlády České republiky. http://www.vyzkum.cz/FrontClanek.aspx?idsekce=16247

[16] Act on Medical Research (9.4.1999/488) [Laki lääketieteellisestä tutkimuksesta (9.4.1999/488) Suomi]. Available from: http://www.finlex.fi/fi/laki/alkup/1999/19990488

[17] Finnish National Ethics Committee Report. Opinion on EU research funding on stem cells. 2006. Available from: http;//www.etene.fi/en/materials/opinions/2006

[18] The Act on the Medical Use of Organs and Tissues (2.2.2001/101) [Laki lääketieteellisestä käytöstä elimien ja kudoksien (2.2.2001/101)]. Available from: http://www.finlex.fi/fi/laki/alkup/2001/20010102

[19] Law on Bioethics, Law no. 2004-800 of 6 August 2004 [Loi n. 2004-800 du 6 Août 2004 relative à la bioéthique]. Available from: http://ec.europa.eu/research/biosociety/pdf/french_law.pdf and http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000441469&dateTexte

[20] Law on Bioethics, LOI n° 2011-814 du 7 juillet 2011 relative à la bioéthique. Available from: http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000024323102&fastPos=2&fastReqId=823265692&categorieLien=cid&oldAction=rechTexte

[21] French ConstCouncil. 27 July 1994, 94-343-344 DC. Available from: http://www.conseil-constitutionnel.fr/decision/1994/94343dc.htm

[22] Act ensuring Protection of Embryos in connection with the importation and use of human embryonic stem cells [Gesetz zur Sicherstellung des Embryonenschutzes im Zusammenhang mit Einfuhr und Verwendung menschlicher embryonaler Stammzellen Stammzellgesetz (StZG- Stem Cell Act]. 14 August 2008. Available from: http://bundesrecht.juris.de/stzg/index.html

[23] Central Ethics Commission for Stem Cell Research [Zentrale Ethik-Kommission für Stammzellenforschung - ZES]. https://www.eurostemcell.org/regulation-stem-cell-research-germany

[24] Research on human embryonic stem research. Opinion of the Austrian Bioethics Commission. 16 March 2009. Bunderkanzleramt Österriech. Austrian Bioethics Commission. Available from: http://www.bka.gv.at/site/3575/default.aspx

[25] Ethical aspects of the development and use of assistive technologies. Opinion of the Austrian Bioethics Commission. 13 July 2009. Bunderkanzleramt Österriech. Austrian Bioethics Commission. Available from: http://www.bka.gv.at/DocView.axd?CobId=39411

[26] Law on the Transplantation of Organs, Tissues and Cells (Promulgated in the State Gazette, issue No. 83/19 September 2003, entered into force on 1 January 2004). Available from: http://bgtransplant.bg/iat/docs/Law%20on%20transplantation.doc

[27] Finnish National Ethics Committee. Opinion on EU Research Funding on Stem Cells. The Act on the Medical Use of Organs and Tissues (2.2.2001/101) [Laki lääketieteellisestä käytöstä elimien ja kudoksien (2.2.2001/101)]. Available from: http://www.finlex.fi/fi/laki/alkup/2001/20010102

[28] Law on Bioethics, Law n.2004-800, 6 Aug 2004 [Loi n. 2004-800 du 6 Août 2004 relative à la bioéthique]. Available from: http://ec.europa.eu/research/biosociety/pdf/french_law.pdf; http://www.legifrance.gouv.fr/affichTexte.do?cidTexte=JORFTEXT000000441469&dateTexte

[29] Law 3089/2002 on Medically Assisted Human Reproduction. Chapter Eight: Medically Assisted Human Reproduction. Official Gazette of the Hellenic Republic [3089/2002 νόμος για την ιατρική υποβοήθηση στην ανθρώπινη αναπαραγωγή, Κεφάλαιο Οκτώ: Ιατρική Υποβοήθηση στην Ανθρώπινη Αναπαραγωγή, 23 του Δεκεμβρίου 2002]. 23 December 2002. Available from: http://www.bioethics.gr/media/pdf/biolaw/human/law_3089_en.pdf; http://www.bioethics.gr/media/pdf/biolaw/human/assisted_reproduction_gr.pdf

[30] Law 3305/2005 on Medically Assisted Reproduction Techniques. Official Gazette of the Hellenic Republic [Νόμος 3305/2005 για την ιατρικώς υποβοηθούμενης αναπαραγωγής (ΦΕΚ Α΄17 27.1.2005)]. 27 January 2005. Available from: http://www.nurs.uoa.gr/fileadmin/nurs.uoa.gr/uploads/Nomothesia_Nosilefton/Nomoi/Nomos_3305_FEK_A__172005.pdf

[31] Hellenic National Bioethics Commission. Report on the Use of Stem Cells in Biomedical Research and Clinical Medicine. Available from: http://www.bioethics.gr/media/pdf/reports/stem_cell_report_eng.pdf

[32] Irish Stem Cell Research Foundation. Irish Public Policy and Human Embryonic Stem Cell Research. Irish Stem Cell Research Foundation. April 2010. Available from: http://www.irishstemcellfoundation.org/docs/policy.pdf

[33] Law on Ethics of Biomedical Research No.VIII-1679 of 11 May 2000 (last amended on 15 November 2007 – No. X-1325). Available from: http://www3.lrs.lt/pls/inter3/dokpaieska.showdoc_l?p_id=326057

[34] Lithuanian Bioethics Committee (LBC). Available from: http://bioetika.sam.lt/index.php?-381039724

[35] Law No 32/2006 of 26 July on Medically Assisted Procreation [Lei no. 32/2006 Procriação medicamente assistida de 26 de Joelho]. Available from: http://www.fd.unl.pt/docentes_docs/ma/tpb_MA_4022.pdf

[36] 47/CNECV/05, Opinion N° 47: Opinion on Stem Cell Research. The National Council of Ethics for the Life Sciences [Conselho Nacional de Ética para as Ciências da Vida (CNECV)]. Available from: http://www.cnecv.pt/pareceres.php

[37] Law on Biomedical Research 14/2007 of July 3 [Ley 14/2007, de 3 de julio, de Investigación biomedical]. Madrid: Boletín Oficial del Estado. Available from: http://www.boe.es/boe/dias/2007/07/04/pdfs/A28826-28848.pdf

[38] Guarantees Commission for the Donation and Use of Human Cells and Tissues, established under the following law: Real Decreto 1527/2010, de 15 de noviembre, por el que se regulan la Comisión de Garantías para la Donación y Utilización de Células y Tejidos Humanos y el Registro de Proyectos de Investigación. Available from: http://www.boe.es/buscar/doc.php?id=BOE-A-2010-18654

[39] Opinion on a preliminary law proposal regulating stem cell research, 2003-05-20. Statins Medinksa -Etisk Rad. Swedish National Council of Bioethics. Available from: http://www.smer.se/yttranden/remissyttrande-angaende-sou-2002119-rattslig-reglering-av-stamcellsforskning/

[40] House of Lords Select Committee on Stem Cell Research. 2002. Available from: http://www.parliament.the-stationery-office.co.uk/pa/ld200102/ldselect/ldstem/83/8301.htm

[41] 21CFR Part 1271—Human Cells, Tissues, and Cellular and Tissue Based Products. Cornell University Document. Available from: https://www.law.cornell.edu/cfr/text/21/part-1271

[42] Human Tissue Act. 2004. Available from: http://www.legislation.gov.uk/ukpga/2004/30/contents

[43] Human Fertilisation and Embryology (Research Purposes) Regulations. 2001. Available from: http://www.legislation.gov.uk/uksi/2001/188/pdfs/uksi_20010188_en.pdf

[44] Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Proliferation. 2008;**41**(Suppl 1):S51-S56

Section 2

Mechanisms of Stem Cell Differentiation and Tissue Engineering

Chapter 2

Current View on Hematopoiesis and Beyond

Jiaying Shen, Hongyan Tao and Zongjin Li

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67050

Abstract

Hematopoietic stem cells (HSCs) have the ability to self-renew and give rise to all lineages of blood cells while remain the capacity of regenerative in hematopoiesis. As the only stem cell type in routine clinical use, HSCs can be isolated from bone marrow, peripheral blood and umbilical cord blood. Stem cells transplantation is mainly used in HSCs while the trans-differentiation ability broadens the research of HSCs in regenerative medicine. Here, we focus on the current view on hematopoiesis and beyond and summarize the clinical application and the regulation of the fate of HSCs. We intend to outline recent advances in the human HSCs research area and review the characteristic of HSCs from definition through development to their clinical applications and future prospect.

Keywords: hematopoietic stem cells, stem cell niche, migration, transplantation, regenerative medicine, clinical application, bone marrow, microenvironment, trans-differentiation

1. Introduction

Hematopoietic stem cells (HSCs) identification was confirmed in the 1950s after the first successful transplant was performed by Thomas et al. [1]. This transplantation involved identical twins, one of whom had leukemia. In 1968, the first major landmark in HSCs transplantation occurred with successful allogeneic transplantations [2]. In 1988, Irving Weissman et al. developed reliable methods to identify HSCs population based on a set of protein markers on the surface of mouse blood cells with flow cytometry and fluorescence-activated cell sorting (FACS) [3]. The technologic advances on HSCs researches, FACS and methods for in vitro assays have extended to the whole field of stem cell researches, such as embryonic stem cells, induced pluripotent stem cells, adult stem cells and cancer stem cells, which directly lead to fast forward the translational applications of stem cell. Four years later, Weissman lab

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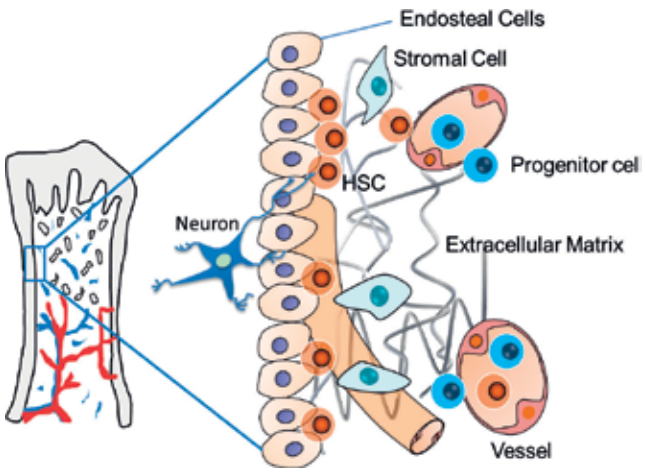

Figure 1. The model of HSCs niche. The hematopoietic niches that hematopoietic stem cells mainly reside are mainly located in the BM during adulthood. They are composed of complex components including HSCs and other functional elements such as vessels, stromal cells, ECM proteins, neural inputs and endothelial cells (ECs). Only through interaction with these components, HSCs can keep self-renewal and differentiation. Under the help of such "blocks," the progenitor cells derived from HSCs locating at the inner surface of BM migrate to blood vessels at the center of the BM cavity when they differentiate into mature blood cells. Reprinted by permission from the publisher.

proposed a comparable set of markers for the human stem cells [4]. Conceptually, human HSCs firstly appear in the earliest embryo, then move to spleen and fetal liver and ultimately migrate to bone marrow (BM) [5]. The specific microenvironments existing within the BM area have been extensively explored, which can host HSCs and other supporting cells, then further organize interaction between cells and cells and cells and growth factors in order to sustain specific aspects of hematopoiesis, such as HSCs survival, self-renewal and differentiation [6]. These special hematopoietic microenvironments are also termed as "Niche," which was first proposed by Schofield in 1978 and significant progresses have gained on hematopoietic niche [7]. Currently, it is gradually established that the main work of hematopoietic microenvironments is undertaking to support hematopoiesis (**Figure 1**). In general, HSCs are mainly maintained in BM microenvironment, which can regulate the proliferation, differentiation and mobilization of HSCs. Nonetheless, said developments in this field will throw light on hematopoietic diseases such as leukemia and aplastic anemia, rapid hematopoietic recovery after chemotherapy or shortening the time for hematopoietic reconstruction after HSCs transplantation.

2. HSCs transplantation

HSCs transplantation is most often performed for patients with malignant blood diseases, such as leukemia and multiple myeloma, or aplastic anemia, inherited blood disorders and many others. The procedure of HSCs transplantation has four steps including cell collection, patient preparation, cells transplantation and recovery. Allogeneic and autologous are the

two main types of HSCs transplantation. Allogeneic HSCs are derived from a matched donor, whereas autologous HSCs are isolated from the patients. After cell collection, patients need chemotherapy, with or without radiation to destroy the hematopoietic function and make space for the transplant. Transplantation will be proceeded with intravenous injection after patient preparation. Recovery after cells transplant can last weeks to months.

However, HSCs transplantation remains a dangerous procedure with many possible complications; it is reserved for patients with life-threatening diseases. Infection and graft-versus-host disease (GVHD) are major complications of allogeneic HSCs transplantation. A major challenge in improving the success of allogeneic hematopoietic stem cells in the treatment of leukemia is to minimize GVHD reactions and simultaneously optimize graft-versus-leukemia (GVL) reactions. Therefore, it remains to see the era of predicting biological behavior based on the knowledge of molecular mechanisms in HSCs transplantation, and how host responds to the transplanted HSCs.

3. Migration of HSCs into and out of marrow and tissues

The progress of stem cell research, along with technological innovation, has brought researchers to focus on the potential role of stem cells in regenerative medicine. Bone marrow-derived stem cells, including HSCs, can potentially restore the function of diseased or damaged tissues/organs, offering significant potential for regenerative medicine [8]. Moreover, trans-differentiation or plasticity of HSCs in repair or rejuvenation of tissues and organs have drawn focused attention [9]. It has been proposed that HSCs continue to migrate via the blood stream throughout adulthood. Continuous migration of HSCs among the organs and circulation likely fill the empty or damaged niches and contribute to the maintenance of normal organ functions and restoring degraded tissues [5, 6, 8–10] (**Figure 2**). Both tissue repair and regeneration are thought to involve resident cell proliferation as well as the selective recruitment of circulating HSCs [11]. Moreover, differentiation of HSCs into cardiomyocytes also give rise to thoughts that the HSCs population is critical for myocardium homeostasis and these repair progress after injury via reprogramming the phenotype of HSCs to induce cardiomyocyte renewal [9]. Furthermore, other HSCs transplantation models have shown that HSCs could transdifferentiate into liver, brain, skeletal muscle, kidney, intestine and many others [9]. Several studies have shown a constant exchange of HSCs between bone and peripheral blood and it has been estimated that up to 400 HSCs circulate in the blood of a mouse at any one time [12] (**Figure 2**). The efficiency of BM cell-based therapy to augment the recovery from damaged tissues depends on not only sufficient amount of stem cells but also efficient delivery of these cells to the desired target tissue. HSCs are unique in their ability to migrate to various sites, ensuring the safety and integrity of their regenerative potential.

Currently, there are some clinical trials about bone marrow-derived stem cells in clinical trial (*clinicaltrials.gov*). A number of trials are focused on the safety and efficacy of autologous/allogeneic stem cell transplantation for treatment of a diverse array of diseases (**Table 1**). The results of these trials have confirmed that HSCs injection is safe and has the capability

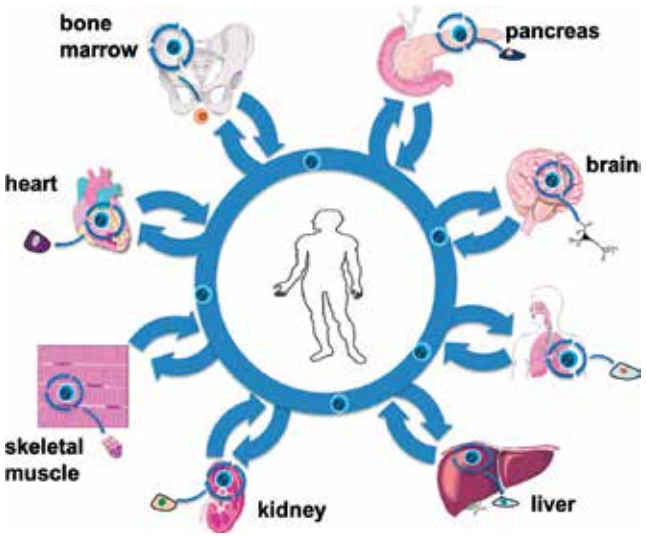

Figure 2. Maintenance and trafficking of HSCs in various tissues and organs. Besides the initial findings that HSCs are located in bone marrow, this extremely rare population of cells is also detected in other adult tissues (e.g. brain, kidney, skeletal muscle and pancreas). HSCs could be a potential back-up source for restoring the function of diseased or damaged tissues/organs. Reprinted by permission from the publisher.

Clinical study	Study phase	Source	Procedure	Year	Disease	Reference
NCT01183728	I, II	Autologous	Articular injection	2014	Knee osteoarthritis	[18]
NCT01775774	I	Allogeneic	Intravenous injection	2015	Acute respiratory distress syndrome	[19]
NCT00768066	I, II	Autologous	Transendocardial injection	2013	Ischemic heart failure	[20]
NCT00629018	II	Autologous	Intracoronary injection	2013	Dilated cardiomyopathy	[21]
NCT01363401	I, II	Autologous	Intrathecal injection	2013	Amyotrophic lateral sclerosis	[22]

Table 1. Completed bone marrow-derived stem cell-based clinical trials in regenerative medicine registered at https://clinicaltrials.gov/

to improve target tissue/organ function. However, the number of trafficking HSCs in blood stream is at an extremely low level [13]. Promoting BM stem cell mobilization is a common strategy to augment the cellular yield of peripheral blood apheresis for clinical stem cell transplantation, and a similar approach has been suggested to increase the number of circulating cells available for homing to the damaged sites following injury [10–14]. The self-renewal and trans-differentiation capacities of BM stem cells are worthless unless their migration to target tissues can be appropriately orchestrated [15].

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4. Present state of art and limitations

Allogeneic and autologous HSCs transplantation is mainly used in in the treatment of hematologic and genetic conditions. According to the data collected by Worldwide Network for Blood & Marrow Transplantation (WBMT), 953,651 HSC transplantations (553,350 [58%] autologous and 400,301 [42%] allogeneic) were reported by 1516 transplant centers from 75 countries [16] between January 1, 2006 and December 31, 2014. The use of HSC transplantations increased from the first transplant in 1957 to almost 1 million by December, 2012. This result suggests an increasing need of HSCs in clinical application. However, there are a number of challenges and limitations still confront the clinical application of HSC transplantation, such as limited HSC numbers in donor grafts and availability of HLA-matched donors. Besides, many complications such as infection, veno-occlusive disease (VOD) and GVHD after HSCs transplantation also influence quality of the patients' life, which limits its use to conditions that are themselves life-threatening. The challenges in the HSCs transplantation field are big for increasing cell survival rate after engraftment and decreasing major complications associated with a high treatment-related mortality in the recipient including infections (sepsis), GVHD and the development of new malignancies.

5. Future perspectives

HSCs are the best characterized adult stem cell type, which are mainly used in hematological disease. Several studies on HSCs biology and transplantation in their clinical therapy. Over recent years, it came as a great surprise that several models had shown that the trans-differentiation of HSCs may cross germ-layer boundaries and differentiate into some mature cells from different layer, which suggest that somatic stem cells, including HSCs, may serve as a cell source for tissue engineering or cell therapy [17]. Besides, induced pluripotent stem (iPS) cell-derived HSCs could be an ideal source for resolving current limitations related to HSCs transplantation. The use of iPS cells to generate HSCs is of considerable therapeutic interest, as traditional HSCs transplantation is limited by the lack of compatible donors, a high risk of engraftment failure and GVHD. In conclusion, these efforts could expand the use of HSCs in several different diseases, reduce the incidence of complications and increase the size of the beneficiary populations.

6. Conclusion

In conclusion, HSCs are somatic stem cells, which are vital in cell-based clinical application. After years of research, HSCs transplantation and therapy from an experimental concept to a safe clinical cure for numbers of diseases. With further study in the trans-differentiation and niche of HSCs, there is no doubt that HSCs have great prospects for application in stem cell transplantation and regenerative medicine.

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References

[1] Thomas ED, Lochte HL Jr, Cannon JH, Sahler OD, Ferrebee JW. Supralethal whole body irradiation and isologous marrow transplantation in man. The Journal of Clinical Investigation. 1959;**38**:1709-1716

[2] Eaves CJ. Hematopoietic stem cells: Concepts, definitions, and the new reality. Blood. 2015;**125**:2605-2613

[3] Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science (New York), N.Y. 1988;**241**:58-62

[4] Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. Proceedings of the National Academy of Sciences of the United States of America. 1992;**89**:2804-2808

[5] Feng G et al. Identification, characterization and biological significance of very small embryonic-like stem cells (VSELs) in regenerative medicine. Histology and Histopathology. 2012;**27**:827-833

[6] He N, Zhang L, Cui J, Li Z. Bone marrow vascular niche: Home for hematopoietic stem cells. Bone Marrow Research. 2014;**2014**:128436

[7] Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1978;**4**:7-25

[8] Voltarelli JC et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2007;**297**:1568-1576

[9] Liu N et al. Bone marrow is a reservoir for cardiac resident stem cells. Scientific Reports. 2016;**6**:28739

[10] Jia X et al. Bone marrow-derived cells can acquire renal stem cells properties and ameliorate ischemia-reperfusion induced acute renal injury. BMC Nephrology. 2012;**13**:105

[11] Rennert RC, Sorkin M, Garg RK, Gurtner GC. Stem cell recruitment after injury: Lessons for regenerative medicine. Regenerative Medicine. 2012;**7**:833-850

[12] Mazo IB, Massberg S, von Andrian UH. Hematopoietic stem and progenitor cell trafficking. Trends in Immunology. 2011;**32**:493-503

[13] Garbern JC, Lee RT. Cardiac stem cell therapy and the promise of heart regeneration. Cell Stem Cell. 2013;**12**:689-698

[14] Feng G et al. The phenotypic fate of bone marrow-derived stem cells in acute kidney injury. Cellular Physiology and Biochemistry. 2013;**32**:1517-1527

[15] Smart N, Riley PR. The stem cell movement. Circulation Research. 2008;**102**:1155-1168

[16] Gratwohl A et al. One million haemopoietic stem-cell transplants: A retrospective observational study. The Lancet. Haematology. 2015;**2**:e91-e100

[17] Sellamuthu S et al. In vitro trans-differentiation of human umbilical cord derived hematopoietic stem cells into hepatocyte like cells using combination of growth factors for cell based therapy. Cytotechnology. 2011;**63**:259-268

[18] Orozco L et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: Two-year follow-up results. Transplantation. 2014;**97**:e66-e68

[19] Wilson JG et al. Mesenchymal stem (stromal) cells for treatment of ARDS: A phase 1 clinical trial. The Lancet. Respiratory medicine. 2015;**3**:24-32

[20] Heldman AW et al. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: The TAC-HFT randomized trial. JAMA. 2014;**311**:62-73

[21] Vrtovec B et al. Effects of intracoronary stem cell transplantation in patients with dilated cardiomyopathy. Journal of Cardiac Failure. 2011;**17**:272-281

[22] Oh KW et al. Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. Stem Cells Translational Medicine. 2015;**4**:590-597

Chapter 3

Stem Cell Aging

Primož Rožman, Katerina Jazbec and Mojca Jež

Additional information is available at the end of the chapter

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Abstract

Stem cells persist throughout life, replacing cells lost to homeostatic turnover, injury, and disease. However, their functions decline with age, which contributes to degeneration and dysfunction. The molecular mechanisms involved in the aging of stem cells are the same as the ones involved in the aging of somatic cells, including telomere shortening, oxidative stress, epigenetic dysregulation, miRNAs changes, alterations of DNA, RNA, proteome, and various cellular organelles. Aging impacts various pathways, such as insulin/insulin-like growth factor 1 (IGF-1), mTOR, FoxO, AMP-activated protein kinase (AMPK), sirtuin, and many others, resulting in senescent stem cells that exhibit functional and numerical impairment. Stem cells have developed special mechanisms to prevent age related damage accumulation and to sustain their stemness properties, however, these mechanisms lose their effectiveness over time. The most fatal consequence of this is found in the immune system, where both innate and adaptive immunity are affected, exhibiting a plethora of defects, including increased autoimmune disease occurrence, elevated tolerance to cancer and chronic inflammatory status. Stem cell therapies call for the best quality of stem cells grafts. Stem cell products should be devoid of cells containing a senescent phenotype, thus a comprehensive knowledge of the biology behind the senescence of stem cells should be taken into account in every cell based therapy.

Keywords: molecular mechanisms of aging, senescence, stem cell niche, epigenetic changes, telomere attrition, stem cell pool, mitochondrial changes, proteostasis, immune deterioration, shortened life span

1. Introduction – Stem cells aid regeneration and longevity

An 70 kg adult human body consists of approximately 3.72×10^{13} cells [1]. These trillions of cells are not permanent and a majority of them are constantly renewed throughout our lifetime, although some of them – such as cells in the lenses of our eyes and some of the neurons

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of our central nervous system – are thought to be an exception. The frequency of renewal depends on the function of the cells and may vary from several hours to several years. A collection of the replacement rates of different cells in our body is given in **Table 1**.

The renewal of adult tissues is enabled by specialized cells that function over the lifetime of an organism, i.e., the stem cells (SCs). They persist throughout life in numerous mammalian

Cell type	Turnover time
Small intestine epithelium	2–4 days
Stomach	2–9 days
Blood neutrophils	1-5 days
White blood cells eosinophils	2-5 days
Gastrointestinal colon crypt cells	3–4 days
Cervix	6 days
Lungs alveoli	8 days
Tongue taste buds (rat)	10 days
Platelets	10 days
Bone osteoclasts	2 weeks
Intestine Paneth cells	20 days
Skin epidermis cells	10–30 days
Pancreas beta cells (rat)	20–50 days
Blood B cells	4–7 weeks
Trachea	1–2 months
Hematopoietic stem cells	2 months
Sperm (male gametes)	2 months
Bone osteoblasts	3 months
Red blood cells	4 months
Liver hepatocyte cells	0.5–1 year
Fat cells	8 years
Cardiomyocytes	0.5–10% per year
Central nervous system	life time
Skeleton	10% per year
Lens cells	life time
Oocytes (female gametes)	life time

Adapted from: http://bionumbers.hms.harvard.edu/bionumber.aspx?&id=107875

Table 1. Cell renewal rates in different tissues of the human body.

tissues, replacing cells lost to homeostatic turnover, injury, and disease. Stem cells reside in specific anatomic reservoirs, such as bone marrow, and circulate in the organism when needed. SCs represent a very small proportion in adult tissues. It is estimated that the bone marrow of a 70 kg adult human contains around $1.5–1.7 \times 10^{12}$ cells, among them only $45–120 \times 10^{6}$ are true hematopoietic stem cells (HSCs) that give rise to more frequent progenitors (Jazbec et al. 2017, submitted). The frequencies of stem cells in other tissues are even lower and still a matter of debate.

Adult SCs can typically self-renew and differentiate into multiple cell types within a developing and adult body. Due to their self-renewal capacity they were regarded as immortal reservoirs of youth, however, they are nonetheless susceptible to the age related damages. To prevent or reverse the accumulation of age related damage and epigenetic changes, SCs developed special mechanisms to maintain long telomeres, enhance proteostasis, avoid ROS production and defend against toxic substances. In spite of that, their functions decline with age in a number of tissues, including blood, forebrain, skeletal muscle, skin and all the other tissues as reviewed by Schultz et al. [2].

Declines in stem cell functions not only contribute to degeneration and dysfunction of aging tissues, but also negatively affect the life span of the organism [3, 4]. Some strong evidence for SCs as regulators of longevity comes from animal studies. For instance, if in *C. elegans* germline stem cells (GSCs) are eliminated, this almost doubles its lifespan [5] and such a phenomenon is highly conserved [6]. Similarly, if the fruit flies are modified with overexpression of a PGC-1α homolog or a heat-shock response transcription factor and moderate repression of insulin/IGF or JNK signaling, this directly extends their life span [7], implying that improved stem cell function leads to better tissue function, and that stem cell aging underlies the aging of tissues and organs.

In humans, there is considerable evidence supporting the fact that young stem cells perform better than old ones. Proof of this concept is best documented is the recent multicenter study on the success of hematopoietic stem cell transplantation, which is currently the most popular and efficient cell therapy for malignant diseases. In more than 6000 cases of allogeneic bone marrow transplantation between 2007 and 2011, it was clearly shown that patient survival was significantly better after grafts from young donors (aged 18–32 years) were used. For every 10-year increment in donor age, there was a 5.5% increase in the hazard ratio for overall mortality [8]. This is probably one of the most important findings in this field, suggesting that for regenerative purposes, and other stem cell therapies, grafted stem cells should be young and devoid of senescent defects.

2. The biology of stem cell aging

Adult stem cells express several characteristic features that are specific to stem cells, as well as certain features that are found in any other somatic cell in the body. They express telomerase – an enzyme required for telomere extension that is essential for repeated self-renewal, they cycle between phases of quiescence and activation needed for the production

of progeny, their chromatin exists in a bivalent state primed for self-renewal or differentiation, they have unique metabolic requirements, they distribute their macromolecules asymmetrically during asymmetric cell divisions, and they reside in niches that regulate their behavior [9].

The molecular mechanisms that are involved in the aging of adult stem cells are the same as the ones involved in the aging of the somatic cells. Traits and mechanisms that are affected by aging are present in various populations of stem cells. The age-related decline of stem cells is mainly functional, but in some cases, a decline in stem cell numbers can also be observed. Since many of these mechanisms appear simultaneously, it is practically impossible to trace or determine a single initial damaging agent that causes the cascade of other detrimental sequences. Therefore authors agree that aging is probably the result of multifactorial derangements caused by several causative factors that act in parallel, including the formation of damaging reactive oxygen species (ROS), telomere attrition, DNA damage and mutations, epigenetic changes (alterations of histones, DNA and the consequent dysregulation of gene expression), mitochondrial DNA mutations with mitochondrial decline, changes of microRNAs, ribosomal changes and defects of RNA splicing, changes of proteostasis, changes in cellular polarity, changes in nutrient sensing and metabolism, niche deterioration, improper accumulation of various circulating factors, stem cell pool exhaustion, cellular senescence with cell cycle arrest, and altered intercellular communication (**Table 2**).

1. Formation of damaging reactive oxygen species (ROS)
2. Mitochondrial DNA mutations, decline of mitochondrial integrity and biogenesis
3. Nuclear damage and nuclear DNA mutations
4. Telomere shortening /attrition
5. Epigenetic changes/alterations of histones and DNA and consequent dysregulation of gene expression
6. Changes of microRNAs
7. Changes of RNA splicing and ribosomal machinery
8. Changes of proteostasis
9. Changes of cell polarity
10. Metabolism and nutrient sensing
11. Niche deterioration
12. Accumulation of various circulating factors
13. Stem cell pool exhaustion
14. Cellular senescence – arrest of the cell cycle
15. Altered intercellular communication

Table 2. Multifactorial causes of stem cell aging.

2.1. Formation of damaging reactive oxygen species (ROS) and oxidative stress

The free radical theory of aging has been long accepted as the most plausible explanation for the aging process. It was first formulated in the 1950s by Harman who hypothesized that an accumulation of endogenous oxygen radicals (reactive oxygen species, or ROS) occurs, which in turn causes further mitochondrial deterioration and the global cellular damage responsible for the aging and death of all living beings [10]. This theory was then revised in 1972 when mitochondria were identified as being responsible for the initiation of most of the free radical reactions [11]. It was also postulated that life span was determined by the rate of free radical damage to the mitochondria. Mitochondrial respiration, the basis of energy production in all eukaryotes, generates ROS by leaking intermediates from the electron transport chain [12]. In all aerobic organisms, age-related oxidative stress is generated either by exposure to endogenous metabolites or exogenous sources such as radiation (UV, X-ray), and ROS accumulation is the result of an imbalance between free radical production and antioxidant defenses, such as superoxide dismutase that is responsible for scavenging superoxide anions [12, 13]. In fact, oxidative modifications have been shown to occur in DNA, protein, and lipid molecules [14].

Whereas young stem cells contain a spectrum of antioxidant mechanisms, aged stem cells display an inadequate anti-oxidant defense that is associated with functional impairment, including decreased responsiveness to physical environmental cues and decreased resistance to oxidative stress [15]. In several studies, aging stem cells from bone marrow and adipose tissue showed a significantly reduced capacity for coping with oxidative stress with increasing donor age [16, 17]. Therefore, oxidative stress is still recognized as the fundamental underlying component of the aging process, leading to dysregulation of various cellular pathways and the subsequent accumulation of toxic aggregates and cellular debris, and ultimately to the activation of cell death/survival pathways leading to apoptosis, necrosis, or autophagy, as reviewed by Haines, et al. [18].

However, recent developments have forced an intense re-evaluation of the mitochondrial free radical theory of aging after the unexpected observation that increased ROS may paradoxically prolong the lifespan of yeast and *Caenorhabditis elegans* [19–21]. In mice, genetic manipulations, which increased mitochondrial ROS and oxidative damage, did not accelerate aging as one would expect [22, 23]. Furthermore, manipulations that increased antioxidant defenses did not extend longevity [24], and lastly, genetic manipulations that impaired mitochondrial function but did not increase ROS, accelerated aging [25, 26]. There has also been other solid evidence that in response to physiological signals and stress conditions, ROS triggered proliferative and survival signals [27].

The mitochondrial theory of aging has also been challenged as it has become clear that there exists a rather complicated interplay between various other cellular compartments [28]. Dysfunctional mitochondria can contribute to aging independently of ROS, as demonstrated by studies with mice deficient in DNA polymerase γ [29, 30]. This could happen through a number of mechanisms, for example, mitochondrial deficiencies may affect apoptotic signaling by increasing the propensity of cell's death through mitochondrial membrane

permeabilization in response to stress [31], and trigger inflammatory reactions by favoring ROS-mediated and/or permeabilization- facilitated activation of inflammasomes [32]. Also, mitochondrial dysfunction may directly impact cellular signaling and interorganellar crosstalk, by affecting mitochondrion-associated membranes that constitute an interface between the outer mitochondrial membrane and the endoplasmic reticulum [33].

The mitochondrial ROS that were considered the main cause of age related defects actually contribute positively to various signaling pathways and normal cellular responses, such as adaptation to hypoxia, cellular differentiation, autophagy, inflammation, and immune responses, as reviewed recently [28, 34], meaning that ROS are also beneficial for cellular biology.

2.2. Mitochondrial DNA mutations, the decline of mitochondrial integrity and biogenesis

Mitochondrial function has a profound impact on the aging process. Mitochondrial dysfunction can accelerate aging in mammals. It was generally believed that age-related pathology was caused by defects of mitochondria related to oxidative stress, leading to the accumulation of irreparable changes of nucleic acids, proteins, and lipid molecules [14, 35]. But there are also other defects of mitochondria that develop during normal aging. Similar to the nuclear DNA, mitochondrial DNA (mtDNA) is exposed to mutations and deletions in aged cells, which are not found in nuclear DNA, and which also contribute to aging [36]. This is aggravated by the oxidative microenvironment of the mitochondria and the limited efficiency of the mtDNA repair mechanisms [37].

The mutations that can lead to mitochondrial dysfunction and death are now detectable in generated induced pluripotent stem cell (iPSC) lines, i.e., expanded clones from each individual skin or blood cell. As a result, every cell in the iPSC line contains the same mitochondrial DNA (mtDNA) mutations as the original adult cell, and can for this reason be easily sequenced. We now know that to ensure healthy mitochondrial genes, we must screen stem cells for mutations or collect them at a younger age. This may help illuminate the role of mutated mitochondria in degenerative diseases and to assess the patient-derived regenerative products destined for clinical applications [38].

Interestingly, most mtDNA mutations in adult or aged cells appear to be caused by replication errors early in life, rather than by oxidative damage. These mutations may undergo polyclonal expansion and cause respiratory chain dysfunction in different tissues [39]. Studies of accelerated aging in HIV-infected patients treated with anti-retroviral drugs, which interfere with mtDNA replication, have supported the concept of clonal expansion of mtDNA mutations that originated early in life [40].

Aging also affects the biogenesis of mitochondria. Mitochondrial biogenesis is the process by which cells increase their individual mitochondrial mass and copy their number to increase the production of ATP, as a response to greater energy needs. With aging, the reduced efficiency of mitochondrial bioenergetics may be a result of multiple converging mechanisms,

including reduced biogenesis of mitochondria. For instance, in telomerase-deficient mice, it can be a consequence of telomere attrition with subsequent p53-mediated repression of PGC-1α and PGC-1β (peroxisome proliferator-activated receptor gamma coactivator 1 – alpha and –beta, which are the master regulators of mitochondrial biogenesis) [41]. This mitochondrial decline also occurs during physiological aging in wild-type mice and can be partially reversed by telomerase activation [42]. Sirtuin 1 (SIRT1) modulates mitochondrial biogenesis through a process involving the transcriptional co-activator PGC-1α [43] and the removal of damaged mitochondria by autophagy [44]. SIRT3, which is the main mitochondrial deacetylase [45], targets many enzymes involved in energy metabolism, including components of the respiratory chain, tricarboxylic acid cycle, ketogenesis and fatty acid β-oxidation pathways [46]. SIRT3 may also directly control the rate of ROS production by deacetylating manganese superoxide dismutase, a major mitochondrial antioxidant enzyme [47]. Collectively, these results support the idea that sirtuins may act as metabolic sensors to control mitochondrial function and play a protective role against age-associated diseases [48, 49].

Interestingly, endurance training and alternate-day-fasting may improve healthspan through the capacity to avoid mitochondrial degeneration [50, 51]. It is tempting to speculate that these beneficial effects are mediated, at least in part, through the induction of autophagy, for which both endurance training and fasting constitute potent triggers [52]. However, autophagy induction is probably not the sole mechanism through which a healthy lifestyle can retard aging, since, depending on the precise diet reduction regime, additional longevity pathways can be activated [53].

The combination of increased damage and reduced turnover in mitochondria, due to lower biogenesis and reduced clearance, may contribute to the aging process [48]. Some other mechanisms can also affect the mitochondrial bioenergetics and contribute to the aging mitochondrial phenotype, among them the mutations and deletions in mtDNA, oxidation of mitochondrial proteins, destabilization of the macromolecular organization of respiratory chain, defects of the lipid membranes, and defective autophagy that targets deficient mitochondria [54].

In conclusion we could say that the importance of mitochondria in the basic biology of aging and the pathogenesis of age-associated diseases is stronger than ever, although the emphasis has moved from ROS to other causative aspects. Obviously, besides the mitochondrial dysfunction due to ROS, there exists a complex interplay of several other factors of aging, such as mDNA mutations, changes of lysosome processing, endoplasmic reticulum stress, genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, altered intercellular communication, mitochondrial biogenesis and turnover, energy sensing, apoptosis, senescence, and calcium dynamics. Mitochondria do play one of the key roles in the pathophysiology of aging and events that lead to the aged phenotype, therefore they will increasingly be targeted to prevent and treat chronic diseases and to promote healthy aging [48, 55, 56]. We expect that future studies will determine whether genetic manipulations that decrease the load of mtDNA mutations and other damaging factors, are able to extend lifespan.

2.3. Nuclear damage and nuclear DNA mutations

It is clear that in aged humans and model organisms, somatic mutations accumulate over time within all cells [57]. Other forms of DNA damage, such as chromosomal aneuploidies, copy-number variations and increased clonal mosaicism for large chromosomal anomalies have also been found to be associated with aging [58, 59]. Each time a stem cell replicates its DNA and divides, the likelihood of DNA defects and oncogenic transformations increases. Therefore the lifetime risk of cancer development in a tissue correlates with the number of divisions the stem cells of this particular tissue have undergone [60]. A variety of these DNA alterations can finally affect the essential genes that control the key transcriptional pathways. Such defect cells should be normally eliminated by apoptosis or senescence, however, if this does not happen it may jeopardize tissue and organismal homeostasis. This is especially important in stem cells because the DNA damage has a detrimental impact on their functional competence, i.e., on their role in tissue renewal [61, 62].

An accumulation in DNA damage and mutations leading to stem cell aging has been one of the earliest theories of aging [63]. DNA damage can be caused by external factors (ionizing radiation, ultraviolet radiation or environmental toxins), or by internal factors (ROS and errors in DNA replication). These factors can lead to various DNA lesions such as modifications of bases or sugar residues, the formation of DNA adducts, cross-linking of the DNA strands or the appearance of single and double-strand breaks. Among these lesions, DNA double-strand breaks (DSBs) are particularly lethal because they result in physical cleavage of the DNA backbone. DSBs can occur through replication fork collapse, during the processing of interstrand crosslinks, or following exposure to ionizing radiation [64, 65]. In spite of the fact that cells have evolved at least six different DNA repair pathways to deal with these distinct types of DNA damage [66], there is convincing evidence that with aging, stem cell DNA is also subject to damage. In HSCs, histone H2AX phosphorylation and comet tails, both of which are measures of DNA damage, increase with age [67, 68]. Phosphorylation of *H2AX*, one of several genes coding for histone H2A (one of the five main histone proteins involved in the structure of chromatin in eukaryotic cells), accumulates with age in satellite cells, i.e., stem cells of the muscles [69]. Moreover, aged HSCs display a history of replication stress and decreased expression of DNA helicases, further sensitizing them to future replication challenges [70].

Since mutations are a common daily occurrence, our cells could not survive without DNA repair mechanisms. There are two groups of repairing mechanisms, the first acting to repair DNA single-strand breaks (mismatch repair mechanism, base excision repair mechanism, nucleotide excision mechanism), and the second acting to synchronously repair DNA double-strand breaks, i.e. homologous recombination and non-homologous end joining (NHEJ). In spite of the repair mechanisms our DNA accumulates mutations, since the genes of repair mechanisms are themselves subject to mutations [71].

Evidence that DNA damage plays a causal role in the aging process includes the observation that mice with defects in DNA damage repair display some aspects of premature aging [72], whereas enhancing DNA repair through increased expression of *SIRT6* increases lifespan [73]. In some situations, DNA damage may also reduce stem cell numbers by causing them

to undergo apoptosis, senescence or differentiation, although it is not yet confirmed whether these effects are due to an increase in stem cell longevity [2].

It is also known that deficiencies in DNA repair mechanisms cause accelerated aging in mice and underlie several human progeroid syndromes such as Werner syndrome, Bloom syndrome, xeroderma pigmentosum, trichothiodystrophy, Cockayne syndrome, or Seckel syndrome [74–76]. Moreover, transgenic mice overexpressing multidomain protein kinase BUBR1 (budding uninhibited by benzimidazole-related 1), a mitotic checkpoint component that ensures the accurate segregation of chromosomes, exhibit an increased protection against aneuploidy and cancer, and display an extended healthy lifespan [77]. These experimental data prove that artificial reinforcement of nuclear DNA repair mechanisms could delay aging [48].

In addition to genomic damage affecting nuclear or mitochondrial DNA, there is evidence that certain defects in the nuclear lamina can also change nuclear architecture and thereby cause genomic instability [78]. Nuclear lamins participate in genome maintenance by providing a scaffold for tethering chromatin and protein complexes that regulate genomic stability [79, 80]. Mutations in genes encoding protein components of this structure, or factors affecting their maturation and dynamics, cause accelerated aging syndromes such as the Hutchinson-Gilford and the Néstor-Guillermo progeria syndromes [81–83]. Alterations of the nuclear lamina and production of an aberrant prelamin A isoform called progerin have also been detected during normal human aging [84]. Since telomere dysfunction also promotes progerin production in normal human fibroblasts upon prolonged *in vitro* culture, this suggests that there exist intimate links between telomere maintenance and progerin expression during normal aging [85]. In addition to these age-associated changes in A-type lamins, lamin B1 levels decline during cell senescence, pointing to its utility as a biomarker of this process [86].

2.4. Telomere shortening

Although accumulation of DNA damage affects the genome near-to-randomly, there are some chromosomal regions that are particularly susceptible to age-related deterioration [87]. Telomeres are repetitive TTAGGG sequences and associated nucleoproteins at the ends of a chromosome that play a critical role in protecting chromosomes from degradation, undesirable recombination, and chromosome fusion [88, 89]. With each somatic cell division, telomeres shorten and this exposes cells to the aging phenotype. Due to inability of the normal DNA replication machinery to completely replicate the telomeric sequences, telomeres in somatic cells shorten with each cell division, and are thus markers for cellular aging and replicative capacity [90].

Mice with shortened or lengthened telomeres exhibit decreased or increased lifespans, respectively [91–93]. Telomere shortening is observed during normal aging both in humans and mice [94]. In humans, recent meta-analyses have indicated a strong relation between short telomeres and mortality risk, particularly at younger ages [95].

In contrast to somatic cells, embryonic and adult stem cells express telomerase, a reverse transcriptase enzyme, which catalyzes the extension of telomeric sequences, thereby avoiding telomere attrition and prolonging cellular proliferative life span. While the telomerases are normally absent from most somatic cells, they are active in the stem cells and most cancer

cells [88, 96]. Mammalian telomerase consists of a telomerase RNA component (TERC) and a telomerase reverse transcriptase (TERT) component. The latter catalyzes the synthesis of new telomeric repeats. Nevertheless, consistent decline in telomere length with age does occur in adult stem cells, suggesting that telomerase activity is insufficient to maintain the replication of these cells indefinitely [90]. So the telomeres of various stem cells, i.e., hematopoietic, neural, germinal and other, do shorten with age [97, 98].

Telomere exhaustion also explains the limited proliferative capacity of some types of *in vitro* cultured cells, the so-called replicative senescence or Hayflick limit [99, 100]. Indeed, as shown already in the 1990s, ectopic expression of telomerase confers immortality to otherwise mortal cells, without causing oncogenic transformation [101]. Similarly, telomerase deficiency in humans is associated with the premature development of diseases, such as pulmonary fibrosis, dyskeratosis congenita and aplastic anemia, which involve the loss of the regenerative capacity of different tissues [102].

Recent evidence also indicates that aging can be reverted by telomerase activation. In particular, the premature aging of telomerase-deficient mice can be reverted when telomerase is genetically reactivated in these aged mice [103]. Moreover, normal physiological aging can be delayed without increasing the incidence of cancer in adult wild-type mice by pharmacological activation or systemic viral transduction of telomerase [104].

This correlation between telomere length, telomerase activity and age is not completely clear. For example, while telomere length is negatively correlated with age in humans up to 75 years, it is positively correlated with age in the elderly, suggesting that long telomeres contribute to survival in old age [105]. Furthermore, telomere length predicted survival in elderly twins, suggesting that telomeres contribute to longevity in humans even when controlling for the influence of genetic background [106].

There is a good correlation between the expression of human TERT mRNA and the presence of telomerase activity in extracts from tissue culture cells, and normal and cancer tissues, suggesting that human TERT expression is the primary and rate-limiting determinant of telomerase activity [107]. This is important in stem cell therapies, so we have already investigated the importance of longer telomere length of the CD34+ cell grafts used for cell therapy and found that longer telomere length and higher telomerase expression agree with CD34+ cell's increased functional capacity, however the patients with longer CD34+ telomere length did not favorably respond to autologous CD34+ cell transplantation therapy [108].

2.5. Epigenetic changes and consequent dysregulation of gene expression

The regulation of the chromatin state is important for stem cell function. In Waddington's epigenetic landscape theory, stem cells stand at an undifferentiated epigenetic summit above multiple cell fates [109]. During the differentiation and aging of cells, numerous chromatin and gene expression changes appear progressively in response to cell stress, most notably in response to DNA damage signals. The changes in epigenetic modification of chromatin and histones lead to dysregulation of gene expression. The epigenetic modifications that are observed to change during aging are histone acetylation, histone methylation, and DNA methylation.

As already mentioned, of all other different types of DNA damage, the one that has the greatest lasting effect on chromatin is the double-strand breaks, which cause a dramatic redistribution of chromatin factors. This is a part of the response to damage that is not fully restored after the repair [110]. Thus, changes in chromatin caused by DNA damage might underlie the skewed lineage phenotypes exhibited by aged stem cells [111].

The epigenetic changes have now been cataloged. In mice, it has been observed that the level of histone deacetylase SIRT1 decreases with age and that decrease of SIRT1 expression correlates with premature aging in mice with increased p53 activity [112].

The expression levels of chromatin modifiers, including components of the SWI-SNF (switch/sucrose non-fermentable) and PRC (polycomb repressive complex) complexes, histone deacetylases (HDACs) including sirtuins, and DNA methyltransferases, also change with age in stem cells [113, 114]. These changes may underpin declining stem cell function. Indeed, the overexpression of enhancer of zeste homolog 2 (EZH2), a component of PRC2, improves long-term repopulating potential in HSCs [115]. Additionally, in aged HSCs, clusters of genes increase in expression levels based on chromosomal location, suggesting that epigenetic dysregulation engenders regional loss of transcriptional silencing [113]. Taken together, these findings suggest that changes in epigenetic modifications are a general trait of stem cell aging, which impacts their function.

It is interesting that with aging appear changes that reinforce self-renewal. Sun et al. conducted a comprehensive integrated genomic analysis of young (4 mo) and old (24 mo) murine HSCs by profiling the transcriptome, DNA methylome, and histone modifications. Transcriptome analysis indicated reduced transforming growth factor beta (TGF-β) signaling and perturbation of genes involved in HSC proliferation and differentiation. Aged HSCs showed increased DNA methylation at transcription factor binding sites associated with differentiation-promoting genes, combined with a reduction at genes associated with HSC maintenance. When they profiled the principal regulatory chromatin marks with the use of chromatin immunoprecipitation sequencing (ChIP-seq) they found that the H3K4me3 mark, an activating histone modification, increases with age at loci that regulate HSC self-renewal, potentially underlying the increase in HSC number observed with aging [116].

In satellite cells of muscles, H3K4me3 levels modestly decrease with age, whereas levels of the repressive modification H3K27me3 significantly increase with age. It has also been shown that the expression levels of histones themselves decrease with age [117]. The levels of H4K16Ac, another activating modification, decrease with age in HSCs; inhibition of cell division control protein 42 homolog (CDC42) restores H4K16Ac levels to that of young HSCs and reverses phenotypes of HSC aging in transplantation assays [118].

It is not known whether the epigenetic changes in stem cell products affect their clinical efficiency. In our recent study we intended to gain insight into the methylation status of CD34+ enriched cell products intended for autologous CD34+ cell transplantation in patients with cardiomyopathy. We found that the global DNA methylation and hydroxymethylation status as well as the target methylation profile of 94 stem cell transcription factor genes in CD34+ enriched cell products did not differ significantly as compared to initial leukapheresis products. The epigenetic landscape of different cell products can tell us little about the functional capacity and regenerative properties of CD34+ cells (Rozman et al. [108]).

2.6. Changes of microRNA

Impairments in stem cell function that occur during aging are globally mirrored in the epigenome and transcriptome of HSCs, including the microRNAs. MicroRNAs (miRNAs) are small noncoding evolutionarily conserved RNAs that regulate gene expression primarily at the posttranscriptional level. They act by binding to specific sequences in the 3′ untranslated region of their target genes and causing the transcripts to be degraded by the RNA-induced silencing complex (RISC). The human genome encodes over 1000 miRNAs that appear to target about 60% of other genes. MiRNAs are important posttranscriptional regulators of gene expression and play important and diverse roles in almost all biological and metabolic processes, including early development, cell proliferation, cell cycle regulation, apoptosis, fat metabolism, signal transduction, aging and diseases, as reviewed recently [119].

In stem cells, miRNAs influence properties such as potency, differentiation, self-renewal, and senescence. Different kinds of stem cells possess distinct miRNA expression profiles. Among other things, miRNAs regulate a number of cell functions such as defense mechanisms against ROS, DNA repair, and apoptosis. These properties, and the assumption that miRNAs act as some kind of general switch, make them highly relevant in research on aging [120], especially since specific miRNA expression profiles could be used to terminally differentiate cells from stem cells in order to treat various diseases, including myocardial infarction, neurodegenerative diseases, blood diseases, and muscle diseases [121].

miRNAs regulate the state of stem cells by directly targeting three prime untranslated region (3′-UTR) of pluripotency factors in the section of messenger RNA. For instance, miR-145 miRNA represses the pluripotency of human embryonic stem cells (ESCs) through targeting octamer-binding transcription factor 4 (*Oct4*; also known as *Pou5f1*), sex determining region Y-box 2 (*Sox2*), and kruppel-like factor 4 (*Klf4*) [122]. In addition, miRNAs target the coding regions of transcription factors to modulate stem cell differentiation. miR-296, miR-470, and miR-134 regulate mouse ESC differentiation by targeting the coding regions of *Nanog*, *Oct4*, and *Sox2* [123]. Other classified miRNAs also regulate pluripotency, self-renewal, reprogramming, and differentiation of stem cells [124–128].

miRNAs act as key regulators of hematopoiesis during the proliferation and differentiation of HSCs in mammals. Ectopic expression of AAAGUGC seed-containing miRNAs enhance the primary hematopoietic progenitors [129]. miR-181, miR-223, and miR-142 are preferentially expressed in hematopoietic tissues, with miR-181 significantly promoting B-lymphocyte differentiation [130]. miR-125a is conservatively expressed in long-term HSCs and can increase the number of HSCs by targeting the apoptosis factor Bax1 [131]. Furthermore, overexpression of miR- 125b leads to lethal myeloid leukemia in mice [132]. See the recent review of Li et al. [119].

Besides regulating the ESCs, miRNAs exert several other actions that indirectly impact stem cells and regeneration. For instance, let-7 family and miR-15a/16-1 cluster function as regulators of the cell cycle and tumor suppressors. While miR-29a and miR-29b regulate progression through the cell cycle [133], miR-9 and miR-124a play a critical role in specification of the neural progenitors from ESCs [134, 135].

On the other hand, miRNAs also modulate development of other tissues, such as cardiovascular differentiation of cardiomyocyte progenitor cells and stem cells, including the differentiation of cardiomyocytes, vascular smooth muscle cells, and endothelial cells. They are involved in the regulation of cardiovascular differentiation of human-derived cardiomyocyte progenitor cells, the cardiovascular differentiation of ESCs and iPSCs, in cardiac differentiation of ESCs after myocardial infarction, vascular endothelial growth factor (VEGF) signaling and angiogenesis, which has great therapeutic value for the future regenerative medicine, as reviewed recently by Li et al. [119]. Some other observations comment on the important role miRNAs play in brain development, as well as in later stages of mammalian neuronal maturation and synapse development. Conversely, dysregulation of miRNAs expression has been implicated in developmental defects, cancers and nervous system diseases, as recently reviewed by Murashov [121].

Lee et al. have measured the expression levels of 521 small regulatory miRNAs in young and old animals of six mouse strains and found that expression levels of three miRNAs (miR-203-3p, miR-664-3p, and miR-708-5p) were associated with lifespan. Pathway analysis of binding sites for these three miRNAs revealed enrichment of key target genes involved in aging and longevity pathways including mechanistic target of rapamycin (mTOR), forkhead box protein O (FOXO) and mitogen-activated protein kinase (MAPK), most of which also demonstrated associations with longevity [136].

In conclusion, one could infer that miRNAs have critical roles in stem cell reprogramming, pluripotency maintenance and differentiation, as well as some other important cellular functions. In the future, miRNAs may greatly contribute to stem cell clinical therapy and have potential applications in regenerative medicine.

2.7. Changes in RNA splicing and ribosomal machinery

RNA splicing is the editing of the nascent precursor messenger RNA (pre-mRNA) transcript into a mature messenger RNA (mRNA). After splicing, introns are removed and exones are joined together. Splicing usually takes place immediately after transcription, and is carried out in a series of reactions catalyzed by the spliceosome, a complex of small nuclear ribonucleoproteins (snRNPs). This results in an mRNA molecule, which can be translated into protein. Splicing enables one gene to generate multiple proteins allowing organisms to generate complexity from a relatively limited number of genes.

In healthy aging, splicing homeostasis takes place, while deregulation of the splicing machinery is linked to several age-related chronic illnesses. Certain studies point out that defective splicing machinery and de-regulation of RNA splicing acts as a driver of the aging process itself. Studies on the roundworm *C. elegans* show that with age they lose muscle mass, their cutickle thickens, they wrinkle, and they experience declines in fertility and immune functions. The pre-mRNA splicing homeostasis is a biomarker and predictor of life expectancy in this worm. Recently, Heintz and her colleagues found that splicing could also play a major role in the aging process of humans. Using transcriptomics and in-depth splicing analysis in young and old animals they found defects in global pre-mRNA splicing

with age that are reduced by caloric restriction via one particular component of the splicing apparatus, called splicing factor 1 (SFA-1)—a factor also present in humans. They also showed that SFA-1 is specifically required for lifespan extension by caloric restriction and by modulation of the target of rapamycin complex 1 (TORC1) pathway components 5′ AMP-activated protein kinase (AMPK), RAGA-1, and ribosomal protein S6 kinase (RSKS-1/S6 kinase), and demonstrated that overexpression of splicing factor 1 (SFA-1) extends lifespan. Together, these data demonstrate a role for RNA splicing homeostasis in caloric restriction longevity and suggest that modulation of specific spliceosome components may prolong healthy aging [137].

The ribosomal machinery that is responsible for protein synthesis (translation), i.e., linking amino acids in the order specified by mRNA molecules, consists of two major components: the small ribosomal subunit, which reads the RNA, and the large subunit, which joins amino acids to form a polypeptide chain. Ribosomes contain ribosomal RNA (rRNA) molecules and a variety of highly conserved ribosomal proteins, and similar to other cellular compartments, these are particular targets of aging. After a comprehensive integrated genomic analysis of young and aged cells, consisting of the profiling of transcriptome, DNA methylome, and histone modifications of young and old murine HSCs, Sun et al. found an increased transcription of ribosomal protein and RNA genes, and hypomethylation of rRNA genes [116]. Indeed, inhibition of ribosomal proteins or their regulators has been shown to extend life span in yeast and worms [138, 139]. Although the research has not been focused on the plicing in stem cells we can expect that the splicing homeostasis in stem cells is similarly affected by aging.

2.8. Proteostasis

The proteostasis or homeostasis of the proteome is a complex system that takes care of the proper folding, functioning, and degradation of cellular proteins. Mechanisms, by which proteostasis is ensured, include regulated protein translation, chaperone assisted protein folding, and protein degradation pathways. Adjusting each of these mechanisms to the requirements of proteins, which need to be correctly folded, is essential for maintaining all cellular functions.

In previous paragraphs it has been already explained that in aged subjects, stem cells display a thoroughly altered proteome. Many studies have demonstrated that proteostasis is altered with aging and that accumulation of misfolded or damaged proteins is an important determinant of the aging process [140]. Indeed, many different proteins involved in cytoskeletal organization, anti-oxidant defense, and other functions are age-dependent and associated with functional impairment of the cell functions, including decreased responsiveness to physical environmental cues and decreased resistance to oxidative stress [15]. Chronic expression of unfolded, misfolded or aggregated proteins contributes to the development of some age-related pathologies, such as Alzheimer's disease, Parkinson's disease and cataracts [141]. Since the passage of altered proteins to progenitor cells during asymmetric division could compromise development and cause aging, proteostasis maintenance in stem cells has an important role in organismal aging [142].

During the evolution the cells developed a variety of mechanisms that maintain and promote proteostasis and slow down the aging. This is performed by an array of quality control mechanisms that preserve the stability and functionality of the proteome. Various mechanisms for the correction of folded proteins have developed, such as the heat-shock family of proteins, as well as the corrective mechanisms for the degradation of misfolded proteins in proteasome or the lysosome [140, 143]. Moreover, there are regulators of age-related proteotoxicity, such as modifier of protein aggregation (MOAG-4), that act through an alternative pathway distinct from molecular chaperones and proteases [144]. The stress-induced synthesis of cytosolic and organelle-specific chaperones is significantly impaired in aging [145]. All these systems function in a coordinated fashion to restore the structure of misfolded polypeptides or to remove and degrade them completely, thus preventing the accumulation of damaged components and assuring the continuous renewal of intracellular proteins.

As previously mentioned, there are several approaches for maintaining or enhancing proteostasis aimed at activating protein folding and stability mediated by chaperones. A number of animal models support a causative impact of chaperone decline on longevity. In particular, transgenic worms and flies overexpressing chaperones are long-lived [146, 147]. Also, mutant mice deficient in a co-chaperone of the heat-shock family exhibit accelerated aging phenotypes, whereas long-lived mouse strains show a marked up-regulation of some heat-shock proteins [148].

Moreover, activation of the master regulator of the heat-shock response, the transcription factor heat shock factor 1 (HSF-1), increases longevity and thermotolerance in nematodes [149], while amyloid-binding components can maintain proteostasis during aging and extend lifespan [150]. Pharmacological induction of the heat-shock protein Hsp72 preserves muscle function and delays progression of dystrophic pathology in mouse models of muscular dystrophy [151].

Small molecules may be also employed as pharmacological chaperones to assure the refolding of damaged proteins and to improve age-related phenotypes in model organisms [152].

For the degradation of unneeded and misfolded proteins there are special protein complexes, named proteasomes, which degrade them with proteolysis, a chemical reaction that breaks peptide bonds. The degradation process yields peptides of about seven to eight amino acids long, which can then be further degraded into shorter amino acid sequences and used in synthesis of new proteins.

Stem cells can also maintain high levels of autophagy and proteasome activity to clear damaged proteins. For example, autophagy is greater in HSCs and skin stem cells than in surrounding differentiated cells [153]. Although proteasome activity has yet to be characterized in adult stem cells, it has been shown that human ESCs exhibit high proteasome activity [142]. Fly oocytes, which require similar long-term proteome-protection mechanisms as stem cells, maintain high activity of large multi-protein complex 26S proteasome with age, despite the decline of its activity in the somatic cells [154].

The activities of the two principal proteolytic systems implicated in protein quality control, namely, the autophagy-lysosomal system and the ubiquitin-proteasome system, decline with

aging [155, 156], supporting the idea that collapsing proteostasis constitutes a common feature of old age. In relation to the proteasome, activation of epidermal growth factor (EGF) signaling extends longevity in nematodes by increasing the expression of various components of the ubiquitin-proteasome system activators accelerates the clearance of toxic proteins in human cultured cells [157]. Moreover, increased expression of the 26S proteasome subunit RPN-6 by the FOXO transcription factor DAF-16 confers proteotoxic stress resistance and extends lifespan in *C. elegans* [158].

Regarding autophagy, transgenic mice with an extra copy of the chaperone-mediated autophagy receptor lysosome-associated membrane protein 2a (LAMP2a) do not experience aging-associated decline in autophagic activity and preserve improved hepatic function with aging [159]. This is a promising example of genetic manipulations that improve proteostasis and delay aging in mammals [159]. Functional decline in the cellular proteolytic machinery leads to the formation of an autofluorescent protein called lipofuscin, which can be used as a biomarker of aging [160]. Based on the given data it is obvious that SCs are a subject of age related changes of proteostasis and further studies will probably focus on proteostasis maintanance in SCs.

2.9. Changes of cell polarity

In order to prevent the accumulation of damaged components, stem cells developed diverse mechanisms such as the asymmetric segregation of damaged proteins and enhanced proteostasis. After a symmetric division, stem cells produce two daughter cells with the same fate, whereas after asymmetric division they produce one daughter stem cell and one differentiating daughter cell. During the asymmetric division, damaged components such as damaged DNA, replicating circular DNA, carbonylated proteins and damaged organelles are distributed into the differentiating cell, whereas the daughter stem cell remains youthful [161, 162]. In a similar way, stem cells have been shown to asymmetrically segregate damaged proteins and mitochondria into the progeny, which retains the stemness of the mother cell [163, 164]. A similar evolutionary principle enables that the parental strand of DNA is always sequestered in the daughter stem cell, whereas the strand synthesized during S phase, which might contain errors from replication, is directed to the differentiating daughter cell [165]. In this way the non-random strand segregation serves to avoid mutations and to control the inheritance of epigenetic state [166]. It was shown that the distribution of epigenetic modifications on mitotic chromosomes differs, which means that the bias is generated non-randomly during chromatid segregation. In *Drosophila* male GSCs, the histone modifications present in the stem cells are distinct from those in the differentiating daughter cells, which helps to retain pre-existing histones in the mother stem cell while imparting newly synthesized histones to the daughter cell. This retention of pre-existing histones in the stem cells is a prerequisite for maintaining their ability to self-renew. Different epigenetic modifications potentially lead to variations in the otherwise equivalent chromatids that segregate during asymmetric cell divisions [167].

There is accumulating evidence that other organelles are also non-randomly distributed between daughter cells. Numerous organelles have been widely studied for their asymmetric segregation in non-mammals and mammals, such as mitochondria, centrioles of the centrosome, and midbody, as well as different protein complexes [168].

The asymmetric division of stem cells first requires that a cell be polarized and several studies demonstrate that aged germinal stem cells (GSCs) and HSCs are less able to perform such polarized divisions, suggesting that loss of polarity contributes to stem cell aging [169]. Other data on HSCs suggest that changes in age-related Wnt signaling are a cause of this loss of polarity [170]. This process also appears to occur in satellite cells [171]. There is certain disagreement as to whether polarized division occurs in other stem cell populations, such as intestinal, hair follicle, neural or germline stem cells, as reviewed by Yennek and Tajbakhsh in 2013 [172].

2.10. Changes in metabolism and nutrient sensing

Metabolic status plays an important role in stem cell aging [2]. Similar to other cells, stem cells generate energy via glycolysis or oxidative phosphorylation. Quiescent stem cells generally rely upon glycolysis, perhaps because this reduces the abundance of ROS [142]. Many adult stem cells also reside in hypoxic niches, perhaps as a part of a mechanism to limit ROS production [173].

For the provision of necessary energy, proliferating stem cells rely on the oxidative phosphorylation, which predisposes them to oxidative damage and cellular dysfunction. Therefore the molecules that scavenge ROS or enable the overexpression of the transcription factor NRF2, which regulates the response to oxidative stress, reduce the aged phenotype of old cell.

2.10.1. Caloric restriction

The most robust longevity-extending intervention across species is caloric restriction (CR). For example, CR increases the abundance of satellite cells in muscles [174] and improves the function of many stem cell populations, including HSCs in mice [175] and GSCs in flies [176].

CR also promotes ISC self-renewal in mice by induction of the enzyme BST1 in Paneth cells, which form the niche. BST1 then converts NAD+ to the paracrine signal cyclic ADP ribose (cADPR), which is sensed by the ISCs [177]. Pathways and factors implicated in mediating the response of stem cells to CR that extend lifespan, include insulin and IGF-1 signaling (IIS) pathway, target of rapamycin (TOR) signaling, AMPK, sirtuins and FOXO transcription factors [178].

2.10.2. Glucose metabolism

Recent studies also show that HSCs and satellite cells increase glucose and glutamine metabolism during activation [179] — an alteration that mimics the Warburg effect in cancer cells. Similarly, in skeletal muscle, aging is associated with pseudohypoxia and Warburg-like metabolism, which compromise cellular function [180] and promote oncogenic transformation [181].

Glucose is the main nutrient in the cell, whereas insulin informs cells about the presence of glucose. The intracellular signaling pathway that governs insulin is the same as that elicited by IGF-1, which is, together with the growth hormone (GH), produced by the anterior pituitary, and is the secondary mediator of the somatotrophic axis in mammals. For this reason, IGF-1 and insulin signaling are known as the "insulin and IGF-1 signaling" (IIS) pathway. GH and IGF-1 levels decline during normal aging, as well as in mouse models of premature aging [182]. Remarkably, the IIS pathway is the most conserved aging-controlling pathway

in evolution and among its multiple targets are the FOXO family of transcription factors and the mTOR complexes, which are also involved in aging and conserved through evolution. Similarly, genetic polymorphisms or mutations that reduce the functions of GH, IGF-1 receptor, insulin receptor or downstream intracellular effectors such as protein kinase B (PKB), also known as AKT, mTOR and FOXO, influence longevity both in humans and in model organisms, further illustrating the major impact these pathways have on longevity [53].

Multiple genetic manipulations of the IIS pathway, which attenuate signaling intensity at different levels, consistently extend the lifespan of worms, flies and mice. Genetic analyses indicate that this pathway mediates part of the beneficial effects of CR on longevity [183].

Mice with an increased dosage of the tumor suppressor protein phosphatase and tensin homolog (PTEN) exhibit a general down-modulation of the IIS pathway and an increased energy expenditure that is associated with improved mitochondrial oxidative metabolism, as well as with an enhanced activity of the brown adipose tissue [184]. In line with other mouse models with decreased IIS activity, PTEN-overexpressing mice, as well as hypomorphic phosphatidylinositol-3-kinase (PI3K) mice show an increased longevity [185].

Organisms with a constitutively decreased IIS pathway can live longer because they have lower rates of cell growth and metabolism, and a lower rates of cellular damage. Similarly, the aged organisms decrease their IIS pathway in an attempt to extend their lifespan. However, defensive responses against aging eventually exhaust and later on they even aggravate aging [186].

2.10.3. Other nutrient-sensing systems: mammalian target of rapamycin (mTOR), AMP-activated protein kinase (AMPK) and sirtuins

Besides the IIS pathway, three additional related and interconnected nutrient-sensing systems that participate in glucose –sensing: mammalian TOR (mTOR), for the sensing of high amino acid concentrations; AMPK that senses low energy states by detecting high AMP levels; and sirtuins, which sense the low energy states by detecting high NAD+ levels [187].

The mTOR kinase is part of two multiprotein complexes, mTORC1 and mTORC2, that regulate essentially all aspects of anabolic metabolism. Genetic down-regulation of mTORC1 activity in yeast, worms and flies extends longevity and attenuates further longevity benefits from CR, suggesting that mTOR inhibition phenocopies CR [188]. In mice, treatment with rapamycin also extends longevity in what is considered the most robust chemical intervention to increase lifespan in mammals [189].

Genetically-modified mice with low levels of mTORC1 activity, but normal levels of mTORC2 activity, have an increased lifespan [190], and mice deficient in ribosomal protein S6 kinase beta-1 (S6 K1), which is a main mTORC1 substrate, are also long-lived [191]. This means that the down-regulation of mTORC1/S6 K1 acts as the critical mediator of longevity in relation to mTOR.

It seems that the intense trophic and anabolic activity, signaled through the IIS or the mTORC1 pathways, is a major accelerator of aging. Although inhibition of TOR activity clearly has beneficial effects during aging, it also has some undesirable side-effects, such as impaired wound healing, insulin resistance, cataract formation and testicular degeneration in mice [192]. In order

to determine the extent to which beneficial and damaging effects of TOR inhibition can be separated from each other, it will be crucial to understand the mechanisms involved.

There are two another nutrient sensors, AMPK and sirtuins, which act in the completely opposite direction of the IIS and mTOR. Instead of signaling nutrient abundance and anabolism, they signal nutrient scarcity and catabolism. Accordingly, their up-regulation promotes a healthy aging. AMPK activation has multiple effects on metabolism and, remarkably, shuts off mTORC1 [193]. There is evidence indicating that AMPK activation may mediate lifespan-extension following metformin administration to worms and mice [194, 195].

The role of sirtuins in lifespan regulation has been discussed above (see section 2.2 on DNA mutations). In addition, SIRT1 can deacetylate and activate the PPARγ co-activator 1α (PGC-1α) [43]. PGC-1α orchestrates a complex metabolic response that includes mitochondriogenesis, enhanced anti-oxidant defenses, and improved fatty acid oxidation [196]. Moreover, SIRT1 and AMPK can engage in a positive feedback loop, thus connecting both sensors of low-energy states into a unified response [197].

Collectively, currently available evidence strongly supports the idea that anabolic signaling accelerates aging, and decreased nutrient signaling extends longevity [183]. Consistent with the relevance of deregulated nutrient-sensing as a hallmark of aging, CR increases lifespan or healthspan in all investigated eukaryote species, which are unicellular and multicellular organisms of several distinct phyla, including non-human primates [198]. What is more, a pharmacological manipulation that mimics a state of limited nutrient availability, such as rapamycin, can extend longevity in mice (Harrison et al. [189]). All of these reflects in stem cells, however, the exact mechanisms in the metabolism of stem cells awaits further clarification.

2.11. Niche deterioration

In the context of a tissue, adult stem cells reside in a special microenvironment referred to as the "niche". The niche allows interaction between the stem cells and different extrinsic signals. In some instances, these signals are mediated via direct cell to cell communication or cell to matrix interaction. Another category of signals comprises of diffusible signaling ligands which regulate various transcription programs in the stem cells. These interactions are crucial, as they are able to regulate whether stem cells are quiescent, self-renew, or commit to differentiation [199].

Similarly to the stem cells themselves, the BM niche changes substantially with age. The niche consists of mesenchymal stem cells (MSCs), stromal cells, osteoblasts, adipocytes, and other cells, as well as extracellular matrix. The proliferative capacity of human MSCs has been shown to decline with age [200]. Certain other authors noticed a prominent increase in adipocytes in the aged BM, which is associated with lower HSC potential [201].

Mechanisms of niche aging are probably the same as in other cells. Khatri et al. recently showed that accumulation of excessive ROS in BM stromal cells suppress BM cellularity by affecting microenvironment in aged mice. Treatment of these mice with a polyphenolic antioxidant curcumin has quenched ROS, rescued stromal cells from oxidative stress-dependent cellular injury, and improved hematopoietic reconstitution in old (18 months) mice. This

implicates the role of ROS in perturbation of stromal cells function upon aging, which in turn affects BM's reconstitution ability in aged mice. Rejuvenation therapy using curcumin, prior to transplantation of HSCs and progenitor cells could be an efficient strategy for successful marrow reconstitution in older mice [202].

The question remains as to whether aged BM niche cells induce age-related changes in HSCs. Evidence suggests that aging in the microenvironment influences HSC engraftment, as aged HSCs demonstrate a lower engraftment after transplantation [203]. Hematopoietic cells engrafted in subcutaneous implantation of BM stroma from both aged and young mice exhibit lower spleen colony-forming units (CFU-S) capacity [204]. Furthermore, young HSCs transplanted to aged niches exhibit impairment in homing and decreased potential for differentiation, failing to efficiently repopulate an old niche [205].

Another characteristic of aged HSCs is an altered differentiation potential tending toward higher myeloid/platelet output and lower lymphoid output. Skewing toward myeloid differentiation is attributed to the niche microenvironment, since the transplantation of young HSCs to aged recipients resulted in a tendency toward higher myeloid output [206]. Transplantation experiments on old recipients show that granulocyte-macrophage progenitor (GMP) expansion is comparable regardless of donor age. Also, the differentiation of B-cells depends on the BM microenvironment [207] and it was shown that aged HSCs occupy different niches to young HSCs [208].

One of the mechanisms of aging in the hematopoietic system are the changes in adhesion between HSCs and niche cells. Expression of various adhesion molecules in HSCs alters with age so the aged HSCs express low levels of integrin α4, integrin α5 and VCAM-1, and high levels of P-selectin and integrin α6 compared to young HSCs [209]. In *Drosophila*, the age-dependent E-cadherin decline in the stem cell-niche junction that regulates the adhesion of GSCs to the niche was shown to contribute to the aging of stem cells [210]. Another authors similarly showed that the aged HSCs exhibit less adhesion to the stromal cells compared to the young ones [211]. Another group has shown that an overexpression of CDC42, a small Rho GTPase that is involved in adhesion signaling, causes premature aging phenotypes in these cells [212].

Age-related changes in niche cells may also be attributed to changes in their metabolic state. MSCs obtained from old human BM have an elevated level of ROS along with p21 and p53 expression, indicating cellular senescence [17]. As already mentioned, high oxygen tension causes senescence in cultured human BM MSCs, whereas the continuous hypoxia make the human MSCs to exhibit higher self-renewal divisions without cellular senescence [213]. Compared to MSCs cultured in low oxygen, MSCs cultured in higher oxygen levels utilize oxidative phosphorylation, suggesting that the generation of ROS might influence MSC senescence.

Age-related changes in the stem cell niches can influence HSC mobilization from the BM, which is extremely important in the clinical settings. Several authors, including ourselves, have noted that the collection of stem cells from aged patients results in low yields of mobilized HSCs intended for therapy [214, 215]. It is interesting that in various animal models an opposite effect was demonstrated since the granulocyte colony-stimulating factor (G-CSF)-induced mobilization resulted in increased numbers of HSCs in aged mice [211]. The authors deduce that differences in mobilization potential according to age are influenced mainly by

the niche in which the HSCs reside and that the clonality of HSCs may largely be influenced by specific niche cells at different anatomical sites [216].

Various studies utilizing heterochronic transplantation and parabiosis experiments showed that aging can be also be caused by extrinsic mechanisms, i.e., it is caused by factors external to the cell itself. This was shown in satellite cells [217], NSCs [218, 219], and GSCs [220]. In flies, the cells that form the niche of the GSCs themselves decline in abundance with age, possibly because of decreased self-renewal [221, 222].

Aged niche cells can also fail to send proper signals to stem cells, namely through morphogen and growth factor signaling, thereby affecting cell fate decisions. For example, increased fibroblast growth factor 2 (FGF2) in the aged satellite cell niche of mouse muscle impairs self-renewal [223]. Markers of inflammation also increase in the aging niche, for example in hair follicle stem cells, and impair stem cell function [224].

Taken together, stem cells require support cells that constitute the niche to maintain proper function. Thus, aging of the stem cell niche can also critically modulate stem cell function.

2.12. Influence of various circulating factors

The concentrations of various circulating factors exerts important influences on stem cell aging. Many of these factors have been identified by rejuvenating effects of blood or plasma derived from either young or calorically restricted animals. Among such factors are insulin and IGF-1, which have been already discussed (see paragraph 2.10.). Reduced signaling from these molecules is believed to mediate much of the longevity-extending effects of CR in mice. An opposite example is the TGF- β molecule, the levels of which increase during aging in mouse and human sera, which impairs the function of satellite muscle cells and NSCs [225]. By contrast, growth differentiation factor 11 (GDF11) has been suggested to improve the function of satellite cells and NSCs, and its levels appear to decrease during aging [218]. The validity of the effects of GDF11 on satellite cells, however, has been questioned by other studies, although it is worth noting that the dose of GDF11 and the skeletal muscle injury models used in the various studies differed [226]. Whether GDF11 actually declines with age has also been questioned, based in part on the argument that GDF11 detection methods cross-react with myostatin (ibid.), although a recent study using additional methods and controls also reports that GDF11 declines with age in mice [227]. Finally, the latest reports infer that high levels of GDF11 cause reductions in body and heart weight in both young and old animals, suggestive of a cachexia effect with the conclusion that elevating blood levels of GDF11 in the aged might cause more harm than good [228].

An important debate regarding the decline in stem-cell function is the relative role of cell-intrinsic pathways compared to cell-extrinsic ones [229]. Recent work has provided strong support for the latter. In particular, CR increases intestinal and muscle stem functions through cell-extrinsic mechanisms [174]. Similarly, when muscle-derived stem cells from young mice are transplanted to progeroid mice, this extends their lifespan and improves degenerative changes even in tissues where donor cells are not detected, suggesting that their therapeutic benefit may derive from systemic effects caused by secreted

factors [230]. Furthermore, parabiosis experiments have demonstrated that the decline in neural and muscle stem cell function in old mice can be reversed by systemic factors from young mice [231, 232].

There is also an ancient system in each cell that relates to the homeostasis of intracellular calcium (Ca2+), which in normal cell sustains a 20,000 fold concentration gradient to the exterior of the cell, resulting in the extracellular Ca2+ acting as cellular regulator when it enters the cell via the Ca2+ channels. This gradient is sustained by specific pumping and transporting mechanisms consisting of protein molecules [233]. Anomalies of these proteins results in an increase of intracellular calcium which can cause various diseases. With age, the hampered calcium homeostasis can lead to different muscle, immune and neural related defects [234].

2.13. Stem cell exhaustion

Although stem cells are regarded as immortal, as they are not subject to replicative senescence, they are susceptible to damage accumulation over time. Besides many other changes, a decline in their relative numbers and changes in subpopulations were observed. The group of dormant and active stem cells, existing in the niches of an organism that can be considered a pool of regenerative reserve, plays an important role in prevention of disease, in regeneration and aging. For instance, a decline in CD34+ circulating progenitor cells was reported with advancing age. When 100 octogenarians were observed for 7 and 10 years it was demonstrated that the number of their circulating CD34+ cells better predicted their lifespan and cardiovascular (CV) issues related mortality then the classic cardiovascular risk factors (hypertension, smoking, hypercholesterolemia), levels of inflammatory markers, or levels of cholesterol, or some other traditional cardiovascular indexes such as FRS and CVFRs The chances of reaching an older age depended on higher numbers of CD34+ cells at baseline, thus the number of CD34+ cells could be considered as a biomarker of longevity in the elderly over 80 years [235].

On the other hand, there are reports that in certain tissues the numbers of adult stem cells even increase with age, however the number of their parent clones decreases, meaning that fewer pluripotent stem cells give rise to more frequent progeny, in order to compensate for the decrease of numbers [236]. Ruzankina and Brown suggest that mammals in fact do have a finite number of stem cell replications per life and that aging of the hematopoietic system, which is due to a finite doubling capacity of stem cells, degrades its regenerative potential as well as the potential for preventing cancer [237].

Verovskaya used cellular barcoding combined with multiplex high-throughput sequencing to demonstrate clonal behavior of young HSCs transplanted to older organisms. In their study, the majority of transplanted clones steadily contributed to hematopoiesis in the long-term, although the clonal output in granulocytes, T cells, and B cells was substantially different. The final pool of old HSCs was composed of multiple small clones, whereas the young HSC pool was dominated by fewer, but larger, clones [238].

Holstege et al. have showed that the contents of a stem cell compartment actually deplete with old age. In the nonrepetitive genome of a 115-year-old centenarian woman they found approximately 450 somatic mutations that accumulated in the last years of her life, and the distribution of these mutations suggested that the majority of her peripheral white blood cells

were offspring of only two HSC clones that were still active in her old age. The telomeres of her white blood cells were significantly shorter than the telomeres from other tissues, suggesting that the HSCs have a finite lifespan, which is the cause of hematopoietic clonal evolution at extreme ages [239].

Several recent studies have confirmed that clonal hematopoiesis is almost a "normal" part of aging, with recent reports showing 0.8%, 11% and 19.5% of normal individuals aged <60, >80 and >90 years, respectively, having demonstrable clonal hematopoiesis – so called age-related clonal hematopoiesis [240, 241]. Clonal hematopoiesis (CH) arises when a substantial proportion of mature blood cells is derived from a single dominant hematopoietic stem cell lineage. It was recently shown, in the study on 11,262 elderly Icelanders which used whole-genome sequencing, that somatic mutations in candidate driver genes are thought to be responsible for at least some cases of CH [242].

At the same time there is ample evidence that there exist many dormant HSCs, and even some other and more "primitive" types of stem cells, such as for instance the VSEL stem cells with "primitive" embryonic characteristics, which co-inhabit the BM [243]. These VSEL cells exhibit some characteristics of long-term repopulating HSCs (LT-HSCs), they may differentiate into organ-specific cells (e.g., cardiomyocytes), and probably have a role in aging since the number of these cells positively correlates with longevity in several murine models [244]. Along with others, we have found similar cells in the reproductive organs [245].

It is now becoming obvious that maintaining robust stem cell pools seems to extend not only lifespan but also healthspan [49].

2.14. Cellular senescence – A stable arrest of the cell cycle

Cellular senescence can be defined as a stable arrest of the cell cycle coupled to typical phenotypic changes [246]. This phenomenon was originally described by Hayflick in human fibroblasts serially passaged in culture [99]. The senescence that was observed by Hayflick was caused by telomere shortening [101] and some other aging-associated stimuli that trigger senescence independently of the telomeric process. It is for instance well known that the non-telomeric DNA damage and de-repression of the *INK4*/*ARF* locus, both of which progressively occur with chronological aging, are also capable of inducing senescence [247].

The accumulation of senescent cells with age is a simple mathematical result of the increase in the rate of generation of senescent cells and/or a decrease in their rate of clearance. In normal physiology this has detrimental consequences, but in some circumstances it also has useful effects. For instance, there is good evidence that the senescent tumor cells are subjected to strict immune surveillance and are efficiently removed by phagocytosis [248].

Among other functions, the senescent cells manifest dramatic alterations in their secretome, which is particularly enriched in pro-inflammatory cytokines and matrix metalloproteinases, which is referred to as the "senescence-associated secretory phenotype" [249, 250]. This pro-inflammatory secretome may contribute to aging (see paragraph 2.15. Intercellular Communication).

Studies on aged mice have revealed an overall decrease in HSC cell cycle activity, with old HSCs undergoing fewer cell divisions than young HSCs [251]. This correlates with the accumulation of DNA damage and with the overexpression of cell cycle-inhibitory proteins such as $p16^{INK4a}$ [252]. In fact, old $p16^{INK4a-/-}$ HSCs exhibit better engraftment capacity and increased cell cycle activity compared with old wild-type HSCs (ibid.). Telomere shortening is also an important cause of stem cell decline with aging in multiple tissues [253].

The accumulation of senescent cells in aged tissues has been often inferred using surrogate markers such as DNA damage. Some studies have directly used senescence-associated β-galactosidase (SABG) to identify senescence in tissues [254]. Of note, a detailed and parallel quantification of SABG and DNA damage in liver produced comparable quantitative data, yielding a total of ~8% senescent cells in young mice and ~17% in very old mice [255]. Similar results were obtained in the skin, lung and spleen, but no changes were observed in heart, skeletal muscle and kidney. Based on these data, it is clear that cellular senescence is not a generalized property of all tissues in aged organisms.

Some authors think that the amount of senescent cells increases with age and that senescence contributes to aging, but this probably undervalues the primary purpose of senescence, which is to prevent the propagation of damaged cells and to trigger their removal by the immune system. They explain that senescence is a beneficial compensatory response that contributes to clearing tissues of damaged and potentially oncogenic cells. This however requires an efficient cell replacement system that involves clearance of senescent cells and mobilization of stem cells and their progenitors to re-establish cell numbers. In aged organisms, this turnover system may become exhausted, resulting in the accumulation of senescent cells that aggravate the damage and contribute to aging [48].

Deficient proliferation of stem and progenitor cells is obviously detrimental for the long-term maintenance of the organism, but excessive proliferation of stem and progenitor cells can also be deleterious by accelerating the exhaustion of stem cell niches, which can be compensated by stem cell quiescence over the long-term. This has been demonstrated in *Drosophila* ISCs, where excessive proliferation leads to exhaustion and premature aging [256] and in p21-null mice, which present premature exhaustion of HSCs and NSCs [257].

Recent studies have shown that an increase in FGF2 signaling in the aged muscle stem cell niche results in the loss of quiescence, stem cell depletion and diminished regenerative capacity, whereas the suppression of this signaling pathway reverses these defects [223]. This opens up the possibility of designing strategies aimed at inhibiting FGF2 signaling to reduce stem cell exhaustion during aging.

As a mechanism to protect themselves from acquiring damage, many stem cells are resting for a long time in a quiescent state. During this time they are protected from replicative damage, but they are more susceptible to mutations [258]. However, although proliferating stem cells are more likely to encounter DNA damages [259], they repair that damages more accurately than do quiescent stem cells.

In addition to DNA damage, excessive mitogenic signaling is the other stress most robustly associated with senescence. A recent account listed more than 50 oncogenic or mitogenic alterations

that are able to induce senescence [260]. The number of mechanisms that implement senescence in response to this variety of oncogenic insults has also grown, but still, the originally reported $p16^{INK4a}$/Rb and $p19^{ARF}$/p53 pathways remain, in general, the most important ones [261]. The relevance of these pathways for aging becomes even more striking when considering that the levels of $p16^{INK4a}$ (and to a lesser extent also $p19^{ARFARF}$) correlate with the chronological age of essentially all tissues analyzed, both in mice and humans [262, 263]. *INK4a*/*ARF* locus was actually determined as being genetically linked to the highest number of age-associated pathologies, including several types of cardiovascular diseases, diabetes, glaucoma, and Alzheimer's disease [264]. Although the activation of p53 and *INK4a*/*ARF* is a beneficial compensatory response that prevents the propagation of damaged cells, under the stress conditions the p53 and *INK4a*/*ARF* responses can become deleterious and even accelerate aging [2].

Taken together, cellular senescence is a beneficial compensatory response to damage, but it becomes deleterious and accelerates aging when tissues exhaust their regenerative capacity. A moderate enhancement of the senescence-inducing tumor suppressor pathways may extend longevity [265], whereas at the same time, elimination of senescent cells in an experimental progeria model delays age-related pathologies [266]. Therefore, two interventions that are conceptually opposite are able to extend healthspan.

2.15. Altered intercellular communication

Beyond intrinsic cellular alterations, aging also involves changes at the level of intercellular endocrine, neuroendocrine or neuronal communication [267, 268]. As during the aging inflammatory reactions increase, immunosurveillance against pathogens and premalignant cells declines, and the composition of the peri- and extracellular environment changes, neurohormonal signaling (i.e., renin-angiotensin, adrenergic, insulin/IGF-1 signaling) is consequently deregulated, which affects various mechanical and functional properties of all tissues [48].

An important age-associated pathological finding in the intercellular communication in mammals is so called "inflammaging," i.e., an appearance of pro-inflammatory phenotype that accompanies aging. Several authors proposed that aging is accompanied by a chronic up-regulation of several pro-inflammatory responses. [35, 269, 270]. Inflammaging may result from multiple causes such as the accumulation of pro-inflammatory substances, tissue damage, the failure of the aged immune system to effectively clear pathogens and remove dysfunctional host cells, the secretion of pro-inflammatory cytokines by aged immune cells, the enhanced activation of the NF-κB transcription factor, or from a defective autophagy response. These defects and alterations result in an enhanced activation of the NLRP3 inflammasome and other pro-inflammatory pathways, finally leading to increased production of interleukin 1ß (IL-1ß), tumor necrosis factor and interferons [271]. Inflammation is also involved in the pathogenesis of obesity and type 2 diabetes, two conditions that contribute to, and correlate with aging in the human population [272]. Likewise, defective inflammatory responses play a critical role in atherosclerosis [273].

Another link between inflammation and aging derives from the finding that inflammatory and stress responses activate NF-κB in the hypothalamus and induce a signaling pathway

that results in reduced production of gonadotropin-releasing hormone (GnRH) by neurons [274]. This GnRH decline can contribute to numerous aging-related changes such as bone fragility, muscle weakness, skin atrophy, and reduced neurogenesis. These findings suggest that the hypothalamus may modulate systemic aging by integrating NF-kB-driven inflammatory responses with GnRH-mediated neuroendocrine effects.

Besides chronic inflammation, aged immune cells are prone to a multitude of deteriorating factors. Age related defects of innate immunity are observed not only in the macrophage/monocyte compartment, which is probably the main "culprit" of inflammaging, but also in other cells, i.e., NK cells, dendritic cells, and granulocytes, whereas the defects of adaptive immunity are observed in both the B-cell and the T-cell compartments. Aging of the immune system or "immunosenescence" is characterized by a time-dependent functional alteration of immunity leading to immunodeficiency [275, 276] that manifests in chronic inflammation [277], reduced resistance to infections [278], poor responses to vaccination [279], and increased incidence of autoimmunity and cancers. Similarly, the involvement of immune processes in clinical conditions, such as atherosclerosis, diabetes, and dementia, have been described [280, 281]. The impairment of the immune system exerts an influence on the increased morbidity and mortality observed in human subjects as they age [282].

There is also accumulating evidence indicating that aging-related changes in one tissue can lead to aging-specific deterioration of other tissues. Typical case are the inflammatory cytokines that can cause so called "contagious aging". In certain bystander effects senescent cells induce senescence in neighboring cells via gap junction-mediated cell-to-cell contacts and processes involving ROS [283]. The microenvironment contributes to the age-related functional defects of CD4 T cells, as assessed by using an adoptive transfer model in mice [284]. Likewise, impaired kidney function can increase the risk of heart disease in humans [285]. Conversely, lifespan-extending manipulations targeting one single tissue can delay the aging process in other tissues [286].

Defective intercellular communication underlying aging processes, including genetic, can be restored by nutritional or pharmacological interventions that may improve the cell–cell communication properties lost with aging [48]. Of special interest in this regard are the CR approaches to extend healthy lifespan [287] and the rejuvenation strategies based on the use of blood-borne systemic factors identified in parabiosis experiments [288, 289]. Moreover, the long-term administration of anti-inflammatory agents, such as aspirin, may increase longevity in mice and healthy aging in humans [290, 291]. Finally, it also appears possible to extend lifespan by manipulating the composition and functionality of the intestinal bacterial ecosystem of the human body [292]. The near future research will undoubtedly bring spectacular results in this field of human physiology that will also be translated to the clinical medicine.

3. Conclusion

Although the stem cells are often considered "a fountain of youth" they are subjected to various aging and degenerative processes. Contrary to somatic cells, they have developed a

plethora of mechanisms that prevent or delay aging and age-related pathology. Over recent decades we have witnessed an immense increase in advanced therapies. The cells used in therapeutic products must meet stringent standards of quality. The huge increase in stem cell based therapies especially demands that we use the most advanced analysis of stem cell grafts to ensure optimal performance.

The aging of stem cells is an important biological factor that contributes to the general aging of an organism. Therefore, senescence and the age related status of grafted stem cells have to be taken into account in every stem cell based therapy, as well as in tissue engineering procedure. Further research on the cellular mechanisms leading to the aging of stem cells will not only answer various burning questions related to current cell based therapies, but also pave the way to designing future counter-aging procedures.

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References

[1] Bianconi E, et al. An estimation of the number of cells in the human body. Annals of Human Biology. 2013;**40**(6):463-471

[2] Schultz MB, Sinclair DA. When stem cells grow old: Phenotypes and mechanisms of stem cell aging. Development. 2016;**143**(1):3-14

[3] Sharpless NE, Depinho RA. How stem cells age and why this makes us grow old. Nature Reviews. Molecular Cell Biology. 2007;**8**:703-713

[4] Sharpless NE, Schatten G. Stem cell aging. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2009;**64**(2):202-204

[5] Wang MC, O'Rourke EJ, Ruvkun G. Fat metabolism links germline stem cells and longevity in *C. elegans*. Science. 2008;**322**(5903):957-960

[6] Kenyon C. A pathway that links reproductive status to lifespan in *Caenorhabditis elegans*. Annals of the New York Academy of Sciences. 2010;**1204**:156-162

[7] Biteau B, et al. Lifespan extension by preserving proliferative homeostasis in drosophila. PLoS Genetics. 2010;**6**:e1001159

[8] Kollman C, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016;**127**(2):260-267

[9] Cheung TH, Rando TA. Molecular regulation of stem cell quiescence. Nature Reviews. Molecular Cell Biology. 2013;**14**(6):329-340

[10] Harman D. Aging: A theory based on free radical and radiation chemistry. Journal of Gerontology. 1956;**11**(3):298-300

[11] Harman D. The biologic clock: The mitochondria? Journal of the American Geriatrics Society. 1972;**20**(4):145-147

[12] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; **408**(6809):239-247

[13] Sastre J, et al. Mitochondria, oxidative stress and aging. Free Radical Research. 2000; **32**(3):189-198

[14] Weinert BT, Timiras PS. Invited review: Theories of aging. Journal of Applied Physiology. 2003;**95**(4):1706-1716

[15] Kasper G, et al. Insights into mesenchymal stem cell aging: Involvement of antioxidant defense and actin cytoskeleton. Stem Cells. 2009;**27**(6):1288-1297

[16] De Barros S, et al. Aging-related decrease of human ASC angiogenic potential is reversed by hypoxia preconditioning through ROS production. Molecular Therapy. 2013;**21**(2):399-408

[17] Stolzing A, et al. Age-related changes in human bone marrow-derived mesenchymal stem cells: Consequences for cell therapies. Mechanisms of Ageing and Development. 2008;**129**(3):163-173

[18] Haines DD, Juhasz B, Tosaki A. Management of multicellular senescence and oxidative stress. Journal of Cellular and Molecular Medicine. 2013;**17**(8):936-957

[19] Van Raamsdonk JM, Hekimi S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. PLoS Genetics. 2009;**5**(2):e1000361

[20] Mesquita A, et al. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H_2O_2 and superoxide dismutase activity. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(34): 15123-15128

[21] Doonan R, et al. Against the oxidative damage theory of aging: Superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. Genes & Development. 2008;**22**(23):3236-3241

[22] Zhang Y, et al. Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2009;**64**(12):1212-1220

[23] Van Remmen H, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiological Genomics. 2003;**16**(1):29-37

[24] Perez VI, et al. The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell. 2009;**8**(1):73-75

[25] Vermulst M, et al. DNA deletions and clonal mutations drive premature aging in mitochondrial mutator mice. Nature Genetics. 2008;**40**(4):392-394

[26] Virant-Klun I, et al. Parthenogenetic embryo-like structures in the human ovarian surface epithelium cell culture in postmenopausal women with no naturally present follicles and oocytes. Stem Cells and Development. 2009;**18**(1):137-149

[27] Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. Molecular Cell. 2012;**48**(2):158-167

[28] Yun J, Finkel T. Mitohormesis. Cell Metabolism. 2014;**19**(5):757-766

[29] Hiona A et al. Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. PLoS One. 2010;**5**(7):e11468

[30] Edgar D, et al. Random point mutations with major effects on protein-coding genes are the driving force behind premature aging in mtDNA mutator mice. Cell Metabolism. 2009;**10**(2):131-138

[31] Kroemer G, Blomgren K. Mitochondrial cell death control in familial Parkinson disease. PLoS Biology. 2007;**5**(7):e206

[32] Green DR, Galluzzi L, Kroemer G. Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science. 2011;**333**(6046):1109-1112

[33] Raffaello A, Rizzuto R. Mitochondrial longevity pathways. Biochimica et Biophysica Acta. 2011;**1813**(1):260-268

[34] Dan Dunn J et al. Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. Redox Biology. 2015;**6**:472-485

[35] Franceschi C, et al. Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of Ageing and Development. 2007;**128**(1):92-105

[36] Park CB, Larsson NG. Mitochondrial DNA mutations in disease and aging. The Journal of Cell Biology. 2011;**193**(5):809-818

[37] Linnane AW, et al. Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet. 1989;**1**(8639):642-645

[38] Kang E, et al. Age-related accumulation of somatic mitochondrial DNA mutations in adult-derived human iPSCs. Cell Stem Cell. 2016;**18**(5):625-636

[39] Ameur A, et al. Ultra-deep sequencing of mouse mitochondrial DNA: Mutational patterns and their origins. PLoS Genetics. 2011;**7**(3):e1002028

[40] Payne BA, et al. Mitochondrial aging is accelerated by anti-retroviral therapy through the clonal expansion of mtDNA mutations. Nature Genetics. 2011;**43**(8):806-810

[41] Sahin E, DePinho RA. Axis of ageing: Telomeres, p53 and mitochondria. Nature Reviews. Molecular Cell Biology. 2012;**13**(6):397-404

[42] Bernardes de Jesus B, et al. Telomerase gene therapy in adult and old mice delays aging and increases longevity without increasing cancer. EMBO Molecular Medicine. 2012;**4**(8):691-704

[43] Rodgers JT, et al. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature. 2005;**434**(7029):113-118

[44] Lee IH, et al. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**(9):3374-3379

[45] Lombard DB, et al. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. Molecular and Cellular Biology. 2007;**27**(24):8807-8814

[46] Giralt A, Villarroya F. SIRT3, a pivotal actor in mitochondrial functions: Metabolism, cell death and aging. The Biochemical Journal. 2012;**444**(1):1-10

[47] Qiu X, et al. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metabolism. 2010;**12**(6):662-667

[48] Lopez-Otin C, et al. The hallmarks of aging. Cell. 2013;**153**(6):1194-1217

[49] Boyette LB, Tuan RS. Adult stem cells and diseases of aging. Journal of Clinical Medicine. 2014;**3**(1):88-134

[50] Castello L, et al. Alternate-day fasting reverses the age-associated hypertrophy phenotype in rat heart by influencing the ERK and PI3K signaling pathways. Mechanisms of Ageing and Development. 2011;**132**(6-7):305-314

[51] Safdar A, et al. Endurance exercise rescues progeroid aging and induces systemic mitochondrial rejuvenation in mtDNA mutator mice. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(10):4135-4140

[52] Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. Cell. 2011;**146**(5):682-695

[53] Kenyon CJ. The genetics of ageing. Nature. 2010;**464**(7288):504-512

[54] Wang K, Klionsky DJ. Mitochondria removal by autophagy. Autophagy. 2011;**7**(3):297-300

[55] Gonzalez-Freire M, et al. Reconsidering the role of mitochondria in aging. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences. 2015;**70**(11):1334-1342

[56] Liu JP. Molecular mechanisms of ageing and related diseases. Clinical and Experimental Pharmacology & Physiology. 2014;**41**(7):445-458

[57] Moskalev AA, et al. The role of DNA damage and repair in aging through the prism of Koch-like criteria. Ageing Research Reviews. 2013;**12**(2):661-684

[58] Forsberg LA, et al. Age-related somatic structural changes in the nuclear genome of human blood cells 144. American Journal of Human Genetics. 2012;**90**(2):217-228

[59] Laurie CC, et al. Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nature Genetics. 2012;**44**(6):642-650

[60] Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science. 2015;**347**(6217):78-81. DOI: 10.1126/science.1260825

[61] Jones DL, Rando TA. Emerging models and paradigms for stem cell ageing. Nature Cell Biology. 2011;**13**(5):506-512

[62] Rossi DJ, Jamieson CH, Weissman IL. Stems cells and the pathways to aging and cancer. Cell. 2008;**132**:681-696

[63] Medawar PB. An Unsolved Problem of Biology. London: H.K. Lewis. Edney, E.B. and Gill, R.W; 1952

[64] Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;**461**(7267):1071-1078

[65] Ciccia A, Elledge SJ. The DNA damage response: Making it safe to play with knives. Molecular Cell. 2010;**40**(2):179-204

[66] Kennedy RD, D'Andrea AD. DNA repair pathways in clinical practice: Lessons from pediatric cancer susceptibility syndromes. Journal of Clinical Oncology. 2006; **24**(23):3799-3808

[67] Beerman I, et al. Quiescent hematopoietic stem cells accumulate DNA damage during aging that is repaired upon entry into cell cycle. Cell Stem Cell. 2014;**15**(1):37-50

[68] Rube CE, et al. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. PLoS One. 2011;**6**(3):e17487

[69] Sinha M, et al. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science. 2014;**344**(6184):649-652

[70] Flach J, et al. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature. 2014;**512**(7513):198-202

[71] Gorbunova V, et al. Changes in DNA repair during aging. Nucleic Acids Research. 2007;**35**(22):7466-7474

[72] Freitas, A.A. and J.P. de Magalhaes, A review and appraisal of the DNA damage theory of ageing. Mutation Research. 2011;**728**(1-2):12-22

[73] Kanfi Y, et al. The sirtuin SIRT6 regulates lifespan in male mice. Nature. 2012;**483**(7388): 218-221

[74] Murga M, et al. A mouse model of ATR-Seckel shows embryonic replicative stress and accelerated aging. Nature Genetics. 2009;**41**(8):891-898

[75] Hoeijmakers JH. DNA damage, aging, and cancer. The New England Journal of Medicine. 2009;**361**(15):1475-1485

[76] Gregg SQ, et al. A mouse model of accelerated liver aging caused by a defect in DNA repair. Hepatology. 2012;**55**(2):609-621

[77] Baker DJ, et al. Increased expression of BubR1 protects against aneuploidy and cancer and extends healthy lifespan. Nature Cell Biology. 2013;**15**(1):96-102

[78] Dechat T, et al. Nuclear lamins: Major factors in the structural organization and function of the nucleus and chromatin. Genes & Development. 2008;**22**(7):832-853

[79] Gonzalez-Suarez I, et al. Novel roles for A-type lamins in telomere biology and the DNA damage response pathway. The EMBO Journal. 2009;**28**(16):2414-2427

[80] Liu B, et al. Genomic instability in laminopathy-based premature aging. Nature Medicine. 2005;**11**(7):780-785

[81] Eriksson M, et al. Recurrent de novo point mutations in lamin a cause Hutchinson-Gilford progeria syndrome. Nature. 2003;**423**(6937):293-298

[82] De Sandre-Giovannoli A, et al. Lamin a truncation in Hutchinson-Gilford progeria. Science. 2003;**300**(5628):2055

[83] Cabanillas R, et al. Nestor-Guillermo progeria syndrome: A novel premature aging condition with early onset and chronic development caused by BANF1 mutations. American Journal of Medical Genetics. Part A. 2011;**155A**(11):2617-2625

[84] Ragnauth CD, et al. Prelamin a acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation. 2010;**121**(20):2200-2210

[85] Cao K, et al. Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. The Journal of Clinical Investigation. 2011;**121**(7):2833-2844

[86] Freund A, et al. Lamin B1 loss is a senescence-associated biomarker. Molecular Biology of the Cell. 2012;**23**(11):2066-2075

[87] Blackburn EH, Greider CW, Szostak JW. Telomeres and telomerase: The path from maize, Tetrahymena and yeast to human cancer and aging. Nature Medicine. 2006;**12**(10): 1133-1138

[88] Blackburn EH. Structure and function of telomeres. Nature. 1991;**350**(6319):569-573

[89] de Lange T. Shelterin: The protein complex that shapes and safeguards human telomeres. Genes & Development. 2005;**19**(18):2100-2110

[90] de Lange T. Protection of mammalian telomeres. Oncogene. 2002;**21**(4):532-540

[91] Tomas-Loba A, et al. Telomerase reverse transcriptase delays aging in cancer-resistant mice. Cell. 2008;**135**(4):609-622

[92] Rudolph KL, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell. 1999;**96**(5):701-712

[93] Armanios M, et al. Short telomeres are sufficient to cause the degenerative defects associated with aging. American Journal of Human Genetics. 2009;**85**(6):823-832

[94] Blasco MA. The epigenetic regulation of mammalian telomeres. Nature Reviews. Genetics. 2007;**8**:299-309

[95] Boonekamp JJ, et al. Telomere length behaves as biomarker of somatic redundancy rather than biological age. Aging Cell. 2013;**12**(2):330-332

[96] Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell. 1985;**43**(2 Pt 1):405-413

[97] Flores I, et al. The longest telomeres: A general signature of adult stem cell compartments. Genes & Development. 2008;**22**(5):654-667

[98] Ferron SR, et al. Telomere shortening in neural stem cells disrupts neuronal differentiation and neuritogenesis. The Journal of Neuroscience. 2009;**29**(46):14394-14407

[99] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Experimental Cell Research. 1961;**25**:585-621

[100] Olovnikov AM. Telomeres, telomerase, and aging: Origin of the theory. Experimental Gerontology. 1996;**31**(4):443-448

[101] Bodnar AG, et al. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998;**279**(5349):349-352

[102] Armanios M, Blackburn EH. The telomere syndromes. Nature Reviews. Genetics. 2012;**13**(10):693-704

[103] Jaskelioff M et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011;**469**:102-106

[104] Bernardes de Jesus B, Blasco MA. Potential of telomerase activation in extending health span and longevity. Current Opinion in Cell Biology. 2012;**24**(6):739-743

[105] Lapham K, et al. Automated assay of telomere length measurement and informatics for 100,000 subjects in the genetic epidemiology research on adult health and aging (GERA) cohort. Genetics. 2015;**200**(4):1061-1072

[106] Bakaysa SL, et al. Telomere length predicts survival independent of genetic influences. Aging Cell. 2007;**6**(6):769-774

[107] Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiology and Molecular Biology Reviews. 2002;**66**(3):407-425, table of contents

[108] Rozman JZ, et al. The effect of CD34+ cell telomere length and hTERT expression on the outcome of autologous CD34+ cell transplantation in patients with chronic heart failure. Mechanisms of Ageing and Development. 2017;**166**:42-47

[109] Waddington CH. The epigenotype. Endeavour. 1942;**1**:18-20

[110] Oberdoerffer P, et al. SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell. 2008;**135**(5):907-918

[111] Beerman I, et al. Functionally distinct hematopoietic stem cells modulate hematopoietic lineage potential during aging by a mechanism of clonal expansion. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(12): 5465-5470

[112] Smith JA, Daniel R. Stem cells and aging: A chicken-or-the-egg issue? Aging and Disease. 2012;**3**(3):260-268

[113] Chambers SM, et al. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biology. 2007;**5**(8):e201

[114] Kofman AE, Huszar JM, Payne CJ. Transcriptional analysis of histone deacetylase family members reveal similarities between differentiating and aging spermatogonial stem cells. Stem Cell Reviews. 2013;**9**(1):59-64

[115] Kamminga LM, et al. The Polycomb group gene Ezh2 prevents hematopoietic stem cell exhaustion. Blood. 2006;**107**(5):2170-2179

[116] Sun D, et al. Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. Cell Stem Cell. 2014;**14**(5):673-688

[117] Liu L, et al. Prognostic value of EZH2 expression and activity in renal cell carcinoma: A prospective study. PLoS One. 2013;**8**(11):e81484

[118] Florian MC, et al. Cdc42 activity regulates hematopoietic stem cell aging and rejuvenation. Cell Stem Cell. 2012;**10**(5):520-530

[119] Li N, et al. microRNAs: Important regulators of stem cells. Stem Cell Research & Therapy. 2017;**8**(1):110. DOI: 10.1186/s13287-017-0551-0

[120] Hodzic M, Naaldijk Y, Stolzing A. Regulating aging in adult stem cells with microRNA. Zeitschrift für Gerontologie und Geriatrie. 2013;**46**(7):629-634

[121] Murashov AK. RNAi and MicroRNA-mediated gene regulation in stem cells. Methods in Molecular Biology. 2017;**1622**:15-25. DOI: 10.1007/978-1-4939-7108-4_2

[122] Xu N, et al. MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell. 2009;**137**(4):647-658

[123] Tay Y, et al. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature. 2008;**455**(7216):1124-1128

[124] Tata PR, et al. Identification of a novel epigenetic regulatory region within the pluripotency associated microRNA cluster, EEmiRC. Nucleic Acids Research. 2011;**39**(9):3574-3581

[125] Neveu P, et al. MicroRNA profiling reveals two distinct p53-related human pluripotent stem cell states. Cell Stem Cell. 2010;**7**(6):671-681

[126] Lin CH, et al. Myc-regulated microRNAs attenuate embryonic stem cell differentiation. The EMBO Journal. 2009;**28**(20):3157-3170

[127] Wang Y, et al. Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. Nature Genetics. 2008;**40**(12):1478-1483

[128] Sinkkonen L, et al. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. Nature Structural & Molecular Biology. 2008;**15**(3):259-267

[129] Reinhart BJ et al. The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature. 2000;**403**(6772):901-906

[130] Chen JF, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetics. 2006;**38**(2):228-233

[131] Brennecke J, et al. Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in drosophila. Cell. 2003;**113**(1):25-36

[132] Xu P, et al. The drosophila microRNA Mir-14 suppresses cell death and is required for normal fat metabolism. Current Biology. 2003;**13**(9):790-795

[133] Hwang HW, Wentzel EA, Mendell JT. A hexanucleotide element directs microRNA nuclear import. Science. 2007;**315**(5808):97-100

[134] Delaloy C, et al. MicroRNA-9 coordinates proliferation and migration of human embryonic stem cell-derived neural progenitors. Cell Stem Cell. 2010;**6**(4):323-335

[135] Sonntag KC, Woo TU, Krichevsky AM. Converging miRNA functions in diverse brain disorders: A case for miR-124 and miR-126. Experimental Neurology. 2012;**235**(2):427-435

[136] Lee BP, et al. MicroRNAs miR-203-3p, miR-664-3p and miR-708-5p are associated with median strain lifespan in mice. Scientific Reports. 2017;**7**:44620

[137] Heintz C, et al. Splicing factor 1 modulates dietary restriction and TORC1 pathway longevity in *C. elegans*. Nature. 2017;**541**(7635):102-106

[138] Kaeberlein M, et al. Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients. Science. 2005;**310**(5751):1193-1196

[139] Syntichaki P, Troulinaki K, Tavernarakis N. eIF4E function in somatic cells modulates ageing in *Caenorhabditis elegans*. Nature. 2007;**445**(7130):922-926

[140] Koga H, Kaushik S, Cuervo AM. Protein homeostasis and aging: The importance of exquisite quality control. Ageing Research Reviews. 2011;**10**(2):205-215

[141] Powers ET, et al. Biological and chemical approaches to diseases of proteostasis deficiency. Annual Review of Biochemistry. 2009;**78**:959-991

[142] Vilchez D, Simic MS, Dillin A. Proteostasis and aging of stem cells. Trends in Cell Biology. 2014;**24**(3):161-170

[143] Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature. 2011;**475**(7356):324-332

[144] van Ham TJ, et al. Identification of MOAG-4/SERF as a regulator of age-related proteotoxicity. Cell. 2010;**142**(4):601-612

[145] Calderwood SK, Murshid A, Prince T. The shock of aging: Molecular chaperones and the heat shock response in longevity and aging--a mini-review. Gerontology. 2009;**55**(5):550-558

[146] Walker GA, Lithgow GJ. Lifespan extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals. Aging Cell. 2003;**2**(2):131-139

[147] Morrow G, et al. Overexpression of the small mitochondrial Hsp22 extends drosophila life span and increases resistance to oxidative stress. The FASEB Journal. 2004;**18**(3):598-599

[148] Swindell WR, et al. Endocrine regulation of heat shock protein mRNA levels in long-lived dwarf mice. Mechanisms of Ageing and Development. 2009;**130**(6):393-400

[149] Chiang WC, et al. HSF-1 regulators DDL-1/2 link insulin-like signaling to heat-shock responses and modulation of longevity. Cell. 2012;**148**(1-2):322-334

[150] Alavez S, et al. Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. Nature. 2011;**472**(7342):226-229

[151] Gehrig SM, et al. Hsp72 preserves muscle function and slows progression of severe muscular dystrophy. Nature. 2012;**484**(7394):394-398

[152] Calamini B, et al. Small-molecule proteostasis regulators for protein conformational diseases. Nature Chemical Biology. 2011;**8**(2):185-196

[153] Salemi S, et al. Autophagy is required for self-renewal and differentiation of adult human stem cells. Cell Research. 2012;**22**(2):432-435

[154] Fredriksson A, et al. Effects of aging and reproduction on protein quality control in soma and gametes of Drosophila Melanogaster. Aging Cell. 2012;**11**(4):634-643

[155] Rubinsztein DC, Marino G, Kroemer G. Autophagy and aging. Cell. 2011;**146**(5):682-695

[156] Tomaru U, et al. Decreased proteasomal activity causes age-related phenotypes and promotes the development of metabolic abnormalities. The American Journal of Pathology. 2012;**180**(3):963-972

[157] Lee JS, et al. Meta-analysis of gene expression in the mouse liver reveals biomarkers associated with inflammation increased early during aging. Mechanisms of Ageing and Development. 2012;**133**(7):467-478

[158] Vilchez D, et al. RPN-6 determines *C. elegans* longevity under proteotoxic stress conditions. Nature. 2012;**489**(7415):263-268

[159] Zhang C, Cuervo AM. Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. Nature Medicine. 2008;**14**(9): 959-965

[160] Brunk UT, Terman A. Lipofuscin: Mechanisms of age-related accumulation and influence on cell function. Free Radical Biology & Medicine. 2002;**33**(5):611-619

[161] Aguilaniu H, et al. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. Science. 2003;**299**(5613):1751-1753

[162] Higuchi R, et al. Actin dynamics affect mitochondrial quality control and aging in budding yeast. Current Biology. 2013;**23**(23):2417-2422

[163] Bufalino MR, DeVeale B, van der Kooy D. The asymmetric segregation of damaged proteins is stem cell-type dependent. The Journal of Cell Biology. 2013;**201**(4):523-530

[164] Katajisto P et al. Asymmetric apportioning of aged mitochondria between daughter cells is required for stemness. Science. 2015;**348**(6232):340-343

[165] Cairns J. Mutation selection and the natural history of cancer. Nature. 1975;**255**(5505): 197-200

[166] Lansdorp PM. Immortal strands? Give me a break. Cell. 2007;**129**(7):1244-1247

[167] Mehrotra S, Deshpande G. All are equal, but some are more equal than others: Epigenetic regulation of germ line stem cell fate in Drosophila Melanogaster. Genes & Genetic Systems. 2017

[168] Moore DL, Jessberger S. Creating age asymmetry: Consequences of inheriting damaged goods in mammalian cells. Trends in Cell Biology. 2017;**27**(1):82-92

[169] Cheng J, et al. Centrosome misorientation reduces stem cell division during ageing. Nature. 2008;**456**(7222):599-604

[170] Florian MC, et al. A canonical to non-canonical Wnt signalling switch in haematopoietic stem-cell ageing. Nature. 2013;**503**(7476):392-396

[171] Conboy MJ, Karasov AO, Rando TA. High incidence of non-random template strand segregation and asymmetric fate determination in dividing stem cells and their progeny. PLoS Biology. 2007;**5**(5):e102

[172] Yennek S, Tajbakhsh S. DNA asymmetry and cell fate regulation in stem cells. Seminars in Cell & Developmental Biology. 2013;**24**(8-9):627-642

[173] Jang YY, Sharkis SJ. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood. 2007;**110**(8):3056-3063

[174] Cerletti M, et al. Short-term calorie restriction enhances skeletal muscle stem cell function. Cell Stem Cell. 2012;**10**(5):515-519

[175] Chen J, Astle CM, Harrison DE. Hematopoietic senescence is postponed and hematopoietic stem cell function is enhanced by dietary restriction. Experimental Hematology. 2003;**31**(11):1097-1103

[176] Mair W, et al. Dietary restriction enhances germline stem cell maintenance. Aging Cell. 2010;**9**(5):916-918

[177] Yilmaz OH, et al. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. Nature. 2012;**486**(7404):490-495

[178] Mihaylova MM, Sabatini DM, Yilmaz OH. Dietary and metabolic control of stem cell function in physiology and cancer. Cell Stem Cell. 2014;**14**(3):292-305

[179] Oburoglu L, et al. Glucose and glutamine metabolism regulate human hematopoietic stem cell lineage specification. Cell Stem Cell. 2014;**15**(2):169-184

[180] Gomes AP, et al. Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. Cell. 2013;**155**(7):1624-1638

[181] Wu LE, Gomes AP, Sinclair DA. Geroncogenesis: Metabolic changes during aging as a driver of tumorigenesis. Cancer Cell. 2014;**25**(1):12-19

[182] Schumacher B, et al. Delayed and accelerated aging share common longevity assurance mechanisms. PLoS Genetics. 2008;**4**(8):e1000161

[183] Fontana L, Partridge L, Longo VD. Extending healthy life span--from yeast to humans. Science. 2010;**328**(5976):321-326

[184] Garcia-Cao I, et al. Systemic elevation of PTEN induces a tumor-suppressive metabolic state. Cell. 2012;**149**(1):49-62

[185] Ortega-Molina A et al. Pten positively regulates brown adipose function, energy expenditure, and longevity. Cell Metabolism. 2012;**15**(3):382-394

[186] Renner O, Carnero A. Mouse models to decipher the PI3K signaling network in human cancer. Current Molecular Medicine. 2009;**9**(5):612-625

[187] Houtkooper RH, Williams RW, Auwerx J. Metabolic networks of longevity. Cell. 2010;**142**(1):9-14

[188] Johnson SC, Rabinovitch PS, Kaeberlein M. mTOR is a key modulator of ageing and age-related disease. Nature. 2013;**493**(7432):338-345

[189] Harrison DE, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature. 2009;**460**(7253):392-395

[190] Lamming DW, et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science. 2012;**335**(6076):1638-1643

[191] Selman C, et al. Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science. 2009;**326**(5949):140-144

[192] Wilkinson JE, et al. Rapamycin slows aging in mice. Aging Cell. 2012;**11**(4):675-682

[193] Alers S, et al. Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: Cross talk, shortcuts, and feedbacks. Molecular and Cellular Biology. 2012;**32**(1):2-11

[194] Anisimov VN, et al. If started early in life, metformin treatment increases life span and postpones tumors in female SHR mice. Aging (Albany NY). 2011;**3**(2):148-157

[195] Onken B, Driscoll M. Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS One. 2010;**5**(1):e8758

[196] Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1alpha, a nodal regulator of mitochondrial biogenesis. The American Journal of Clinical Nutrition. 2011;**93**(4):884S-8890

[197] Price NL, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metabolism. 2012;**15**(5):675-690

[198] Colman RJ, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science. 2009;**325**(5937):201-204

[199] Morrison SJ, Spradling AC. Stem cells and niches: Mechanisms that promote stem cell maintenance throughout life. Cell. 2008;**132**:598-611

[200] Stenderup K, et al. Aging is associated with decreased maximal life span and accelerated senescence of bone marrow stromal cells. Bone. 2003;**33**(6):919-926

[201] Katayama Y, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell. 2006;**124**(2):407-421

[202] Khatri R, et al. Reactive oxygen species limit the ability of bone marrow stromal cells to support hematopoietic reconstitution in aging mice. Stem Cells and Development. 2016;**25**(12):948-958

[203] Chambers SM, Goodell MA. Hematopoietic stem cell aging: Wrinkles in stem cell potential. Stem Cell Reviews. 2007;**3**(3):201-211

[204] Hotta T, et al. Age-related changes in the function of hemopoietic stroma in mice. Experimental Hematology. 1980;**8**:933-936

[205] Woolthuis CM, de Haan G, Huls G. Aging of hematopoietic stem cells: Intrinsic changes or micro-environmental effects? Current Opinion in Immunology. 2011;**23**(4):512-517

[206] Liang Y, Van ZG, Szilvassy SJ. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. Blood. 2005;**106**(4):1479-1487

[207] Li F, et al. Impaired regeneration of the peripheral B cell repertoire from bone marrow following lymphopenia in old mice. European Journal of Immunology. 2001;**31**(2):500-505

[208] Kohler A, et al. Altered cellular dynamics and endosteal location of aged early hematopoietic progenitor cells revealed by time-lapse intravital imaging in long bones. Blood. 2009;**114**(2):290-298

[209] Geiger H, Koehler A, Gunzer M. Stem cells, aging, niche, adhesion and Cdc42: A model for changes in cell-cell interactions and hematopoietic stem cell aging. Cell Cycle. 2007;**6**(8):884-887

[210] Boyle M, et al. Decline in self-renewal factors contributes to aging of the stem cell niche in the drosophila testis. Cell Stem Cell. 2007;**1**:470-478

[211] Xing Z, et al. Increased hematopoietic stem cell mobilization in aged mice. Blood. 2006;**108**(7):2190-2197

[212] Wang L, et al. Cdc42 GTPase-activating protein deficiency promotes genomic instability and premature aging-like phenotypes. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(4):1248-1253

[213] Pattappa G, et al. Continuous and uninterrupted oxygen tension influences the colony formation and oxidative metabolism of human mesenchymal stem cells. Tissue Engineering. Part C, Methods. 2013;**19**(1):68-79

[214] Pozotrigo M, et al. Factors impacting stem cell mobilization failure rate and efficiency in multiple myeloma in the era of novel therapies: Experience at memorial Sloan Kettering cancer Center. Bone Marrow Transplantation. 2013;**48**(8):1033-1039

[215] Kresnik PK, et al. Collection and immunoselection of CD34+ cells: The impact of age, sex, and diabetes in patients with chronic heart failure. Transfusion. 2016;**56**(7):1792-1800

[216] Nakamura-Ishizu A, Suda T. Aging of the hematopoietic stem cells niche. International Journal of Hematology. 2014;**100**(4):317-325

[217] Brack AS, Rando TA. Intrinsic changes and extrinsic influences of myogenic stem cell function during aging. Stem Cell Reviews. 2007;**3**(3):226-237

[218] Katsimpardi L, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;**344**(6184):630-634

[219] Villeda SA, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nature Medicine. 2014;**20**(6):659-663

[220] Ryu BY, et al. Effects of aging and niche microenvironment on spermatogonial stem cell self-renewal. Stem Cells. 2006;**24**(6):1505-1511

[221] Pan L, et al. Stem cell aging is controlled both intrinsically and extrinsically in the drosophila ovary. Cell Stem Cell. 2007;**1**(4):458-469

[222] Toledano H, et al. The let-7-imp axis regulates ageing of the drosophila testis stem-cell niche. Nature. 2012;**485**(7400):605-610

[223] Chakkalakal JV, et al. The aged niche disrupts muscle stem cell quiescence. Nature. 2012;**490**(7420):355-360

[224] Doles J, et al. Age-associated inflammation inhibits epidermal stem cell function. Genes & Development. 2012;**26**(19):2144-2153

[225] Pineda JR, et al. Vascular-derived TGF-beta increases in the stem cell niche and perturbs neurogenesis during aging and following irradiation in the adult mouse brain. EMBO Molecular Medicine. 2013;**5**(4):548-562

[226] Egerman MA, et al. GDF11 increases with age and inhibits skeletal muscle regeneration. Cell Metabolism. 2015;**22**(1):164-174

[227] Poggioli T, et al. Circulating growth differentiation factor 11/8 levels decline with age. Circulation Research. 2016;**118**(1):29-37

[228] Harper SC, et al. Is growth differentiation factor 11 a realistic therapeutic for aging-dependent muscle defects? Circulation Research. 2016;**118**(7):1143-1150 discussion 1150

[229] Conboy IM, Rando TA. Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches 5. Cell Cycle. 2012;**11**(12):2260-2267

[230] Lavasani M, et al. Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model. Nature Communications. 2012;**3**:608-608

[231] Conboy IM, et al. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005;**433**(7027):760-764

[232] Villeda SA, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature. 2011;**477**(7362):90-94

[233] Case RM, et al. Evolution of calcium homeostasis: From birth of the first cell to an omnipresent signalling system. Cell Calcium. 2007;**42**(4-5):345-350

[234] Nedergaard M, Verkhratsky A. Calcium dyshomeostasis and pathological calcium signalling in neurological diseases. Cell Calcium. 2010;**47**(2):101-102

[235] Mandraffino G, et al. CD34+ cell count predicts long lasting life in the oldest old. Mechanisms of Ageing and Development. 2017;**164**:139-145

[236] Pang WW, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(50):20012-20017

[237] Ruzankina Y, Brown EJ. Relationships between stem cell exhaustion, tumour suppression and ageing. British Journal of Cancer. 2007;**97**(9):1189-1193

[238] Verovskaya E, et al. Heterogeneity of young and aged murine hematopoietic stem cells revealed by quantitative clonal analysis using cellular barcoding. Blood. 2013; **122**(4):523-532

[239] Holstege H, et al. Somatic mutations found in the healthy blood compartment of a 115-yr-old woman demonstrate oligoclonal hematopoiesis. Genome Research. 2014; **24**(5):733-742

[240] Xie M, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nature Medicine. 2014;**20**(12):1472-1478

[241] McKerrell T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Reports. 2015;**10**(8):1239-1245

[242] Zink F, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. Blood. 2017;**130**(6):742-752

[243] Ratajczak MZ, et al. Very small embryonic/epiblast-like stem cells (VSELs) and their potential role in aging and organ rejuvenation--an update and comparison to other primitive small stem cells isolated from adult tissues. Aging. 2012;**4**(4):235-246

[244] Ratajczak MZ, et al. Very small embryonic/epiblast-like stem cells (VSELs) and their potential role in aging and organ rejuvenation--an update and comparison to other primitive small stem cells isolated from adult tissues. Aging (Albany NY). 2012;**4**(4): 235-246

[245] Virant-Klun I, et al. Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes. Differentiation. 2008;**76**(8):843-856

[246] Campisi J, d'Adda di Fagagna F. Cellular senescence: When bad things happen to good cells. Nature Reviews. Molecular Cell Biology. 2007;**8**(9):729-740

[247] Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;**130**(2):223-233

[248] Hoenicke L, Zender L. Immune surveillance of senescent cells—biological significance in cancer- and non-cancer pathologies. Carcinogenesis. 2012;**33**(6):1123-1126

[249] Kuilman T, et al. The essence of senescence. Genes & Development. 2010;**24**(22):2463-2479

[250] Rodier F, Campisi J. Four faces of cellular senescence. The Journal of Cell Biology. 2011;**192**(4):547-556

[251] Rossi DJ, Bryder D, Weissman IL. Hematopoietic stem cell aging: Mechanism and consequence. Experimental Gerontology. 2007;**42**(5):385-390

[252] Janzen V. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. Nature. 2006;**443**:421-426

[253] Flores I, Blasco MA. The role of telomeres and telomerase in stem cell aging. FEBS Letters. 2010;**584**(17):3826-3830

[254] Dimri GP, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proceedings of the National Academy of Sciences of the United States of America. 1995;**92**(20):9363-9367

[255] Wang C, et al. DNA damage response and cellular senescence in tissues of aging mice. Aging Cell. 2009;**8**(3):311-323

[256] Rera M, et al. Modulation of longevity and tissue homeostasis by the drosophila PGC-1 homolog. Cell Metabolism. 2011;**14**(5):623-634

[257] Cheng T. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science. 2000; **287**:1804-1808

[258] Kanaar R, Wyman C, Rothstein R. Quality control of DNA break metabolism: In the 'end', it's a good thing. The EMBO Journal. 2008;**27**(4):581-588

[259] Walter D, et al. Exit from dormancy provokes DNA-damage-induced attrition in haematopoietic stem cells. Nature. 2015;**520**(7548):549-552

[260] Gorgoulis VG, Halazonetis TD. Oncogene-induced senescence: The bright and dark side of the response. Current Opinion in Cell Biology. 2010;**22**(6):816-827

[261] Serrano M. The tumor suppressor protein p16INK4a. Experimental Cell Research. 1997;**237**(1):7-13

[262] Krishnamurthy J, et al. Ink4a/Arf expression is a biomarker of aging. The Journal of Clinical Investigation. 2004;**114**:1299-1307

[263] Ressler S, et al. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell. 2006;**5**(5):379-389

[264] Jeck WR, Siebold AP, Sharpless NE. Review: A meta-analysis of GWAS and age-associated diseases 29. Aging Cell. 2012;**11**(5):727-731

[265] Matheu A, et al. Anti-aging activity of the Ink4/Arf locus. Aging Cell. 2009;**8**(2):152-161

[266] Baker DJ, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature. 2011;**479**(7372):232-236

[267] Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;**149**(2):274-293

[268] Rando TA, Chang HY. Aging, rejuvenation, and epigenetic reprogramming: Resetting the aging clock. Cell. 2012;**148**(1-2):46-57

[269] Salvioli S, et al. Inflamm-aging, cytokines and aging: State of the art, new hypotheses on the role of mitochondria and new perspectives from systems biology. Current Pharmaceutical Design. 2006;**12**(24):3161-3171

[270] De Martinis M, et al. Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. FEBS Letters. 2005;**579**(10):2035-2039

[271] Salminen A, Kaarniranta K, Kauppinen A. Inflammaging: Disturbed interplay between autophagy and inflammasomes. Aging (Albany NY). 2012;**4**(3):166-175

[272] Barzilai N, et al. The critical role of metabolic pathways in aging. Diabetes. 2012;**61**(6): 1315-1322

[273] Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. Nature Reviews. Immunology. 2010;**10**(1):36-46

[274] Zhang G, et al. Hypothalamic programming of systemic ageing involving IKK-beta, NF-kappaB and GnRH. Nature. 2013;**497**(7448):211-216

[275] Ginaldi L, et al. The immune system in the elderly: Activation-induced and damage-induced apoptosis. Immunologic Research. 2004;**30**(1):81-94

[276] Sansoni P, et al. The immune system in extreme longevity. Experimental Gerontology. 2008;**43**(2):61-65

[277] Cesari M, et al. Oxidative damage and platelet activation as new predictors of mobility disability and mortality in elders. Antioxidants & Redox Signaling. 2005;**8**(3-4):609-619

[278] High KP. Infection as a cause of age-related morbidity and mortality. Ageing Research Reviews. 2004;**3**(1):1-14

[279] Goronzy JJ, et al. Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. Journal of Virology. 2001;**75**(24):12182-12187

[280] Chung HY, et al. The inflammation hypothesis of aging: Molecular modulation by calorie restriction. Annals of the New York Academy of Sciences. 2001;**928**:327-335

[281] McGeer EG, McGeer PL. Brain inflammation in Alzheimer disease and the therapeutic implications. Current Pharmaceutical Design. 1999;**5**(10):821-836

[282] Grubeck-Loebenstein B, Wick G. The aging of the immune system. Advances in Immunology. 2002;**80**:243-284

[283] Nelson G, et al. A senescent cell bystander effect: Senescence-induced senescence. Aging Cell. 2012;**11**(2):345-349

[284] Lefebvre JS, et al. The aged microenvironment contributes to the age-related functional defects of CD4 T cells in mice 44. Aging Cell. 2012;**11**(5):732-740

[285] Sarnak MJ, et al. Kidney disease as a risk factor for development of cardiovascular disease: A statement from the American Heart Association councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. Hypertension. 2003;**42**(5):1050-1065

[286] Durieux J, Wolff S, Dillin A. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell. 2011;**144**(1):79-91

[287] Sanchez-Roman I, et al. Effects of aging and methionine restriction applied at old age on ROS generation and oxidative damage in rat liver mitochondria. Biogerontology. 2012;**13**(4):399-411

[288] Conboy IM, Rando TA. Aging, stem cells and tissue regeneration: Lessons from muscle. Cell Cycle. 2005;**4**(3):407-410

[289] Loffredo FS, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell. 2013;**153**(4):828-839

[290] Strong R, et al. Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. Aging Cell. 2008;**7**(5):641-650

[291] Rothwell PM, et al. Effect of daily aspirin on long-term risk of death due to cancer: Analysis of individual patient data from randomised trials. Lancet. 2011;**377**(9759):31-41

[292] Claesson MJ, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012;**488**(7410):178-184

Chapter 4

Monitoring of Chimerism Following Hematopoietic Stem Cell Transplantation

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Additional information is available at the end of the chapter

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Abstract

One of the most important events in the posthematopoietic stem cell transplantation is the immune system reconstitution—a process characterized by a considerable dynamic. During this period, patients are exposed to different life threatening complications. In this chapter, we consider chimerism levels in relation to the conditioning regimens and disease type. Furthermore, the predictive role of chimerism analysis as an important method in monitoring the early diagnosis of graft versus host disease (GVHD), minimal residual disease (MRD), graft failure or rejection, and disease relapse has been discussed.

Keywords: chimerism, HSCT, STR, conditioning

1. Introduction

In March 1969, Prof. Thomas and his team performed the first transplantation of hematopoietic stem cells (HSCs). Since then, the transplantation of bone marrow or peripheral stem cells has become a routine method for treatment of a number of malignant and nonmalignant hematologic diseases [1–6]. Allogeneic stem cell transplantation is effective in restoring normal hematopoiesis and is a preferred therapeutic method for malignant diseases of the blood, due to its graft-versus-leukemia (GVL) effect. This effect is mainly due to donor T cells that exhibit immunoreactivity against the minor histocompatibility antigens (mHags) of the recipient or epitopes specific for leukemic cells. The immune system recovery after allogeneic hematopoietic stem cells transplantation is of crucial importance during the postoperative period. This process lasts for months to years and depends mainly on the ability of the donor hematopoietic stem cells to take over the recipient cells.

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The determination of the genetic origin of hematopoiesis is referred to as chimerism analysis. The term "chimera" was introduced in medicine by Anderson and coworkers [7] in 1951, and in transplantology—by Ford [8] in 1956. It is used for people who have cell populations from different individuals of the same or a different type that arise occasionally during pregnancy or blood transfusions or are targeted—by transplantation of tissues, organs, and cells. The term chimerism refers to the coexistence of cells from two different organisms in a body.

Each stage of the transplant process potentially exposes the patient to complications and life-threatening events; this is largely due to a lack of understanding of the mechanisms of engraftment, as well as the genetic differences that exist between donor and recipient. Additional factor for consideration is the sensitivity of the methods used. The most widely used one—PCR-STR, is thought to have 1–5% sensitivity. Factors, such as type and stage of disease, patient age, donor type, HLA compatibility, number of transplanted $CD34^+$ cells, graft T cell depletion, and many others, influence the postSCT immune reconstitution, as well as all subsequent complications.

The recovery of hematopoiesis depends mainly on the possibility of the donor hematopoietic stem cells to generate progenitor cells and repopulate the bone marrow niches. It creates a dynamic donor-recipient chimera, the exploration of which—qualitatively or quantitatively, has become an important component of the posttransplant monitoring of the patients. Chimerism is an important indicator for relapse, graft rejection, minimal residual disease (MRD), and graft versus host disease (GVHD). The presence of persistent or emerging recipient cells could mean surviving leukemic cells that could lead to a recurrence of a malignant branch by inhibiting immunocompetent donor cells. Therefore, the accurate assessment of chimerism in the patient's blood or bone marrow provides important information about the engraftment process and aids in providing a more adequate treatment to the recipient. Initially, full donor hematopoiesis was thought to be essential for the survival of the graft after allogeneic stem cell transplantation [9], but in recent years it became clear that posttransplantation chimerism is a dynamic process, and patients with full donor chimerism (FDC) in a posttransplant period may subsequently develop mixed chimerism and vice versa—patients with mixed can develop complete chimerism.

2. Chimerism and different therapeutic strategies

Patients undergoing allogeneic SCT are given chemotherapy alone or in combination with radiotherapy prior to reinfusion of blood or a bone marrow graft. In autologous transplantations, the conditioning aims to eradicate tumor cells, whereas in allogeneic transplantations, it aims to immunosuppress the recipient (for the purposes of preventing graft rejection), provide better control of possible GVHD, eradicate tumor cells (which correlates mainly with the intensity of the regimen used), and allow immune reconstitution. There are some exceptions, like recipients with severe aplastic anemia or those with severe combined immunodeficiency who do not require full immune system eradication.

Treatment with chemotherapy or radiation therapy results in severe depletion of all hematopoietic cells of the immune system. Both alkylating chemotherapeutic agents and irradiation target highly proliferative cells [10–12], including developing and naïve lymphocytes, making

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them particularly depleted following treatment [13]. Conditioning regimens cause neutropenia, which are likely to last up to approximately 30 days depending on the source of stem cells [14]. Delayed recovery following immunodepletion is associated with a high degree of morbidity and mortality [13, 15, 16]. Lymphoid recovery is critically dependent on primary immune system organs—the younger the patient, the faster the recovery of $CD4^+$ T cells, B cells, and NK cells [17–20]. Recovery of NK cells after HSCT depends on an expansion of the cytokine-producing $CD56^{bright}$ NK cell subset. Initial recovery of the T cell compartment relies on the peripheral expansion of memory T cells, driven by cytokines and the presence of alloreactive antigens [14]. Interestingly, $CD8^+$ T cells recover at similar rates in young and aged patients, which could be due to extrathymic clonal expansion [20–23]. The recovery of B cells takes longest time—up to 2 years and is preceded by expansion of transitional $CD19^+CD24^{++}CD38^{++}$ B cells [14]. Furthermore, immunodepletion from pretransplant conditioning causes enhanced senescence of the hematopoietic system coupled with an upregulation in the cyclindependent kinase inhibitors $p19^{Arf}$ and $p16^{ink4A}$ mimicking some of the age-related effects [24, 25].

With the development of less intensive conditioning regimens, the frontiers of hematopoietic stem cell transplantations have become even broader covering more diseases and more people becoming eligible for this treatment. The myeloablative regimens (MAs) are designed to fully eradicate the host immune system and facilitate the engraftment of donor cells. In contrast, reduced-intensity conditioning (RIC) and nonmyeloablative (NMA) regimens aim to suppress the immune system in view of preventing donor cell rejection rather than ablate it. Protocols using lower intensity conditioning regimens have been developed to treat hematological disorders in patients with medical comorbidities or elderly people who are not considered appropriate candidates for more intense and toxic conditioning. Thus, the duration of cytopenia is shorter and initial mixed donor chimerism (DC) is more likely to occur in those patients.

In 2009, Bacigalupo et al. published a report proposing a categorization of conditioning regimens into three categories—myeloablative conditioning, reduced-intensity conditioning, and nonmyeloablative conditioning. These categories were distinguished on the basis of cytopenia duration and the need of stem cell support [26]. The terminology reflects the early regimen-related toxicity to the host bone marrow cells, but not the biological effect of the transplant.

- *Myeloablative conditioning regimens*

The MA protocols include administration of high doses of total body irradiation (TBI) and/or alkylating agents, which do not allow autologous hematologic recovery and should benefit a faster donor cell engraftment. Since it is unlikely to fully eradicate a person's immune system, the term myeloablation should be considered as an operational definition, indicating a regimen usually causing an irreversible pancytopenia [26]. The myeloablative conditioning of patients results in higher transplant-related mortality (TRM) and higher frequency of GVHD, compared to other protocols.

- *Reduced-intensity conditioning regimens*

RIC regimens are considered an intermediate category of regimens, which cannot be classified as either NMA or MA—they cause cytopenia that may be prolonged, but the dose of

alkylating agents or TBI is reduced by at least 30%, as compared to MA, and they do require stem cell support [26].

- *Nonmyeloablative conditioning regimens*

NMAs typically cause minimal cytopenia—they do not require stem cell support, but are immunosuppressive to the extent that, when followed by G-CSF mobilized peripheral blood stem cells (PBSC), they usually result in full donor cell engraftment. According to Bacigalupo et al., the NMA regimens are more "immune-ablative" than myelo-ablative [26]. The NMA conditioning results in lower TRM, as compared to MA but requires a larger amount of donor cells to facilitate a full replacement of the recipient's hematopoiesis [27, 28]. Acute GVHD after NMA is delayed and may develop after day +100, at a time when chronic GVHD is usually diagnosed after MA regimen. GVHD remains a significant cause of morbidity and mortality after the application of both MA and NMA [29].

Many studies address the importance of chimerism monitoring after allo-SCT, but the data are controversial [30–36]. Moreover, chimerism monitoring in patients with MA conditioning is considered to be less informative and unnecessary, since the aggressive pretransplant preparation is thought to result in irreversible pancytopenia and achievement of stable full donor chimerism [30, 37, 38]. The chimerism status seems to be even more complicated as new data show the vague borders between the postSCT effects of the different conditioning protocols [31, 39, 40].

The aggressive pretransplant therapy is more effective against the disease, but its use is limited to younger and healthier patients who are able to tolerate concomitant toxicity. MA conditioning aims to facilitate normal hematopoietic reconstitution with FDC by day +30, after allo-SCT. In unmanipulated grafts, chimerism analysis shows mostly FDC, as its early establishment may be an indication of GVHD. Mixed chimerism is more likely to be observed in patients who had received T cell-depleted graft. T cell depletion is used to reduce the frequency of GVHD—Mickelson et al. showed a significantly increased risk of developing acute or chronic GVHD in patients who reached levels of donor chimerism in T cells >90% (HR = 1.92, P = 0.08 and HR = 2.26, and P = 0.07, respectively) [31]. Moreover, speed and extent of donor chimerism have been shown to be influenced by additional factors including primary diagnosis and previous treatment [37].

2.1. Investigation of chimerism in myeloablative HSCT

From a historical point of view, ablative chemotherapy and total body irradiation were accepted as a treatment for elimination of malignant cells and targeting immunosuppression with view of facilitating donor cell engraftment. High dosage protocols aiming to achieve antileukemic effect are still in use for patients with aggressive malignant diseases. The main treatment includes cyclophosphamide and TBI or busulfan (BU), and more often a combination of busulfan and fludarabine at dosages eliminating all myeloid cells [41]. The aggressive pretransplant conditioning is very effective for the primary disease, but it is limited to younger patients who are able to tolerate treatment-related toxicity. Myeloablative conditioning schemes could facilitate reconstitution of hematopoiesis with a complete lymphocyte chi-

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merism at day 30 after HSCT. In such type of HSCT, cases of GVHD are more frequent, while relapses have been rarely observed. Chimerism analysis shows complete donor chimera [37]. In 50–100% of the patients transplanted with T cell-depleted bone marrow grafts, mixed chimera have been observed. In these transplant procedures very often donor-lymphocyte infusion is performed, especially in CML patients.

2.2. Investigation of chimerism in nonmyeloablative/reduced-intensity HSCT

One of the main factors allowing increase in the number of HSCT is the development of nonmyeloablative conditioning and reduced-intensity conditioning regimens. The introduction of these protocols is related to the lower treatment-related mortality, allowing the application of HSCT as a treatment for elderly people and patients in a severe clinical condition. Another substantial advantage of these protocols is the better immune reconstitution due to the lower degree of thymus injury, despite the fact that studies showed similar outcomes for HSCT. This allows better regeneration of naïve T cells and proliferation of immune-competent cells of the recipient, which have survived after conditioning. The majority of RIC protocols are based on a combination of purine analogs, usually fludarabine with alkylating agents, such as busulfan or cyclophosphamide, and represent a different intensity of myelosuppression [42–44]. Another approach is to use low doses of TBI, alone or in combination with fludarabine [45]. This approach relies on the GVL effect and is associated with minimal toxicity. Several studies have shown the association between patient conditioning and posttransplant chimerism levels [46, 47].

Unlike HSCT with myeloablative conditioning, nonmyeloablative conditioning is related to a mixed chimerism [6]. Additionally, the probability to develop aGVHD is lower, while probability to develop cGVHD is comparable for both conditioning protocols. The immunobiology of allo-SCT after NMA conditioning differs from that after MA mainly by the release of less inflammatory cytokines. Tissue damage caused by myeloablation is translated into proinflammatory cytokines that are supposed to provide a milieu for the development of GVHD. As a result, aGVHD after NMA is delayed and may develop after day +100, remaining a significant cause of morbidity and mortality in NMA SCT patients. Mixed chimerism does not mean grim prognosis but could be associated with increased risk of relapses.

Relatively decreased aGVHD after RIC HSCT could be explained by a combination of pretransplant conditioning and initial mixed chimerism that could contribute to the development of tolerance [29, 45]. Some patients demonstrate late development of aGVHD that coincides with the transition from mixed to complete donor T cell chimera [46]. In patients who do not develop GVHD, donor lymphocyte infusion could be performed in order to achieve complete chimera and GVL effect. Therefore, in HSCT with nonmyeloablative conditioning, it is very important to monitor chimerism level in order to assess correctly the need of donor lymphocyte infusion and the effect of this treatment [37]. Lower levels of T and NK cells in the first 30 days following HSCT are associated with increased risk of graft rejection. Granulocyte and monocyte chimerism levels have limited relevance for graft rejection [46]. In contrast, high levels of donor T cells are predictive for development of aGVHD grade 2–4 [31, 46, 48]. Additionally, levels above 90% donor T and/or NK cells are associated with antitumour response and disease-free survival [46,

49]. Similar results have been reported by Mohty et al. (n = 102) [48]. In contrast, some studies do not reveal an association between the level of donor T lymphocytes and the development of GVHD [50, 51]. In their study, Lim et al. showed that rapid conversion to complete donor chimera is associated with decreased overall survival and increased TRM when compared with persistence of mixed chimerism. No association with relapse frequency was observed [52]. Mixed chimera is related to better survival—this observation could be explained by the persisting recipient cells, associated with resistance to infections and overall survival [52, 53]. Patients with low absolute count of dendritic cells, one month following HSCT, have a higher TRM (60% versus 12%, $P < 0.02$) and decreased overall survival (15% versus 45%, $P < 0.002$) [54]. There is also an association between decreased $CD16^+$ dendritic cells and increased infection-related mortality (50% versus 0%, $P < 0.05$). The possibility that the infection itself is likely to decrease the number of these cells cannot be excluded [54].

2.3. Chimerism following donor lymphocyte infusion (DLI)

Donor lymphocyte infusion is a new therapeutic strategy, applied mainly in cases of nonmyeloablative, T cell-depleted myeloablative HSCT, or haploidentical transplantations due to its GVL effect. These patients have delayed immune reconstitution, increasing the risk of disease relapse. Application of standard DLI is limited due to GVHD and aplasia. New protocols using G-CSF-stimulated blood progenitor cells, allodepleted donor T cells and mHag-specific $CD8^+$ cytotoxic T lymphocytes have been currently developed allowing wider application of this type of therapy. A limitation of the latter is estimating the right time for DLI. Lymphocyte infusions in the early relapses are associated with favorable outcome [55, 56]. Therefore, chimerism should be monitored regularly in order to detect recipient-specific hematopoiesis [6, 57, 58]. Infused lymphocytes are sensitized by surface antigens that are associated with leukemia or polymorphic minor histocompatibility antigens, which are expressed by the leukemic cells. This induces their transformation into cytotoxic lymphocytes killing cancer cells. DLI is most effective in patients with CML (approximately 75%), which could be explained by the antigen-presenting cells found in malignant clones. This therapy is not effective in patients with AML or multiple myeloma. The development of new therapeutic protocols has decreased aplasia and GVHD.

3. Chimerism in nonmalignant diseases

Currently, a major part of inherited or acquired nonmalignant diseases, such as thalassemia, aplastic anemia, and immune deficiencies, can be successfully treated by HSCT. The aim of such type of transplantation is to achieve stable engraftment, enabling hematopoiesis, enzyme activity, or immune competence. Therefore, establishment of complete donor chimera is not necessary and the recipient can be conditioned by nonmyeloablative therapy. This results more often in the establishment of mixed chimera, increased risk of graft rejection, and decreased risk of GVHD. On the other hand, rapid development of complete chimera in NK and T cells is very important for the successful engraftment, although it increases the frequency of acute GVHD [59–61].

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4. Chimerism in malignant diseases

Monitoring MRD and chimerism levels in patients with malignant diseases is very important. Allogeneic HSCT is an effective therapy in both low- and high-risk CLL. The analysis of 44 high-risk patients with 17p deletion and RIC transplantation showed a four-year cumulative frequency of disease progression of 34% [62]. Establishment of T cell mixed chimera 90 days following transplantation and chemorefractory disease are associated with increased risk of disease progression. Establishment of complete donor chimera and lack of MRD are associated with prolonged DFS. Limited data on the relapse outcome in CLL are available and some patients have positive response to DLL treatment and immunosuppression withdrawal. A better response has been observed in patients with 100% donor T cells. Many studies have shown that conversion from mixed to complete donor chimera predicts development of GVHD [46, 62–64].

In patients with acute leukemia (AML and ALL), mixed chimera may be associated with increased risk of graft rejection. A greater risk has been demonstrated in patients with high levels of residual cells and/or rapid increase of recipient cells [2, 4, 5]. Monitoring of the level of expression of WT1 and the kinetics of nonseparated CD34 chimerism allows early detection of relapse in MDS and AML patients. In patients with stable $CD34^+$ levels and WT1 expression under predefined cut off, relapse could be excluded within the following 28 days [34, 65, 66].

Establishment of mixed chimera can be associated with many factors such as conditioning intensity, T cell-depleted grafts, CD34 cell dosage, techniques used for monitoring, and primary disease. Due to the dynamics in chimerism development, chimerism should be monitored at shorter intervals, especially in the first 1–2 months following transplantation when the risk of rejection is higher. In the early posttransplant period, the persistent mixed chimera could indicate graft rejection or early relapse. On the other hand, the increased number of recipient cells at a later stage after transplantation is associated with a relapse or late rejection.

Due to its low sensitivity of about 1%, chimerism analysis could be used as a prognostic rather than an indirect indicator for MRD. With the introduction of RQ-PCR technique, the sensitivity of chimerism monitoring has improved to 0.1%, resulting in improvement of its prognostic value for MRD detection.

5. Chimerism analysis: technical considerations

Expanding the curability of hematological diseases with stem cell transplantation, as well as introducing new conditioning protocols allows more patients to be eligible for this treatment. This raises the need for better posttransplant chimerism monitoring, since the different quantitative or semiquantitative methods have their advantages and disadvantages. While the significance of the absolute value is still under discussion, the relative changes in engraftment

kinetics are a reliable sign for acceptance or rejection of the graft. This, in its turn, requires a determination of the factors intrinsic for the platform, which may influence the observed changes in chimerism result [67, 68]. Some of the most important characteristics of the methods for postSCT chimerism monitoring are their sensitivity, precision, and reproducibility [69–71].

Investigating microsatellites (STR) and minisatellites (VNTR) is considered the most sensitive and informative technique to study the levels of chimerism after HSCT. Micro (2–5 bp) and minisatellites (9–80 bp) are tandemly repeated blocks of noncoding DNA that are widespread throughout the human genome. The repeat-pattern of these blocks varies greatly among individuals in a population. There is a large number of STR (short tandem repeats) systems that have been mapped throughout the human genome, and they are found on almost every chromosome (**Table 1**).

Chromosome	STR markers	RFLP-based VNTR markers	Other PCR-based markers
1	F13B, RENA4, D1S1171, D1S1627, D1S1656, D1S1677, D1S2142, and D1GATA113	D1S7 and D1S339	D1S80
2	APOB, TPOX, D2S410, D2S441, D2S436, D2S1242, D2S1338, D2S1360, D2S1772, and D2S1776	D2S44	ApoB
3	ACPP, D3S1349, D3S1352, D3S1358, D3S1359, D3S1545, D3S1744, D3S3053, and D3S4529		
4	FABP, FGA (FIBRA), GABARB15, D4S2364, D4S2366, D4S2368, and D4S2408	D4S139	GC (PM) and GYPA (PM)
5	CSF1PO, D5S373, D5S815, D5S818, and D5S2500	D5S110	
6	F13A1, FOLP23, SE33 (ACTBP2), D6S366, D6S474, D6S477, D6S502, D6S965, D6S1017, and D6S1043	D6S132	DQa
7	D7S460, D7S809, D7S820, D7S821, D7S1517, D7S1520, and D7S3048	D7S21, D7S22, D7S467	D7S8 (PM)
8	LPL (LIPOL), D8S306, D8S320, D8S323, D8S344, D8S347, D8S639, D8S1132, D8S1115, and D8S1179		
9	D9S52, D9S302, D9S304, D9S925, D9S1122, and D9S2157		
10	D10S89, D10S1248, D10S1435, D10S2325, and D10S2326	D10S28	
11	APOAI1, TH01 (TC11), UGB, D11S488, D11S554, and D11S4463		HBGG (PM)
12	CD4, PLA2A1, VWA, D12S67, D12S391, D12S1090, and D12ATA63	D12S11	

http://dx.doi.org/10.5772/67383

Chromosome	STR markers	RFLP-based VNTR markers	Other PCR-based markers
13	D13S308, D13S317, and D13S1492		
14	D14S306, D14S608, and D14S1434	D14S13	
15	CYAR04 (P450), FES/FPS, Penta E, D15S659, and D15S822		
16	D16S537, D16S539, and D16S3253	D16S85	
17	D17S974, D17S976, and D17S1301	D17S79, D17S26	D17S5, YNZ22
18	MBP, D18S51, D18S535, D18S849, D18S853, and D18S1270		
19	D19S253, D19S433		LDLR (PM)
20	D20S85, D20S161, D20S470, D20S482, and D20S1082		
21	Penta D, D21S11, D21S1437, and D21S2055		
22	D22S683 and D22S1045		
X	HPRTB, ARA, STRX1, DXYS156, DXS101, DXS981, DXS6789, DXS6795, DXS6797, DXS6800, DXS6801, DXS6803, DXS6807,DXS6809, DXS7130, DXS7132, DXS7133, DXS7423, DXS7424, DXS8377, DXS8378, DXS9895, DXS9898, DXS9905, DXS9908, DXS10011, GATA31E08, GATA165B12, and GATA172D05		Amelogenin
Y	DYS19, DXYS156, DYS385 a/b, DYS388, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYF406S1, DYS437, DYS438, DYS439, DYS444, DYS446, DYS447, DYS448, DYS449, DYS456, DYS458, DYS460, DYS461, DYS462, DYS463, DYS464 a/b/c/d, DYS481, DYS485, DYS495, DYS505, DYS508, DYS520, DYS522, DYS525, DYS531, DYS532, DYS533, DYS534, DYS540, DYS549, DYS556, DYS557, DYS570, DYS576, DYS578, DYS589, DYS594, DYS617, DYS635, DYS643, DYS724 (CD a/b), GATA-H4, and YCAII a/b		Amelogenin and Y-SNPs
mtDNA			HV1 and HV2

Table 1. Chromosome location of mini- and microsatellites [72].

The method is semiquantitative and has a moderate level of sensitivity—1–5%; neither gender nor HLA compatibility of donor and recipient can influence its informativity; it requires a very small number of cells, which makes it applicable in the first postSCT days.

Multiplex platforms use STR/VNTR (variable number tandem repeats) loci with identical amplification protocols and different allelic lengths, using fluorescently labeled primers. Due to its large size, VNTR more often showed discrepancies in the evaluation of chimerism between markers [73]. All loci are amplified together in a single PCR reaction and amplification products are electrophoretically separated on an automated DNA sequencer—fragment analysis. Further analysis is carried out by specialized software. Microsatellites are more widely used, since they are polymorphic, more sensitive, faster to work with, and cheaper. Despite their excellent performance in forensic science, their application in the study of chimerism has its limitations. The most important one results from the very design of the platform—the amount of DNA is indirectly evaluated based on measurement of fluorescence during electrophoresis. The various fluorophores differ in their efficiency to emit light, and the platform has no internal calibration for measurement. As a result, the measurement of one and the same absolute value of DNA will not be equally estimated in all loci. However, this fluorescence-based technology is considered the gold standard in the study of chimerism [58]. Another feature of the STR system is the presence of additional signals—stutter peaks, which are mainly n − 1 and to a lesser degree n + 1 signals [74, 75]. These artifacts are the result of slipped-strand mispairing during amplification. Their size depends on the size and type of the allele, and is in the range 2–13% of their respective base peaks. Stutter is less pronounced with larger repeat units (dinucleotides > tri- > tetra- > penta-). Longer repeat regions generate more stutter, and each successive stutter product is less intense. If stutter peaks match the corresponding donor or recipient peaks, this would affect the estimate of chimerism. This is especially important at low levels of the host residual cells, which would hamper the assessment of minimal residual disease. In heterozygous loci, the two alleles should be equal in amount, but due to stochastic effects during PCR amplification, an imbalance in the two detected alleles is established—this is especially true when the amount of DNA being amplified is limited. Under conditions of extreme imbalance, one allele may "drop-out".

6. Conclusion

Different protocols have been used to condition patients prior to HSCT. They differ from each other in intensity of myeloablation. Myeloablative conditioning is very aggressive and leads to high toxicity and TRM, so its application is limited to younger patients and patients in good medical condition. The risk of TRM decreases with time, although the causes for this are not fully understood. Most likely this is due to advances in technology for HLA typing, better understanding of the role of HLA compatibility, particularly with regard to unrelated donors, and better medical services for patients. Various conditioning protocols are deemed to have varying degrees of ablation of the recipient immune system resulting in different profiles of posttransplantation recovery.

The levels of chimerism after HSCT depend on a number of factors, including the intensity of pretransplant conditioning, the use of T cell-depleted grafts, the number of transplanted stem cells, the sensitivity of the technique used for detection and the interval of study, and as well as the type of the disease.

The main objective in posttransplantation monitoring is early diagnosis of adverse events. Due to the dynamic nature of the development of chimerism, its monitoring should be carried out at short intervals, especially in the first 1–2 months after transplantation, when the risk of disease recurrence or graft failure/rejection is greatest. In the earliest posttransplantation period, the presence of persistent mixed chimerism or the disappearance of donor alleles are associated with both graft rejection and early relapse of the underlying disease. On the other hand, the increasing number of recipient cells in the later periods after the transplantation is associated with upcoming relapse or late allograft rejection. The mechanisms that direct hematopoietic recovery toward one or another course are not yet fully understood. The analysis of chimerism kinetics allows early differentiation between the lack of engraftment and its delay, as well as early detection of patients at high risk of developing GVHD or susceptible to a relapse. In this context, the study of chimerism is undoubtedly an important method for monitoring the outcome of HSCT.

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References

[1] Kvasnicka H, Wickenhauser C, Thiele J, Varus E, Hamm K, et al. Mixed chimerism of bone marrow vessels (endothelial cells, myofibroblasts) following allogeneic transplantation for chronic myelogenous leukemia. Leuk Lymphoma. 2003;**44**(2):321–328. DOI: 10.1080/1042819021000035699

[2] Bader P, Hancock J, Kreyenberg H, Goulden N, Niethammer D, Oakhill A, et al. Minimal residual disease (MRD) status prior to allogeneic stem cell transplantation is a powerful predictor for post-transplant outcome in children with ALL. Leukemia. 2002;**16**(9):1668–1672. DOI: 10.1038/sj.leu.2402552

[3] Elmaagacli A, Peceney R, Steckel N, Trenschel R, Ottinger H, Grosse-Wilde H, et al. Outcome of transplantation of highly purified peripheral blood CD34+ cells with T cells add-back compared with unmanipulated bone marrow or peripheral blood stem cells from HLA-identical sibling donors in first chronic phase CML. Blood. 2003;**101**(2):446–453. DOI: 10.1182/blood-2002-05-1615

[4] Bader P, Beck J, Frey A, Schlegel P, Hebarth H, Handgretinger R, et al. Serial and quantitative analysis of mixed hematopoietic chimerism by PCR in patients with acute leukemias allows the prediction of relapse after allogeneic BMT. Bone Marrow Transplant. 1998;**21**(5):487–495. DOI: 10.1038/sj.bmt.1701119

[5] Bader P, Holle W, Klingebiel T, Handgretinger R, Benda N, Schlegel P, et al. Mixed hematopoietic chimerism after allogeneic bone marrow transplantation: the impact of quantitative PCR analysis for prediction of relapse and graft reject ion in children. Bone Marrow Transplant. 1997;**19**(7):697–702. DOI: 10.1038/sj.bmt.1700721

[6] Mapara MY, Kim YM, Marx J, Sykes M. Donor lymphocyte infusion-mediated graft-versus-leukemia effects in mixed chimeras established with a nonmyeloablative conditioning regimen: extinction of graft-versus-leukemia effects after conversion to full donor chimerism. Transplantation. 2003;**76**(2):297–305. DOI: 10.1097/01.TP.0000072014.83469.2D

[7] Anderson D, Billingham RE, Lampkin GH, Medawar PB. The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity. 1951;**5**:379–397. DOI: 10.1038/hdy.1951.38

[8] Ford C, Hamerton J, Barnes D, Loutit J. Cytological identification of radiation-chimaeras. Nature. 1956;**177**:452–454. DOI: 10.1038/177452a0

[9] McCann S, Lawler M. Mixed chimerism: detection and significance following BMT. Bone Marrow Transplant. 1993;**11**(2):91–94.

[10] Brock N. The history of the oxazaphosphorine cytostatics. Cancer. 1996;**78**(3):542–547. DOI: 10.1002/(SICI)1097-0142(19960801)78:3<542::AID-CNCR23>3.0.CO;2-Y

[11] Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol. 2008;**8**(1):59–73. DOI: 10.1038/nri2216

[12] Trobaugh FE Jr, Husseini S. Effects of radiation on hematopoietic tissue. Am J Med Technol. 1973;**39**(4):119–131.

[13] Mackall CL, Fleisher TA, Brown MR, Magrath IT, Shad AT, Horowitz ME, et al. Lymphocyte depletion during treatment with intensive chemotherapy for cancer. Blood. 1994;**84**(7):2221–2228.

[14] Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood. 2010;**115**(19):3861–3868. DOI: 10.1182/blood-2009-12-234096

[15] Pizzo PA, Rubin M, Freifeld A, Walsh TJ. The child with cancer and infection. II. Nonbacterial infections. J Pediatrics. 1991;**119**(6):845–857. DOI: 10.1016/S0022-3476(05)83032-X

[16] Mackall CL. T-cell immunodeficiency following cytotoxic antineoplastic therapy: a review. Stem Cells. 2000;**18**(1):10–18. DOI: 10.1634/stemcells.18-1-10

[17] Sfikakis PP, Gourgoulis GM, Moulopoulos LA, Kouvatseas G, Theofilopoulos AN, Dimopoulos MA. Age-related thymic activity in adults following chemotherapy-induced lymphopenia. Eur J Clin Invest. 2005;**35**(6):380–387. DOI: 10.1111/j.1365-2362.2005.01499.x

[18] Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, Feuerstein IM, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med. 1995;**332**(3):143–149. DOI: 10.1056/NEJM199501193320303

[19] Storek J, Witherspoon RP, Storb R. T cell reconstitution after bone marrow transplantation into adult patients does not resemble T cell development in early life. Bone Marrow Transplant. 1995;**16**(3):413–425.

[20] Dudakov J, van den Brink M. Greater than the sum of their parts: Combination strategies for immune regeneration following allogeneic hematopoietic stem cell transplantation. Best Pract Res Clin Haematol. 2011;**24**(3):467–476. DOI: 10.1016/j.beha.2011.05.003.

[21] Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, Feuerstein IM, et al. Distinctions between CD8+ and CD4+ T-cell regenerative pathways result in prolonged T-cell subset imbalance after intensive chemotherapy. Blood. 1997;**89**(10):3700–3707.

[22] Fagnoni FF, Lozza L, Zibera C, Zambelli A, Ponchio L, Gibelli N, et al. T-cell dynamics after high-dose chemotherapy in adults: elucidation of the elusive CD8+ subset reveals multiple homeostatic T-cell compartments with distinct implications for immune competence. Immunology. 2002;**106**(1):27–37. DOI: 10.1046/j.1365-2567.2002.01400.x

[23] Heitger A, Neu N, Kern H, Panzer-Grumayer ER, Greinix H, Nachbaur D, et al. Essential role of the thymus to reconstitute naive (CD45RA+) T-helper cells after human allogeneic bone marrow transplantation. Blood. 1997;**90**(2):850–857.

[24] Meng A, Wang Y, Van Zant G, Zhou D. Ionizing radiation and busulfan induce premature senescence in murine bone marrow hematopoietic cells. Cancer Res. 2003;**63**(17):5414–5419.

[25] Wang Y, Schulte BA, LaRue AC, Ogawa M, Zhou D. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood. 2006;**107**(1):358–366. DOI: 10.1182/blood-2005-04-1418

[26] Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;**15**(12):1628–1633. DOI: 10.1016/j.bbmt.2009.07.004

[27] Ballen K, Colvin G, Porter D, Quesenberry P. Low dose total body irradiation followed by allogeneic lymphocyte infusion for refractory hematologic malignancy--an updated review. Leuk Lymphoma. 2004;**45**(5):905–910. DOI: 10.1080/10428190310001628167

[28] Sorror ML, Maris MB, Storer B, Sandmaier BM, Diaconescu R, Flowers C, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation. Blood. 2004;**104**(4):961–968. DOI: 10.1182/blood-2004-02-0545

[29] Mielcarek M, Martin PJ, Leisenring W, Flowers ME, Maloney DG, Sandmaier BM, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. Blood. 2003;**102**(2):756–762. DOI: 10.1182/blood-2002-08-2628

[30] Doney K, Loken M, Bryant E, Smith A, Appelbaum F. Lack of utility of chimerism studies obtained 2–3 months after myeloablative hematopoietic cell transplantation for ALL. Bone Marrow Transplant. 2008;**42**(4):271–274. DOI: 10.1038/bmt.2008.155

[31] Mickelson DM, Sproat L, Dean R, Sobecks R, Rybicki L, Kalaycio M, et al. Comparison of donor chimerism following myeloablative and nonmyeloablative allogeneic hematopoietic SCT. Bone Marrow Transplant. 2011;**46**(1):84–89. DOI: 10.1038/bmt.2010.55

[32] Bader P, Kreyenberg H, Hoelle W, Dueckers G, Handgretinger R, Lang P, et al.Increasing mixed chimerism is an important prognostic factor for unfavorable outcome in children with acute lymphoblastic leukemia after allogeneic stem-cell transplantation: possible role for pre-emptive immunotherapy? J Clin Oncol. 2004;**22**(9):1696–1705. DOI: 10.1200/JCO.2004.05.198

[33] Qin X-Y, Li G-X, Qin Y-Z, Wang Y, Wang F-R, Liu D-H, et al. Quantitative chimerism: an independent acute leukemia prognosis indicator following allogeneic hematopoietic SCT. Bone Marrow Transplant. 2014;**49**(10):1269–1277. DOI: 10.1038/bmt.2014.158

[34] Koreth J, Kim HT, Nikiforow S, Milford EL, Armand P, Cutler C, et al. Donor chimerism early after reduced-intensity conditioning hematopoietic stem cell transplantation predicts relapse and survival. Biol Blood Marrow Transplant. 2014;**20**(10):1516–1521. DOI: 10.1016/j.bbmt.2014.05.025

[35] Tang X, Alatrash G, Ning J, Jakher H, Stafford P, Zope M, et al. Increasing chimerism following allogeneic stem cell transplantation is associated with longer survival time. Biol Blood Marrow Transplant. 2014;**20**(8):1139–1144. DOI: 10.1016/j.bbmt.2014.04.003

[36] Baron F, Maris M, Sandmaier BM, Storer BE, Sorror M, Diaconescu R, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol. 2005;**23**(9):1993–2003. DOI: 10.1200/JCO.2005.08.136

[37] Antin JH, Childs R, Filipovich AH, Girald S, Mackinnon S, Spitzer T, et al. Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem meetings. Biol Blood Marrow Transplant. 2001;**7**(9):473–485. DOI: 10.1053/bbmt.2001.v7.pm11669214

[38] Mossallam GI, Kamel AM, Storer B, Martin PJ. Prognostic utility of routine chimerism testing at 2 to 6 months after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2009;**15**(3):352–359. DOI: 10.1016/j.bbmt.2008.12.496

[39] Huisman C, de Weger R, de Vries L, Tilanus MG, Verdonck LF. Chimerism analysis within 6 months of allogeneic stem cell transplantation predicts relapse in acute myeloid leukemia. Bone Marrow Transplant. 2007;**39**(5):285–291. DOI: 10.1038/sj.bmt.1705582

[40] Michallet AS, Furst S, Le QH, Dubois V, Praire A, Nicolini F, et al. Impact of chimaerism analysis and kinetics on allogeneic haematopoietic stem cell transplantation outcome after conventional and reduced-intensity conditioning regimens. Br J Hematol. 2005;**128**(5):676–689. DOI: 10.1111/j.1365-2141.2005.05372.x

[41] Gyurkocza B, Rezvani A, Storb R. Allogeneic hematopoietic cell transplantation: the state of the art. Expert Rev Hematol. 2010;**3**(3):285–299. DOI: 10.1586/ehm.10.21

[42] Giralt S, Thall PF, Khouri I, Wang X, Braunschweig I, Ippolitti C, et al. Melphalan and purine analogs containing preparative regimens: reduced-intensity conditioning for

patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood. 2001;**97**(3):631–637.

[43] Khouri IF, Saliba RM, Giralt SA, et al. Nonmyeloablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-vs-host disease, and treatmentrelated mortality. Blood. 2001;**98**(13):3595–3599. DOI: 10.1182/blood.V98.13.3595

[44] Martino R, Caballero MD, Simon-Perez JA, Canals C, Solano C, Urbano-Ispizua A, et al. Evidence for a graft-versus-leukemia effect after allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning in myelogenous leukemia and myelodisplastic syndromes. Blood. 2002;**100**(6):2243–2245. DOI: 10.1182/blood-2002-02-0400

[45] McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-vs-tumor effects. Blood. 2001;**97**(11):3390–3400.

[46] Baron F, Baker JE, Storb R, Gooley TA, Sandmaier BM, Maris MB, et al. Kinetics of engraftment in patients with hematological malignancies given allogenic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood. 2004;**104**(8):2254–2262. DOI: 10.1182/blood-2004-04-1506

[47] Svenberg P, Mattsson J, Ringden O, Uzunel M. Allogeneic hematopoietic SCT in patients with non-malignant diseases, and importance of chimerism. Bone Marrow Transplant. 2009;**44**(11):757–763. DOI: 10.1038/bmt.2009.82

[48] Mohty M, Avinens O, Faucher C, Viens P, Blaise D, Eliaou JF. Predictive factors and impact of full donor T-cell chimerism after reduced intensity conditioning allogeneic stem cell transplantation. Haematologica. 2007;**92**(7):1004–1006.

[49] Keil F, Prinz E, Moser K, Mannhalter C, Kalhs P, Worel N, et al. Rapid establishment of long-term culture-initiating cells of donor origin after nonmyeloablative allogeneic hematopoietic stem-cell transplantation, and significant prognostic impact of donor T-cell chimerism on stable engraftment and progression-free survival. Transplantation. 2003;**76**(1):230–236. DOI: 10.1097/01.TP.0000071862.42835.76

[50] Kapp M, Rasche L, Einsele H, Grigoleit GU. Cellular therapy to control tumor progression. Curr Opin Hematol. 2009;**16**(6):437–443. DOI: 10.1097/MOH.0b013e32832f57d4

[51] Schmaltz C, Alpdogan O, Kappel BJ, Muriglan SJ, Rotolo JA, Ongchin L, et al. T cells require TRAIL for optimal graft-versus-tumor activity. Nat Med. 2002;**8**(12):1433–1437. DOI: 10.1038/nm797

[52] Lim ZY, Pearce L, Ho AY, Barber L, Ingram W, Tobal K, et al. Delayed attainment of full donor chimaerism following alemtuzumab-based reduced-intensity conditioning haematopoeitic stem cell transplantation for acute myeloid leukaemia and myelodysplastic syndromes is associated with improved outcomes. Br J Haematol. 2007;**138**(4):517–526. DOI: 10.1111/j.1365-2141.2007.06676.x

[53] Ting DT, Spitzer TR, Chaudhary A, Muzikansky A, Colby C, Power K, et al. Clinical outcomes of late rather than early full-donor chimerism in patients with advanced lymphomas receiving nonmyeloablative allogeneic hematopoietic SCT. Bone Marrow Transplant. 2008;**42**:329–335. DOI: 10.1038/bmt.2008.167

[54] Talarn C, Urbano-Ispizua A, Martino R, Perez-Simon JA, Battle M, Herrera C, et al. Kinetics of recovery of dendritic cell subsets after reduced-intensity conditioning allogeneic stem cell transplantation and clinical outcome. Haematologica (http://www.haematologica.org/content/92/12/1655.long). 2007;**92**(12):1655–1663. DOI: 10.3324/haematol.11076

[55] Wang Y, Liu DH, Xu LP, Liu KY, Chen H, Zhang XH, et al. Prevention of relapse using granulocyte CSF-primed PBPCs following HLA-mismatched/haploidentical, T-cell-replete hematopoietic SCT in patients with advanced-stage acute leukemia: a retrospective risk-factor analysis. Bone Marrow Transplant. 2012;**47**(8):1099–1104. DOI: 10.1038/bmt.2011.213

[56] Wang Y, Liu DH, Fan ZP, Sun J, Wu XJ, Ma X, et al. Prevention of relapse using DLI can increase survival following HLA-identical transplantation in patients with advanced-stage acute leukemia: a multi-center study. Clin Transplant. 2012;**26**(4):635–643. DOI: 10.1111/j.1399-0012.2012.01626.x

[57] Qin XY, Li GX, Qin YZ, Wang Y, Liu DH, Xu LP, et al. Quantitative chimerism kinetics in relapsed leukemia patients after allogeneic hematopoietic stem cell transplantation. Chin Med J. 2012;**125**(11):1952–1959.

[58] Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, et al. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood. 2002;**99**(12):4618–4625.

[59] Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebeil T. How and when should we monitor chimerism after allogeneic stem cell transplantation? Bone Marrow Transplant. 2005;**35**(2):107–119. DOI: 10.1038/sj.bmt.1704715

[60] McCann SR, Crampe M, Molloy K, Lawler M. Hemopoietic chimerism following stem cell transplantation. Transfus Apher Sci. 2005;**32**(1):55–61. DOI: 10.1016/j.transci.2004.10.006

[61] Stikvoort A, Gertow G, Sundin M, Remberger M, Mattsson J, Uhlin M. Chimerism patterns of long-term stable mixed chimeras posthematopoietic stem cell transplantation in patients with nonmalignant diseases: follow-up of long-term stable mixed chimerism patients. Biol Blood Marrow Transplant. 2013;**19**(5):838–844. DOI: 10.1016/j.bbmt.2013.02.015

[62] Schetelig J, van Biezen A, Brand R, Caballero D, Martino R, Itala M, et al. Allogeneic hematopoietic stem-cell transplantation for chronic lymphocytic leukemia with 17p deletion: a retrospective European group for blood and marrow transplantation analysis. J Clin Oncol. 2008;**26**(31):5094–5100. DOI: 10.1200/JCO.2008.16.2982

[63] Khouri IF, Saliba RM, Admirand J, O'Brien S, Lee MS, Korbling M, et al. Graft-versus-leukaemia effect after non-myeloablative haematopoietic transplantation can overcome the unfavourable expression of ZAP-70 in refractory chronic lymphocytic leukaemia. Br J Hematol. 2007;**137**(4):355–363. DOI: 10.1111/j.1365-2141.2007.06591.x

[64] Shaffer BC, Modric M, Stetler-Stevenson M, Arthur DC, Steinberg SM, Liewehr DJ, et al. Rapid complete donor lymphoid chimerism and graft-versus-leukemia effect are important in early control of chronic lymphocytic leukemia. Exp Hematol. 2013;**41**(9):772–778. DOI: 10.1016/j.exphem.2013.04.015

[65] Lange T, Hubmann M, Burkhardt R, Franke GN, Cross M, Scholz M, et al. Monitoring of WT1 expression in PB and CD34(1) donor chimerism of BM predicts early relapse in AML and MDS patients after hematopoietic cell transplantation with reduced-intensity conditioning. Leukemia. 2011;**25**:498–505. DOI: 10.1038/leu.2010.283

[66] Woehlecke C, Wittig S, Sanft J, Kreyenberg H, Gruhn B. Detection of relapse after hematopoietic stem cell transplantation in childhood by monitoring of WT1 expression and chimerism. J Cancer Res Clin Oncol. 2015;**141**(7):1283–1290. DOI: 10.1007/s00432-015-1919-0

[67] Gineikiene E, Stoskus M, Griskevicius L. Recent advances in quantitative chimerism analysis. Expert Rev Mol Diagn. 2009;**9**(8):817–832. DOI: 10.1586/erm.09.66

[68] Thiede C. Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers. Am J Pharmacogenomics. 2004;**4**(3):177–187.

[69] Kristt D, Klein T. Reliability of quantitative chimerism results: assessment of sample performance using novel parameters. Leukemia. 2006;**20**:1169–1172. DOI: 10.1038/sj.leu.2404191

[70] Kristt D, Stein J, Klein T. Frontiers of stem cell transplantation monitoring: capturing graft dynamics through routine longitudinal chimerism analysis. Isr Med Assoc J. 2007;**9**(3):159–162.

[71] Lukanov T, Shivarova-Ivanova M, Naumova E. Chimerism analysis after allogeneic stem cell transplantation: quantitative real-time or short tandem repeat PCR? Compt Rend Acad Bulg Sci. 2015;**68**(4):521–528.

[72] Butler JM, Reeder DJ. Chromosomal locations for DNA typing markers [Internet]. [Updated: 17.11.2011]. Available from: http://www.cstl.nist.gov/strbase/chrom.htm [Accessed: 15.8.2016].

[73] Butler JM. Commonly used short tandem repeat markers. In: Forensic DNA Typing. London, UK: Academic Press; 2001. pp. 53–79. ISBN 10: 012147951X.

[74] Butler JM. Biology of STRs: stutter products, non-template addition, microvariants, null alleles, and mutation rates. In: Forensic DNA Typing. London, UK: Academic Press; 2001. pp. 81–98. ISBN 13: 9780121479510.

[75] Walsh PS, Fildes NJ, Reynolds R. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. Nucleic Acids Res. 1996;**24**(14):2807–2812.

Section 3

Advances on Stem Cells in Medical Practice

Chapter 5

Human-Induced Pluripotent Stem Cell-Derived Engineered Cardiac Tissues

Takeichiro Nakane, Hidetoshi Masumoto and Bradley B. Keller

Additional information is available at the end of the chapter

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Abstract

By combining tissue engineering techniques with human-induced pluripotent stem cell (hiPSC) technology, human-derived engineered cardiac tissues (ECTs) have been developed using several cell lineage compositions and 3-dimensional geometries. Although hiPSC ECTs are relatively immature compared with native adult heart tissues, they have promising potential as a platform technology for drug-screening and disease modeling, and as grafts for hiPSC-based regenerative heart therapy. This chapter provides the focused overview of the current status of cardiac tissue engineering technology and its possible application.

Keywords: iPS cell, engineered cardiac tissue, tissue engineering, drug screening, disease modeling, cardiac regeneration

1. Introduction

With the progress of tissue engineering technology in the last decade, many kinds of engineered cardiac tissues (ECTs) have been developed and reported. These tissues possess striated myofibers which recapitulate unique contractile function of heart tissues. Takahashi, Yamanaka, and colleagues developed iPSCs from mice in 2006 and from human next year [1, 2]. Human iPS cells (hiPSCs) have the potential to differentiate into cardiomyocytes and other cardiac lineage cells. Recently, the efficiency of cardiac differentiation has rapidly improved, which makes it possible to robustly induce cardiac lineage cells [3–6]. By using cardiac cells derived from hiPSCs as a cell source for ECT generation, the potential of ECTs has expanded. The present chapter overviews the current status of ECT technology and its possible application.

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2. Methods for generating engineered cardiac tissues

2.1. Cell sheet technology

Okano, Shimizu and colleagues have developed a culture surface grafted with temperature-responsive polymer, poly(N-isopropyl acrylamide) (PIPAAm) [7]. This system enables confluent cells to detach themselves from the surface while maintaining a sheet structure simply by reducing the culture temperature. The cell sheets are fabricated without the use of exogenous matrices. It has endogenous matrix layer at the bottom side, and it secures biological attachment to the recipient heart surface within 30 min [8]. This scaffold-free tissue is a thin sheet, and three-dimensional thicker structure can be obtained by stacking several sheets. However, stacking more than three layers (approximately 80 μm thickness) triggers cell death at the center due to hypoxia [9]. At our lab, we overcame this limit by stacking cell sheets with gelatin hydrogel microspheres (GHM) [10]. Five-layered cell sheets with GHM displayed better cell viability *in vitro* compared with the control cell sheet without GHM. Furthermore, cell sheet modification with GHM improved the retention of grafted cell sheets on the rat heart epicardial surface after myocardial infarction. We succeeded in generating over 1 mm thick constructs from 15 sheets using this technology.

2.2. Biomaterials

Cardiomyocytes and other cells are embedded in biomaterials, such as collagen I, matrigel, and/or fibrin in a casting mold. Exogenous gel matrix promotes self-assembly of cells leading to form a tissue structure. The first successful cardiac tissue fabrication by this method was reported in the year 1997 [11]. Fixed anchors in these molds generate static strain, which enhances the cell alignment and contractile function. The geometry of mold controls the final tissue architecture, and a variety of tissue shapes have been formulated, including linear [12–16], circular [17], and mesh structures (**Figure 1**) [18–20]. By applying engineering manufacturing methods, it may be possible to automate the process of generating small-sized ECTs, leading to the development of high throughput *in vitro* analysis systems [21, 22]. In fact, recently, 3D bio-printing technology has been applied and provided the possibility of creating more complicated structures reproducibly by printing both scaffold matrix and living cells [23].

2.3. Prefabricated matrix

Biomaterials provide a 'soft' environment for the cells to grow in. On the other hand, synthetic microporous ('spongy') scaffolds made from alginate, collagen, and gelatin or other stiffer materials, such as polystyrene, PLLA (Poly-L-Lactide Acid), PLGA (Poly(lactic-co-glycolic acid)) and PCL (Polycaprolactone), have also been tested [24, 25]. The advantage of this method is that these rigid scaffolds contribute to engineering any desired structure and size with mechanical stability. However, the maturation of various mesodermal cells is matrix stiffness dependent, and therefore both the biomaterial gel and the supporting mold may impact cell maturation and survival [26].

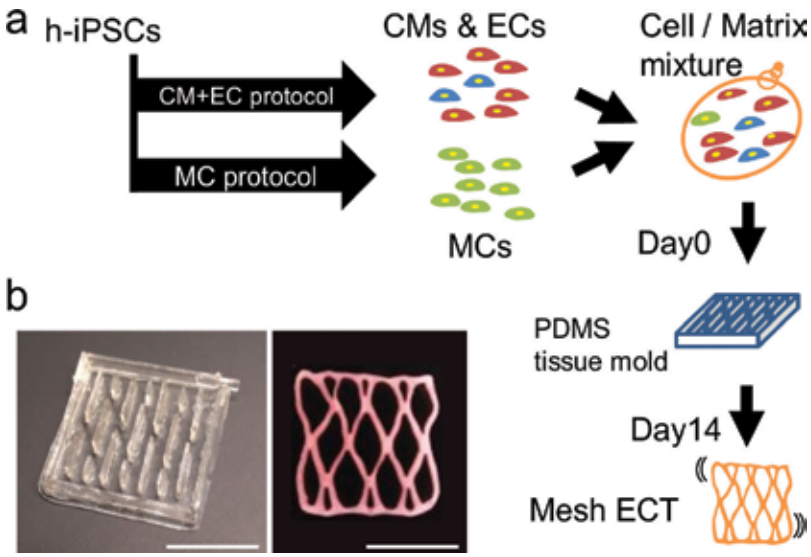

Figure 1. Generation of a mesh ECT by biomaterial-based technique. (a) Schematic diagram for a mesh ECT generation. Cardiomyocytes (CM), endothelial cells (EC), and mural cells (MC) are induced from human iPS cells (h-iPSCs) using two different protocols. Cells and collagen-based matrix were combined and poured into custom polydimethylsiloxane (PDMS) molds at day 0 and cultured for 14 days. (b) Representative image of a PDMS mold (left) and a mesh ECT (right). Scale bar: 10 mm. (Reproduced from Ref. [20] with permission).

2.4. Decellularized tissue

Decellurization of a whole heart is achieved by the treatment with sodium dodecyl sulfate (SDS) and Triton X-100 under Langendorf perfusion [27]. The decellularized tissue maintains most of the tissue contents and function of the extracellular matrix. Hence, decellularized tissue provides a native scaffold which can support repopulation by mesoderm lineage cells. These decellularized tissues may be advantageous in the generation of *in vitro* cardiac tissues [28, 29].

3. Maturation of the tissue

3.1. Cellular contents

A heart tissue is composed of multiple cell types including cardiomyocytes, fibroblasts, endothelial cells, and smooth muscle cells. In human hearts, cardiomyocytes account for 25-50% of total cells, occupying around 70% of the whole volume [30]. Many reports have demonstrated that non-myocytes support the maturation of cardiomyocytes and play a significant role in both myocardial structure and function [13, 16, 19].

We induced cardiomyocytes (CM), endothelial cells (EC), and vascular mural cells (MC) from hiPSCs, and generated ECTs from several formulations of cardiomyocytes and non-myocytes,

such as CM+EC, CM+MC, and CM+EC+MC [16]. According to in vitro force measurement, CM+EC+MC ECTs showed most advanced electrophysiological properties. Furthermore, histological analysis revealed CM+EC+MC possessed more unidirectionally aligned myofiber with mature sarcomeric structures (**Figure 2**).

The mechanism for the improvement of tissue maturation as the result of a more complex lineage mixture is still unknown. Evidence is emerging that cardiomyocytes and fibroblasts are electrically coupled, which may modulate electrophysiologic function [31]. Direct interaction of different cell types or paracrine effects can be considered. It is reported that extracellular matrix derived from cardiac fibroblast supported the proliferation *in vitro* and indicated the usefulness of the coculture [32].

3.2. Effects of extended culture duration on CM and ECT maturation and function

Cardiomyocytes derived from pluripotent stem cells mature early in culture but are arrested at the late embryonic stage under 2-dimensional (2D) culture condition [33]. Meanwhile, it is widely recognized that cardiomyocytes in a 3-dimensional (3D) cardiac tissue acquire a more mature phenotype than those in 2D culture [25, 34].

In our study, we prolonged culture duration of mesh ECTs from 14 to 28days [20]. Long-cultured constructs showed the tendency to augment the active contractile force along with the increase of beating frequency from 1.5Hz to 2.5Hz and maintained greater force compared to 14-day constructs. The shift from a negative to a neutral force-frequency relationship in 28-day constructs represents sustained functional maturation as well as more rapid force generation and relaxation cycle, and the ability to capture higher pacing frequency. Additional culture duration enhanced myofiber alignment along with the expression of several genes related to ion channels and gap junction (**Figure 3**).

3.3. Impact of culture condition on ECT maturation and function

In order to expand the dimension of ECTs, it is necessary to improve the distribution of oxygen and nutrient throughout tissues. Direct perfusion of culture medium reduces the gradients associated with diffusional mass transport between the construct surfaces and the inner phase and improves the microenvironmental conditions within ECTs. [35–37]. In addition to the increase of cellularity at the center of ECTs, perfusion system contributes to the maturation of cardiomyocytes and the tissue structure. It is reported that even the simple rocking dynamic culture yields engineered myocardium with near-adult functional output [38]. The system accelerates force generation and conduction velocity probably due to the activation of mTOR signaling.

For generating ECTs, especially from biomaterial-based technique, materials from other sources, such as collagen derived from rat or matrigel, have been widely used with xeno-serum containing culture medium. Tiburcy and colleagues modified their methods toward clinical application and developed a formulation for generating ECTs by using medical grade bovine collagen and maintaining them under serum-free defined condition [19]. This modification provided a proof-of-concept for a universally applicable technology for the engineering

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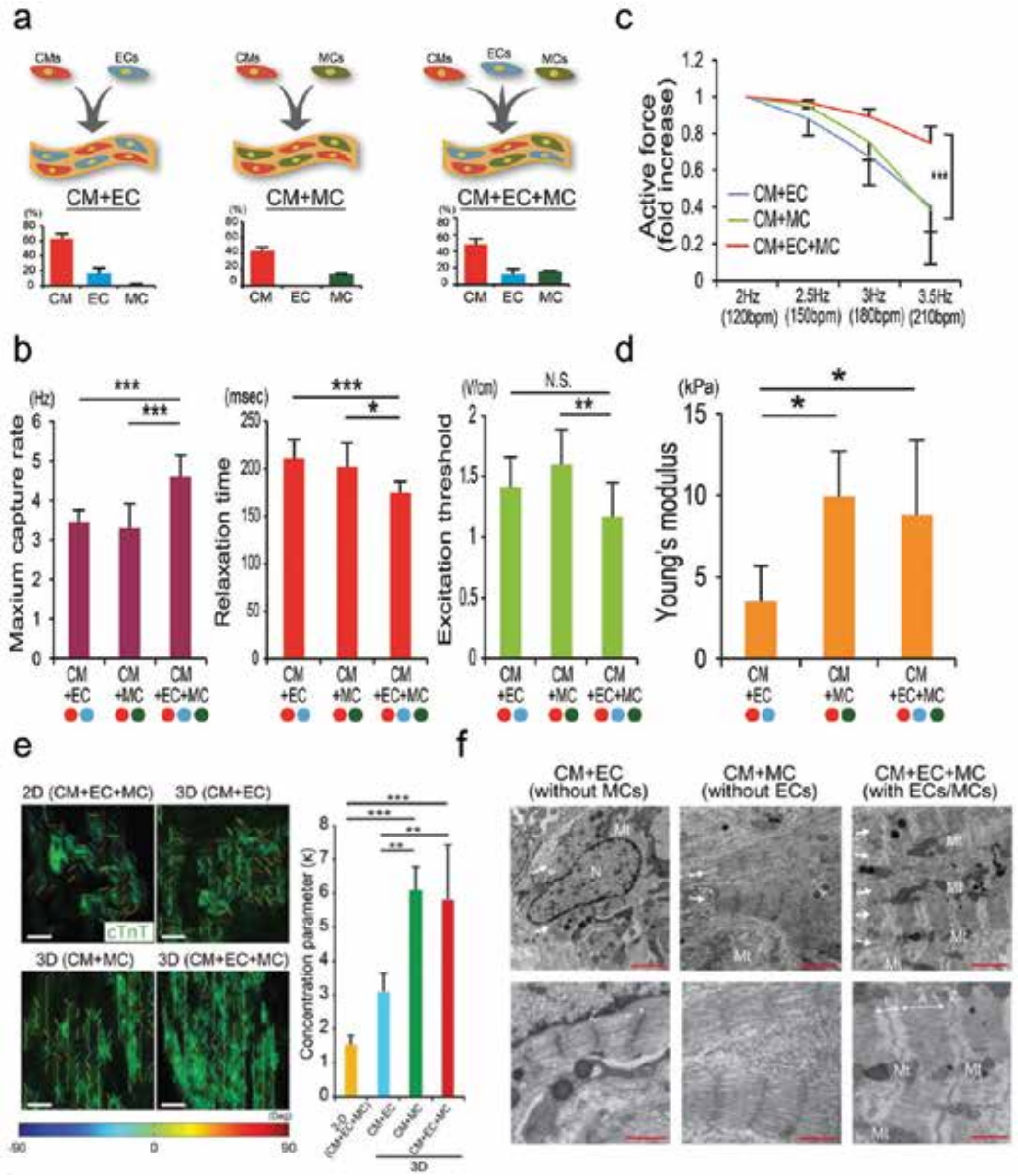

Figure 2. Electromechanical properties and structural maturation of hiPSC-ECT**s**. (a) Schematic diagrams for generationg 3 types of ECTs containing cardiomyocytes (CM), endothelial cells (EC), and/or mural cells (MC) (upper) and the proportions of each cell type (lower) [n = 8 (CM+ EC), 7 (CM+ MC), and 12 (CM+ EC+ MC). (b–d) Results of contractile force measurements [n = 8 (CM+ EC), 7 (CM+ MC), and 12 (CM+ EC+ MC)]. (b) Maximum capture rate (left), relaxation time (center) and excitation threshold voltage (right) (c) Relationship between active force and pacing frequency (2Hz to 3.5Hz). (d) Young's modulus of ECTs. NS, not significant; *P < 0.05,**P < 0.01, ***P < 0.001. (e) Representative alignment analysis using cTnT-stained images after 2-dimensional (2D) and 3-dimensional (3D) culture for 3 types of ECTs (left). Calculated concentration parameter (κ) (right) [n =3 (2D) and 5 (each 3D)]. Larger values of κ represent greater alignment to a single direction. cTnT, cardiac troponin T; Deg, degree. (f) Representative transmission electron microscopic images for each type of ECT cultured for 4 weeks. Arrows indicate myofibers. N, nucleus; Mt, mitochondria; I, I-band; A, A-band; Z, Z-line. (Reproduced from Ref. [16] with permission).

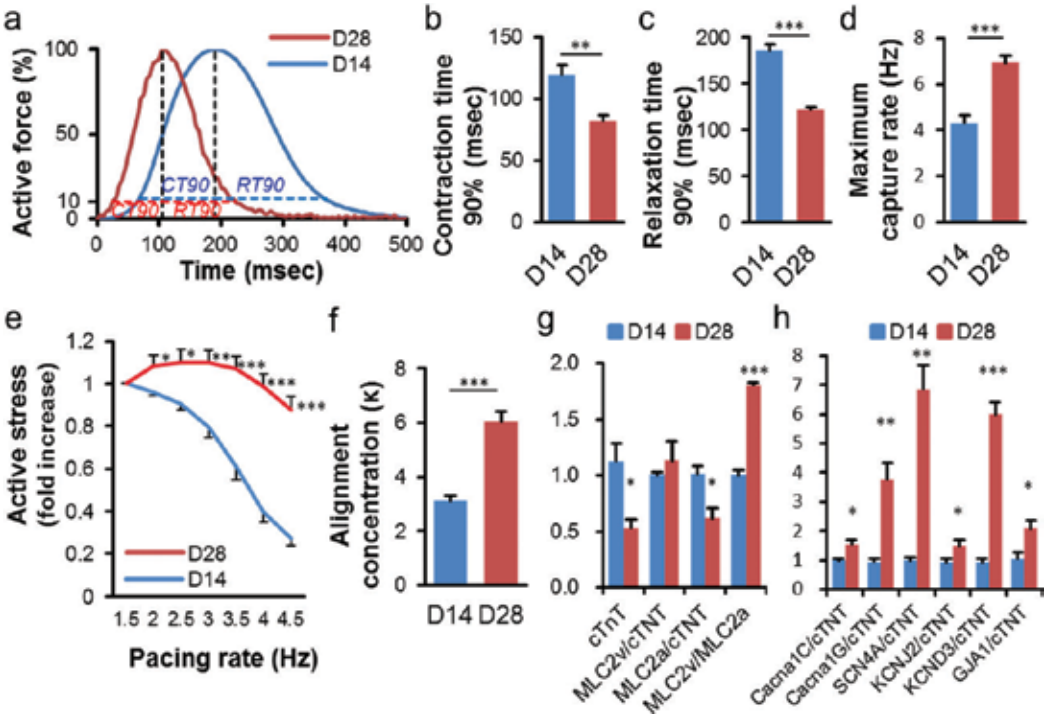

Figure 3. Effects of extended culture duration on mesh ECT maturation. (a) Averaged normalized active force-time curves for mesh ECT cultured for either 14 (blue, D14) or 28 (red, D28) (n = 6) days under 2 Hz electrical stimulation. Contraction time (CT) or relaxation time (RT) represents the time of 90% increase or decrease of force respectively. Comparison of (b) contraction time 90% (n = 6; ***P < 0.01), (c) relaxation time 90% (***P < 0.001) at 2 Hz pacing, (d) maximum capture rate (n = 6; ***P < 0.001), (e) force-frequency relationship (n = 6; *P < 0.05 to ***P < 0.001 vs. D14), and (f) cardiomyocyte alignment concentration (κ), [n = 4 (D14) and n = 3 (D28); ***P < 0.001]. (g), (h) Q-PCR analysis (n = 3; *P < 0.05 to ***P < 0.001). cTnT level was lower in D28, and other genes were normalized to the value of cTnT expression. (Reproduced from Ref. [20] with permission).

cardiac tissue. In addition, ECTs generated by this condition displayed advanced cardiomyocyte maturation compared to the conventional methods [19].

3.4. Role of mechanical loading on ECT maturation and function.

Several *in vitro* ECT studies revealed the impact of uniaxial mechanical loading on the alignment of cardiomyocytes and functional maturation. The simplest system for that is the two fixed anchors which generate static strain on the tissue between them. Furthermore, cyclic stress conditioning markedly increases cardiomyocyte hypertrophy and proliferation rates versus unconditioned constructs [13]. We demonstrated that ECTs displayed a broad spectrum of altered gene expression in response to cyclic stretch, reflecting a complex regulation of proliferation, differentiation, and architectural alignment of cardiomyocytes and non-cardiomyocytes within ECTs [39].

Electrical stimulation is considered to be another important cue for further maturation of tissues [40]. Nunes and colleagues subjected their engineered tissues, named biowires,

on electrical stimulation at up to 6 Hz [41]. Biowires subjected to electrical stimulation had markedly increased myofibril ultrastructural organization, elevated conduction velocity and improved both electrophysiological and Ca^{2+} handling properties compared to non-stimulated controls. These changes were in agreement with cardiomyocyte maturation and were dependent on the stimulation frequency.

Another group created a system which applied combined electromechanical stimulation mimicking isovolumic contraction and confirmed the improvement of functional properties over electrical or mechanical stimulation alone. In the report, the timing of the combined stimulation greatly affected the electrophysiological properties of ECTs [42].

4. Applications of ECTs

4.1. Drug screening

Cardiotoxicity is one of the main causes of drug withdrawal from the market [43, 44]. To evaluate the safety and effectiveness of drugs, several kinds of pre-clinical studies are performed using non-human models. However, these models occasionally show incorrect results, such as pseudo-negative, due to the difference of electrophysiology in other species [45]. By using hiPSC technology, effects to humans can be examined from the early stage [46]. Moreover, hiPSC-derived ECTs are expected to be more specific and sensitive platforms for drug screening [14].

We have developed a 3D cardiac tissue model which reproduces Torsade de Pointes (TdP) showing both a typical polymorphic extracellular field potential and meandering spiral wave re-entry upon treatment with IKr channel blockers [47]. It is generated from hiPSC-derived cardiomyocytes and mesenchymal cells using the cell sheet technology. The appearance of TdP-like waveforms was significantly higher in this 3D model compared with 2D monolayer conditions indicating that the multilayered 3D structure is an essential factor for this arrhythmia along with the coexistence of non-cardiomyocytes.

Many groups have developed various high throughput screening formats, such as strip format [48] and heart-on-chip [21, 49], and confirmed the similarity of hiPSC-derived engineered tissues with native heart tissues [14, 50–52]. Video optical recording system set up in a usual CO_2 incubator is also useful to continuously monitor contractile abilities of tissues [48].

Cardiomyocytes from hiPSCs are at the premature stage, and even ECTs generated to date remain immature compared to native adult myocardium. Hence, further cues for maturation as described above is necessary to imitate native tissues more precisely [51].

4.2. Disease modeling

iPS cells derived from a patient with a known genetic mutation for the disease can provide the disease model, which may offer a useful strategy for understanding the mechanism of the disease and exploring a new treatment modality.

Cardio-facio-cutaneous syndrome (CFCS) is one of the RASopathies, and cardiac abnormalities are the most common findings among CFCS, including hypertrophic cardiomyopathy (HCM) in approximately 40% of patients. Nearly 75% of patients with CFCS exhibit mutations in *BRAF*, which encodes a serine/threonine kinase and a direct effector of Ras. Cashman and colleagues created 3D human engineered cardiac tissue model of HCM using human cardiomyocytes yielded by directed differentiation of iPSCs established from a patient with CFCS carrying an activated BRAF mutation [53].

Chronic catecholamine overstimulation contributes to heart failure progression. Overstimulation of ECTs with norepinephrine provides a simulation of a human heart failure

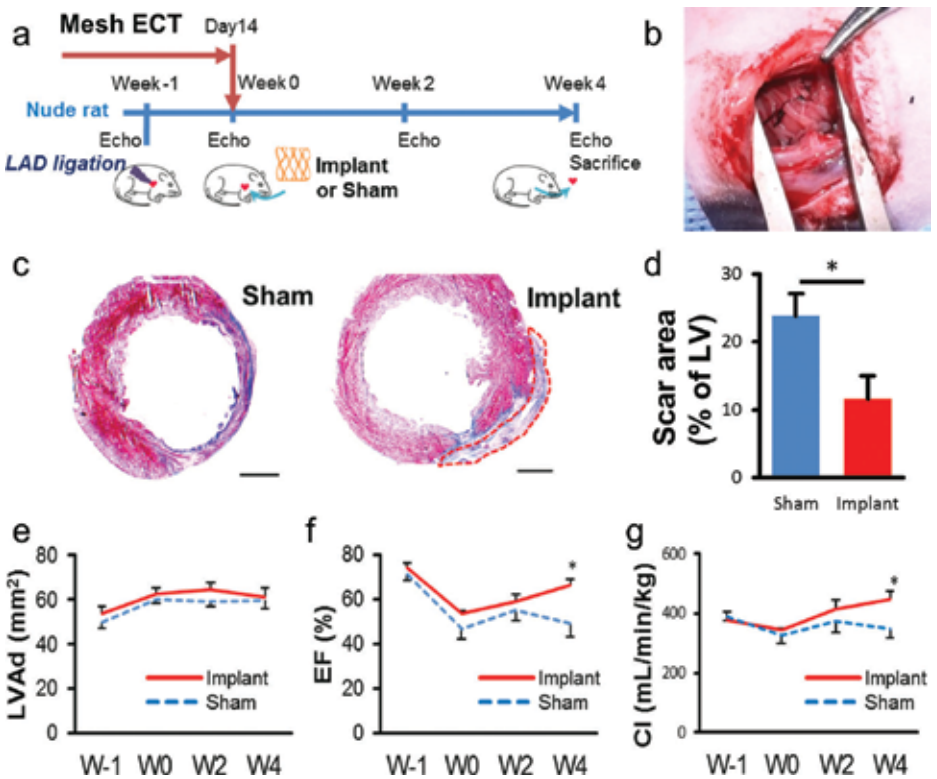

Figure 4. Therapeutic effects of hiPSC-mesh ECT implantation in a rat myocardial infarction model. (a) Schematic timeline of surgery. A mesh ECT matured *in vitro* for 14 days (or sham suture) is implanted in a nude rat (week 0) 1 week after the induction of myocardial infarction by ligating left anterior descending artery (LAD). Echocardiogram (Echo) is performed prior to LAD ligation at week-1 (W-1), prior to implantation at week 0 (W0), then week 2 (W2) and week 4 (W4). (b) Grafted mesh ECT on the heart surface covering infarction site. (c) Representative Masson's trichrome staining images of sham treated (left) and mesh ECT implanted (right) rat hearts at W4. Scale bar: 2 mm. Red dotted line indicates engrafted area. (d) Comparison of scar area (% of LV area) at W4 (n=5, *P<0.05 Implant versus Sham). (**e-g**) Results of echocardiogram [n=5 (Implant, red solid line) and 5 (Sham, blue dotted line)]. **(e)** Left ventricular end diastolic area (LVAd; mm^2), **(f)** ejection fraction, EF (%), and **(g)** cardiac index, CI (mL/min/kg) (*P<0.05 Implant versus Sham at W4). (Reproduced from Ref. [20] with permission).

phenotype [19]. Tissues responded to chronic catecholamine toxicity with contractile dysfunction, cardiomyocyte hypertrophy, cardiomyocyte death, and NT-proBNP release, which are classical hallmarks of heart failure. Notably, the pathological phenotype could be partially or fully prevented by β1- or α1-adrenoreceptor blockade, demonstrating the applicability of ECTs in the *in vitro* simulation of heart failure and its prevention by pharmacological means.

4.3. Transplantation therapy

HiPSCs are now one of the most promising cell sources for cardiac regenerative cell therapy [54–56]. There are major methods of cell delivery, including intracoronary or intramuscular injection of dispersed cells and epicardial transplantation of engineered tissues [57]. It is possible to deliver a large number of differentiated cells with organized architecture by ECT implantation. The grafted tissues survive and support the heart wall, which overcomes the problem of poor retention rate following cell injection [34].

A variety of studies has revealed the efficacy of ECT implantation for myocardial structural and functional recovery in injured hearts of several animal models [6, 10, 58, 59]. We implanted ECTs in an athymic nude rat myocardial infarction model. ECTs with vascular cells displayed the invasion of vasculature from the host heart to the tissue and their perfusion. Survived ECTs replaced the ventricular wall in the injured area and prevented the scar formation after myocardial infarction and improved cardiac function (**Figure 4**). ECTs survived during 4-week follow-up period [16, 20]. However, further work is required to identify the underlying mechanism for the functional recovery. Meanwhile, the first transplantation of cardiac progenitor patch derived from human embryonic stem cells in a severe heart failure patient was performed in France, offering an encouraging result [60].

5. Conclusion

In this chapter, we have reviewed several aspects of current cardiac tissue engineering technologies and presented the possible applications of these tissues for *in vitro* drug toxicity testing, human disease modeling, and paradigms for myocardial recovery with muscle replacement following injury. This rapidly evolving new field is now incorporating manufacturing process to expand the scalability and reduce the cost of generating these novel engineered *in vitro* myocardial tissues.

Conflict of interest

None.

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References

[1] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;**126**:663-676

[2] Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;**131**:861-872

[3] Laflamme MA, Chen KY, Naumova AV, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. Nature Biotechnology. 2007;**25**:1015-1024

[4] Kattman SJ, Witty AD, Gagliardi M, et al. Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. Cell Stem Cell. 2011;**8**:228-240

[5] Uosaki H, Fukushima H, Takeuchi A, et al. Efficient and scalable purification of cardiomyocytes from human embryonic and induced pluripotent stem cells by VCAM1 surface expression. PLoS One. 2011;**6**:e23657

[6] Masumoto H, Ikuno T, Takeda M, et al. Human iPS cell-engineered cardiac tissue sheets with cardiomyocytes and vascular cells for cardiac regeneration. Scientific Reports. 2014;**4**:6716

[7] Shimizu T, Yamato M, Isoi Y, et al. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circulation Research. 2002;**90**:40-48

[8] Chang D, Shimizu T, Haraguchi Y, et al. Time course of cell sheet adhesion to porcine heart tissue after transplantation. PLoS One. 2015;**10**:1-14

[9] Shimizu T, Sekine H, Yang J, et al. Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues. The FASEB Journal. 2006;**20**:1-20

[10] Matsuo T, Masumoto H, Tajima S, et al. Efficient long-term survival of cell grafts after myocardial infarction with thick viable cardiac tissue entirely from pluripotent stem cells. Scientific Reports. 2015;**5**:16842

[11] Eschenhagen T, Fink C, Remmers U, et al. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: A new heart muscle model system. The FASEB Journal. 1997;**11**:683-694

[12] Tobita K, Liu LJ, Janczewski AM, et al. Engineered early embryonic cardiac tissue retains proliferative and contractile properties of developing embryonic myocardium. American Journal of Physiology. Heart and Circulatory Physiology. 2006;**291**: H1829-H1837

[13] Tulloch NL, Muskheli V, Razumova MV, et al. Growth of engineered human myocardium with mechanical loading and vascular coculture. Circulation Research. 2011; **109**:47-59

[14] Schaaf S, Shibamiya A, Mewe M, et al. Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. PLoS One. 2011;**6**(10):e26397. DOI: 10.1371/journal.pone.0026397

[15] Kensah G, Lara AR, Dahlmann J, et al. Murine and human pluripotent stem cell-derived cardiac bodies form contractile myocardial tissue in vitro. European Heart Journal. 2013;**34**:1134-1146

[16] Masumoto H, Nakane T, Tinney JP, et al. The myocardial regenerative potential of three-dimensional engineered cardiac tissues composed of multiple human iPS cell-derived cardiovascular cell lineages. Scientific Reports. 2016;**6**:29933

[17] Zimmermann W-H, Melnychenko I, Wasmeier G, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nature Medicine. 2006; **12**:452-458

[18] Bian W, Liau B, Badie N, et al. Mesoscopic hydrogel molding to control the 3D geometry of bioartificial muscle tissues. Nature Protocols. 2009;**4**:1522-1534

[19] Tiburcy M, Hudson JE, Balfanz P, et al. Defined engineered human myocardium with advanced maturation for applications in heart failure modeling and repair clinical perspective. Circulation. 2017;**135**:1832-1847

[20] Nakane T, Masumoto H, Tinney JP, et al. Impact of cell composition and geometry on human induced pluripotent stem cells-derived engineered cardiac tissue. Scientific Reports. 2017;**7**:45641

[21] Sidorov VY, Samson PC, Sidorova TN, et al. I-Wire Heart-on-a-Chip I: Three-dimensional cardiac tissue constructs for physiology and pharmacology. Acta Biomaterialia. 2017; **48**:68-78

[22] Schroer AK, Shotwell MS, Sidorov VY, et al. I-Wire Heart-on-a-Chip II: Biomechanical analysis of contractile, three-dimensional cardiomyocyte tissue constructs. Acta Biomaterialia. 2017;**48**:79-87

[23] Gaetani R, Feyen DAM, Verhage V, et al. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. Biomaterials. 2015;**61**:339-348

[24] Asthana A, Kisaalita WS. Biophysical microenvironment and 3D culture physiological relevance. Drug Discovery Today. 2013;**18**:533-540

[25] Hirt MN, Hansen A, Eschenhagen T. Cardiac tissue engineering: State of the art. Circulation Research. 2014;**114**:354-367

[26] Young JL, Engler AJ. Hydrogles with time-dependent material properties enhance cardiomyocyte differentiation in-vitro. Biomaterials. 2011;**32**:1002-1009

[27] Ott HC, Matthiesen TS, Goh S-K, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. Nature Medicine. 2008;**14**:213-221

[28] Guyette JP, Charest J, Mills RW, et al. Bioengineering human myocardium on native extracellular matrix. Circulation Research. 2015 CIRCRESAHA.115.306874

[29] Blazeski A, Kostecki GM, Tung L. Engineered heart slices for electrophysiological and contractile studies. Biomaterials. 2015;**55**:119-128

[30] Jugdutt BI. Ventricular remodeling after infarction and the extracellular collagen matrix: When is enough enough? Circulation. 2003;**108**:1395-1403

[31] Quinn TA, Camelliti P, Rog-Zielinska EA, et al. Electrotonic coupling of excitable and nonexcitable cells in the heart revealed by optogenetics. Proceedings of the National Academy of Sciences. 2016;**113**:14852-14857

[32] Zeng Q, Guo Y, Liu L, et al. Cardiac fibroblast-derived extracellular matrix produced in vitro stimulates growth and metabolism of cultured ventricular cells. International Heart Journal. 2013;**54**:40-44

[33] Uosaki H, Cahan P, Lee DI, et al. Transcriptional landscape of cardiomyocyte maturation. Cell Reports. 2015;**13**:1705-1716

[34] Pecha S, Eschenhagen T, Reichenspurner H. Myocardial tissue engineering for cardiac repair. The Journal of Heart and Lung Transplantation. 2016;**35**:294-298

[35] Radisic M, Yang L, Boublik J, et al. Medium perfusion enables engineering of compact and contractile cardiac tissue. American Journal of Physiology. Heart and Circulatory Physiology. 2004;**286**:H507-H516

[36] Carrier RL, Rupnick M, Langer R, et al. Effects of oxygen on engineered cardiac muscle. Biotechnology and Bioengineering. 2002;**78**:617-625

[37] Carrier RL, Rupnick M, Langer R, et al. Perfusion improves tissue architecture of engineered cardiac muscle. Tissue Engineering. 2002;**8**:175-188

[38] Jackman CP, Carlson AL, Bursac N. Dynamic culture yields engineered myocardium with near-adult functional output. Biomaterials. 2016;**111**:66-79

[39] Ye F, Yuan F, Li X, et al. Gene expression profiles in engineered cardiac tissues respond to mechanical loading and inhibition of tyrosine kinases. Physiological Reports. 2013; **1**:e00078

[40] Korolj A, Wang EY, Civitarese RA, et al. Biophysical stimulation for in vitro engineering of functional cardiac tissues. Clinical Science. 2017;**131** http://www.clinsci.org/content/131/13/1393 [Accessed 5 September 2017]

[41] Nunes SS, Miklas JW, Liu J, et al. Biowire: A platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nature Methods. 2013;**10**:781-787

[42] Morgan KY, Black LD. Mimicking isovolumic contraction with combined electromechanical stimulation improves the development of engineered cardiac constructs. Tissue Engineering. Part A. 2014;**20**:1654-1667

[43] Ferri N, Siegl P, Corsini A, et al. Drug attrition during pre-clinical and clinical development: Understanding and managing drug-induced cardiotoxicity. Pharmacology & Therapeutics. 2013;**138**:470-484

[44] Li X, Zhang R, Zhao B, et al. Cardiotoxicity screening: A review of rapid-throughput in vitro approaches. Archives of Toxicology. 2016;**90**:1803-1816

[45] Gintant G. An evaluation of hERG current assay performance: Translating preclinical safety studies to clinical QT prolongation. Pharmacology & Therapeutics. 2011;**129**: 109-119

[46] Navarrete EG, Liang P, Lan F, et al. Screening drug-induced arrhythmia events using human induced pluripotent stem cell-derived cardiomyocytes and low-impedance microelectrode arrays. Circulation. 2013;**128**:S3-13. DOI: 10.1161/CIRCULATIONAHA.112.000570

[47] Kawatou M, Masumoto H, Fukushima H, et al. Modelling Torsade de Pointes arrhythmias in vitro in 3D human iPS cell-engineered heart tissue. Nature Communications. 2017;**8**:1078

[48] Hansen A, Eder A, Bönstrup M, et al. Development of a drug screening platform based on engineered heart tissue. Circulation Research. 2010;**107**:35-44

[49] Grosberg A, Alford PW, McCain ML, et al. Ensembles of engineered cardiac tissues for physiological and pharmacological study: Heart on a chip. Lab on a Chip. 2011;**11**: 4165-4173

[50] Tzatzalos E, Abilez OJ, Shukla P, et al. Engineered heart tissues and induced pluripotent stem cells: Macro- and microstructures for disease modeling, drug screening, and translational studies. Advanced Drug Delivery Reviews. 2015:1-11

[51] Feric NT, Radisic M. Towards adult-like human engineered cardiac tissue: Maturing human pluripotent stem cell-derived cardiomyocytes in human engineered cardiac tissues. Advanced drug delivery reviews. 2016;**96**:110-134. DOI: 10.1016/j.addr.2015.04.019

[52] Mannhardt I, Breckwoldt K, Letuffe-Brenière D, et al. Human engineered heart tissue: Analysis of contractile force. Stem Cell Reports. 2016;**7**:29-42

[53] Cashman TJ, Josowitz R, Johnson BV, et al. Human engineered cardiac tissues created using induced pluripotent stem cells reveal functional characteristics of BRAF-mediated hypertrophic cardiomyopathy. PLoS One. 2016;**11**:1-17

[54] Yamashita JK. ES and iPS cell research for cardiovascular regeneration. Experimental Cell Research. 2010;**316**:2555-2559

[55] Masumoto H, Sakata R. Cardiovascular surgery for realization of regenerative medicine. General Thoracic and Cardiovascular Surgery. 2012;**60**:744-755

[56] Masumoto H, Yamashita JK. Pluripotent Stem Cells for Cardiac Cell Therapy: The Application of Cell Sheet Technology, Pluripotent Stem Cells, Dr. Deepa Bhartiya (Ed.), InTech; 2013. DOI: 10.5772/56326. Available from: https://www.intechopen.com/books/pluripotent-stem-cells/pluripotent-stem-cells-for-cardiac-cell-therapy-the-application-of-cell-sheet-technology

[57] Lalit PA, Hei DJ, Raval AN, et al. Induced pluripotent stem cells for post-myocardial infarction repair: Remarkable opportunities and challenges. Circulation Research. 2014; **114**:1328-1345

[58] Kawamura M, Miyagawa S, Miki K, et al. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. Circulation. 2012;**126**:29-37

[59] Weinberger F, Breckwoldt K, Pecha S, et al. Cardiac repair in guinea pigs with human engineered heart tissue from induced pluripotent stem cells. Science Translational Medicine. 2016;**8**:363ra148-363ra148

[60] Menasché P, Vanneaux V, Hagège A, et al. Human embryonic stem cell-derived cardiac progenitors for severe heart failure treatment: First clinical case report: Figure 1. European Heart Journal. 2015;**36**:2011-2017

Chapter 6

Stem Cells in Treatment of Coronary Heart Disease and Its Monitoring: Tissue Engineering and Clinical Evaluation

Rakesh Sharma

Additional information is available at the end of the chapter

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Abstract

Cardiovascular and coronary heart diseases involve molecular and tissue level damage of blood vessels and heart. Coronary Heart Disease and heart failure are the leading cause of mortality worldwide. Stem cell transplantation is emerging as a new treatment option. Stem cells are capable to reach and settle down at damaged cardiac tissue. This stem cell option also repairs the myocardial infarction area in heart or vascular territories and ultimately reduces the infarct-related mortality. Non-invasive cardiovascular imaging monitors the real-time status of cardiovascular remodeling or differentiated stem cell autografting. Cardiac magnetic resonance imaging (MRI) and bioluminescence are robust non-invasive monitoring techniques to visualize cardiovascular structure changes due to myocardial dysfunction or restorative myocardial recovery. The present chapter highlights the sources, types, delivery methods of stem cells in cardiovascular treatment, advantages and current limitations of stem cell monitoring, scopes of ultra-high field cardiac 900 MHz MRI and bioluminescence methods applied in stem cell transplantation, to translate stem cell molecular events into clinical success and evaluation of rejuvenation rate with future perspectives. ***In conclusion***, right choice of stem cells, pluripotent stem cell delivery, transplantation and real-time monitoring of stem cell trafficking enhances the stem cell therapeutic efficacy in cardiac engraftment and differentiation.

Keywords: stem cell delivery, transplantation, magnetic resonance imaging, coronary disease, cardiac tissue engineering

1. Introduction

Cardiovascular diseases (CVD) and coronary heart disease (CHD) are worldwide leading causes of present mortality as high as 32.8% [1]. Last 5-year American Heart Association

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(AHA) data show high prevalence of heart failure as high as 50% and mortality as high as 32.8%. Coronary artery bypass grafting (CABG) was routine interventional and surgical treatment to bring low morality of coronary heart disease and now stem cell therapy is a new option. Still, major life threat is myocardial necrosis of myocardial tissue that cannot restore the original function of myocardium. Currently, stem cell research has opened vista in transplantation therapy, and its feasibility and effectiveness are well proven in animal experiments as well as in small-scale clinical trials [2–15].

The present chapter is divided into six sections. Section 1 introduces the evolution of stem cell therapy and its mechanism in regeneration and heart restoration. Section 2 introduces different stem cells and their purpose in repair and remodeling myocardium. Section 3 defines transplantation. Section 4 describes different modes of stem cell delivery. Section 5 highlights the purpose of rapid noninvasive real-time monitoring the myocardial repair and evaluation of heart territories. Section 6 reviews different clinical trials, available current nanotechnology and tissue engineering tools, and new approaches with future perspectives. A sketch of metabolic regulation during rejuvenation is presented for exploring new thoughts on secretory molecules regulating remodeling stem cells to explain regeneration of heart with possibility of better regeneration outcome. The chapter is written for interested physicians, surgeons, tissue engineers, scientists, and entrepreneurs.

1.1. Evolution of stem cell therapy: regeneration and healing

History records two types of bone marrow cells (BMC): hematopoietic stem cells [red blood cells (RBC), white blood cells (WBC), lymphocytes, macrophages] and bone marrow stroma mesenchymal stem cells (osteogenic cells for bone formation, chondrogenic cells for cartilage formation, adipogenic cells for fat tissue, and myoblast cells for heart regeneration). Stroma mesenchymal cells are sources of stem cells. Now, stem cell treatment is emerging in heart regeneration and restoration by using intracoronary, intramyocardial, and epicardial injections.

Stem cell transplantation therapy was reported useful first time in recovering myocardial viability after myocardial infarction in ischemic heart disease [16]. Later, autologous intracoronary delivery of mononuclear bone marrow cells 5–9 days after percutaneous transluminal coronary angioplasty [(PTCA); performed within 12 hours of myocardial infarction) was successful in 10 patients. Patients showed improved wall motion [2]. These clinical trials showed angiogenesis, decreased perfusion defects, and improved ejection fraction by endocardial injection of bone marrow cells (BMC) directly in hibernating myocardium useful in heart failure patients. Later 'Myoblast Autologous Grafting in Ischemic Cardiomyopathy MAGIC-cell-5-combination cytokine clinical trial' using intracoronary blood stem cells or induced pluripotent stem cells along with granulocyte-colony-stimulating factor therapy recorded improved angiogenesis and cardiac function [17]. Currently, induced pluripotent stem cells bearing specific membrane surface marker proteins are emerging as potential engineered cells useful for constructing 3D matrices in cardiac repair.

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1.2. What should be the goal of stem cell therapy?

Stem cells are used as autograft (self-renewing, undifferentiated clonigenic) transplantation in myocardial repair or regeneration to bring tissue functionality back to normal for long-term survival in patients with permanent myocardial damage. Some stem cells are used as allograft (multipotent) daughter cells that give rise to multiple progenies [18]. The result of this asymmetric replication of stem cells is that after each division of stem cells, some progeny enters into the differentiation phase. Bone marrow cells and embryonic stem cells have differentiation plasticity and capacity. Goal is achieved by stimulating blood stem cells to cardiomyocytes providing a continuous supply of cardiac stem cells by trans-differentiation. Other exciting option is 'therapeutic cloning' means transplanted stem cells reprogram into induced pluripotent stem (iPS) cells at target organ. Still many issues are: (1) How many number of optimized stem cells needed when performing cell transplantation therapy?; (2) How survival time of transplanted cells can be best monitored?; (3) How do transplanted stem cells undergo differentiation into cardiomyocytes, smooth muscle cells, or endothelial cells?; (4) Do transplanted stem cells produce electrochemical coupling closer to normal myocardial tissue and normal cardiac cells and their functions really recover?; (5) What is the mechanism of cell transplantation in the treatment of myocardial perfusion and cardiac function after a short enhancement (myocardial cell regeneration or paracrine or other)? In nutshell, regeneration and healing of damaged cardiac tissue by myocardial repair is critical in survival. For interested readers, myocardial repair refers to the restoration of tissue architecture is shown in **Figure 1** and its remodeling of metabolic functions after injury is described in **Figure 7** in detail. Regeneration is defined as 100% myocardial repair and recovery.

Currently, cardiac stem cell therapy researchers are exploring new differentiation and surface protein expression markers. Main focus is to design stem cell therapy supports using different 3D extracellular matrices, polymeric scaffolds. Most intriguing advances are in innovative new methods of safe stem cell delivery with subsequent repair monitoring and follow up of stem cell therapy [19]. If incomplete repair, it leaves myocardial scar or fibrosis of collagen or necrosis after inflammation. After repair, myocardial recovery involves the proliferation of stem cells and interaction with native tissue cells to fill up myocardial mass and extra cellular matrix (remodeling) with its improved cellular paracrine function. The improved function is monitored by non-invasive MRI and bioluminescent techniques. Moreover, total success depends on choice of supporting engineered stem cell delivery method and monitoring the extent of repair or regenerating cardiac territories with its visualization by physiochemical methods during follow up of post-stem cell therapy benefits.

1.3. Potential mechanisms

Basic mechanism is 'myocardial revascularization and regeneration' to combat little or no blood supply left to slowly dying heart after myocardial ischemia or infarct or hypoxia. If sufficient oxygen diffusion from endocardium and collateral vessels provide sufficient oxygen

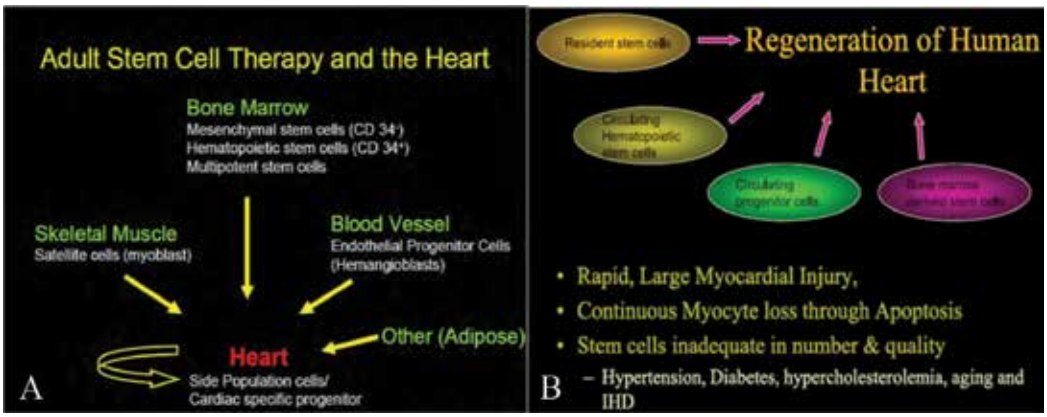

Figure 1. Different sources of adult stem cells are shown in heart regeneration in panel A and different conditions of myocardial injury are shown for the need of heart regeneration in panel B.

to preserve progenitor cells, cardiac repair is done by progenitor cell migration from healthy adjacent myocardium or from the blood circulation.

During regeneration, in fact, initially myoblasts, hemingioblasts, multipotent BMCs and adipocytes transform into cardiac specific progenitor cells. These resident stem cells, circulating hematopoietic cells, progenitor cells and BMCs collectively repair the dying heart by establishing revascularization and regeneration of heart as shown in **Figure 1**. These progenitor cells mainly differentiate into endothelial phenotype and cardiac phenotype to produce paracrine factors for perivascular incorporation and fusion to develop into myocytes and coronary vessels as shown in **Figure 2**. In this process, it requires specific transcription factors. In nutshell, bone marrow mononuclear stem cells, mesenchymal stem cells, endothelial stem cells, and hematopoietic stem cells undergo local neovascularization, neoangiogenesis and paracrine function to have positive effect on endogenous cell angiogenesis and energy metabolism by secretary molecules to inhibit myocyte apoptosis [20] as shown in **Figure 2**. As a result, heart left ventricle ejection fraction, arteriole, ventricular walls, end-diastolic and end-systolic ejection volumes, perfusion rate, contractility are improved with oxygen sufficiency. Revascularization and differentiation are mainly triggered by cycline dependent myocyte membrane surface proteins and remodeling factors as described in detail (see **Figure 7** in section 6).

1.4. Cardiovascular tissue has progenitor differentiating cells to replenish dead or dying cells

Stem cells can be mobilized from bone marrow, fat tissue, or blood, and then cultured to produce large numbers of pluripotent stem cells to transplant into the area of heart injury. It can be explained by the concept of 'cardiac chimerism' that explains the role of putative stem cells and progenitor cells present in transplanted heart during regeneration from circulating stem cells. For example, human circulating endothelial progenitor cells from bone cells are rich in membrane surface proteins such as CD34, CD31, KDR, and c-kit positive myocardial

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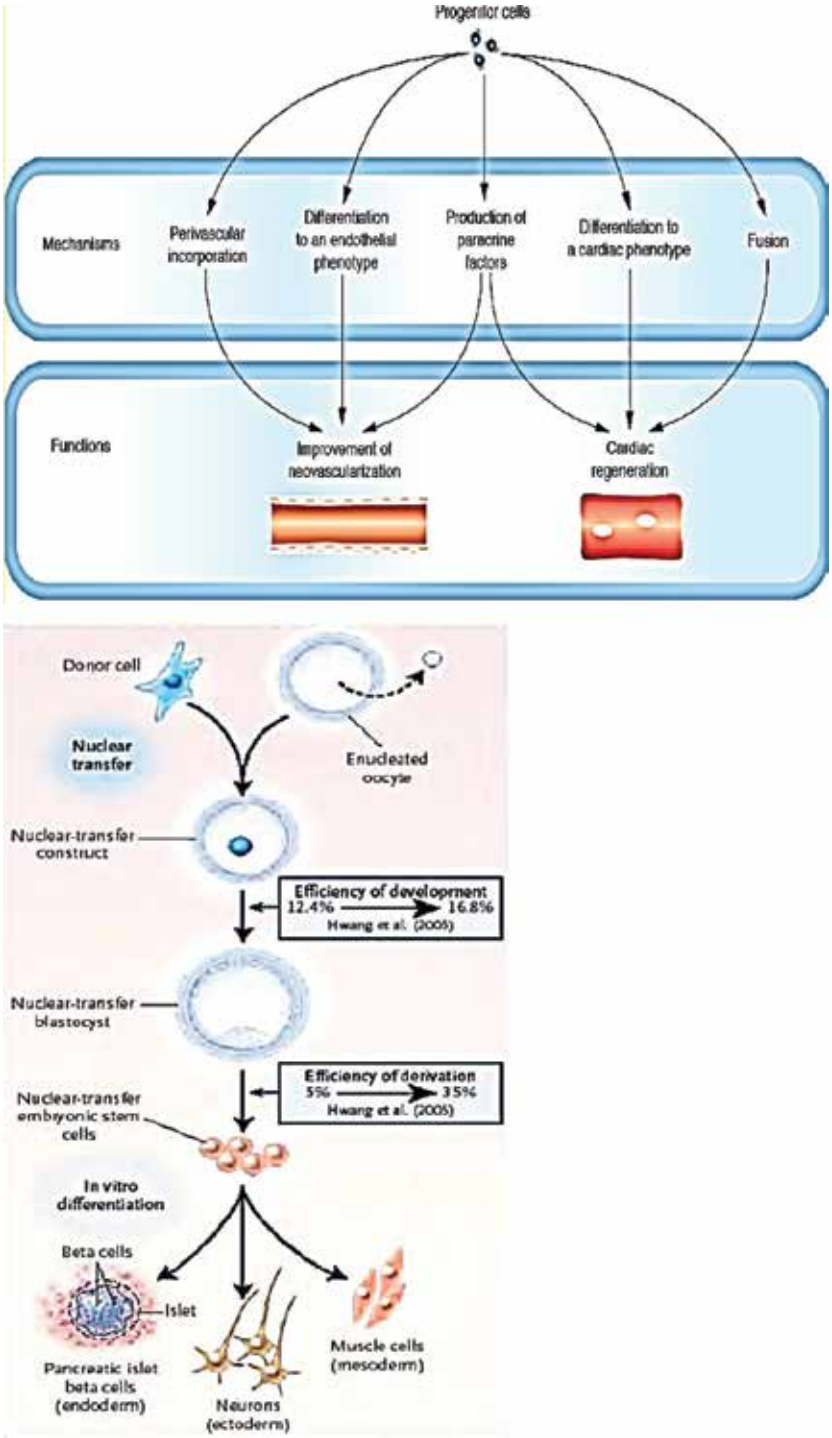

Figure 2. Panel on top: Different mechanisms are shown for differentiation of stem cells to improve revascularization and cardiac regeneration after stem cell therapy. Panel on bottom: Somatic nuclear transfer mechanism is shown for differentiated cells. Induced pluripotent cells have potential of cardiac repair and used in treatment.

differentiation proteins visible in myocardial cell biopsy or cultures [21]. Bone-derived endocardial progenitor cells also do cardiac repair of functional myocardium by declining angiogenic activity. Bearzi et al. reported chimeric heart containing human myocardium with myocytes, coronary arterioles, and capillaries formed in mice injected with human cardiac stem cells [22]. It also supported the view of human stem cell therapy of cardiomyopathy [22]. The following description introduces readers with stem cell types, sources, stem cell engineering, and clinical application in heart repair.

2. Stem cell types and regeneration

Human body has continuously dividing tissues, stable tissues, or permanent tissues. Hematopoietic cells in bone marrow continuously divide and readily regenerate. These regenerating matured cells are short-lived and continuously replenished by stem cells to maintain a constant equilibrium between replicating and dying mature cells, for example, skin and Gastrointestinal tract (GIT). Stable tissues with least replicating cells are heart, liver, kidney cells, endothelial cells, fibroblasts, and smooth muscle cells. Permanent cells are neuron and cardiac muscle cells. They can replicate but cannot terminally differentiate.

2.1. Sources of stem cells

- **Embryonic stem cells** originate from endoderm of embryo after fertilization. Endoderm cells produce 220 kinds of specialized cells during mammalian development by irreversible differentiation process [23]. Later, embryonic precursor cells differentiate into adult muscle or bone marrow cells, fat stem cells or multipotent cells.
- **iPS cells** are formed from regular adult cells by a "cocktail" of inducers or transcription factors so called "induced" pluripotent stem cells (iPS). These transform into the embryo-like state, without eggs or embryos. The iPS cells are pluripotent and make any type of tissue in human body because iPS cells can resemble genetically and immunologically matched with the recipient body. Now, transplantation of these cells into the desired organ offers regenerative therapy of that tissue. However, turning back the biological clock of adult cells to an embryonic state is myth or miraculous escape from aging "immortal divinity". Interested readers may read comprehensive review on pluripotent cells [23, 24]. Yamanaka, 2007 introduced a combination of genes into adult cells changed their behavior as embryonic stem cell, hence called them 'pluripotent' stem cells. In fact, four gene trans-acting factors Oct3/4, Sox2, c-Myc, and Klf4 in adult myocyte cells possibly transformed them pluripotent stem cells [24]. Yu et al. 2007 reported the delivery of trans-acting factors Oct4, NANOG, Sox2, and LIN28 sufficient to reprogram a human somatic fibroblast cell into pluripotent cell bearing same telomerase and surface markers as embryonic cells [25]. Now, cellular programming by somatic nuclear transfer or cloning enables iPS cells behaving like embryonic cells [25]. Cloning develops embryo by the injection of new DNA material from an adult stem cell to an egg cell whose DNA is removed. This enucleated oocyte is the best source of pluripotent stem cells [24] as shown in **Figure 2**. The said engineered

egg rejuvenates the DNA of adult donor cells means restores telomere length without DNA loss during advancing age. This hypothetical idea poses ethical questions. On the other side of coin, iPS cells may treat or correct harmful mutations or diseases such as sickle cell hemoglobin.

- **Cardiac stem cells are** composed of four types including: resident stem cells, circulating hematopoietic cells, circulating progenitor cells, bone marrow cells. These all cells have significant role in cardiac regeneration after myocardial infarction. Urbanek et al. 2005 reported high number of activated stem cells (myocytes, smooth muscle cells, and embryonic cells) formed after cell regeneration in acute myocardial infarcts over chronic infarcts. Poor cell regeneration caused predisposition to chronic congestive heart failure [19]. Answer lies in telomere attrition, leading to decreased telomerase levels in chronic infarct and higher telomerase activity in acute infarcts. Telomerase enzyme is a marker showing growth potential of myocytes, endothelial cells, and smooth muscle cell lineages. Telomerase protects the DNA at the end of a chromosome during mitosis. Autologous transplantation raises hope of increasing telomerase activity to correct end stage cardiomyopathy.
- **Mesenchymal stem cells** are nonhematopoietic cells in adult bone marrow and adipose tissues. These differentiate or modify *in vitro* to adopt phenotypic characters of cardiomyocytes and vascular cells by mesenchymal stem cell allogeneic therapy or cardiac repair by paracrine function [4].
- **Allogeneic stem cells** are "off the shelf" mesenchymal stem cell products from bone marrow of healthy donor. These are useful in therapy phase I trials as they target the myocardial injury site due to the presence of several stromal cell-derived factor-1 (SDF-1), major histocompatibility antigen class 2 molecules, and phenotypes CD145+, CD166+, and CD45− protein markers. These cells can differentiate into bone, tendon, fat, and muscle tissues. These cells also secrete immunosuppressive cytokines. Moreover, these cells can be administered by intravenous route. These stem cells also target and differentiate into cardiac myocytes and blood vessels [26].

3. Ideal stem cell transplantation to treat cardiovascular diseases

In stem cell transplantation methods, ideally adult stem cells, embryonic stem cells (ESC), or induced pluripotent stem cells (iPSC) are locally fixed at dying myocardial tissue sites. However, major challenge is to monitor them timely and confirm the real-time improvement in dying or recovering myocardial tissue physiology efficiently to treat the ischemic heart disease. In other words, capability of MR imaging and monitoring heart metabolism visualize the anterior wall in acute myocardial infarction patients to detect improved myocardial perfusion and myocardial recovery status. Real-time cell imaging also confirms the efficacy of injected bone marrow stem cells (BMC cells) in the recovery of myocardial fragility and viability without any increase in left ventricular ejection fraction (LVEF) [27]. In initial experimental study, the success of embryonic stem cell transplantation in rat myocardial ischemia model showed

significant recovery as reduced left ventricular expansion and reduced area of myocardial infarction after 3–6 weeks. However, in this recovery process, stem cell transcription factors such as Oct3/4, Sox2, Klf4, and c-Myc transformed the embryonic stem cells into induced pluripotent stem cells or iPSCs [28]. These pluripotent cells form regenerative myocardial tissue, smooth muscle, or endothelial vascular cells *in situ* to repair myocardial infarction in heart or increased ventricular wall thickness and electrical stability [29]. Recently, different clinical centers claim their success differently to transplant pluripotent stem cells in remodeling myocardial muscle or endothelial vascular cells [10, 28–35]. In fact, stem cell treatment centers follow the strategy that pluripotent stem cells may be stable rather than terminally differentiated as meta-analysis of randomized controlled clinical trials on stem cell therapy also indicated clearly that intracoronary adult bone marrow stem cells improve left ventricular function and reduce the risk of recurrent heart failure soon after acute myocardial infarction (AMI) [36]. **Table 1** shows the major stem cell types commonly used in medical practice using autograft or allograft transplantation in myocardial repair. Mainly adult stem cells, embryonic stem cells (ESC), or induced pluripotent stem cells (iPSC) are choice.

Allogenic origin of stem cells	Fate of stem cells	Autologous origin of adult stem cells	Fate of stem cells
Embryonic stem cells	Fetal cardiomyocytes	Mesenchymal stem cells	Endothelial progenitor cells
Adipose derived stem cells	Umblical cord derived cells	Endothelial progenitor cells	Multipotent adult progenitor cells
Resident cardiac stem cells	Fetal cardiomyocytes	Endothelial progenitor cells	Induced pluripotent stem cells
Skeletal myoblast cells	Bone marrow mononuclear $CD34^+$		

Table 1. Potential applications of different stem cell types for cardiomyocytes in heart transplantation for myocardial repair.

4. Delivery protocols of stem cell therapy

Each protocol differs in cell retention and regeneration rates depending upon method and site of injection, i.e., intracoronary, intramyocardial, transendocardial, or via coronary sinus delivery (see **Figure 3**), time of delivery, inflammatory response. Other factor is timing of administration rapid or slow injection rates. The early administration of cells facilitates better retention of stem cells or rejuvenating homing signals evidenced in TIME trial [6], while a long delay may cause scar formation in the LateTIME trial [7] as highlighted in **Tables 2** and **3**.

4.1. Intracoronary stem cell therapy

It is done by cell transplantation through transcoronary passage of cells at infarct site along with a standard percutaneous transluminal coronary angioplasty (PTCA) procedure or coronary artery bypass grafting (CABG) procedure, with the use of an over-the-wire balloon

http://dx.doi.org/10.5772/intechopen.70229

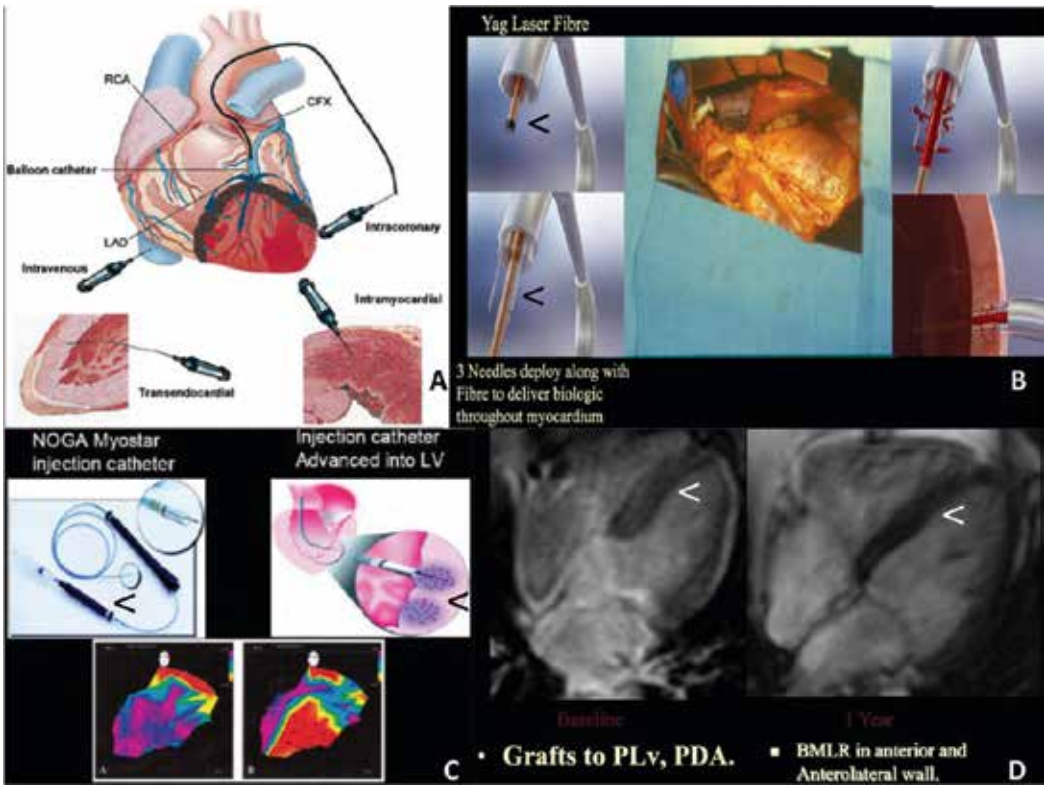

Figure 3. Different delivery sites of stem cell injections are shown in panel A. Yag laser with three needles is shown for BM Laser Repair procedure to deliver stem cells and rejuvenation molecules in panel B. NOGA Myostar catheter is shown for delivery of stem cells in left ventricle in panel C. The evaluation of heart recovery as improved anterolateral wall after stem cell therapy by MRI is shown in panel D.

with central lumen placed at a desired position (see **Figure 3**). It allows intracoronary cells to "home-in" or retention of stem cells by extravasation of BMC to the infarcted area in the presence of chemokines and adhesion molecules, SDF-1, and beta-2-integrin factors induced by ischemic cell injury [32, 35–37].

Coronary infusion of cells is performed four to six times, with 3-minute sequential balloon inflations followed by 3-minute rest periods, to create a "stop flow" situation for maximal retention period to come into contact with the microcirculation of the infarct-related artery. It maximizes the migration and retention of cells into the infarct and peri-infarct tissues for successful transplantation. After transplantation, baseline and post procedure LV angiograms are monitored for 24 hours, with cardiac markers checked at every 6 and 12 hours. Injection of stem cells into a contralateral artery may increase retention in ischemic area if there are well-formed collaterals. Imaging studies further confirm the success of contralateral stem cell injections to increase the retention of cells in occluded artery territories. The crucial issues are: retention of cells, improved ejection fraction, improved regional wall LV function, microvascular plugging, biodistribution, homing to myocardium, proapoptotic factors in the ischemic myocardium, CD34+ cells [35, 38].

Study name	Published	n	Days	Primary outcome	Imaging modality
A. Proven stem cell treatment and evaluation					
TOPCARE-AMI	2002	59	4–5.5	-Global LVEF improved 51–60% in 3 months	-SPECT, echo, MRI
BOOST	2004	60	5–6.3	-Global LVEF improved in large infarcts after 6 months(18 m follow up)	-Cardiac MRI
REPAIR-AMI	2006	187	3–6	-LVEF improved 2.5% in 4 months	-LV angiography
ASTAMI	2006	97	6–7	-No change in global LVEF in 1 year	-SPECT, MRI, echo
LEUVEN-AMI	2006	66	1	-No change in LVEF in 4 months but regional contractility improved & infarct size less	-Cardiac MRI, echo
FINCELL	2008	77	3	-LVEF improved 5% in 6 months but global LVEF same after 4 months	-Cardiac MRI, echo
HEBE	2010	200	3–8	-LVEF improved 6% in 6 months	-Cardiac MRI
B. Other clinical stem cell trials using different stem cell types					
Autologous BMNCs	CABG + SC	5	1y	>5 days old MI	Improved perfusion
Autologous BMNCs	PTCA + SC	13	3m	5–9 days post MI	Better perfusion, wall motion, less infarct size
BMNCs+EPCs	PTCA + SC	23	4m	<3 days post MI	Better LVEF, EDV, perfusion, % contractile function
BMCs+AC133	CABG + SC	12	3–9 m	0–3 m post MI	Better EF, better perfusion
BMNCs	EMM + SC	8	3 m	severe IHD	Improved perfusion, angina, contractile function
Autogous BMNCs	EMM + SC	14	2 m	CHF	Improved LVEF, perfusion, contractile function
Myoblasts	CABG + SC	10	11 m	CHF	Improved EVEF, contractile function
Autologous skeletal myoblasts	CABG + SC	12	12 m	Old MI + ischemic CAD	improved LVDF, regional contractility
Autologous skeletal myoblasts	IM SC injection at LVAD site	5	6 m	IHD	improved LVEF, wall thicker at the injection site
Autologous BMCs	IC infusion + PTCA	30	6 m	<5 days post MI	Improved LVEF, contractile function
Autologous BMSCs	IC infusion + 18 days post PTCA	34	3–6 m	10 days post MI	Better perfusion, high EDV, ESV, wall movement, LVEF
Autologous blood SCs + inj G-CSF	IC infusion + PTCA	10	6 m	>48 hours AMI(old)	Higher stent restenosis in G-CSF group

Table 2. Randomized control trials showing administration of pluripotent stem cells with primary outcome of improved cardiac mass by monitoring improvement in left ventricle ejection function by imaging.

Clinical trial	Administration	Engineered tissue construct used	Reference
Hirsch et al. 2011 HEBE clinical trial	Intracoronary	No change in LVEF in 4 months followup	[12]
Roncalli et al. 2011 BONAMI Trial	Intracoronary	Pluripotent cells	[13]
Traverse et al. 2011 LateTIME Trial	Intracoronary	Pluripotent cells	[7]
Bolli et al. 2011 SCIPIO Trial	Intracoronary	Pluripotent cells	[5]
Makkar et al. 2012 CADUCEUS Trial	Intracoronary	Pluripotent cells	[89]
Zhao et al. 2013	Intracoronary	Pluripotent cells	[90]
Kurbonov et al. 2013	Intracoronary	Engineered stem cells	[91]
Forcillo et al. 2013	Via CABG+i.m.	Stem cells	[92]
Assmann et al. 2013	Via CABG+epicardial	Engineered stem cells	[93]
Nasseri et al. 2014	i.m	Stem cells	[94]
Brickwedel et al. 2014	Via CABG	Engineered stem cells	[95]
Hong et al. 2014	Intracoronary + retrograde coronary sinus	Engineered stem cells	[96]
Hao et al. 2015	Intracoronary	Stem cells	[97]
Chang et al. 2015	Intracoronary	Stem cells	[98]
Gao et al. 2015	Intracoronary	Stem cell engineering	[99]
Fiarresga et al. 2015	Intracoronary	Stem cell engineering	[100]
Helseth et al. 2015	Intracoronary	Stem cell engineering	[101]
Eirin et al. 2015	Intrarenal	Pluripotent cells	[102]
Lee et al. 2015	Intracoronary	Engineered stem cells	[103]
Tseliou et al. 2016	Intracoronary	Stem cell engineering	[104]
Hasan et al. 2016	Intracoronary	Stem cell engineering	[105]
Xiao et al. 2017	Intracoronary	Stem cell engineering	[106]
Gao et al. 2017	Intracoronary	Pluripotent cells in 3D scaffold	[107]

Table 3. A chronology of clinical trials using different stem cell delivery and engineered constructs.

How success in heart recovery after heart transplantation is assessed? After heart recovery, improvement in cardiac functions is the success key. Important cardiac parameters are improved ejection fraction (LVEF), improved contractile function, improved regional wall thickness reduction or improved LV function, Ejection Diastolic and Ejection Systolic Volumes, improved perfusion along with decreased adverse perfusion defects, all these events within less than a week as shown in **Table 2** and **Figure 3** (see panel D). In support, several randomized trials clearly shown that administration of intracoronary autologous bone marrow nuclear cells in patients soon after myocardial infarction improved the ejection fraction within 5 days [8, 39] shown in **Table 2** and illustrated in **Figure 3** (see panel D). Other randomized

trials showed clear evidence of improved regional wall LV function [9]. The Repair-AMI trial showed a significant decrease in major adverse events [10]. However, several clinical trial and pilot studies have failed to demonstrate that bone marrow nuclear cells really improve LV function in the setting of acute myocardial infarction because of empirical calibration or lack of preclinical results[7, 11–13, 31, 40]. Other critical issue is successful cardiac recovery or revived myocardial function rapidly and fast as much as possible. In the previous studies, most of the autologous bone marrow mononuclear cell implantations were performed within week following ST elevation myocardial infarction event. Specific mention here is the evidence of most favorable cardiac recovery effect on LV function obtained on the fifth day after delivery of stem cells in small cohort of patients in the Repair-AMI trial [10] as shown in **Figure 3**.

In the light of above, it is very important that timing of 'appropriate stem cell conditioned delivery' in right manner soon after myocardial infarction may have an influence on stem cell treatment as highlighted in **Figure 3** (see panel C). This timing and delivery issue has debated over the stem cell choice, delivery mode of stem cells, and timing of stem cell implantation after acute myocardial infarction. Two factors need attention here for successful implantation and its action on recovery of myocardium: (1) Release rate of circulating progenitor mononuclear cells from bone marrow within hours of acute myocardial infarction [27, 41, 42]; (2) Release of enormous hematopoietic stem cells, endothelial progenitor stem cells, mesenchymal stem cells, and a very small number of embryonic-like pluripotent cells with cardiorejuvenating properties [43]. Moreover, other factors are also determinant in the success of cardiac rejuvenation such as inadequate cell count, improper processing, and timing of stem cell administration.

Important concern in regard to negative findings is timing of stem cell administration. The National Heart Lung and Blood Institute sponsored Cardiovascular Cell Therapy Research Network reported two prospective clinical trials, TIME [6] and LateTIME [7]. The TIME trial was proposed to compare the effects of bone marrow mononuclear source cells delivered at 3–7 days in patients with predominantly ST elevation myocardial infarction. The LateTIME trial proved the hypothesis that delayed delivery of autologous bone marrow cells at 2–3 weeks following acute myocardial infarction may improve global LV systolic function. LateTIME trial calibrated the cell count and processing issues but did not show any detectable improvement in LV function over a period of 2 years [44]. For interested readers, intramyocardial stem cell therapy protocol is described in following section.

4.2. Intramyocardial stem cell therapy protocol

In open heart surgery, direct visualization of the heart is a preferred method as an endocardial approach during supervised intramyocardial injection of stem cells. Using endocardial approach for intramyocardial stem cell therapy, a transmyocardial injection of stem cells is guided by LV electromechanical mapping with NOGA™ software (Biologics Delivery Systems, Diamond Bar, CA) to deliver stem cells in target infarct area [15] as shown in **Figure 3**. For instance, in routine stem cells are injected into nonviable myocardium soon after an observed

low cardiac output, by an 8Fr MYOSTAR™ catheter (Biologics Delivery Systems) with nitinol tubing and retractable needle set up at a depth of 4.5–6 mm inside cardiac tissue and placed at an appropriate angle 45° under fluoroscopy observation as shown in **Figure 3**. Volumes of approximated 0.3 cc of stem cells are injected by manually advancing the needle initially at several different space volumes of 1 cm^3 in areas of thinned myocardium (<0.5 mm^2 by MRI). Without motion, still patient is kept under observation. Later, patient is monitored for 18–24 hours attached with cardiac life support device and recovery, and myocardial viability is monitored by continuous real-time LV angiography. First time, Federal Drug Agency (FDA) approved the protocol of autologous BMC stem cells as milestone showing salvaged hibernating myocardium with improved angiogenesis, 75% decreased perfusion defects, and improved 20–29% ejection fraction [17]. Now, improved protocols in clinical trials are in practice throughout the US and Europe as shown in **Tables 2** and clinical trials in recent 5 years shown in **Table 3**.

4.3. Retrograde coronary sinus injection

It is other approach to deliver potentially therapeutic stem cells in coronary sinus. A double lumen catheter attached with a larger proximal and a smaller distal balloons is used for delivery of cells in distal lumen. The stem cells are injected and their transport is confirmed angiographically in the mid- to distal interventricular vein that runs parallel to the left anterior descending artery, as shown in **Figure 3** panel A.

4.4. Intravenous delivery

This approach depends upon the intravenous access site as shown in **Figure 3** panel A. The cells get trapped in the lungs, liver, and spleen, so that only a small number may enter in coronary circulation, and myocardial homing is minimal [15]. Myocardial homing depends on the expression of adhesion molecules, cytokines, and homing receptors. In following sections, growing interest of real-time noninvasive monitoring of pre- and post-cardiac recovery of myocardium tissue by advanced 900 MHz MRI methods in preclinical studies and real-time stem cell behavior are discussed. 900 MHz MRI facility is available only in laboratory at our place in the light of less known facts, limitations and challenges to use this facility.

5. Need of noninvasive in vivo monitoring stem cells in preclinical studies

Molecular events by imaging methods offer excellent opportunity to visualize and track stem cell behavior in vivo to evaluate their efficacy of cardiac cell recovery or therapy in preclinical studies. Monitoring the settled home-in rejuvenated stem cells functioning well at cardiac infarct site is based on the fact that active myocardial metabolite protons and water relaxation dynamics is characteristic while 31P MR peaks predict the settled stem cell physiology [45]. Ultrasound imaging, positron emission tomography/single photon emission computed

tomography (PET/ SPECT), magnetic resonance imaging (MRI), optical imaging, and CT imaging are routine molecular imaging techniques. Magnetic resonance of odd-numbered protons in cardiac tissue molecules with resonant radiofrequency in high magnetic field generates the physiological MR cardiac MRI fingerprint as most promising in clinical transformation to provide the structural-functional information of resettled cardiac mass with superior resolution and high sensitivity relatively safer and without radiation [45, 46].

Other major challenges are visualizing myocardial functionality and real-time monitoring the status of transplanted stem cell behavior within native tissue as true representative of altered or improved visible myocardial territories or metabolic recovery. For this purpose, the smart imaging contrast agents or contrast labeling of stem cells offer to visualize the behavior of transplanted stem cells in tissue in situ. Different techniques of cell MRI, bioluminescence, chemiluminescence, myofibril scanning, and DNA end-labeling are routine methods to track myocardial functionality, viability, and fragility [47, 49]. Recently, nanoparticle-labeled stem cells have been developed to achieve dephasing susceptibility contrast and monitoring the stem cell behavior, physiological changes and molecular events by 900 MHz MR imaging stem cells [50].

5.1. MRI contrast labeling of stem cells: source of contrast in images

Tracking of transplanted stem cells and their behavior in native tissue is done using stem cell MRI contrast agents such as gadolinium (Gd) chelating agents (Gd-DTPA) and manganese chloride ($MnCl_2$) [51–53]. Mostly, these image contrast agents provide longitudinal relaxation constant (T1) enhanced positive contrast effects of stem cell originated proton nuclear magnetic resonance (NMR) relaxation as shown in **Figure 4**. Recently, several stem cell specific iron oxide paramagnetic/super paramagnetic contrast agents emerged as negative contrast agents.

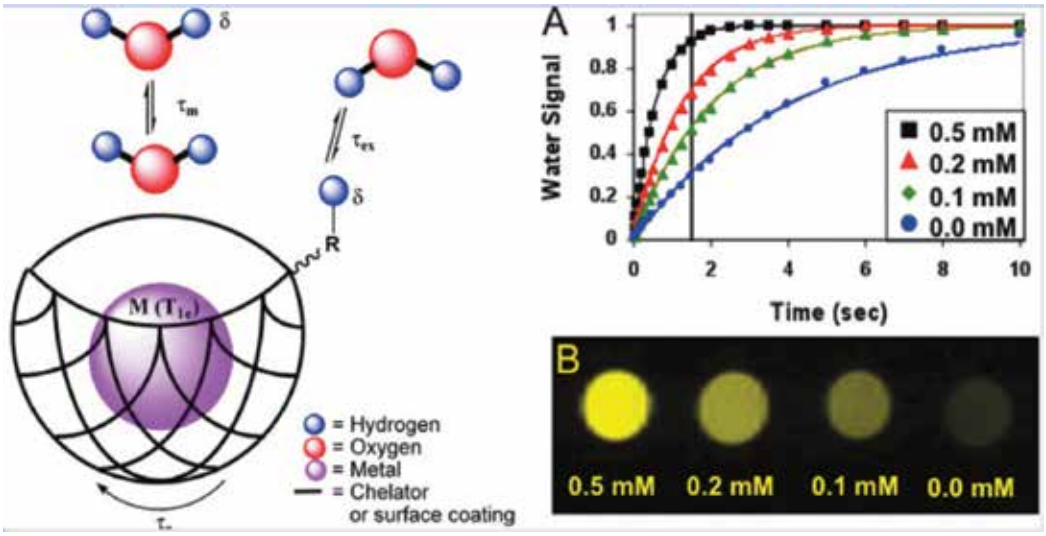

Figure 4. The metal (M) is encapsulated in chelator coat (Gd-DTPA in dextran coated SPIO). The water accessibility (τ_m), rotational tumbling time (τ_r), electron spin state T_{1e} of superparamagnetic metal, chemical exchange rate (τ_{ex}), and MR frequency (δ) are shown as source of contrast. On right: Distinct longitudinal relaxation T1 constants as graphs at different contrast agent concentrations are shown in panel A and respective T1 images are shown in panel B. Reproduced from [52] with permission.

Iron oxide nanoparticles produce strong transverse relaxation constant/dephased transverse relaxation constant (T2/T2*) ratio as negative contrast effect due to dephasing effect [54–56].

5.2. Iron oxide particle stem cell labeling

Super paramagnetic iron oxide nanoparticles (SPION) are family of paramagnetic/ superparamagnetic contrast agents. It consists of a ferrite (maghemite or magnetite) core and a polymer coating. Depending on the diameter size (including both metal core and polymer coat), the nanoparticles can be divided into the SPION (diameter size 60–150 nm), USPION (diameter size 10–40 nm), and MION (diameter size 10–30 nm) categories [57]. Ferucarbotran (Resovist®) and ferumoxides (Endorem or Feridex®) are MRI enhancement contrast agents approved by FDA for clinical diagnosis of liver tumors and metastatic lymph nodes. High concentrations of ferromagnetic material can shorten both the T1/T2 constants as well as the effect of T2*, resulting in a significant reduction in MR relaxation with higher biological safety of stem cells [48, 58–60].

5.3. Positively charged polymer transfection agents

Most of these labeled stem cells do not internalize SPIONs and they need endocytosis polymer boosters. Positively charged polymer transfection agents (TAs) or polycations enhance the endocytosis across negatively charged membrane surface. So, they can be coated on the surface of magnetic iron oxide particles to boost SPION endocytosis or stem cells nonspecifically uptake these SPION particles through the negatively charged membrane surface. At present, composites of SPION and polycation TAs are the most commonly used methods to enhance the endocytosis of iron oxide particles [49, 60–62].

- Negative charge on stem cell membrane surface does not permit ferric oxide particles to attach them with the stem cells. To accomplish it, iron oxide particle surface modifications can enhance cellular endocytosis. In this direction, several surface modification approaches of polycation binding, incubation with hematopoietic cells, monoclonal antibody-antigen binding, receptor binding, magnetoelectric perforation and others are used in but these are still in infancy.
- Positive charged polycation TAs macromolecules such as polylysine or protamine sulfate are used in making SPION/TA composites with strong positive/negative interaction or cationic polymer material coating [63]. These SPIO/TA composites easily adhere to the surface of stem cell membranes and persuade the phagocytosis of iron oxide particles without aggregation of SPION particles [51]. Ferumoxides (Feridex) with USPIO (MION-46 L) and added polycationic TAs have been in use to raise the concentration of intracellular SPION particles [32]. After 4–48-hour incubation with 25 μg Fe/mL TA-(USPION), target stem cells demonstrated a significant reduction in T2 signal intensities due to dephasing effect [52]. Ferumoxides mixed with protamine sulfate (50:3) μg/ml offers an optimized protocol [53].
- Overnight incubation of the human mesenchymal stem cells with hematopoietic $CD34^+$ stem cells and specific mammalian cells, increases the iron content in the stem cells 1.47–17.31 pg/cell [64].

- Monoclonal antibodies of pancreatic cancer specific antigen (PAP2a) fused with dextran-modified SPIONs show antigen-antibody reaction to target the iron oxide particles in pancreatic cancer cells and promote the receptor-mediated SPIO endocytosis [65].
- Iron oxide particle surfaces can be modified by specific target receptors such as vascular cell adhesion molecule-1 and membrane mucin A5. These nanoparticles target specific tissues or organs, but the presence of specific target receptors limits the application of modified nanoparticles in cell tracking [66, 67].

5.4. Magnetoelectric perforation method

It increases the efficiency of nanoparticle endocytosis. Toxicity testing of mesenchymal stem cells, neural stem cells and adipose cells in vitro all indicate safer use of magnetoelectric perforation because of less cell incubation time and effective safer SPION contrast agent to target cells approved by the FDA [68, 69]. Still, stem cell transplantation biological safety considerations need attention and further research.

5.5. Biosafety of iron oxide particle labeling on stem cells

Cell labeling with iron oxide requires intensive toxicity evaluation tests for every protocol and characterization of cell type before translating them in clinical application. Feridex®, Resovist®, and Endorem® are FDA-approved agents. These agents in stem cells are cleared by the reticuloendothelial system. Peripheral blood mononuclear cells labeled in vitro with Ferumoxide® upon administrated these cells through intravenous injection in organs showed localized T2*-weighted images and R2* maps of cell migration at the tissue inflammation damage areas [65]. However, extensive experiments are needed to verify the bio-safety of paramagnetic SPION contrast agents.

5.6. Sensitivity of in vivo MRI detection of labeled stem cells

The intracellular iron distribution in stem cells influences greatly the MRI detection signal from labeled stem cells. Several inherent factors are determinant of image quality such as MRI sequence selection, spatial resolution, magnetic field intensity, and surrounding stem cell or cardiac tissue heterogeneity to affect the molecule sensitive signal. Known factors are: (1) Higher intracellular iron content in cells shortens the relaxation time; (2) The T_2*-weighted image is highly sensitive for iron oxide particle labeling load; (3) Field inhomogeneity and surrounding tissues; and (4) MRI sensitivity can reach 3000 times that of T_1 weighting or 60 times that of T_2 weighting due to iron oxide-induced dephasing effect [54]. To nullify the iron-induced MRI signal sensitivity, specific techniques are chosen. T_2* sequence or steady-state free precession (SSFP) is a choice to detect SPION-labeled cells. However, the T_2* sequence gets artifact by intracellular magnetic field inhomogeneity and interference of the surrounding normal tissues at high magnetic field. Fast 3D gradient echo (GE) sequences balance this effect of T_2* sensitivity, spatial resolution within imaging time. At the present time, the best choice is **g**radient **e**cho **a**cquisition for **s**uperparamagnetic particles with **p**ositive contrast (GRASP), to create a positive contrast of SPION free from T_2* artifacts and high sensitive and specific hyperintense signal of cell tracking even for smaller imaging voxel size in the high field MRI [55].

5.7. Limitations of tracking SPION labeling stem cells

SPION particle cell tracking method for cell labeling has some shortcomings. The MRI signal in preclinical or clinical studies is usually generated from surrounding tissue areas of noninterest cardiopulmonary junction [56]. The paramagnetic material usually accumulates in hemorrhagic infarction. So, hemoglobin shows false low signal intensity on T2*-weighted image [70]. In case of death and rupture of transplanted cells, targeted SPIO nanoparticles can be trapped in surrounding tissue cells or reticuloendothelial cells. Subsequently, SPION are redistributed, deposited, or differentiated in extracellular environment to generate false positive signal. In author's opinion, direct iron oxide labeling is only suitable for short-term stem cell tracking in vivo or in vitro experiments. Other reason of false negative signal can be partial volume effects or low concentrations of cells in one imaging voxel. After every cell division, intracellular iron content remains half. So, every cell division evidences gradual reduction in cell detection sensitivity. The said fact was reported as MRI nonvisible heart cells after 6 weeks post-transplant stem cell administrated to the heart [71]. Despite these limitations and shortcomings, paramagnetic/superparamagnetic iron oxide particles are still highly popular in the field of stem cell tracking because of their high sensitivity.

5.8. Reporter gene labeling in stem cells

Reporter gene labeling is other method based on fusion of an MRI reporter gene to a target gene in stem cells. In transfection of a target stem cell, genes are incorporated into the cellular DNA via transgenic methods. These products of reporter genes are expressed in living stem cells and produces reporter gene expression as indirectly MRI visible in vivo. Transgenic gene labeling methods are highly valuable in long-term studies of labeled stem cell survival, proliferation, and differentiation in vivo. The MRI reporter gene expression can make two products of its expression in stem cells: (1) Intracellular enzymes including β-galactosidase, cytosine deaminase, creatinine kinase, tyrosinase, and arginine kinase [72]; (2) Ferritin or transferrin receptors [73]. Recently, a MRI reporter gene (a ferritin receptor) has emerged as a choice of robust contrast. Excessive expression of ferritin can increase iron uptake. Inside cells, redistribution of intracellular iron enhances transverse relaxation rates and reduces T2 relaxation constants. Recently, adenovirus-ferritin reporter gene injection into murine corpus striatum generated robust contrast on T2 and T2*-weighted imaging within 5–39 days [74].

Clearly MRI reporter gene imaging is still a choice, but it cannot rule out the potential damage to cell proliferation and differentiation. Still open issues are the sources and safety of cells, issues relating to gene mutation and sensitivity [75]. Now, MR microimaging technology has advanced with available 900 MHz magnetic fields to visualize cardiovascular myofibrillar territories up to 30 micrometer resolution using SPION and SPOIT nanoparticle-enhanced relaxation susceptibility signal intensities of revived cardiac muscles enough to decipher the insight of stem cells as shown in **Figure 5**. Author developed mice beating heart microimages using antibody-coated nanoparticles to visualize cardiac muscle orientation angles as fingerprints of cardiac revival and rejuvenation [76]. It can be easily noticed that dying heart left ventricle wall clearly shows the damage sites with clear muscle mass with altered orientation of angle proportional to degree of distortion.

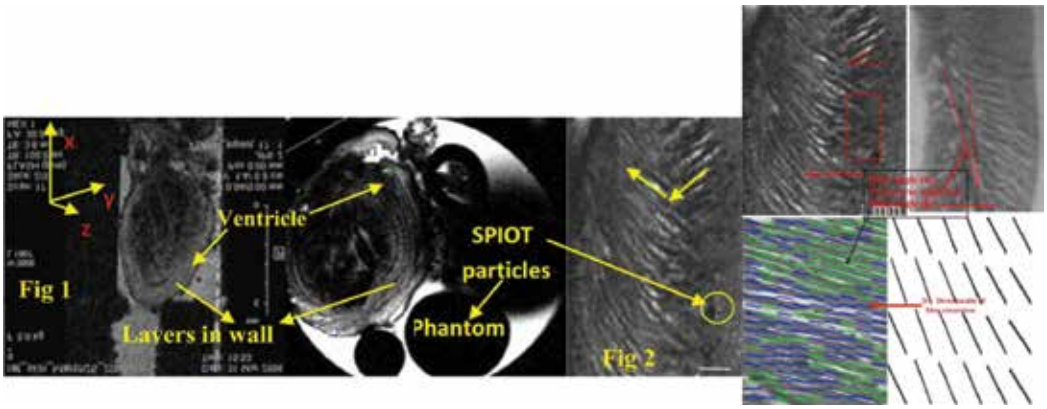

Figure 5. Monitoring cardiac cells by 900 MHz MR microimaging on left panels. Axial image shows details of muscle fibers in ventricle wall (shown as arrows). Cardiac muscle fibers are shown on right panels with superparamagnetic iron oxide troponin nanoparticles (shown in circle) to indicate angles of muscle fiber orientation on right panels. On top right, change in muscle fiber orientation angle is shown before and after infarction (shown as vector directions).

5.9. Regenerating stem cell *in vivo* dual optical imaging

Cellular engraftment may be monitored by reporter gene construct (fluc-mrfp-ttk) visualization by optical methods such as bioluminescence (BLI), chemiluminescence combined with MRI, PET, fusion multimodal imaging (FMI), near infra-red (NIR), and radionuclide methods [77]. The dual-modality imaging has unique strength to monitor cell delivery, survival status, graft morphology, and impact on post-MI remodeling on same platform in less time [78]. Recently, application of BLI for tracking transplanted stem cells was reviewed on the association of stem cell viability with the therapeutic efficacy of stem cell evaluated in preclinical disease models of vascular disease [79]. Reporter gene technology with BLI provides

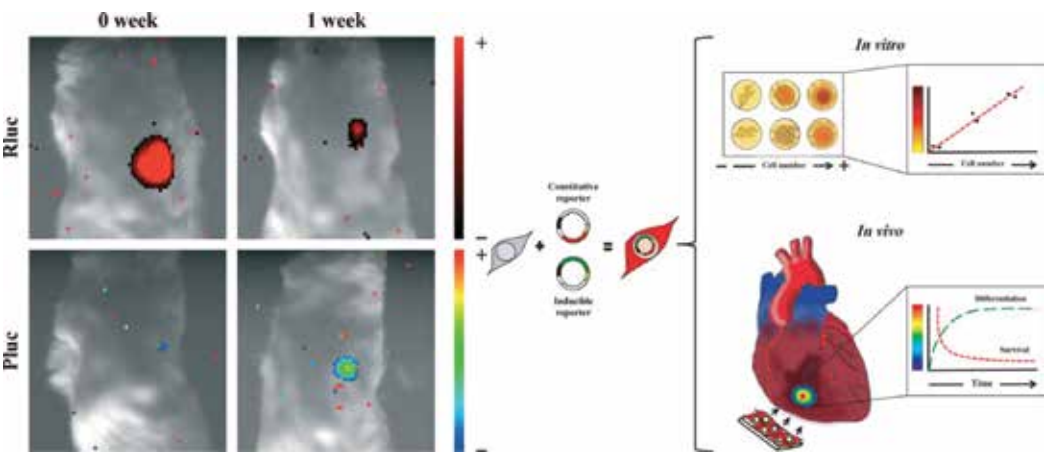

Figure 6. Noninvasive bioluminescence (BLI) monitoring of cardiac differentiation in the experimental model of acute myocardial infarction shows BLI images showing decrease in RLuc by CMV promoter and increase in PLuc by cardiac-specific cTnI promoter in adipose tissue–derived progenitor cells after myocardial implantation. BLI can finely quantify cardiac regeneration degree relative to the number of surviving cells under ischemic conditions. See Ref. [80].

valuable information about the location and functional status of regenerative cells implanted into numerous animal models of disease to define the effectiveness and underlying mechanisms of cardiac cell therapy. The light-emitting capability of BLI illustrates the insights of cardiac regeneration [80]. Recently, survival kinetics of induced pluripotent stem cell and engraftment of viable cells was monitored by BLI imaging by visualizing the retention of bioluminescent agents in adult stem cells as shown in **Figure 6** to monitor stem cells [34]. Efforts are still continuing for regenerating the heart and using myocardial stem cells in cardiovascular system in treatment of heart disease or remodeling [81]. Now, new trend of noninvasive in vivo MR imaging with spectroscopy is emerging to visualize cardiac muscle metabolites [81] and products of gene expression or imaging reporter gene induced inhomogeneity signal peaks from regenerating stem cells [82].

6. Future perspectives

Future research may focus on conversion of adult cells into iPS cells, and conversion of these iPS cells to relevant cell types to treat individual diseases. In near future, multimodal single platform bioluminescent/NIR/FRET optical cum MRI/CT/PET microimaging techniques will emerge to track the pluripotent stem cell sensitive superior detection methods by monitoring the distribution of molecular events in differentiating myocardial progenitor cells in less time. It remains to see in coming years if differentiating stem cells remain safe and stem cells are not affected by radionuclide, chelators, contrast agents, and electromagnetic radiations used to image these cells. To expose these stems cells in different preservative media solutions for storage without any effect on their capability to remodeling is also a challenge in tissue engineering art. Before transplantation and regrafting, it needs thorough investigation of perfect autograft and metabolic compatibility, myocardial contractility to remain viable longer. Larger double blinded placebo-controlled clinical trials are needed on trans-aortic or trans-septal approach to reach different zones of endocardial necrosis. In cases of intramyocardial or epicardial necrosis, epicardial approach should be compared with endocardial one. Brachial can be an alternate option for patients who have peripheral vascular disease with difficult femoral approach. Safer delivery of stem cells to the heart opens vista of transplantation of stem cells as tissue-engineered constructs.

Throughout life, every person experiences many injuries and recovers with time spontaneously by wound healing, organ recovery, or repair mechanisms without even realizing the past injuries in the first place. In this repair and wound healing process, proliferation of existing stem cells makes an individual capable of repairing or restoring the injured tissue(s). In fact, these pluripotent stem cells contain the genetic fingerprint or molecule metabolic blueprint as memory of tissue origin how a particular tissue cell was assembled from biomolecules and functionalized into physiochemical units of organs constructed to begin with from embryonic progenitor cells. If these pluripotent stem cells are maintained artificially in physiological cultures, rejuvenation potential of stem cells maintains all properties of biotransforming and differentiating into organ cells. This potential offers an excellent opportunity of clinical applications. In fact, these restorative potentials in stem cells are possible due to simultaneous multiple functions of stem cells, such as self-renewal, multipotency, and paracrine functions. Of mention, paracrine secretion releases colony-stimulating factors,

growth factors, regulatory energy molecules, and stimulatory cytokines from a number of retained stem cells during regenerative processes at tissue sites as shown in recent clinical trials in **Table 3**. These secretary molecules lead to further mobilization of endogenous progenitor cells. We do not understand the complete sequence of underlying mechanisms of stem cell during regeneration and cardiac healing, even though everyone experiences the benefits of cardiac rejuvenation even without complete knowledge of origin of electrophysiology of heart, cardiomyocyte functions, and mechanism of molecular events.

The embryonic stem cells have excellent capacity to differentiate into virtually any type of tissue cells [83]. Presently, investigators and government agencies have intensified the detailed search for a similar cell lineage or stem cell rejuvenate database in adults [84]. However, many challenges remain to understand how these adult stem cells over-ride the complex tasks (failed heart in to beating heart again) to take up residence quickly when placed in just the right place to gain control and restore or correct the necessary cardiomyocyte shape to assume paracrine functions to perform their multiple plasticity functions in a complex different cellular environments (rejuvenation). Other major challenge is perfect retention of these cells after implantation via intracoronary, intramyocardial, and retrograde coronary sinus approach. In fact, a significant percent of stem cells leaves the heart soon after implantation and stem cell administration before they stick at damage site [84]. So, the clinical ramifications may be significant but they are limited. One fact is clear that remarkable universal nature of stem cells offers the exciting possibility of a universal stem cell transformation capability into any tissue cell or organ that can circulate throughout the body and reside wherever needed to promote regeneration or repair of local tissue if retention of stem cells is good. These stem cells have multiple functions and behave proangiogenic and proparacaine, thereby stem cells may consume or produce potentially detrimental substances as indicated in recent clinical trials shown in **Figure 7**, while stem cells may also survive in nontarget organs [85].

From clinical practice standpoint, the major hurdles to the clinical application and translational research in regard to adult stem cells are the limited small number of stem cells isolated from any adult tissue with successful propagation and harvesting of multipotent adult stem cells [86]. Other hurdle is the development of perfect "stem cell cocktails" to optimize the proliferation and of adult stem cells and differentiation in timely manner [30]. These hurdles indicate the urgent attention on supervised expansion of adult stem cells in cultures uniformly keeping stem cell intrinsic properties intact may be the answer to stable retention [87]. Although extensive cultures of human adult cells may suddenly change the intrinsic properties of stem cells in vivo [88], putting them unfit rendering them with no restoring capability to repair or reverse the injured or diseased tissue in prospective heart failure patients.

Author offers his opinion that cardiac stem cell therapy in future will have an acceptable wide spectrum of preclinical and double blinded placebo-controlled clinical trials on trans-aortic or trans-septal approaches solving the issue of epicardial or endocardial necrosis in cardiovascular regenerative medicine as shown in **Table 3** with emphasis on intracoronary and retrograde coronary methods or possibly combined with 3D scaffold biomatrices delivery. In development of engineered and constructed scaffold, intensive investigations will introduce new rejuvenator secretory molecules in remodeling and metabolic regulation to provide insight of right choice and optimization for best cardiac repair. Researchers may explore

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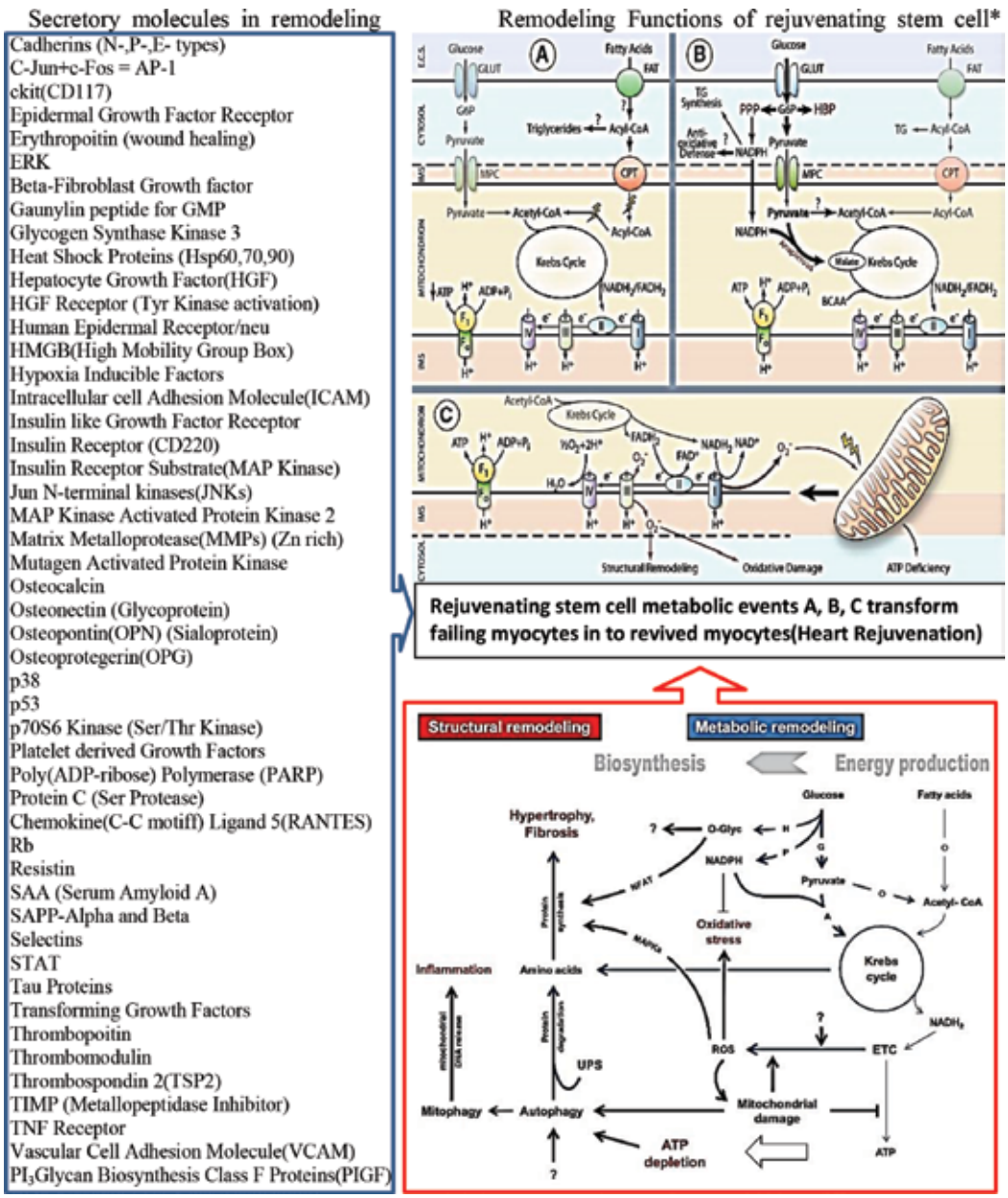

Figure 7. Different secretory molecules are shown either synthesized or released from damaged myocardium from altered cardiomyocyte metabolic pathways (shown in left panel). The process of rejuvenation in stem cells is shown to correct the metabolic events (see at top on right) to lead repair, rejuvenation and restoration of cardiomyocyte viability with improved functions by remodeling in metabolic steps (shown in bottom at right).

more options of differentiated stem cell remodeling in addition to the engineered constructs, rejuvenative molecules and regenerative metabolic pathways highlighted in clinical trials shown in **Figure 7**. What secretory molecules and metabolic regulatory events are common in

differentiated stem cell remodeling?. During remodeling, substrates are transported across the extracellular membrane into the cytosol and are metabolized in various ways. For oxidation, the respective metabolic intermediates [e.g., pyruvate or acylcoenzyme A (CoA)] are transported across the inner mitochondrial membrane by specific transport systems. Once inside the mitochondrion, substrates are oxidized or carboxylated (anaplerosis) and fed into the Krebs cycle for the generation of reducing equivalents [reduced nicotinamide adenine dinucleotide $(NADH)_2$; reduced flavin adenine dinucleotide (FADH)] and GTP. The reducing equivalents are used by the electron transport chain to generate a proton gradient, which in turn is used for the production of ATP. This principal functionality can be recovered in various ways during reverting heart failure (HF), thereby regaining ATP production or improving cellular function in other many ways. Researchers may explore more molecular options of remodeling in addition to the molecules and regenerative metabolic pathways shown in **Figure 7**.

Mainly two types of cardiovascular tissue biomaterials synthetic (polymer, ceramic, or metals) and biologic (cell-based, extracellular matrix-based, whole tissue) and hybrid biomaterials will be available. Advanced therapy medicinal products (ATMP), cardiomyocytes, ECM hydrogels and scaffolds, urinary bladder matrix (UBM) scaffolds, glycosaminoglycans (GAGs), collagen, fibronectin and laminin matrix, endothelial cells (ECs) and vascular smooth muscle cells(VSMCs), poly-tetra fluoro ethylene (PTFE), cardiac patches, cell-seeded matrices, and pericytes seeding with biodegradable tissue engineering-based graft (PLLA/PCL, designed to be degraded in 3–5 years) are currently used methods for dynamic seeding with total BMCs or selected bone marrow aspirate mononuclear cells (BMMNCs). Electrospinning is routine to design scaffolds. For heart regeneration, cell delivery vehicle is implanted using smooth muscle cells (SMC), fibroblasts, endothelial progenitor cells, embryonic CD 34 stem cells, BM cells, tissue-engineered vascular grafts are becoming promised biomaterials. Vascular CorMatrix® patch, vascular grafts made of PG/PGA, PCL/PLA polymers offer clinical use [107–109]. The stem cell treatment will have a universal role in reversing the aging process, although a natural phenomenon. Naturally with the aging process, there is a continuous decline in stem cell number and their viability or physiochemical cardiac capability with time. Due to these facts, aging and heart diseases are interlinked and advancing age promotes organ diseases. Therefore, restorative repairing capability of stem cells may provide a renewable life, and a "fountain of youth" as evidenced by jelly fish rejuvenation.

7. Conclusion

The in vivo imaging techniques are useful in dynamic monitoring of cardiac stem cell therapy following myocardial infarction. Choice of stem cells and mode of delivery are very crucial in getting successful stem cell therapy positive outcome. Cardiovascular remodeling evaluation by MRI has merits because it is safe, sensitive, lacks radiation, provides good resolution, generates a real-time events' blueprint or first-hand information of myocardial viability with functional information of cardiac territories and their physiochemical changes in cardiac functions during stem cell rejuvenating process and after myocardial repair. Present time, ultrahigh magnetic field CMR possibly has preclinical prospects as in vivo noninvasive molecular

imaging or restorative monitoring reporter of rejuvenating stem cell genes to evaluate success of transplantation and cardiac repair. On the other side of coin, researchers are continuously developing new real-time physiological cum functional MRSI options to explore new stem cell molecular probes and smart MRS imaging sequences with improved MRI sensitive specific stem cell differentiation and rejuvenating detection by targeting energy metabolites, myocardial viability, and vital physiochemical molecules. Noninvasive monitoring is necessary and bioluminescence or other radionuclide methods may be alarming because the potential biological damage caused by radionuclide exposed reporter genes and bioluminescence induced immune responses is concern in differentiating stem cells. Seriously, all these issues need research to minimize artifacts within safe limits. With the help of stem cell imaging and monitoring, transplantation of stem cells sooner or later will be optimized for the effective long-lasting therapy of myocardial infarction and heart failure on some day.

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References

[1] Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics—2012 update: A report from the American Heart Association. Circulation. 2012;**125**(1):e2-e220

[2] Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation. 2002;**106**(24):3009-3017. [PubMed]

[3] Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: The MAGIC cell randomised clinical trial. Lancet. 2004;**363**(9411):751-756. [PubMed]

[4] Hare JM, Traverse JH, Henry TD, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. Journal of American College of Cardiology. 2009;**54**(24):2277-2286. [PMC free article][PubMed]

[5] Bolli R, Chugh AR, D'Amario D, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): Initial results of a randomised phase 1 trial. Lancet. 2011;**378**(9806): 1847-1857. [PMC free article] [PubMed]

[6] Traverse JH, Henry TD, Vaughan DE, et al. Cardiovascular Cell Therapy Research Network (CCTRN) Rationale and design for TIME: A phase II, randomized, double-blind, placebo-controlled pilot trial evaluating the safety and effect of timing of administration of bone marrow mononuclear cells after acute myocardial infarction. American Heart Journal. 2009;**158**(3):356-363. [PMC free article] [PubMed]

[7] Traverse JH, Henry TD, Ellis SG, Pepine CJ, Willerson JT, Zhao DXM, et al. Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function. JAMA: The Journal of the American Medical Association. 2011;**306**(19);2110-2119. [PMC free article] [PubMed]

[8] Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: The BOOST randomised controlled clinical trial. Lancet. 2004;**364**(9429):141-148. [PubMed]

[9] Janssens S, Dubois C, Bogaert J, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: Double-blind, randomised controlled trial. Lancet. 2006;**367**(9505):113-121. [PubMed]

[10] Schächinger V, Erbs S, Elsässer A, et al. REPAIR-AMI investigators improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: Final 1-year results of the REPAIR-AMI trial. European Heart Journal. 2006;**27**(23):2775-2783. [PubMed]

[11] Tendera M, Wojakowski W, Ruzyłło W, et al. REGENT investigators intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: Results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. European Heart Journal. 2009;**30**(11):1313-1321. [PubMed]

[12] Hirsch A, Nijveldt R, van der Vleuten PA, et al. HEBE investigators intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: Results of the randomized controlled HEBE trial. European Heart Journal. 2011;**32**(14):1736-1747. [PubMed]

[13] Roncalli J, Mouquet F, Piot C, et al. Intracoronary autologous mononucleated bone marrow cell infusion for acute myocardial infarction: Results of the randomized multicenter BONAMI trial. European Heart Journal. 2011;**32**(14):1748-1757. [PMC free article] [PubMed]

[14] Traverse JH, McKenna DH, Harvey K, et al. Results of a phase 1, randomized, double-blind, placebo-controlled trial of bone marrow mononuclear stem cell administration in patients following ST-elevation myocardial infarction. American Heart Journal. 2010;**160**(3):428-434. [PMC free article] [PubMed]

[15] Traverse JH, Henry TD, Ellis SG, et al. Cardiovascular cell therapy research network effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function: The LateTIME randomized trial. JAMA: The Journal of the American Medical Association. 2011;**306**(19):2110-2119. [PMC free article] [PubMed]

[16] Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002;**106**(15):1913-1918. [PubMed]

[17] Kang HJ, Kim MK, Kim MG, Choi DJ, Yoon JH, Park YB, Kim HS, A multicenter, prospective, randomized, controlled trial evaluating the safety and efficacy of intracoronary cell infusion mobilized with granulocyte colony-stimulating factor and darbepoetin after acute myocardial infarction: Study design and rationale of the MAGIC cell-5-combination cytokine trial. Trials. 2011;**12**:33. DOI: 10.1186/1745-6215-12-33

[18] Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation. 2003;**107**(18): 2294-2302. [PubMed]

[19] Urbanek K, Torella D, Sheikh F, et al. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(24):8692-8697. [PMC free article] [PubMed]

[20] Ratajczak MZ, Kucia M, Jadczyk T, et al. Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: Can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? Leukemia. 2012;**26**(6):1166-1173

[21] Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circulation Research. 2004;**95**(9):911-921. [PubMed]

[22] Bearzi C, Rota M, Hosoda T, et al. Human cardiac stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(35):14068-14073. [PMC free article] [PubMed]

[23] Shevde N. Stem cells: Flexible friends. Nature. 2012;**483**(7387):S22-S26. [PubMed]

[24] Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. Cell Proliferation. 2008;**41**(Suppl 1):S51-S56. [PubMed]

[25] Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007;**318**(5858):1917-1920

[26] Quevedo HC, Hatzistergos KE, Oskouei BN, et al. Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating

capacity. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(33):14022-14027. [PMC free article][PubMed]

[27] Leone AM, Rutella S, Bonanno G, et al. Mobilization of bone marrow-derived stem cells after myocardial infarction and left ventricular function. European Heart Journal. 2005;**26**(12):1196-1204. [PubMed]

[28] Takahashi K, Tanabe K, Ohnuki M, Narita O, Ichisaka T et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;**131**(5):861-872. DOI: http://dx.doi.org/10.1016/j.cell.2007.11.019

[29] Lee H, Park J, Forget BG, Gaines P. Induced pluripotent stem cells in regenerative medicine: an argument for continued research on human embryonic stem cells. Regenerative Medicine. 2009;**4**(5):759-769. [PubMed]

[30] Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature. 2002;**418**(6893):41-49. [PubMed]

[31] Wöhrle J, Birkemeyer R, Markovic S, et al. Prospective randomised trial evaluating a paclitaxel-coated balloon in patients treated with endothelial progenitor cell capturing stents for de novo coronary artery disease. Heart. 2011;**97**(16):1338-1342. [PubMed]

[32] De Falco E, Porcelli D, Torella AR, et al. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. Blood. 2004;**104**(12): 3472-3482. [PubMed]

[33] Szmitko PE, Fedak PW, Weisel RD, Stewart DJ, Kutryk MJ, Verma S. Endothelial progenitor cells: New hope for a broken heart. Circulation. 2003;**107**(24):3093-3100. [PubMed]

[34] Lepperhof V, Polchynski O, Kruttwig K, Brüggemann C, Neef K, Drey F, et al. Bioluminescent imaging of genetically selected induced pluripotent stem cell-derived cardiomyocytes after transplantation into infarcted heart of syngeneic recipients. PLoS One. 2014;**9**(9):e107363. DOI:10.1371/journal.pone.0107363

[35] Geng YJ. Molecular mechanisms for cardiovascular stem cell apoptosis and growth in the hearts with atherosclerotic coronary disease and ischemic heart failure. Annals of the New York Academy of Sciences. 2003;**1010**:687-697. [PubMed]

[36] Delewi R, Andriessen A, Tijssen JG, Zijlstra F, Piek JJ, Hirsch A. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: A meta-analysis of randomised controlled clinical trials. Heart. 2013;**99**(4):225-232. DOI: 10.1136/ heart jnl-2012-302230

[37] Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nature Medicine. 2004;**10**(8):858-864. [PubMed]

[38] Blocklet D, Toungouz M, Berkenboom G, et al. Myocardial homing of nonmobilized peripheral-blood CD34+ cells after intracoronary injection. Stem Cells. 2006;**24**(2):333-336. [PubMed]

[39] Chavakis E, Aicher A, Heeschen C, et al. Role of beta2-integrins for homing and neovascularization capacity of endothelial progenitor cells. Journal of Experimental Medicine. 2005;**201**(1):63-72. [PMC free article] [PubMed]

[40] Schächinger V, Erbs S, Elsässer A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. New England Journal of Medicine. 2006;**355**(12):1210-1221. [PubMed]

[41] Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. New England Journal of Medicine. 2006;**355**(12): 1199-1209. [PubMed]

[42] Shintani S, Murohara T, Ikeda H, et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation. 2001;**103**(23):2776-2779. [PubMed]

[43] Massa M, Rosti V, Ferrario M, et al. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. Blood. 2005;**105**(1):199-206. [PubMed]

[44] Wojakowski W, Landmesser U, Bachowski R, Jadczyk T, Tendera M. Mobilization of stem and progenitor cells in cardiovascular diseases. Leukemia. 2012;**26**(1):23-33. [PubMed]

[45] Perin EC, Geng YJ, Willerson JT. Adult stem cell therapy in perspective. Circulation. 2003;**107**(7):935-938. [PubMed]

[46] Sosnovik DE, Nahrendorf M. Cells and iron oxide nanoparticles on the move: Magnetic resonance imaging of monocyte homing and myocardial inflammation in patients with ST-elevation myocardial infarction. Circulation. Cardiovascular Imaging. 2012;**5**(5):551-554

[47] Ye Y, Bogaert J. Cell therapy in myocardial infarction: Emphasis on the role of MRI. European Radiology. 2008;**18**(3):548-569

[48] Nejadnik H, Henning TD, Castaneda RT, Boddington S, Taubert S, Jha P, et al. Somatic differentiation and MR imaging of magnetically labeled human embryonic stem cells. Cell Transplantation. 2012;**21**(12):2555-2567. DOI: 10.3727/096368912X653156

[49] Lee JH, Jung MJ, Hwang YH, Lee YJ, Lee S, Lee DY, et al. Heparin-coated superparamagnetic iron oxide for in vivo MR imaging of human MSCs. Biomaterials. 2012;**33**(19):4861-4871. DOI: 10.1016/j.biomaterials. 2012.03.035

[50] Sharma R. Human coronary lesions: 900 mhz magnetic resonance microimaging, nmr spectroscopy, histopathology and oxidative stress markers. Journal of Cardiology & Cardiovascular Therapy. 2017;**3**(1):1-9. https://juniperpublishers.com/jocct/pdf/JOCCT.MS.ID.555602.pdf

[51] Frank JA, Anderson SA, Kalsih H, Jordan EK, Lewis BK, Yocum GT, et al. Methods for magnetically labeling stem and other cells for detection by in vivo magnetic resonance imaging. Cytotherapy. 2004;**6**(6):621-625

[52] Bulte JW, Douglas T, Witwer B, Zhang SC, Strable E, Lewis BK, et al. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nature Biotech nology. 2001;**19**(12):1141-1147

[53] Frank JA, Zywicke H, Jordan EK, Mitchell J, Lewis BK, Miller B, et al. Magnetic intracellular labeling of mammalian cells by combining (FDA-approved) superparamagnetic iron oxide MR contrast agents and commonly used transfection agents. Academic Radiology. 2002;**9**(Suppl 2):S484-S487

[54] Bowen CV, Zhang X, Saab G, Gareau PJ, Rutt BK. Application of the static dephasing regime theory to superparamagnetic iron-oxide loaded cells. Magnetic Resonance in Medicine. 2002;**48**(1):52-61

[55] Mani V, Briley-Saebo KC, Itskovich VV, Samber DD, Fayad ZA. Gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP): Sequence characterization in membrane and glass superparamagnetic iron oxide phantoms at 1.5T and 3T. Magnetic Resonance in Medicine. 2006;**55**(1):126-135

[56] Hill JM, Dick AJ, Raman VK, Thompson RB, Yu ZX, Hinds KA, et al. Serial cardiac magnetic resonance imaging of injected mesenchymal stem cells. Circulation. 2003;**108**(8): 1009-1014

[57] Ruggiero A, Thorek DL, Guenoun J, Krestin GP, Bernsen MR. Cell tracking in cardiac repair: What to image & how to image. European Radiology. 2012;**22**(1):189-204. DOI: 10.1007/s00330-011-2190-7

[58] Richards JM, Shaw CA, Lang NN, Williams MC, Semple SI, MacGillivray TJ, et al. In vivo mononuclear cell tracking using superparamagnetic particles of iron oxide: Feasibility and safety in humans. Circulation. Cardiovascular Imaging. 2012;**5**(4):509-517. DOI: 10.1161/CIRCIMAGING. 112. 972596

[59] Jasmin, Torres AL, Jelicks L, de Carvalho AC, Spray DC, Mendez-Otero R. Labeling stem cells with superparamagnetic iron oxide nanoparticles: Analysis of the labeling efficacy by microscopy and magnetic resonance imaging. Methods in Molecular Biology. 2012;**906**:239-252. DOI: 10.1007/978-1-61779-953-2_18

[60] Mcfadden C, Mallett CL, Foster PJ. Labeling of multiple cell lines using a new iron oxide agent for cell tracking by MRI. Contrast Media & Molecular Imaging. 2011;**6**(6):514-522. DOI: 10.1002/cmmi.456

[61] Ramaswamy S, Schornack PA, Smelko AG, Boronyak SM, Ivanova J, Mayer JE Jr, et al. Superparamagnetic iron oxide (SPIO) labeling efficiency and subsequent MRI tracking of native cell populations pertinent to pulmonary heart valve tissue engineering studies. NMR in Biomedicine. 2012;**25**(3):410-417. DOI: 10.1002/nbm.1642

[62] Ramaswamy S, Greco JB, Uluer MC, Zhang Z, Zhang Z, Fishbein KW, et al. Magnetic resonance imaging of chondrocytes labeled with superparamagnetic iron oxide nanoparticles in tissue-engineered cartilage. Tissue Engineering. Part A. 2009;**15**(12):3899-3910. DOI: 10.1089/ ten.tea. 2008.0677

[63] Li L, Jiang W, Luo K, et al. Superparamagnetic iron oxide nanoparticles as MRI contrast agents for non-invasive stem cell labeling and tracking. Theranostics. 2013;**3**(8):595-615. DOI: 10.7150/thno.5366

[64] Arbab AS, Yocum GT, Kalish H, Jordan EK, Anderson SA, Khakoo AY, et al. Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI. Blood. 2004;**104**(4):1217-1223

[65] Koyama T, Shimura M, Minemoto Y, Nohara S, Shibata S, Iida Y, et al. Evaluation of selective tumor detection by clinical magnetic resonance imaging using antibody-conjugated superparamagnetic iron oxide. Journal of Controlled Release. 2012;**159**(3):413-418. DOI: 10.1016/j.jconrel. 2012.01.023

[66] Aikawa E, Nahrendorf M, Sosnovik D, Lok VM, Jaffer FA, Aikawa M, et al. Multimodality molecular imaging identifies proteolytic and osteogenic activities in early aortic valve disease. Circulation. 2007;**115**(3):377-386

[67] van Tilborg GA, Vucic E, Strijkers GJ, Cormode DP, Mani V, Skajaa T, et al. Annexin A5-functionalized bimodal nanoparticles for MRI and fluorescence imaging of atherosclerotic plaques. Bioconjugate Chemistry. 2010;**21**(10):1794-1803. DOI: 10.1021/bc100091q

[68] Walczak P, Ruiz-Cabello J, Kedziorek DA, Gilad AA, Lin S, Barnett B, et al. Magnetoelectroporation: Improved labeling of neural stem cells and leukocytes for cellular magnetic resonance imaging using a single FDA-approved agent. Nanomedicine. 2006;**2**(2):89-94

[69] Walczak P, Kedziorek DA, Gilad AA, Lin S, Bulte JW. Instant MR labeling of stem cells using magnetoelectroporation. Magnetic Resonance in Medicine. 2005;**54**(4):769-774

[70] Tallheden T, Nannmark U, Lorentzon M, Rakotonirainy O, Soussi B, Waagstein F, et al. In vivo MR imaging of magnetically labeled human embryonic stem cells. Life Sciences. 2006;**79**(10):999-1006

[71] Winter EM, Hogers B, van der Graaf LM, Gittenberger-de Groot AC, Poelmann RE, van der Weerd L. Cell tracking using iron oxide fails to distinguish dead from living transplanted cells in the infarcted heart. Magnetic Resonance in Medicine. 2010;**63**(3):817-821. DOI:10.1002/mrm.22094

[72] Lee SW, Lee SH, Biswal S. Magnetic resonance reporter gene imaging. Theranostics. 2012;**2**(4):403-412. DOI: 10.7150/thno.3634

[73] Naumova AV, Reinecke H, Yarnykh V, Deem J, Yuan C, Murry CE. Ferritin overexpression for noninvasive magnetic resonance imaging-based tracking of stem cells transplanted into the heart. Molecular Imaging. 2010;**9**(4):201-210

[74] Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET. A new transgene reporter for in vivo magnetic resonance imaging. Nature Medicine. 2005;**11**(4):450-454

[75] See F, Johnson LL. Radionuclide approaches to imaging stem cells and their biological effects on myocardium. In: Kraichman DL, Wu JC, editors. Stem Cell Labeling for Delivery and Tracking Using Noninvasive Imaging. Series in Medical Physics and Biomedical Engineering. CRC Press: Roca Baton, FL; 2012. Ch 7. pp. 123-138

[76] Sharma R. Bioimaging techniques by Antibodies. Chapter 15. In: Benita S, Pathak Y, editors. Antibody Mediated (mAB) Drug Delivery Systems (DDS): Concepts, Technology and Applications. John Wiley & Sons. Springer Science, NY; 2010. 290

[77] Zhang H, Qiao H, Bakken A, et al. Utility of dual-modality bioluminescence and mri in monitoring stem cell survival and impact on post myocardial infarct remodeling. Academic Radiology. 2011;**18**(1):3-12. DOI: 10.1016/j.acra.2010.09.006

[78] Sharma R.,Katz JK. Evaluation criteria of carotid artery atherosclerosis: Non-invasive multimodal imaging and molecular imaging. Chapter 27. In: Suri JS, Kathuria C, Molinari F, editors. Atherosclerosis Disease Management. Springer Verlag Publishers;2011. pp. 817-877. http://www.springer.com/biomed/book/978-1-4419-7221-7

[79] Huang NF, Okogbaa J, Babakhanyan A, Cooke JP. Bioluminescence imaging of stem cell-based therapeutics for vascular regeneration. Theranostics. 2012;**2**(4):346-354. DOI: 10.7150/thno.3694

[80] Roura S, Gálvez-Montón C, Bayes-Genis A. Bioluminescence imaging: A shining future for cardiac regeneration. Journal of Cellular and Molecular Medicine. 2013;**17**(6):693-703. DOI: 10.1111/jcmm.12018

[81] Fu Y, Kraichman DL. Tracking stem cells in vivo. Chap. 25. In: Cohen IS, Gaudette GR, editors. Regenerating the Heart: Stem Cells and the Cardiovascular System. Humana Press, NY 2011. pp. 499-522

[82] Pagel MD, Basilion JP. Noninvasive imaging of gene expression with magnetic resonance imaging and magnetic resonance spectroscopy. Chap 4. In: Gambhir SS, Yaghoubi SS, editors. Molecular Imaging with Reporter Genes. NY: Cambridge University Press; 2010. p. 88

[83] Rosenthal N. Prometheus's vulture and the stem-cell promise. New England Journal of Medicine. 2003;**349**(3):267-274

[84] Hou D, Youssef EA, Brinton TJ, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: Implications for current clinical trials. Circulation. 2005;**112**(Suppl 9):I150-I156

[85] Doenst T, Nguyen TD, Dale Abel E. Cardiac metabolism in heart failure: Implications beyond ATP production. Circulation Research. 2013;**113**:709-724. [PubMed]

[86] Smits PC, van Geuns RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: Clinical experience with six-month follow-up. Journal of the American College of Cardiology. 2003;**42**(12):2063-2069. [PubMed]

[87] Deasy BM, Qu-Peterson Z, Greenberger JS, Huard J. Mechanisms of muscle stem cell expansion with cytokines. Stem Cells. 2002;**20**(1):50-60. [PubMed]

[88] Quyyumi AA, Waller EK, Murrow J, Esteves F, Galt J, Oshinski J, et al. CD34(+) cell infusion after ST elevation myocardial infarction is associated with improved perfusion and is dose dependent. American Heart Journal. 2011;**161**(1):98-105

[89] Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LEJ, Berman D, et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): A prospective, randomised phase 1 trial. The Lancet. 2012;**379**(9819):895-904

[90] Zhao X, Huang L. Cardiac stem cells. A promising treatment option for heart failure. Experimental and Therapeutic Medicine. 2013;**5**:379-383

[91] Kurbonov U, Dustov A, Barotov A et al. Intracoronary infision of autologous CD133 cells in myocardial infarction and tracing TC99MIBI scintigraphy of the heart areas involved in cell homing. Stem Cells International. 2013;**2013**:8257

[92] Forcillo J, Stevens L-M, Mansour S, et al. Implantation of CD133+stem cells in patients undergoing coronary bypass surgery: IMPACT-CABG pilot trial. Canadian Journal of Cardiology. 2013;**29**:441-447

[93] Assmann A, Heke M, Kropil P, et al. Laser-supported CD133+ cell therapy in patients with ischemic cardiomyopathy: Initial results from a prospective phase I multicenter trial. PLoS One. 2014;**9**:Ide101449

[94] Nasseri BA, Ebell W, Dandel M, et al. Autologous CD133+bone marrow cells and bypass grafting for regeneration of ischaemic myocardium: The Cardio133 trial. European Heart Journal. 2014;**35**:1263-1274

[95] Brickwedel J, Gulbins H, Reichenspurner H. Long-term follow-up after autologous skeletal myoblast transplantation in ischaemic heart disease. Interactive Cardiovascular and Thoracic Surgery. 2014;**18**:61-66

[96] Hong SJ, Hou D, Brinton TJ, Johnstone B, Feng D et al. Intracoronary and retrograde coronary venous myocardial delivery of adipose-derived stem cells in swine infarction lead to transient myocardil trapping with predominant pulmonary distribution. Catheterization and Cardiovascular Interventions. 2014;**83**:E17-E25

[97] Hao L, Hao J, Fang W, Han C, Zhang W, Wang X. Dual isotope simultaneous imaging to evaluate the effects of intracoronary bone marrow derived mesenchymal stem cells on perfusion and metabolism in canines with acute myocardial infarction. Biomedical Reports. 2015;**3**:447-452

[98] Chung ES, Miller L, Patel AN, et al. Changes in ventricular remodeling and clinical status during the year following a single administration of stromal cell-derived factor 1 non-viral gene therapy in chronic heart failure patients: The STOP-HF randomized phase II trial. European Heart Journal. 2015;**36**:2228-2238

[99] Gao LR, Chen Y, Zhang NK, Yang XL et al. Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: Double-blind, randomized controlled trial. BMC Medicine. 2017;**13**:162

[100] Fiarresga A, Mata MF, Cavaco-Goncalyes S, et al. Intracoronary delivery of human mesenchymal/stromal stem cells: Insight from coronary microcirculation invasive assessment in a swine model. PLoS One. 2015;**10**:e139870

[101] Helseth R, Opstad T, Solheim S, et al. The effect of intracoronary stem cells injection on markers of leukocyte activation in acute myocardial infarction. Cardiology Research. 2015;**6**:209-215

[102] Eirin A, Zhu XY, Ferguson CM et al. Intra-renal delivery of mesenchymal stem cells attenuate myocardial injury after reversal of hypertension in porcine renovascular disease. Stem Cell Research & Therapy. 2015;**6**:7

[103] Lee HW, Lee HC, Park JH et al. Effects of intracoronary administration of autologous adipose tissue-derived stem cells on acute myocardial infarction in porcine model. Yonsei Medical Journal. 2015;**56**:1522-1529

[104] Tseliou E, Kanazawa H, Dawkins J et al. Widespread myocardial delivery of heart-derived stem cells by nonoclusive triple-vessel intracoronary infusion in porcine ischemic cardiomyopathy: Superior attenuation of adverse remodeling documented by magnetic resonance imaging and histology. PLoS One. 2016;**11**:e0144523

[105] Hasan A, Waters R, Roula B, Dana R, Yara S, Alexandre T, Paul A. Engineered biomaterials to enhance stem cell-based cardiac tissue engineering and therapy. Macromolecular Bioscience. 2016;**16**(7):958-977

[106] Xiao W, Guo S, Gao C, Dai G, Gao Y et al. A randomized comparative studyon the efficacy of intracoronary infusion of autologous bone marrow mononuclear cells and mesenchymal stem cells in patients with dilated cardiomyopathy. International Heart Journal. 2017;58(3):238-244. DOI: 10.1536/ihj.16-328

[107] Gao L, Kupfer ME, Jung JP, Yang L, Zhang P, et al.Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. Circulation Research. 2017;**120**(8):1318-1325

[108] Lee AY,Lee YU, Mahler N, Best C,Tara S, Breurer C. Regenerative implants for cardiovascular tissue engineering. Chapter 4. In: Laurence J, Baptista P, Atala A, editors. Translating Regenerative Medicine to the Clinic; 2016:39-69

[109] Zhang J. Engineered tissue patch for cardiac therapy. Current Treatment Options in Cardiovascular Medicine. 2015;**17**(8):399. DOI: 10.1007/s11936-015-0399-5

Chapter 7

Impact of the Donor KIR Genotype on the Clinical Outcome of Hematopoietic Stem Cell Unrelated Transplants: A Single Center Experience

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Additional information is available at the end of the chapter

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Abstract

In recent years, the anti-leukemic potential of Natural Killer (NK) cells and their role in hematologic malignancies and in Hematopoietic Stem Cell Transplants (HSCT) has attracted greater interest and a recent study by Cooley S. et al. showed a better clinical outcome when patients with Acute Myeloid Leukemia received a transplant from unrelated Group B KIR haplotypes donors. As a consequence of these results, an algorithm called "KIR B-content score" was formulated. The aim of our research is a retrospective analysis of HSC unrelated transplants performed in our center to analyze the effect of the donor KIR B status on the clinical-outcome. Our results showed a better overall survival-rate in the AML recipients, HLA mismatched with the donor, when the donor KIR B content status is Best/Better (37% vs 18% at three years P=0,028). Moreover, we observed that AML recipients, whose Donors KIR B status was Best/Better, had more incidence of aGvHD grade I and II than patients whose Donors KIR B status was Neutral (70% vs 26%) and also a lower rate of relapse (36% vs 58%) and a better Disease Free Survival-rate (58% vs 38% at three years P=0,1) because of a better GvL effect.

Keywords: UDHSCT, NK cells, donor KIR B status, AML, GvL effect

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1. Introduction

1.1. Hematopoietic stem cell transplantation (HSCT)

Hematopoietic stem cell transplantation (HSCT) has emerged as one of the most useful therapeutic strategies in the treatment of hematologic malignancies (acute or chronic myeloid or lymphoid leukemia) or hereditary (Thalassemia major) for which conventional therapies offer little or no chance of cure. HSCT consists of the replacement of the diseased or non-functional bone marrow with healthy stem cells capable of regenerating all blood cells and restoring the normal hematologic and immunologic functions. The source of hematopoietic stem cells (HSC) can be bone marrow (BMT), peripheral blood after appropriate stimulation (PBHSCT) or cord blood (CBHSCT). The transplant can be autologous (the patient's own HSC transplant after suitable treatment) or allogeneic (HSC transplant from a healthy donor). In the latter case, it is essential to find a donor with genetic characteristics similar to those of the receiver because one of the most important variables that influence the success of HSCT is the compatibility of the genes of the HLA system. The Class I and II HLA matching between donor and recipient is crucial to prevent alloreactivity and, consequently, rejection, graft failure and, above all, the graft versus host disease (GVHD), because the transplanted cells from the donor are immunologically competent and can attack cells and tissues of the recipient [1–3].

A very important aspect of the HSC transplant is the graft versus leukemia effect (GvL), which is a GvHD directed toward the leukemic cells: donors' T lymphocytes attack the cells of the tissues causing GvHD but they can also attack and eliminate residual leukemic cells [4]. If the donor-recipient HLA compatibility is high, the GvL effect is low, consequently, the possibility of relapse is high; on the contrary, when the HLA compatibility is low, the GvHD reaction will be great. A major challenge in improving the success of allogeneic hematopoietic stem cells in the treatment of leukemia is to minimize GvH reactions and simultaneously preserve and optimize GvL reactions. It has also been demonstrated that the GvL effect may be mediated by NK cells [5].

It has been shown that only 25% of patients who need a HSC transplant find an HLA-identical sibling donor; the remaining 75% do not have an HLA-identical donor in their own family; in this case, the alternative is to look for a HLA-matched, unrelated volunteer donor (matched unrelated donor (MUD)) in the worldwide register of bone marrow donors. If there is no compatible donor, the choice of the haploidentical donor is a viable alternative, because there might often be half-identical donors such as parents or siblings [6]. Recently, the number of so-called alternative donor transplants, MUD and haploidentical transplants with one or more HLA mismatches has significantly increased. This is due to the rise in the average age of the patients and therefore the impossibility of finding a compatible donor in their own family. For this reason research has steered toward the study of factors other than HLA matching, which could have a positive influence on the outcome of the transplants, including the role of natural killer (NK) cells in hematopoietic stem cell transplants and, consequently, the study of killer-cell immunoglobulin-like receptors (KIRs) genes.

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1.2. Natural killer cells and KIRs

The human NK cells are a part of the immune system with an important role in the host's defense against infections from pathogens and in "immune surveillance" against cancer cells. Their function depends on several families of activating and inhibitory receptors including KIR (killer-cell immunoglobulin-like receptors). The "missing-self" concept [7, 8], put forward by Karré and colleagues in the 1980s, formed the basis for understanding the mechanisms of the action of NK cells (**Figure 1**). According to this hypothesis, the function of NK cells is to recognize and kill autologous cells which are deficient in MHC Class I expression, a frequent event in tumor or virus-infected cells.

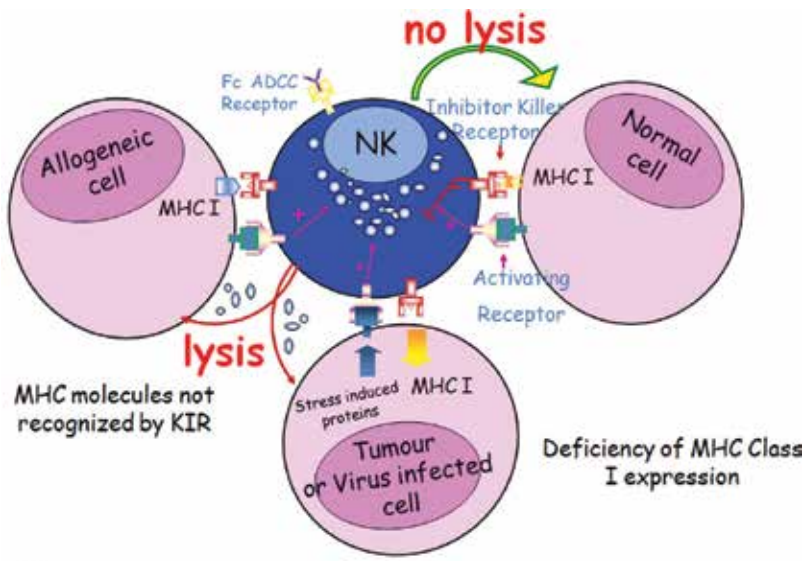

Figure 1. "Missing-self hypothesis" explains the mechanism of action of NK cells.

KIRs belong to the immunoglobulin superfamily and are structurally characterized by two or three extracellular immunoglobulin-like domains. KIRs recognize the MHC Class I molecules such as HLA-A, HLA-B and HLA-C. There are two distinct groups of KIRs: inhibitors and activators. The family of KIR genes, located on chromosome 19q13.4, includes 15 different loci [9]. Receptors are monomeric (single chain) with two (KIR2D) or three (KIR3D) immunoglobulin domains, which can be further divided into those with a long (Long) cytoplasmic tail (KIR2DL and KIR3DL) and those with a short (Short) one (KIR2DS and KIR3DS). The short tail generates an activation signal, while the long one generates an inhibition signal. Each group has the same extracellular domain, and consequently each group binds the same ligands. However, due to differences in their transmembrane and intracellular domains, a group of KIRs determines an inhibitory response, while the other group determines an activating response [10]. KIRs and their ligands are summarized in **Table 1**.

KIRs	KIR ligands
KIR2DL1	Group 2 HLA-C Asn77 Lys80 (w2, w4, w5, w6)
KIR2DS1	
KIR2DL2	Group 1 HLA-C Ser77 Asn80 (w1, w3, w7, w8)
KIR2DL3	
KIR2DS2	
KIR3DL1	HLA-Bw4
KIR3DL2	HLA-A3,-A11
KIR2DL4	HLA-G
KIR2DL5	Unknown
KIR3DL7	
KIR2DS4	
KIR2DS5	
KIR3DS1	

Table 1. KIRs and their ligands.

In normal cells the expression of Major Histocompatibility Complex (MHC) Class I molecules and their binding to NK inhibitory receptors inhibits the lysis, while in the virus-infected or cancer cells the lack of expression of self MHC molecules determines their susceptibility to lysis mediated by NK cells. In tumor or virus-infected cells, stress-induced molecules are expressed on the cell surface and are recognized by the activating receptors that enhance the lysis. NK cells can also lyse allogeneic cells expressing HLA Class I antigens that are not recognized by their inhibitory NK receptors.

The KIR genes are polymorphic in humans and there are different allelic variants; each individual possesses his own KIRs repertoire depending on the allelic variants possessed. Different haplotypes contain a different number of KIR genes; some have only one or a few activating receptors, others have more activating receptors. In humans, two groups of KIR haplotypes have been identified, A and B, based on the content of different KIR genes. Haplotypes of group A are present in the entire population and consist of six KIR inhibitory genes KIR3DL3, KIR2DL3, KIR2DL1, KIR2DL4, KIR3DL1 and KIR3DL2 and one activator KIR2DS4 that is often present in a null allele variant that is not expressed on the cell surface. Group B comprises haplotypes with a different genetic content including genes KIR2DS2, KIR2DL2, KIR2DL5, KIR2DS3, KIR2DS1, KIR2DS5 and KIR3DS1 that are not part of the haplotypes of Group A. As a consequence, many haplotypes in Group B encode for a greater number of activating receptors compared to haplotypes in Group A.

All individuals can be classified on the basis of the two KIR genotypes possessed: A/A, which is homozygous for the haplotypes of Group A, B/x, which contains one (heterozygous A/B) or two haplotypes of Group B (homozygous B/B). Individuals B/x possessing a larger number of activating receptors theoretically should have a better response against cancer or virus-infected cells.

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1.3. Role of NK Cells in HSCT

In recent years, the antileukemic potential of natural killer (NK) cells and their role in hematologic malignancies and in HSCT has been attracting ever greater interest; however, it has not yet been clearly established whether the typing of KIR genes may be a useful tool in the selection of an HSC donor to promote a better outcome of the transplant [11].

The beneficial GvL effect, promoted by alloreactive NK cells derived from the donor, was demonstrated for the first time in the HSC haploidentical transplants in patients with acute myeloid leukemia (AML); the best donor is NK mismatched with the recipient and therefore the donor's KIRs do not recognize HLA class I molecules expressed by the host. In this way, the donor's NK cells are responsive to the direction GvH and this results in a better antileukemic effect with a better overall survival rate and a lower rate of relapse of leukemia [12].

More recently, Cooley et al. [13] have demonstrated that AML patients who received HSC transplants from unrelated Group B KIR haplotype donors had a better outcome after transplant, whereas recipient KIR genotype had no effect. The clinical outcome was better when the donors had one or two KIR B haplotypes (KIR B/x donors) rather than donors who had two KIR A haplotypes (KIR A/A donors); with a KIR B/x donor, relapse was reduced and the leukemia-free survival rate (LFS) was increased. This is because the haplotypes of Group B have a larger number of activating receptors, and this translates into better antileukemic activity. A subsequent study [14] sought to determine whether the protective effect of KIR B could be mapped to either the centromeric or the telomeric region of the KIR locus. The centromeric region contains genes encoding the inhibitory receptors for the C1 and C2 epitopes of HLA-C, whereas the telomeric region contains genes encoding the inhibitory receptors for the Bw4 and A3/11 epitopes and the activating C2 receptor. It was found that both the centromeric and telomeric regions of KIR B correlated with protective effect, but the much stronger association was with the centromeric region. As a consequence of these results, an algorithm was formulated, called "KIR B-content score", based on the number of centromeric and telomeric Group B KIR haplotypes gene-content motifs. The KIR B-content score defines three categories of donors: neutral donors have none or one KIR B motifs, better donors have two or more B motifs without KIR Cen B/B and best donors have two or more B motifs with KIR Cen B/B. A calculator for classification of the donor KIR B status (best, better and neutral) may be found at http://www.ebi.ac.uk/ipd/kir/.

Recently, these results have been confirmed in HSCT from HLA-identical sibling donor [15]. Together, these results highlight the need for further studies of KIR polymorphisms, possibly at allelic level, to determine whether the typing of the donor KIR genes may be useful in donor selection for HSCT.

2. Research aims

The aim of our research is a retrospective analysis of HSC transplants performed at the Bone Marrow Transplant Unit (UTMO) of the "V. Cervello" Hospital in Palermo. The category of HSC transplants to be studied is the one from MUD.

The objectives of this study are the following:

1. Typing of the donors' KIR genes of the transplants performed.
2. Donors classification in accordance with the KIR B Content Score in three categories: neutral, better and best.
3. Correlation of the donor KIR B status with the clinical outcome of the transplants considering overall survival, the degrees of graft versus host disease (GvHD) graft versus leukemia (GvL), relapse and disease-free survival (DFS).

3. Materials and methods

3.1. Patient cohort

We analyzed 89 patients, who received a URD HSCT between 1996 and 2013. The median age of the 89 patients was 43 years (14–65). The transplanted patients had different hematological malignancies: 33 acute myeloid leukemia (AML), 19 acute limphoblastic leukemia (ALL), 9 myelodysplastic syndromes (MDS), 6 non-Hodgkin lymphoma (NHL), 6 multiple myeloma (MM), 5 Hodgkin lymphoma (HL), 4 chronic myeloid leukemia (CML), 3 chronic lymphocytic leukemia (CLL), 1 aplastic anemia syndrome (AAS), 1 Fanconi syndrome, 1 ematodermic neoplasia and 1 biphenotypic acute leukemia (BAL). The HSC source was: bone marrow no. 19, peripheral blood no. 70. Complete high-resolution, allele-level HLA-A, B, C, DRB1 and DQB1 typing of the 89 pairs of recipients and donors was previously performed: 32 pairs were 10/10 matched, 33 pairs were 9/10 and 24 were ≤8/10. Patient and transplant characteristics are summarized in **Tables 2** and **3**.

Median age (range)	**43 (14–65)**
Diagnosis	
AML	33
ALL	19
MDS	9
NHL	6
MM	6
LH	5
CML	4
CLL	3
AAS	1
Fanconi syndrome	1
Ematodermic neoplasia	1
BAL	1

Table 2. Patient characteristics.

Graft type	
Bone marrow	19
Peripheral blood progenitor cells	70
Conditioning	
Busilvex-ciclofosfamide	15
Busilvex-fludarabine	15
Fludarabine-melphalan	4
Thiotepa + others	7
Ciclofosfamide + others	14
Endoxan	4
Unknown	32
TBI	6
ATG	12
Donor/recipient HLA-allele matching at A, B, C, DRB1 and DQB1	
10/10	32
9/10	33
≤8/10	24

Table 3. Transplants characteristics.

3.2. KIR genotyping

The donors' DNA that has already been typed for HLA has been preserved in our biobank; about 80% of the samples is still available. The DNA was extracted from buffy coat or whole blood by the automatic extractor Maxwell 16 (Promega) or by the salting out method.

Typing of KIR genes was performed with PCR-SSO Luminex, the same method already used in our laboratory to type the HLA genes. The kit used for KIR genes typing was the SSO KIR Genotyping Test (One Lambda Inc., Canoga Park, CA) and the method was performed in accordance with the manufacturer's instructions. Luminex technology is based on the ability to soundly measure multiple analytes simultaneously in a single reaction and this is done through the use of plastic microbeads (microspheres), which are color-coded with two fluorescent dyes so as to emit two different wave lengths in red and infrared. In this way a set of over 100 different microspheres can be created. The microspheres represent the solid support of oligonucleotide probes which can bind amplified DNA that is labeled with a fluorescent labeled reporter. Subsequently, the microbeads are acquired by the Luminex machine through a precision sheath fluid system, based on classical flow cytometry, which aligns them in single file, where they pass through two lasers: the red laser excites the colors inside the microbeads to identify which microbead is currently being read, while the green laser excites the color on the microbead surface, that is, the labeled reporter tag. Finally, the color signals are detected by an advanced optical system, and the signals are processed into data for each reaction [16].

The PCR using specific primers for the KIR genes is first conducted with a specific amplification program via the GeneAmp PCR System 9700 (Applied Biosystems, Forster City, CA). The amplification products are tested by electrophoresis on 2% agarose gel and then observed and photographed under a UV transilluminator. The PCR product is biotinylated, which allows it to be detected by strepavidin-conjugated with PE (SAPE). Subsequently, the PCR products are denatured facilitating rehybridization to complementary DNA probes bound to the microbeads with a specific sequence (SSO) to recognize the polymorphic sites within KIR genes. The amplified products which have undergone a hybridization reaction are labeled with streptavidin conjugated with R-Phycoerythrin and then acquired with the flow cytometry system LABScanTM 100 (Luminex), which measures the fluorescence intensity of phycoerythrin on each microsphere. The data are then analyzed with the software HLA Fusion.

4. Results

4.1. Donor classification

We typed KIR genes of the 89 selected donors of the transplanted patients and we classified them on the basis of the KIR haplotypes possessed. KIR gene frequency among the donor population was similar to that of published data [17, 18]: no. 30 (33.7%) donors were A/A and no. 59 (66.3 %) were B/x.

Then we classified them using the donor KIR B Content Score Calculator at http://www.ebi.ac.uk/ipd/kir/: no. 63 (70.8%) donors were neutral, no. 18 (20.2%) better and no. 8 (9%) best. In our analysis we combined the better and best groups to form the KIR better/best donor group (with two or more B motifs). The classification of the donors in accordance with the KIR B content score is summarized in **Table 4**.

Donor/ recipient HLA-allele matching at A, B, C, DRB1 and DQB1	**Total n = 89**			**AML recipients n = 33**			**ALL recipients n = 19**			**Other hematological malignancies n = 37**		
		Donor KIR B status	Donor KIR B status		Donor KIR B status	Donor KIR B status		Donor KIR B status	Donor KIR B status		Donor KIR B status	Donor KIR B status
	Total (89)	**Neutral (63)**	**Best/ better (26)**	**Tot (33)**	**Neutral (21)**	**Best/ better (12)**	**Tot (19)**	**Neutral (14)**	**Best/ better (5)**	**Tot (37)**	**Neutral (28)**	**Best/better (9)**
10/10	32	24	8	10	9	1	7	4	3	15	11	4
9/10	33	22	11	14	7	7	8	7	1	11	8	3
≤8/10	24	17	7	9	5	4	4	3	1	11	9	2

Table 4. Donor classification in accordance with the KIR B content score.

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4.2. Clinical outcome analysis

4.2.1. Overall survival, relapse and disease-free survival

The overall survival rate was analyzed generating the Kaplan-Meyer curves, using the GraphPad6 Demo software; the data were compared using the log-rank test 95%CI.

We observed no significant difference in the overall survival rate of those patients transplanted from A/A donor and those from B/x donor, while we found a better overall survival rate in the AML recipients, HLA mismatched with the donor, when the donor KIR B content status is best/better (37 vs. 18% at three years log-rank test P = 0.028) (**Figure 2**), whereas there is no beneficial effect in recipients with other hematological malignancies (**Figure 3**).

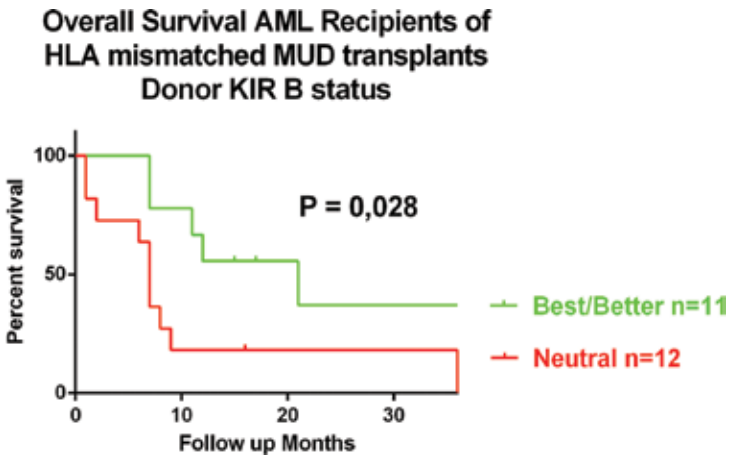

Figure 2. Overall survival rate of AML recipients of HLA mismatched MUD transplants.

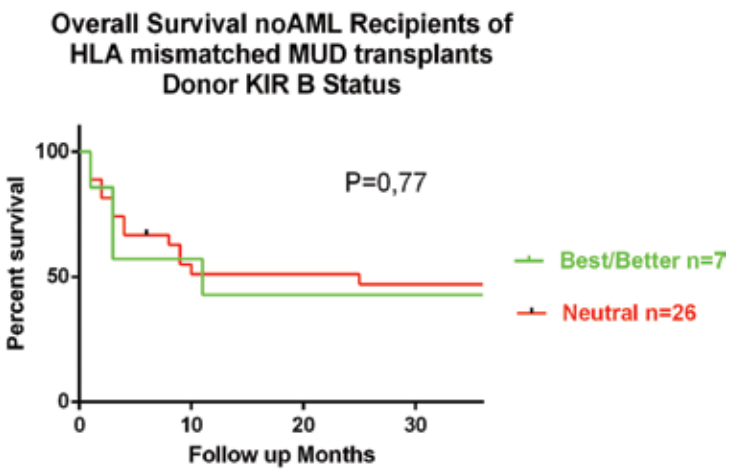

Figure 3. Overall survival rate of recipients with other hematologic malignancies of HLA mismatched MUD transplants.

Moreover, we observed that AML recipients, whose donor KIR B status was best/better, had a lower incidence of relapse than patients whose donor KIR B status was neutral (36 vs. 58%) (**Figures 4** and **5**) and a better disease-free survival rate (58 vs. 38% at three years P = 0.1) (**Figure 6**).

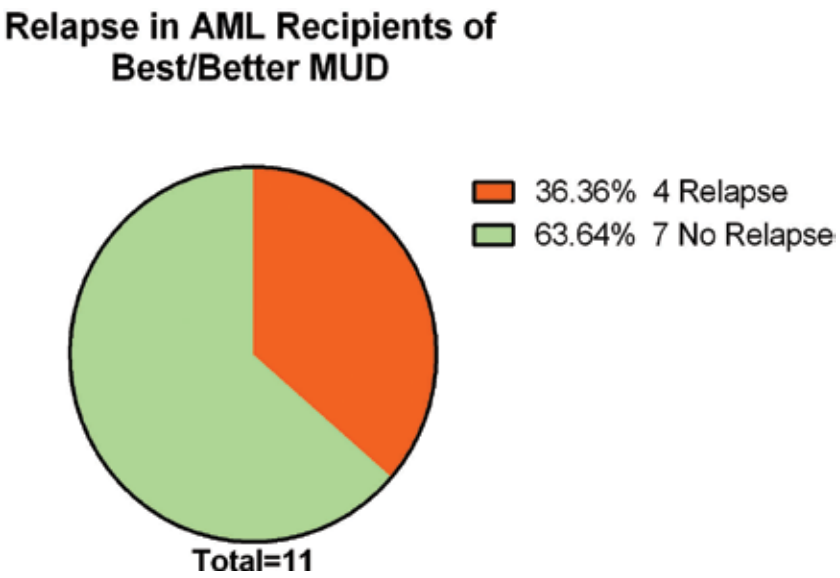

Figure 4. Incidence of relapse in AML recipients of best/better MUD.

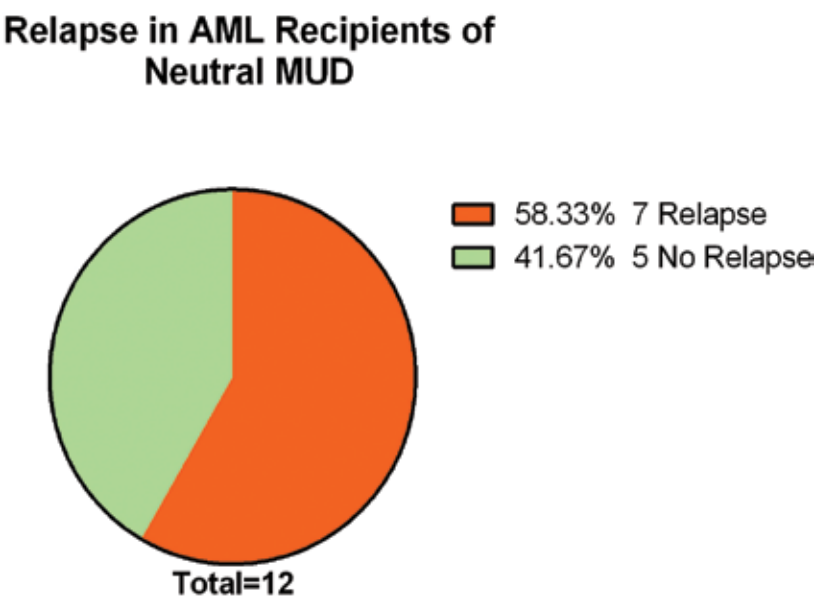

Figure 5. Incidence of relapse in AML recipients of neutral MUD.

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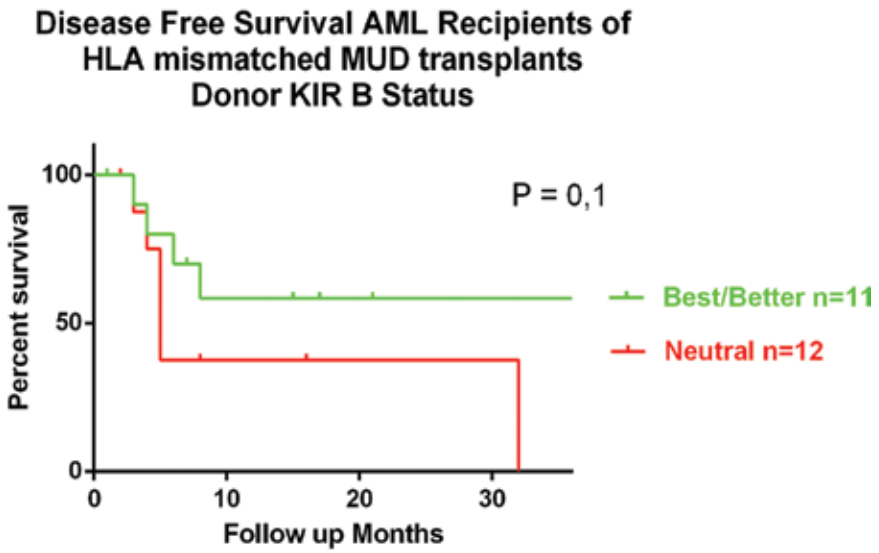

Figure 6. Disease-free survival rate of AML recipients of HLA mismatched MUD transplants.

We have not been able to make a comparison of the 10/10 HLA matched AML recipients because only one donor was best/better.

4.2.2. aGvHD

The incidence of aGvHD in AML patients was: 55.17% (no. 16/29) of no aGvHD, 41.38% (no. 12/29) of aGvHD grade I and II, 3.45% (no. 1/29) of aGvHD grade III and IV (**Figure 7**).

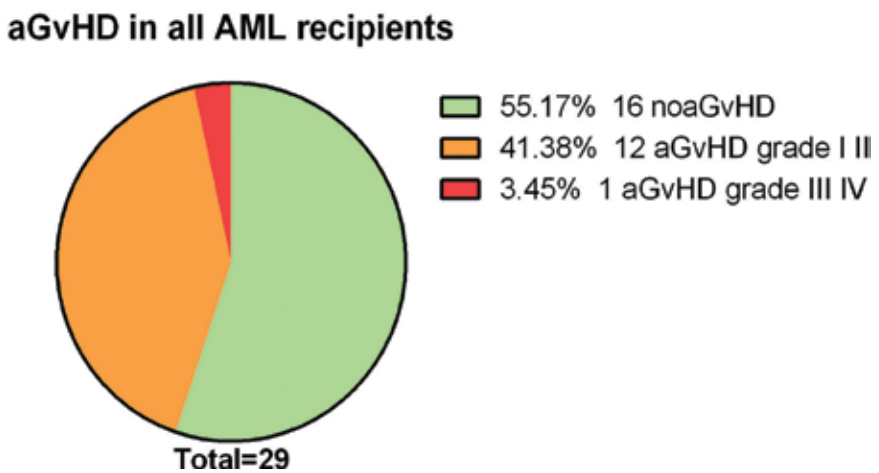

Figure 7. The incidence of aGvHD in AML patients.

We observed that AML recipients, whose donor KIR B status was best/better, had more incidence of aGvHD grade I and II than patients whose donor KIR B status was neutral: 70 vs. 26% (**Figures 8** and **9**).

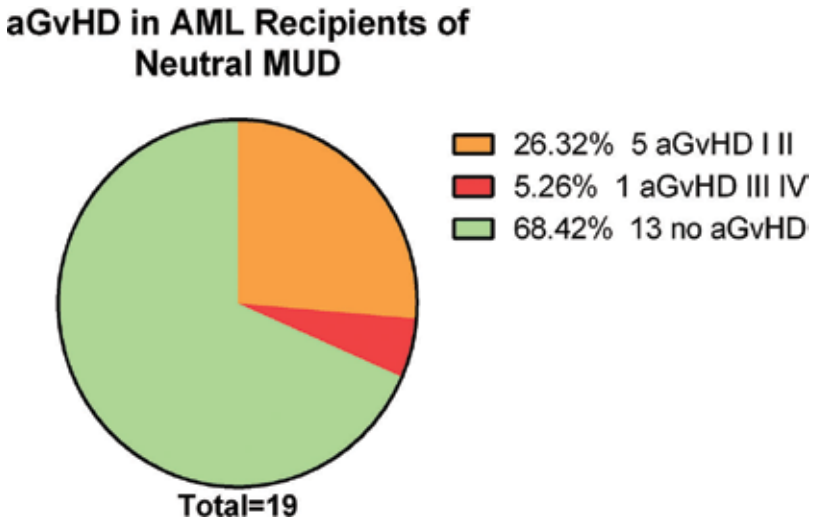

Figure 8. Incidence of aGvHD in AML recipients of neutral MUD.

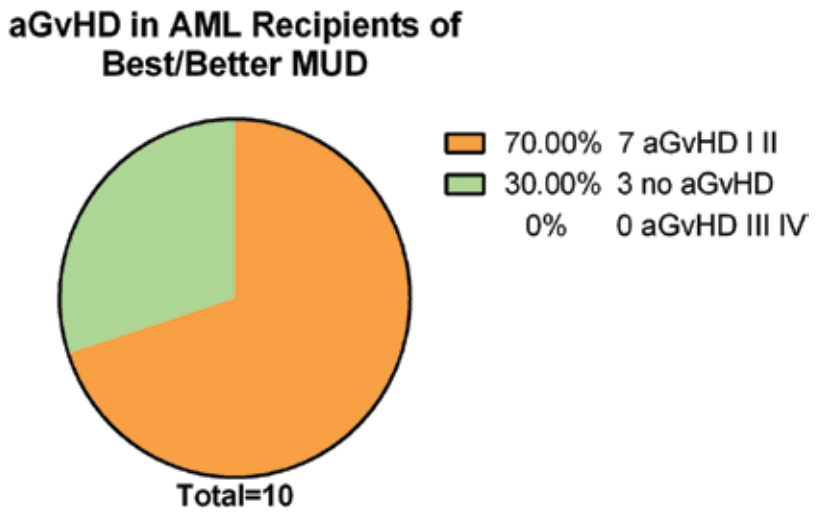

Figure 9. Incidence of aGvHD in AML recipients of best/better MUD.

Actually, as regards patients who have aGvHD grade I and II, this results in a beneficial effect on the overall survival rate. In fact, we observed that patients with aGvHD grade I and II had a significantly better overall survival rate than patients with noGvHD (51.6 vs. 38% at three years $P = 0.04$) (**Figure 10**). This agrees with those studies [4] showing that patients with aGvHD I and II have a better overall survival rate and a lower rate of relapse due to a GvL effect.

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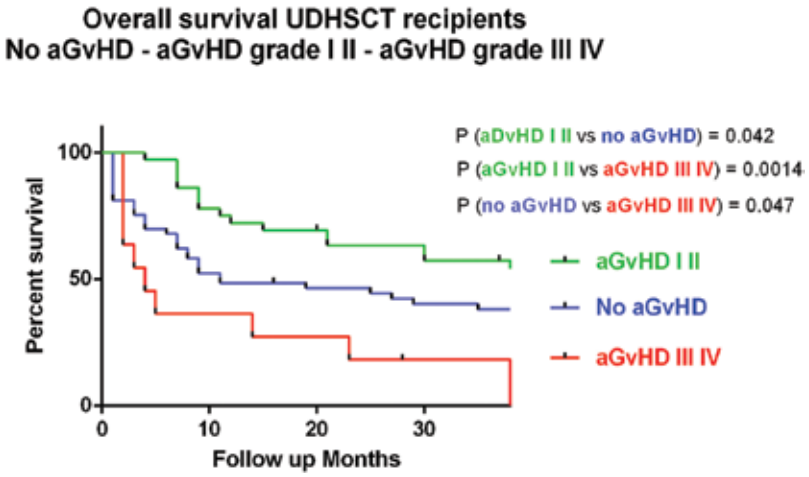

Figure 10. Patients with aGvHD grade I and II had a significantly better overall survival rate than patients with noGvHD (51.6 vs. 38% at three years P = 0.04).

5. Discussion

In recent years, various studies have shown that the beneficial GvL effect promoted by alloreactive NK cells derived from the donor can improve the outcome of the HSCT from haploidentical, unrelated and HLA identical sibling donors. In particular Cooley et al. [12] have demonstrated that AML patients who received HSC transplants from unrelated Group B KIR haplotypes donors, had a better outcome after transplant because the haplotypes of Group B have a larger number of activating receptors, and this translates into better antileukemic activity. In a subsequent study [13], they found that some particular KIRs had a stronger effect and they defined three categories of donors: neutral (none or one KIR B motifs), better (two or more B motifs without KIR Cen B/B) and best (two or more B motifs with KIR Cen B/B).

In our study we analyzed the impact of the donor KIR B status on the outcome of the unrelated HSC transplants performed in our center. We observed no significant difference in overall survival of those patients transplanted from A/A donor and those from B/x donor, probably because we do not have a large enough cohort of transplanted patients, and we were not able to make a meaningful comparison; on the other hand, despite this small cohort of patients, we have been able to observe the powerful effect of the best/better donor KIR B status in the AML recipients HLA mismatched with the donor. They had a significantly better overall survival rate if the donor KIR B content status was best/better and they also had a higher incidence of aGvHD grade I and II, and had a lower rate of relapse due to GvL effects.

Even though our cohort was small, our results confirmed that the presence of more activator KIR genes in donors can improve the outcome of UDHSC HLA mismatched transplants in AML recipients whereas there is no benefit in recipients with other hematologic malignancies. Our next target is to increase the number of cases by adding the other transplants performed and to study the effect of GvL in relapse in the same patients.

In any case, our results confirm that NK cells derived from donors with two or more KIR B motifs have an improved ability to kill residual leukemic blasts in AML recipients of UDHSCT. As a consequence of this, the KIR genotype of the donor is another important criterion to be taken into account for the choice of the best possible donor in unrelated, sibling and haploidentical HSC transplant settings, together with all the other important factors, such as HLA matching, CMV status, blood group, age and gender. Future studies should be aimed at finding the right alloreactive subsets among donor NK cell repertoires, opening up the possibility for successful NK cell–based immunotherapy. Antileukemic NK cells, either allogeneic or unlicensed autologous NK cells, emerge as a feasible therapy option and might improve the clinical outcome in myeloid leukemia.

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References

[1] Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, Fernandez-Vina M, Flomenberg N, Horowitz M, Hurley CK, Noreen H, Oudshoorn M, Petersdorf E, Setterholm M, Spellman S, Weisdorf D, Williams TM, Anasetti C. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007;**110**(13):4576-83. Epub 2007 Sep 4

[2] Marsh SGE, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, Fernández-Vina M, Geraghty DE, Holdsworth R, Hurley CK, Lau M, Lee KW, Mach B, Mayr WR, Maiers M, Müller CR, Parham P, Petersdorf EW, Sasazuki T, Strominger JL, Svejgaard A, Terasaki PI, Tiercy JM, Trowsdale J. Nomenclature for factors of the HLA system. Tissue Antigens. 2010;**75**:291-455

[3] Robinson J, Mistry K, McWilliam H, Lopez R, Parham P, Marsh SGE. The IMGT/HLA database. Nucleic Acids Research. 2011;**39**(Suppl 1):D1171-6

[4] Petersen SL. Alloreactivity as therapeutic principle in the treatment of hematologic malignancies. Studies of clinical and immunologic aspects of allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. Danish Medical Bulletin. 2007;**54**(2):112

[5] Pende D, Moretta A, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. Blood. 2009;**113**(13):3119-29. doi: 10.1182/blood-2008-06-164103. Epub 2008 Oct 22

[6] Rocha V, Locatelli F. Searching for alternative hematopoietic stem cell donors for pediatric. Patients Bone Marrow Transplantation. 2008;**41**:207-14

[7] Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. Immunology Today. 1990;**11**:237-44

[8] Ljunggren HG, Malmberg KJ. Prospects for the use of NK cells in immunotherapy of human cancer. Nature Reviews Immunology. 2007;**7**:329-38

[9] Jiang W, Johnson C, Jayaraman J, Simecek N, Noble J, Moffatt MF, Cookson WO, Trowsdale J, Traherne JA. Copy number variation leads to considerable diversity for B but not A haplotypes of the human KIR genes encoding NK cell receptors. Genome Research. 2012;**22**(10):1845-54. doi: 10.1101/gr.137976.112. Epub 2012 Sep 4

[10] Farag SS, Fehniger TA, Ruggeri L, Velardi A, Caligiuri MA. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood. 2002;**100**(6): 1935-47

[11] Florian B, Fischer JC, Uhrberg M. The role of KIR genes and ligands in leukemia surveillance. Frontiers in Immunology. 2013. doi: 10.3389/fimmu. 2013.00027

[12] Ruggeri L, Mancusi A, Capanni M, Urbani E, Carotti A, Aloisi T, Stern M, Pende D, Perruccio K, Burchielli E, Topini F, Bianchi E, Aversa F, Martelli MF, Velardi A. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood. 2007;**110**(1):433-40. Epub 2007 Mar 19

[13] Cooley S, Trachtenberg E, Bergemann TL, Saeteurn K, Klein J, Le CT, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. Blood. 2009;**113**:726-32

[14] Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010;**116**:2411-9

[15] Impola U, Turpeinen H, Alakulppi N, Linjama T, Volin L, Niittyvuopio R, Partanen J, Koskela S. Donor haplotype B of NK KIR receptor reduces the relapse risk in HLA-identical sibling hematopoietic stem cell transplantation of AML patients. Frontiers in Immunology. 2014 Aug 25;**5**:405

[16] Heinemann FM. HLA genotyping and antibody characterization using the Luminex™ multiplex technology. Transfusion Medicine and Hemotherapy. 2009;**36**:273-8

[17] Witt CS, Dewing C, Sayer DC, Uhrberg M, Parham P, Christiansen FT. Population frequencies and putative haplotypes of the killer cell immunoglobulin-like receptor sequences and evidence for recombination. Transplantation. 1999;**68**:1784-9

[18] Cook MA, Milligan DW, Fegan CD, et al. The impact of donor KIR and patient HLA-C genotypes on outcome following HLA-identical sibling hematopoietic stem cell transplantation for myeloid leukemia. Blood. 2004;**103**:1521-6

Chapter 8

Hematopoietic Stem Cell Transplantation for Acute Lymphoblastic Leukemia in the Era of Novel Therapies

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Khalid Ahmed Al-Anazi

Additional information is available at the end of the chapter

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Abstract

Recently, the outcomes of patients with acute lymphoblastic leukemia have improved significantly due to the progresses achieved in diagnostics and various therapeutic interventions. In particular, the availability of several novel agents and targeted therapies as well as the provision of safer modalities of stem cell transplants have yielded higher responses and improved survival rates. The role of hematopoietic stem cell transplantation is reviewed in children and adults with acute lymphoblastic leukemia in the era of novel agents and targeted therapies. Various modalities of stem cell therapies in different types of acute lymphoblastic leukemia as well as closely related issues such as graft versus tumor effect, minimal residual disease, and conditioning therapies are discussed thoroughly. In addition, various modalities of novel therapies have been discussed to be efficacious in clinical practice.

Keywords: acute lymphoblastic leukemia, hematopoietic stem cell transplantation, reduced intensity conditioning, monoclonal antibodies, immunotherapies

1. Introduction

Acute lymphoblastic leukemia (ALL) is a clonal expansion or malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood, and extramedullary sites [1, 2]. It is a highly heterogeneous disease comprising several entities that have distinct clinical manifestations, therapeutic strategies as well as prognostic implications [2]. ALL can occur at any age, but 80% cases occur in children [1, 3]. The incidence of ALL follows a bimodal distribution with the first peak occurring in children 2–5 years of age, while the second peak

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is encountered around the age of 50 years [1, 3]. While most children with ALL are potentially curable, the prognosis in infants, adults, and elderly individuals remains poor [3].

Worldwide, different induction chemotherapeutic regimens are utilized in the treatment of patients with ALL [4–7]. Examples are—(1) USA and Canada: CCG series in children and CALGB in adults, modified DFCI 91-01 and 95-01, in addition to hyper-fractionated hyper-CVAD (cyclophosphamide, vincristine, cytarabine, dexamethasone, methotrexate, and doxorubicin); (2) UK: ALL-97, revised ALL-99 for children and UKALL XII for adults; (3) France: FRALLE-93 in children, LALA-94 in adults and GRAALL-2003; (4) Germany: pediatric DCOG-ALL or Berlin-Frankfurt-Munster [standard or augmented regimen]; (5) Italy: AIEPO [pediatric] and GIMEMA [adults]; (6) Holland and Belgium: HOVON-70; (7) Spain: PETHEMA ALL-96; (8) Sweden and Finland: pediatric NOPHO-92 and adult Nordic protocols; and (9) Mexico: LALIN (pediatric) and LALA (adult) [4–7]. Sometimes, different chemotherapeutic regimens are used in the same geographic location or even in the same country [4–7]. Additionally, these treatment regimens undergo modifications or even total replacement once new literature data or results of large studies become available [4–8]. Despite the development of several induction regimens, there is no single best regimen for induction therapy in ALL [4–8]. The main constituents of these chemotherapeutic regimens are almost similar with different dosing and treatment schedules and they include: daunorubicin, doxorubicin, or idarubicin; prednisolone or dexamethasone; vincristine; L-asparaginase; cyclophosphamide; 6-mercaptopurine; and intrathecal (IT) as well as intravenous (IV) methotrexate [4–8].

Recently, the more intensified pediatric ALL induction regimens have been used in adolescents and young adults (AYAs), 15–40 years of age, having ALL, and their use has been associated with superior response rates [4, 6, 9]. Several studies have shown that AYAs treated with adult chemotherapeutic regimens have poorer outcome compared with patients belonging to the same age group treated with pediatric-inspired regimens [4, 6, 9]. Additionally, certain cancer centers, such as Dana-Farber Cancer Center, USA, are currently treating patients between the ages of 1 and 50 years with the same regimens of chemotherapy [5]. The recent incorporation of novel and targeted therapies, such as tyrosine kinase inhibitors (TKIs), nelarabine and rituximab, into the induction therapy of ALL has further improved the response rates and the outcomes in general [4, 5, 10–12].

2. HSCT in ALL patients

2.1. GVL effect in ALL

Several studies have shown that (1) in adults with ALL receiving cytotoxic chemotherapy, the high incidence of relapse is the main cause of treatment failure, hence post-remission therapy particularly the efficacy of allogeneic hematopoietic stem cell transplantation (HSCT) is a critical issue; (2) the rates of relapse of ALL following HSCT are higher than those encountered in other hematologic malignancies; (3) relapse of ALL post-allogeneic HSCT is a major cause of treatment failure as it is associated with an extremely poor prognosis; (4) graft versus host disease (GVHD) encountered in the post-HSCT period has a protective effect against disease

relapse; and (5) graft versus leukemia (GVL) effect plays a major role in curing patients with ALL subjected to allogeneic HSCT [13–17]. However, there is considerable evidence for the existence of GVL effect after HSCT in patients with ALL: (1) relapse rates are lower in recipients of allogeneic HSCT compared with recipients of autologous grafts, (2) relapse rates are lower in patients who develop acute or chronic GVHD following allografts, and (3) the use of interferon immediately post-allogeneic HSCT may reduce relapse rate through stimulation of an immunological response [15, 18]. Unfortunately, the efficacy of GVL effect in the context of donor lymphocyte infusion (DLI) of ALL in the post-allogeneic HSCT setting is quite unimpressive as response rates to DLI in ALL patients receiving allografts have been reported to range from 0 to 18% [15, 18].

GVL effect in ALL is influenced by the extent of leukemia burden [15]. Minimal residual disease (MRD) studies after HSCT have found a strong correlation between the presence of MRD and relapse of leukemia [16]. Frequent MRD monitoring post-allogeneic HSCT may predict ALL relapse early enough, thus allowing the implementation of various approaches such as: (1) reduction of immunosuppressive therapy, (2) DLI, and (3) adoptive T-cell therapy, but such approaches may be ineffective in the presence of high disease burden [15, 16].

The development of GVHD following allogeneic HSCT in patients with B-cell ALL is associated with a lower probability of leukemia relapse due to a non-specific inhibition of B-lymphocytosis [19]. Also, the improved survival in recipients of allogeneic HSCT who develop acute or chronic GVHD is attributed to the beneficial GVL effect of GVHD [14]. Chronic GVHD, particularly limited form, is associated with a significant GVL effect [13]. However, the correlation between GVHD and GVL is mainly seen in non-T cell-depleted allografts [14]. The influence of chronic GVHD on the risk of relapse has been found to be prominent in patients with chromosomal translocations or normal cytogenetics [13].

Studies have shown that in ALL patients subjected to allogeneic HSCT, relapse rates are higher in: (1) patients receiving dual or effective GVHD prophylaxis as the intensity of the GVHD prophylactic regimen inversely correlates with the incidence of acute GVHD and (2) recipients of matched sibling donor (MSD) allografts compared with those receiving matched unrelated donor (MUD) grafts as MSD allogeneic HSCT is associated with reduced likelihood of GVHD and reduced treatment-related mortality (TRM), while MUD allografts are associated with higher incidence of GVHD and lower relapse rates due to the pronounced GVL effect of GVHD [14, 17, 20].

In ALL patients receiving allogeneic HSCT, GVL effects are associated with detectable Wilms tumor-1-specific T lymphocyte (WT1) [18]. These results support the immunogenicity of WT1 after HSCT for ALL and highlight the potential for WT1 vaccines to boost GVL effect after HSCT for ALL [18]. The lower relapse rates encountered in ALL patients receiving HSCT may indicate that viral antigens play a role in the induction of anti-leukemic effect [14].

2.2. MRD in ALL

Several studies have shown the prognostic relevance of detection of MRD in patients with ALL [21–23]. MRD identified prior to allogeneic HSCT is the strongest predictor of post-HSCT

relapse in ALL patients [23]. Thus, elimination of pre-HSCT MRD in patients with ALL by novel therapeutic approaches or drug combinations may decrease the risk of post-HSCT relapse and improve overall survival (OS) [23].

MRD evaluation or monitoring in ALL patients can be performed by: (1) flow cytometry, (2) real-time quantitative polymerase chain reaction (RT-Q-PCR), and (3) next-generation sequencing (NGS) [21, 22]. Currently, analysis of MRD is mostly performed by PCR analysis of immunoglobulin (IG) and T-cell receptor (TCR) gene rearrangements, and this method has sensitivity of 10^{-4} in patients with ALL [21, 24]. However, despite the broad clinical usefulness of MRD evaluation, false-positive MRD results can be obtained due to massive B-lymphocyte regeneration after HSCT [24].

NGS enables precise and sensitive detection of multiple antigen receptor rearrangements, thus providing more specific readout compared to RT-Q-PCR, and this will reflect positively on the treatment interventions in ALL patients undergoing HSCT [24]. MRD determines the outcome of autologous HSCT in patients with HR-ALL [25]. In patients with ALL planned for autologous HSCT, MRD evaluation by PCR or NGS may play a role in the direction of therapy as it can predict long-term relapse-free survival [26, 27]. For example, patients with SR-ALL who do not have HR features at diagnosis and who have pre-transplantation negative MRD can be offered autologous HSCT combined with maintenance therapy [26].

2.3. Autologous HSCT in ALL

Complete remission (CR) can be achieved in approximately 80% of adults with ALL, but relapse occurs frequently leading to poor long-term disease-free survival that ranges between 25 and 40% [28, 29]. The post-remission therapies for patients with ALL generally include: (1) consolidation followed by maintenance chemotherapy, (2) allogeneic HSCT for high-risk (HR) patients, and (3) autologous HSCT for standard-risk (SR) patients or HR patients who do not have an HLA identical sibling donor [26, 29]. Therefore, after achieving first CR, intensive therapies, such as allogeneic HSCT and autologous HSCT, are generally offered to patients who are eligible for HSCT [28].

Autologous HSCT was first introduced as a treatment for ALL patients nearly 60 years ago [29]. However, autologous HSCT has been underutilized in ALL patients [29, 30]. Autologous HSCT performed in patients with ALL in CR1 has produced leukemia-free survival ranging between 45 and 65% [29]. Strategies to enhance autologous GVL effect after HSCT may enhance long-term survival in ALL patients subjected to autologous HSCT [31].

In patients with ALL, factors identifying patients who are at high risk of relapse include: (1) age more than 35 years, (2) T-cell type of ALL, (3) elevated white blood cell (WBC) count at presentation, (4) elevated serum lactic dehydrogenase (LDH) level at diagnosis, (5) extramedullary disease (EMD) prior to HSCT, (6) specific cytogenetic abnormalities, (7) blast cell proportion ≥5% on day 15 of induction therapy, and (8) having MRD at various stages during therapy [26, 27]. These factors indicate poor prognosis and decreased OS as well as disease-free survival (DFS) [26, 27]. Factors such as the risk features at the time of diagnosis and MRD following induction therapy greatly affect the outcome of autologous HSCT in ALL patients [29]. SR patients with

ALL who lack poor prognostic factors will benefit from autologous HSCT. Also, HR patients with ALL who are likely to benefit from autologous HSCT include: (1) rapid responders who achieve CR after the first induction therapy and (2) those with negative pre-HSCT MRD [25, 26, 28, 29]. In ALL patients, autologous HSCT should be performed after completion of consolidation chemotherapy as an alternative to maintenance chemotherapy [25]. Autologous HSCT combined with post-transplantation maintenance therapy could be a valid therapeutic option in adult patients with ALL [26, 30]. Adoptive immunotherapy and maintenance therapy after autologous HSCT reduce relapse rate and improve outcome in patients with ALL [30, 32]. Post-autologous HSCT maintenance therapy can be in the form of: (1) 6-mercaptopurine, methotrexate, vincristine, and prednisolone or (2) TKIs in patients with Philadelphia chromosome-positive (Ph+) ALL [27, 30]. Novel therapies, such as blinatumomab, may reduce the burden of MRD before stem cell collection prior to autologous HSCT, thus making the combination of novel therapies and autologous HSCT a real alternative to allogeneic HSCT and prolonged maintenance therapy for ALL patients [27]. Improved DFS and low relapse rates can be achieved after autologous HSCT in adults with ALL who (1) rapidly respond to the first induction chemotherapy and (2) achieve MRD prior to autologous HSCT [26, 28]. Long-term outcome of allogeneic HSCT is superior to autologous HSCT or maintenance chemotherapy [27–29]. Several studies have failed to demonstrate the superiority of autologous HSCT over chemotherapy in adult patients with ALL [27–29]. Before the era of novel therapies and haploidentical HSCT, the prognosis of patients with ALL who relapse post-autologous HSCT was reported to be dismal due to the few available therapeutic options [29].

2.4. Allogeneic HSCT conditioning therapies

Allogeneic HSCT cures hematologic malignancies through two major mechanisms: (1) pre-transplantation conditioning therapy that kills leukemic cells directly and (2) graft versus tumor (GVT) effect [33]. Over the past 25–30 years, the outcome of HSCT has been steadily improving due to improvements in: (1) conditioning therapies, (2) GVHD prophylaxis and therapy, (3) supportive care facilities, (4) new antifungal agents, (5) better diagnostic tools, (6) incorporation of novel and targeted therapies such as TKIs into conventional therapeutic regimens, and (7) donor selection by improvement of human leukocyte antigen (HLA) typing methods and the increased use of MUDs [13, 34, 35]. Also, the use of flow cytometry and PCR for evaluation of MRD and monitoring of early relapse has improved the outcome of HSCT further [34]. Pre-transplantation detection of MRD by flow cytometry or PCR has been associated with lower OS and relapse-free survival [34].

For the past 40 years, the standard myeloablative conditioning (MAC) regimen for ALL is composed of total body irradiation (TBI) 1200 cGy and IV cyclophosphamide 120 mg/Kg body weight [36, 37]. In patients with ALL, relapse is common after HSCT [37]. Attempts to decrease the risk of relapse following HSCT include: (1) modulation of the conditioning regimen by increasing TBI dose to >1200 cGy or adding a second chemotherapeutic agents such as etoposide and (2) decreasing the intensity of the conditioning regimen by relying on immune modulation, GVL effect, for disease control [36]. However, the optimal conditioning therapy for transplant-eligible patients with ALL has not been defined yet [38].

The MAC regimens for ALL consist of: TBI (1200–1400 cGy) in addition to one or more chemotherapeutic agents [36]. In children with ALL, the MAC therapies include: (1) TBI 1000–1200 cGy + cyclophosphamide, (2) TBI 1000–1200 cGy + cyclophosphamide + etoposide, (3) TBI 1320–1400 cGy + cyclophosphamide, and (4) TBI 1320–1400 cGy + cyclophosphamide + etoposide [36]. Studies have shown that (1) etoposide + fractionated TBI and cyclophosphamide + fractionated TBI are equally effective, (2) effectiveness of chemotherapy alone, such as IV busulfan + melphalan, conditioning therapy in patients with HR ALL, (3) there is an advantage of substituting etoposide for cyclophosphamide or increasing the TBI dose to ≥13 Gy when cyclophosphamide is used, and (4) treosulfan, etoposide, and cyclophosphamide conditioning regimen has favorable toxicity profile with lower NRM [33, 35, 39, 40].

In comparison to TBI-containing conditioning regimens, fludarabine and pharmacokinetic-targeted busulfan conditioning therapy appears to be safer and equally effective in controlling ALL [41]. Fludarabine + amsacrine + cytarabine (FLAMSA) + anti-thymocyte globulin (ATG) + fractionated TBI conditioning regimen followed by allogeneic HSCT is feasible and effective in patients with HR or relapsed ALL, thus presenting a potential alternative to the classical TBI and cyclophosphamide MAC therapy [37]. Studies have shown that (1) combination of busulfan and clofarabine provides an effective control while maintaining a favorable safety profile and has produced OS and NRM rates comparable to those achieved with traditional TBI-based conditioning regimens and (2) busulfan + fludarabine + ATG + TBI conditioning therapy has achieved excellent outcomes in all patients with ALL except older patients with comorbidities [38, 42, 43].

The incorporation of etoposide into the intensified conditioning regimens has been associated with improved disease control but at the expense of higher rates of toxicity and TRM [37]. Medium-dose etoposide, cyclophosphamide, and TBI conditioning therapy is suitable for adults with HR-ALL in CR1 and SR-ALL in CR2, below the age of 50 years, as it has been shown to be associated with: lower relapse rate, no increase in toxicity, and better OS [44, 45]. In children and adolescents with ALL, the addition of etoposide to TBI + cyclophosphamide conditioning regimen should be avoided due to increased risk of mortality. Also, TBI dose >1300 cGy should be avoided due to increased risk of second malignancy [36]. In children with ALL in CR1 and CR2, the incorporation of alemtuzumab, anti-CD52 monoclonal antibody, into the MAC therapy in MUD allografts has produced durable engraftment with low rates of GVHD and comparable rates of DFS to recipients of MSD transplants [46]. Thiotepa-based conditioning regimen for allogeneic HSCT in patients with ALL is feasible and effective, and it has produced main outcomes comparable to those achieved by other conditioning therapies [47]. In children with ALL transplanted in second CR, the 3-year DFS using MAC followed by allogeneic HSCT has been reported to range between 30% and 70% [48].

Reduced intensity conditioning (RIC) regimens have been used extensively in adults with hematologic malignancies including ALL [48]. In MAC regimens, relapse protection is provided by dose-intensive chemotherapy ± TBI, while in RIC regimens, relapse protection is provided by GVL effect [44, 48]. The indications for RIC-allogeneic HSCT include: (1) old age, (2) poor performance status, (3) active infection, (4) significant organ dysfunction, and

(5) presence of comorbid medical conditions [44, 48]. Compared with MAC regimens, RIC regimens have been associated with acceptable rates of donor engraftment and lower rates of TRM [48]. In children with ALL, the use of RIC regimens has achieved long-term DFS, but it has been associated with high rates of TRM, acute and chronic GVHD, myelosuppression, and disease relapse [44, 48]. In adult patients with HR-ALL receiving umbilical cord blood transplantation (UCBT): (1) MAC regimens have been associated with DFS comparable to that reported with other stem cell sources and (2) the results of RIC regimens are encouraging [49]. New therapeutic strategies for adults with ALL are increasingly utilized with better outcomes and they include: (1) various TKIs for Ph+ ALL, (2) pediatric inspired chemotherapeutic regimens for Philadelphia chromosome-negative (Ph−) ALL, and (3) HLA-haploidentical HSCT [44]. However, the optimal therapeutic modality should be selected after taking the following factors into consideration: age of the patient, Philadelphia chromosome positivity, donor availability, disease risk stratification, and efficacy as well as safety of the therapeutic measure [44].

2.5. Allogeneic HSCT in ALL

Cytogenetic abnormalities occur in 60–85% of patients with ALL. However, numerical chromosomal abnormalities, alone or in association with structural changes, occur in 50% of ALL patients [2, 50, 51]. The most common chromosomal abnormalities that are encountered in patients with ALL are listed in **Table 1** [2, 50, 51]. In patients with ALL, certain HR features predict poor long-term outcome even in patients receiving intensive chemotherapy. These HR features are shown in **Table 2** [2, 3, 50, 52–55]. Patients having HR features, including HR cytogenetic abnormalities and genetic mutations, are less likely to respond well to chemotherapy and are more likely to relapse. Hence, this category of patients may require not only more intensified chemotherapeutic regimens but also novel therapies as well as HSCT in order to have optimal control of their leukemia [2, 3, 50, 52–55]. The main indications of allogeneic HSCT in children and adults with ALL are shown in **Table 3** [7, 23, 51–54, 56–65].

In adults with ALL, post-remission therapies include: consolidation chemotherapy followed by maintenance therapy, autologous HSCT, and allogeneic HSCT [66, 67]. There is controversy regarding the role of frontline allogeneic HSCT for patients with ALL in CR1 [68]. However, three meta-analyses showed potential benefit of allogeneic HSCT in CR1 [67–69]. These three meta-analyses included 41 studies and they came to the following conclusions: (1) myeloablative MSD allografts had absolute survival benefit of 10–15% at 5 years compared to chemotherapy alone or chemotherapy followed by autologous HSCT, (2) MSD allografts improve survival in patients younger than 35 years and are the optimal post-remission therapy in ALL patients ≥15 years old, (3) no beneficial effect of autologous HSCT in comparison to chemotherapy, and (4) MSD allografts offer superior OS as well as DFS and significantly reduce the risk of relapse but carry increased risk of NRM [67–69]. For patients with relapsed and refractory ALL, allogeneic HSCT is the only potentially curative therapeutic modality [68]. Three major studies that included 1419 patients with relapsed ALL in adults showed that the prognosis of relapsed ALL in adults was very poor and that the 5-year OS of adults with relapsed ALL not subjected to allogeneic HSCT ranged between 0.0 and 10% [70–72].

1. **Philadelphia chromosome [t9,22]:** The commonest cytogenetic abnormality. Encountered in 15–30% of adults and 5% of children with ALL

2. **Chromosomal abnormalities that are associated with higher risk of central nervous system involvement:**

   - t4,11
   - t8,14
   - t14q+

3. **Chromosomal abnormalities that are associated with:**

   - High white cell and blast cell counts at presentation
   - High risk of relapse
   - t9,22
   - t4,11

4. **Other common cytogenetic encountered in patients with ALL:**

   - t10,14
   - t1,14
   - Deletion 11q22
   - Deletion 11q23 [MLL]
   - Hypodiploidy
   - Hyperdiploidy

ALL, acute lymphoblastic leukemia and MLL, mixed lineage leukemia.

Table 1. The most common chromosomal abnormalities in patients with ALL.

Either MAC therapies or RIC regimens can be offered according to the age and comorbid medical conditions of the HSCT recipient [73, 74]. In patients with acute leukemia, the following stem cell sources have been utilized in allogeneic HSCT: MSD, MUD, and UCB [73, 75].

2.6. Focus on haploidentical HSCT

Haploidentical HSCT evolved several decades ago, and it has undergone several modifications and remarkable developments in relation to: conditioning therapy, post-transplantation immunosuppression, and graft manipulation [73, 76]. Historically, the main obstacles to successful haploidentical HSCT were graft failure, intractable GVHD, decreased GVL effect, and delayed immune reconstitution [75, 76]. This form of HSCT is readily available for the majority of patients with acute leukemia and is an acceptable alternative to other donor sources of stem cells [75–77]. Techniques that are used to improve the outcome of haploidentical HSCT include: (1) CD3/CD19 depletion to reduce GVHD, (2) KIR B haplotype donors

1. **Age:** <1 year and >35 years
2. **White blood cell count at presentation:**
   - > 30,000 in B-lineage
   - > 100,000 in T-lineage
3. **Cell type:** pro-B; early and mature T
4. **Immunophenotyping:**
   - CD 20 positivity
   - CD 10 negative pre-B ALL.
5. **Poor performance status:** >1
6. **Poor response to prednisolone**
7. **Peripheral blood blasts ≥5% on day: 8–15**
8. **Failure to achieve remission >4 weeks of induction chemotherapy**
9. **Involvement of central nervous system**
10. **Clinical relapse:** >First complete remission
11. **Minimal residual disease:** Detectable molecular and immunophenotypic evidence of disease while in morphologic remission.
12. **High-risk cytogenetic and molecular abnormalities:**
    - +8
    - –7
    - Deletion 6q
    - Low hypodiploidy
    - Near triploidy
    - Immunoglobulin H gene rearrangement
    - Intrachromosomal amplification of chromosome 21
    - Translocation 8,14
    - Translocation 4,11
    - Translocation 1,19
    - Translocation 9,22
    - Philadelphia-like
    - Complex cytogenetics

13. High-risk genetic mutations:
• IKZF1 deletion [IKAROS]
• Unmutated NOTCH1
• MLL [mixed lineage leukemia] gene rearrangement: 11q23
• RAS-PTEN altered
• JAK 2 mutation
• KMT2A rearrangement
• CREBBP
• CRLF2
• ETP
• PBX-E2A+
• BAALC+

Table 2. High-risk features that predict poor long-term outcome even with intensive chemotherapy in patients with acute lymphoblastic leukemia.

confer rapid natural killer cell proliferation soon after HSCT resulting in lower relapse rates due to GVL effect, and (3) infusion of high numbers of CD34+ cells to improve immune reconstitution [77].

Historically, mega doses of stem cells had been used after T-cell depletion in order to avoid development of GVHD, but this maneuver had been associated with high incidence of graft failure and disease relapse [73, 78]. Recently, unmanipulated allografts and post-transplantation cyclophosphamide have been used with high success [73, 74, 78]. Also, it has been shown that the outcome of unmanipulated haploidentical HSCT in adult patients with acute leukemia are comparable to those of unrelated UCBT and MUD allografts [74, 78].

Adults	Children
1. Philadelphia chromosome-positive ALL in CR1	**1.** Severe hypodiploidy
2. ALL with Philadelphia-like molecular signature	**2.** Persistent MRD
3. High-risk or very high-risk ALL in CR1	**3.** T-cell ALL with poor response to prednisolone
4. Relapsed ALL [ALL in CR2 or beyond]	**4.** Primary induction failure
5. Primary refractory disease [ALL refractory to induction or first-line chemotherapy]; once CR is achieved following salvage therapy, allogeneic HSCT can be performed	**5.** MLL gene rearrangement in infants with ALL
6. Presence of MRD at any stage during the course of the disease, regardless their initial risk group [standard risk or high risk]	**6.** Relapsed ALL [ALL in CR2]: bone marrow or extramedullary relapse
7. MLL [mixed lineage leukemia] gene rearrangement	

ALL, acute lymphoblastic leukemia; CR, complete remission; MLL, mixed lineage leukemia; and MRD, minimal residual disease.

Table 3. Indications of allogeneic HSCT in patients with ALL.

In patients with Ph− ALL in CR1, studies have shown that (1) outcomes of haploidentical HSCT are comparable to MSD and MUD allografts and (2) outcomes of HR patients are comparable to those of low-risk patients once haploidentical allografts are used. Therefore, haploidentical HSCT represents a valuable alternative for patients with Ph− ALL lacking MSDs [79, 80]. In adult patients with HR-ALL in CR1, haploidentical HSCT performed with post-transplantation cyclophosphamide-based GVHD prophylaxis has produced 52% DFS at 3 years, thus providing a suitable alternative to HLA-matched transplantation [68].

Also in adults with HR-ALL in CR1 and CR2 or beyond, unmanipulated haploidentical transplants have been associated with 3-year OS and DFS of 33 and 31%, respectively; thus, unmanipulated haploidentical allografts can be considered a valid option for adults with HR-ALL lacking HLA-identical donors particularly if performed in early disease status [74]. Compared to HLA-matched related donor allografts, haploidentical HSCT has produced similar rates of long-term survival and NRM but lower relapse rates in patients with Ph+ ALL, thus haploidentical HSCT represents a valid therapeutic option for patients who lack a suitable HLA-matched donor [81, 82].

2.7. HSCT in T-cell ALL

T-cell ALL is an aggressive neoplasm derived from malignant transformation of lymphoblasts committed to T-cell lineage [83]. It accounts for 10–25% of all cases of ALL [84–86]. Given the rarity of T-cell ALL, patients are typically treated in a similar fashion to B-cell ALL with pediatric inspired dose-intense multi-agent chemotherapy regimens in addition to central nervous system (CNS) prophylaxis [83–85]. T-cell ALL carries a poor prognosis compared with B-cell ALL due to: (1) higher relapse rates even if they respond to chemotherapy and achieve CR and (2) more extensive involvement of bone marrow and extramedullary sites, particularly the mediastinum [83, 84, 86]. Even with the current intensive chemotherapeutic regimens, the 5-year event-free survival (EFS) is 80%, whereas the 10-year EFS is only 15% [84].

Allogeneic HSCT is a potentially curative therapeutic option for patients with T-cell ALL, but relapse after allogeneic HSCT is a major cause of treatment failure [86]. Patients with T-cell ALL who lack an HLA-matching donor for allogeneic HSCT should preferably have prolonged maintenance chemotherapy [83]. Studies have shown that (1) in children with T-cell ALL in CR2 subjected to allogeneic HSCT, the 3-year OS is about 50% and (2) in adults with T-cell ALL including the aggressive early-thymic precursor (ETP) subtype subjected to allogeneic HSCT in CR1, the 3-year OS is 62% and those transplanted in CR2 or beyond, the 3-year OS is 24% indicating the better outcome of allogeneic HSCT in CR1 in adults [84, 85]. Other studies on adults with T-cell ALL subjected to allogeneic HSCT have shown the following results: (1) allogeneic HSCT is safe and effective in overcoming the poor prognosis particularly when applied early, (2) there was controversy regarding the use of TBI in the conditioning therapies as some studies highlighted the importance of having TBI as part of the conditioning regimen, while other studies showed no difference between TBI-based and busulfan-based conditioning therapies, and (3) MRD status at transplantation is highly predictive of disease relapse, suggesting that novel therapies

can be offered before and after allogeneic HSCT to improve the outcome of this group of patients [85–88]. Patients with refractory T-cell ALL can be treated successfully with unmanipulated allografts from HLA-mismatched haploidentical siblings as haploidentical HSCT offers higher GVL effect [89, 90].

Nelarabine, a prodrug of Ara-G, has shown selective cytotoxicity against T-cell lymphoblasts and is usually used in relapsed and refractory T-cell ALL [83, 91]. Nelarabine is a valuable therapeutic option in patients with T-cell ALL relapsing after allogeneic HSCT as it has shown response rates reaching 81%; hence it should be considered in: (1) treating relapses post-allogeneic HSCT and (2) maintenance therapy following transplantation in HR patients [91].

Gamma delta subtype of precursor T-cell ALL is usually treated with the same intensive chemotherapeutic regimens like other types of ALL (T- and B-cell types) [92]. In this rare type of T-cell ALL, the preferred therapy is usually induction chemotherapy to achieve CR followed by upfront allogeneic HSCT to eradicate the potential residual disease by the GVT effect of allogeneic HSCT [92].

2.8. HSCT in Ph+ ALL

Philadelphia chromosome positivity is the most common recurrent cytogenetic abnormality observed in adult patients with ALL [93–95]. Approximately 20–25% of adults and only about 2% of children with ALL harbor Philadelphia chromosome and express the bcr-abl transcript [93, 96, 97]. In recent years, the most significant therapeutic advancement has been the introduction of TKIs into the therapeutic regimens of Ph+ ALL [94]. In the era before TKIs, patients with Ph+ ALL were having poor prognosis after standard chemotherapy with DFS rates ranging between 0.0% and 20% [94, 96]. Historically, adult Ph+ ALL had been associated with high relapse rates and short DFS and OS [98].

Results of autologous HSCT in adults with Ph+ ALL are still disappointing [99]. Studies have shown that (1) Ph+ ALL is incurable without allogeneic HSCT and (2) in patients with Ph+ ALL, particularly adults, who are eligible for HSCT and who have achieved CR allogeneic HSCT, has remained the consolidation therapy of choice and the only proven curative therapeutic strategy [94, 96, 98–101]. However, patients with Ph+ ALL planned for allogeneic HSCT can be divided into three risk groups or categories: (1) HR; positive MRD or overt clinical disease, (2) intermediate risk; molecular disease but without morphologic disease, and (3) low risk; no evidence of MRD [96].

In children and adolescents with Ph+ ALL, allogeneic HSCT is a controversial issue and there is increasing reluctance to offer allogeneic HSCT to children in the era of TKIs [94, 97, 102]. The children's Oncology Group reported the following results on the use of TKIs in children and adolescents with Ph+ ALL: (1) excellent outcomes of the combination of TKI and chemotherapy with OS of 88% at 3 years and 70% at 5 years, (2) achievement of complete hematological remission in approximately 95% of cases and molecular remission in >50% of patients, and (3) no advantage of subjecting patients to allogeneic HSCT [103, 104]. On the contrary, in children and young adults with Ph+ ALL, studies have shown that (1) results

of allogeneic HSCT are superior to chemotherapy alone, (2) MSD and MUD allografts have yielded equivalent outcomes, (3) in patients subjected to allogeneic HSCT, age, WBC count at presentation, and early response to treatment have been shown to be independent prognostic indicators, (4) early allogeneic HSCT is recommended once morphologic remission is achieved as this form of treatment has shown to produce durable remissions in patients with CR1, and (5) in children with Ph+ ALL, two studies showed advantage of allogeneic HSCT in protection against late disease relapses and in achieving 5-year OS and DFS of 80.2 and 72.9%, respectively [105–108].

In patients with Ph+ ALL, achievement of complete molecular remission (CMR) prior to allogeneic HSCT reduces the risk of leukemia relapse post-allogeneic HSCT even though TKIs may still rescue some patients who relapse after transplantation [109, 110]. Without TKIs, 30–50% of patients with Ph+ ALL relapse after allogeneic HSCT [96]. Even in the era of TKIs, relapse is still the main cause of allogeneic HSCT failure in HR patients Ph+ ALL [111]. Ph+ ALL patients, subjected to allogeneic HSCT, should ideally be (1) in CR and without MRD prior to transplantation and (2) below 60 years of age. However, only carefully selected patients ≥60 years old are candidates for allogeneic HSCT [100, 112]. In patients with Ph+ ALL, RIC allogeneic HSCT can be offered to older patients and those with comorbidities, that is, patients who are ineligible for MAC therapy [100, 112].

Additional cytogenetic abnormalities, such as monosomy 7, and abnormalities of chromosomes 8 and 9 are common in patients with Ph+ ALL as they are encountered in two thirds of cases [113]. Pre-existing mutations in the ABL kinase domain are frequently associated with resistance to TKIs and are a common cause of post-HSCT relapse in patients with Ph+ ALL [101]. Pre-transplantation risk factors for relapse in patients with Ph+ ALL include: (1) expression of P190 transcript, (2) evidence of morphologic disease at the time of transplantation, and (3) type of donor, with recipients of autologous HSCT or MSD having the highest risk of relapse [96]. Post-transplantation risk factors for relapse in patients with Ph+ ALL include: (1) expression of P190 transcript which carries the highest risk of relapse, (2) detection of MRD by reverse transcription PCR for bcr-abl transcript, and (3) lack of chronic extensive GVHD [96]. These risk factors can be utilized to improve risk stratification for patients with Ph+ ALL undergoing HSCT in order to develop specific strategies or therapeutic plans [96]. In patients with Ph+ ALL subjected to allogeneic HSCT, TKI therapy tailored according to pre-transplantation TKI response, anticipated toxicities, and Abl-1 domain mutations is feasible and may reduce relapse rate and improve the outcome of patients [111].

2.9. Relapse of ALL before and after HSCT

Approximately 20–25% of ALL patients experience relapses of their disease at 5 years from diagnosis and initial therapy despite receiving the standard chemotherapeutic regimens [114, 115]. The prognosis of children and young adults with relapsed ALL is poor [114, 115]. Only a minority of adults with ALL who relapse after first line therapy can be rescued [116]. Salvage chemotherapy in patients with relapsed ALL includes: (1) mitoxantrone, etoposide, and cytarabine or (2) fludarabine, cytarabine, pegylated-asparaginase, and granulocyte colony stimulating factor [116]. Salvage chemotherapy alone is not curative in relapsed ALL [115].

Allogeneic HSCT offers the best and may be the only chance for cure in relapsed ALL, particularly in adult patients [114–116]. Allogeneic HSCT can be performed using either a sibling donor or an unrelated donor [114, 115].

In patients with ALL, relapse after HSCT remains the main cause of treatment failure due to the limited therapeutic options and the associated poor outcome [117–119]. Factors that affect the occurrence as well as the outcome of ALL relapse after HSCT include: GVHD, MRD, intrinsic factors of the disease, and transplantation characteristics [120]. The prognosis of patients with ALL who relapse after HSCT is extremely poor with long-term survival <10%, and there is no difference in short-term survival between patients with isolated EMR and systemic relapse, suggesting that all disease relapses should be treated systemically [118]. EMR after allogeneic HSCT poses significant challenge for transplantation physicians as it carries poor outcome and has limited therapeutic options [119]. The risk factors for EMR, particularly CNS relapse, after allogeneic HSCT include: HR cytogenetics, advanced disease status, male gender, history of EMD before HSCT, hyperleukocytosis at diagnosis, and receiving peripheral blood stem cells [119]. However, prophylactic IT chemotherapy and maintenance treatment after HSCT may reduce the rate of CNS relapse post-HSCT [119].

Treatment of ALL relapse post-HSCT includes: (1) continuation of low-dose immunosuppressive therapy may be the optimal approach as abrupt discontinuation of immune suppression does not lead to any clinical benefit and may result in aggravation of GVHD, (2) re-induction or salvage therapy followed by second allogeneic HSCT in highly selected patients may offer the best chance of prolonged survival, and (3) other interventions, such as frequent MRD monitoring, pre-emptive immunotherapy in the form of DLI, post-transplantation maintenance therapy, use of novel and targeted therapies in post-HSCT, and enrollment in clinical trials [117, 118, 120].

Prerequisites for successful management of ALL post-HSCT relapse by either second allogeneic HSCT or experimental non-transplant approaches include: good clinical condition of the patient and the capacity to achieve hematological remission by the salvage therapy [117]. For patients with acute leukemia who relapse after the first allogeneic HSCT, only a second allograft can provide a realistic chance of long-term disease remission [121]. The second allogeneic HSCT used in the treatment of relapse after the first allograft can be obtained from HLA-MSD, MUD, or HLA-mismatched alternative donor [122]. Based on the rapid donor availability as compared to MUD, a haploidentical second allogeneic HSCT might be considered as an alternative therapeutic option for relapse after the first allograft [122, 123].

3. Novel and targeted therapies in ALL

3.1. Tyrosine kinase inhibitors

The introduction of TKIs has revolutionized the therapy of patients with Ph+ ALL [94, 107]. Over the past 12 years, administration of TKIs before allogeneic HSCT has significantly improved the long-term outcome of allogeneic HSCT in adults with Ph+ ALL [93, 107]. In

patients with Ph+ ALL, TKIs have been incorporated into: (1) the induction phase in conjunction with cytotoxic chemotherapy, (2) in the consolidation and maintenance phases in conjunction with cytotoxic chemotherapy in patients who are not eligible for allogeneic HSCT, and (3) in the post-transplantation maintenance therapy in recipients of allogeneic HSCT and their incorporation into the treatment regimens at various stages of the disease has significantly improved the outcomes of patients [10–12, 93, 124].

3.1.1. Imatinib

Studies in children with Ph+ ALL have shown that (1) the incorporation of imatinib into the chemotherapeutic regimens has improved the prognosis and (2) TKIs administered in the early phases of therapy can dramatically reduce MRD and improve the outcome of patients [12, 94, 124, 125]. Also, several groups have reported that the combination of imatinib and high dose chemotherapy has significantly improved the outcome of children and adults with Ph+ ALL with CR rates reaching 86–95% and 1-year OS reaching 75% [94, 124]. The 4-year OS in Ph+ ALL subjected to allogeneic HSCT in the era of imatinib has increased significantly to reach 52% [94]. Imatinib maintenance following allogeneic HSCT may further improve the outcome of patients with Ph+ ALL [125].

3.1.2. Dasatinib

Dasatinib is a second-generation TKI with dual Src and Abl kinase inhibition activity [126, 127]. It is active against all imatinib-resistant kinase domain mutations apart from T315I mutation, thus having superior potency for inhibiting bcr-abl fusion protein compared to imatinib [126, 127]. The use of dasatinib is associated with the following adverse effects: bone marrow suppression, fluid retention, pleural effusion, skin eruptions, cardiac conduction disturbances, and colitis that may be hemorrhagic, immune-mediated, or cytomegalovirus-induced [127].

Dasatinib is usually used in combination with cytotoxic chemotherapy such as hyper-CVAD regimen to control Ph+ ALL prior to allogeneic HSCT [126]. It can also be used in the setting of post-HSCT as maintenance therapy to prevent disease relapse or to eliminate MRD [127]. In older adults who are not candidates for allogeneic HSCT or younger patients who are unable to tolerate intensive chemotherapy, an induction regimen composed of targeted therapies such as dasatinib and corticosteroids may offer the potential of greater and longer responses, thus avoiding the morbidity associated with the use of cytotoxic chemotherapy [128, 129]. In this combination, dasatinib inhibits Scr-Abl kinase while corticosteroids modulate Bcl2 family of proteins leading to apoptosis [128]. The combination of dasatinib and corticosteroids is associated with relapses mainly due to T315I mutations that can be further treated with ponatinib [129].

3.1.3. Nilotinib

The use of TKIs, including the second-generation drugs such as nilotinib, in the post-HSCT setting in patients with Ph+ ALL could potentially reduce relapse rates [130, 131]. Studies

on the use of nilotinib have shown the following results: (1) safety and efficacy of nilotinib prophylaxis to prevent relapse of Ph+ ALL after allogeneic HSCT, (2) effectiveness of nilotinib to control MRD and to convert patients to CMR, and (3) achievement of prolongation of survival without jeopardizing immune function or reconstitution following HSCT [130–133]. However, side effects such as prolongation of QT interval on electrocardiogram and early disease relapse post-HSCT may limit its use [132, 134].

3.1.4. Ponatinib

Ponatinib is a pan-bcr/abl TKI which is capable of inhibiting T315I kinase domain mutation that confers resistance to other TKIs and dismal prognosis [95, 129]. Ponatinib can be given prior to HSCT as bridging therapy to control disease and to prevent disease relapse following HSCT [95, 135]. Rapid hematological responses can be obtained in almost all patients but morphologic and molecular responses can unfortunately be short-lived [95]. One study showed that patients with Ph+ ALL who underwent allogeneic HSCT had better survival than those who received ponatinib alone [136].

3.2. Nelarabine

Nelarabine is a purine nucleoside analogue and a soluble prodrug of Ara-G with specific cytotoxicity against T-lymphocytes [136–145]. It has significant activity in patients with T-cell ALL and T-cell lymphoblastic lymphoma (LBL) [137, 140, 142, 143, 146]. In October 2005, nelarabine gained an accelerated approval by the food and drug authority (FDA) in the USA for the treatment of children and adults with T-cell ALL and T-cell LBL who are in relapse or refractory to at least two chemotherapeutic regimens [137–145]. It has been used as a single agent or in combination with intensive chemotherapy or P13K inhibitors [138, 142, 143, 146–148]. The use of nelarabine in the treatment of patients having T-cell ALL relapsing after allogeneic HSCT has been associated with 90% OS at 1 year [144]. The adverse effects of nelarabine include: (1) myelosuppression with neutropenia and thrombocytopenia and (2) neurotoxicity, which is the commonest side effect, may be transient and reversible and can manifest as depression in the level of consciousness, sensory and motor neuropathies, and Guillain-Barre syndrome [139–145]. The mechanisms of resistance to nelarabine include: (1) reduced drug incorporation into DNA and (2) anti-apoptosis [149]. Other novel purine analogues, such as clofarabine and forodesine, have demonstrated significant anti-tumor activity in relapsed/refractory T-cell ALL and T-cell LBL [138].

3.3. Blinatumomab

Blinatumomab is a bispecific T-cell engager monoclonal antibody construct that is designed to direct cytotoxic T-cells to CD19-expressing B-cells [150, 151]. It is indicated (1) in the treatment of Ph− relapsed/refractory pre-B ALL, (2) to induce GVL reaction in patients with pre-B ALL relapsing post-allogeneic HSCT, and (3) in the treatment of HR patients with Ph+ ALL [150, 151]. In a phase II single arm multicenter study that included 45 patients with HR-Ph+ ALL patients who had relapsed or were refractory to TKIs, single agent blinatumomab showed remarkable anti-leukemic activity as 88% of patients achieved CR or CR with partial hematologic recovery and a median OS of 7.1 months [151]. The adverse effects

of blinatumomab include: fever, febrile neutropenia, headache, neurotoxicity, such as aphasia, and cytokine release syndrome (CRS) [140].

3.4. CAR T-cells

Chimeric antigen receptors (CAR) consist of an extracellular antigen recognition domain linked to an intracellular signaling domain [152, 153]. An important part of CAR design in choosing an optimal tumor-associated antigen to target [152]. A patient's own T-cells may be genetically modified to express an artificial T-cell receptor termed CAR designed to be specific to a tumor-associated antigen [154]. CARs are artificial receptors that redirect antigen specificity, activate T-cells, and further enhance T-cell function through their costimulatory component [155]. Ideally, the target antigen should only be expressed on tumor cells and not on normal cells in order to ensure that there is no (on-target-off tumor) activity that could result in toxicity [152]. The most extensively investigated CAR in clinical setting targets CD19, which is expressed not only in most B-cell malignancies but also in normal B-cells. Thus, CAR-mediated tumor destruction is accompanied by CAR-mediated destruction of normal B-cells resulting in B-cell aplasia [152].

Center	Patients (number and diagnosis)	Viral transduction	Conditioning therapy	Cytokine release syndrome (%)	Outcome
Memorial Sloan Kettering Cancer Center	**27** Relapsed B-ALL	Retroviral transduction	Cyclophosphamide	**18**	• 88% CR • Molecular CR: 70%
University of Pennsylvania and Children Hospital of Philadelphia	**25** Pediatric R/R B-ALL	Lentiviral transduction	No conditioning therapy	**27**	• 90% morphologic remission • 73% MRD by flow cytometry • 6 patients relapsed
Fred Hutchinson Cancer Research Center	**9** Phase I	Lentiviral transduction	Cyclophosphamide	**33.3**	• 7 patients had CR with 5 having MRD
MD Anderson Cancer Center	**10** CAR T-cells infused following allogeneic HSCT	Sleeping beauty transposon electroporation	No conditioning therapy	**30**	• 10 patients achieved CR at 5 months

ALL, acute lymphoblastic leukemia; R/R, relapsed/refractory; CR, complete remission; HSCT, hematopoietic stem cell transplantation; CAR, chimeric receptor antigen; and MRD, minimal residual disease.

Table 4. Clinical trials on the use of CAR-T cells in ALL patients.

1. Monoclonal antibodies, immunotoxins, and immunoconjugate antibodies:

- CD20:
  - Rituximab
  - Obinutuzumab
  - Ofatumumab
  - REGN 1979
- CD 22:
  - Epratuzumab
  - Inotuzumab ozogamicin (IO)
  - Moxetumomab pasudotox, reformulation of BL22
- CD 19:

a. Single CD 19 monoclonal antibodies:

- Coltuximab ravtansine [SAR3419]
- Denintuzumab mafodotin [SGN-CD19A]
- ADC-402, newest CD19 monoclonal antibody

b. Dual monoclonal antibodies including anti-CD19 activity:

- Combotox: Anti-CD19 and anti- CD22
- Bispecific T cell engager (BITE) construct: Blinatumomab [anti-CD3; CD 19 construct]

2. Proteasome inhibitors: Bortezomib

3. JAK inhibitors: Ruxolitinib

4. Hypomethylating agents: Decitabine

5. P13K- mTOR inhibitors:

- NVP-BEZ 235
- NCT01756118
- NCT 02484430
- NCT 01523977
- NCT 01403415
- NCT 01614197
- NCT 01184885

6. Chimeric antigen receptor T-cells (CAR T-cells)

Table 5. Novel and targeted therapies in acute lymphoblastic leukemia.

CAR T-cell therapy involves several laboratory, technical, and clinical procedures that include: (1) obtaining peripheral blood mononuclear cells by leukapheresis, (2) CD3 (T-cell) separation, (3) engineering of T-cells to express CAR by gene transfer technology, viral transduction, or

genetic modification with CD19-specific CAR to target tumor in addition to cell expansion, (4) lymphodepletion by administration of pre-infusion conditioning therapy in the form cyclophosphamide or cyclophosphamide and fludarabine, (5) CAR T-cell infusion to target CD 19+ B-cells, and (6) cell death or apoptosis of CD19+ lymphoblasts [152–154, 156]. Indication for CAR T-cell therapy include: (1) relapsed and refractory B-cell ALL, (2) chronic lymphocytic leukemia, (3) acute myeloid leukemia, (4) follicular lymphoma, (5) diffuse large B-cell lymphoma, (6) multiple myeloma, (7) Waldenstrom's macroglobulinemia, and (8) treatment of relapse post-allogeneic HSCT for B-cell malignancies [152, 157, 158]. Studies have shown that treatment options in relapse after allogeneic HSCT for lymphoid malignancies including ALL include: (1) DLI, (2) salvage chemotherapy followed by a second allogeneic HSCT, and (3) CAR T-cell infusions, a cell-based immunotherapy that can effectively enhance and maintain antitumor GVL response after transfusion into patients [152, 157, 158].

Adverse effects or complications of CAR T-cell therapy in relapsed and refractory ALL include: (1) CRS, which can be severe and life-threatening, may manifest with: hyperpyrexia, hypotension, capillary leak syndrome, neurological manifestations, myalgia, and respiratory as well as renal insufficiency, (2) neurotoxicity in the form of delirium and seizures, (3) macrophage activation syndrome, (4) aplasia of normal B-lymphocytes, and (5) death that may occasionally be encountered [155, 159–165]. Serum biochemical markers of CRS following CAR T-cell therapy in relapsed and refractory ALL include C-reactive protein and ferritin [155, 159, 165]. Management of CRS includes: supportive care, corticosteroids, vasopressors, ventilatory support, and anti-interleukin-6 receptor antibody (tocilizumab) therapy [165]. The main clinical trials on the use of CAR T-cells are shown in **Table 4** [152, 156]. Novel and targeted therapies that can be used in the treatment of patients with ALL are shown in **Table 5** [1–3, 56, 160, 161].

4. Future directions

Recently, management of patients with ALL has improved dramatically due to several reasons such as improvements in our understanding of the disease biology; improvements in the diagnostic techniques, including molecular genetics, that help in disease stratification; improvements in supportive care; adoption of dose-intense pediatric-inspired chemotherapeutic regimens in AYAs; progress in HSCT including donor selection, conditioning therapies, and prevention as well as treatment of GVHD; monitoring of MRD; and the availability of several novel agents and targeted therapies in addition to cellular and immunotherapies. The availability of the modern therapeutic interventions has translated into improved response rates and outcomes including OS. The integration of various novel and targeted therapies before and after transplantation has further improved the outcomes of patients with ALL.

Different therapeutic interventions available for treating children and adults with ALL should be considered complementary to each other. Future studies should focus on the optimal integration of these novel therapies into the treatment paradigm of this malignancy in order to achieve higher rates of response, disease control as well as long-term survival. Risk stratification of ALL will help in tailoring the management of patients according to their risk category taking into consideration their clinical manifestations, laboratory findings, including cytogenetic and molecular profiles, as well as responses to therapeutic interventions.

In children with ALL, the role of HSCT has decreased due to the use of intensified chemotherapeutic regimens and the incorporation of novel and targeted therapies into the upfront treatment. However, patients with HR features, those with MRD, and patients with relapsed or refractory disease should be considered for HSCT and novel therapies should be administered whenever indicated.

5. Conclusion

Intensified pediatric chemotherapeutic regimens show poor outcome of ALL in adults compared to children particularly in patients with HR features or disease relapse. Thus, allogeneic HSCT has more indications in adults than in children. However, the integration of other therapeutic interventions into the management of ALL, before and after transplantation, is likely to improve the outcome of patients further.

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References

[1] Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: A comprehensive review and 2017 update. Blood Cancer Journal. 2017;**7**(6):e577. DOI: 10.1038/bcj.2017.53

[2] Faderl S, O'Brien S, Pui CH, Stock W, Wetzler M, Hoelzer D, et al. Adult acute lymphoblastic leukemia: Concepts and strategies. Cancer. 2010;**116**(5):1165-1176. DOI: 10.1002/cncr.24862

[3] Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet. 2013;**381**(9881): 1943-1955. DOI: 10.1016/ S0140-6736(12)62187-4 (Epub Mar 22, 2013)

[4] Ribera JM, Ribera J, Genescà E. Treatment of adolescent and young adults with acute lymphoblastic leukemia. Mediterranean Journal of Hematology and Infectious Diseases 2014;**6**(1):e2014052. DOI: 10.4084/ MJHID.2014.052. eCollection 2014

[5] DeAngelo DJ. The treatment of adolescents and young adults with acute lymphoblastic leukemia. Hematology. American Society of Hematology. Education Program. 2005; **2005**(1):123-130. DOI: 10.1182/ asheducation-2005.1.123

[6] Larson RA. Induction therapy for Philadelphia chromosome negative acute lymphoblastic leukemia in adults. Edited by: Loweberg B, Rosmarin AG. Up To Date 2017

[7] Spiekerman K. Therapeutic management of acute lymphoblastic leukemia. In: Hiddemann W, editor. Handbook of Acute Leukemia. Switzerland: Springer International Publishing; 2016. DOI: 10.1007/798-3-319-26772-2_7

[8] Rowe JM, Buck G, Burnett AK, Chopra R, Wiernik PH, Richards SM, et al., ECOG; MRC/NCRI Adult Leukemia Working Party. Induction therapy for adults with acute lymphoblastic leukemia: Results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. Blood. 2005;**106**(12):3760-3767. DOI: 10.1182/blood-2005-04-1623 (Epub Aug 16, 2005)

[9] Nachman JB, La MK, Hunger SP, Heerema NA, Gaynon PS, Hastings C, et al. Young adults with acute lymphoblastic leukemia have an excellent outcome with chemotherapy alone and benefit from intensive postinduction treatment: A report from the Children's oncology group. Journal of Clinical Oncology. 2009;**27**(31):5189-5194. DOI: 10.1200/JCO.2008.20.8959 (Epub Oct 5, 2009)

[10] Mathisen MS, O'Brien S, Thomas D, Cortes J, Kantarjian H, Ravandi F. Role of tyrosine kinase inhibitors in the management of Philadelphia chromosome-positive acute lymphoblastic leukemia. Current Hematologic Malignancy Reports. 2011;**6**(3):187-194. DOI: 10.1007/s11899-011-0093-y

[11] Larson RA. Post-remission therapy for Philadelphia chromosome positive acute lymphoblastic leukemia.in adults. Edited by: Lowenberg B, Rosmarin AG. Up To Date 2016

[12] Jeha S, Coustan-Smith E, Pei D, Sandlund JT, Rubnitz JE, Howard SC, et al. Impact of tyrosine kinase inhibitors on minimal residual disease and outcome in childhood Philadelphia chromosome-positive acute lymphoblastic leukemia. Cancer 2014;**120**(10):1514-1519. DOI: 10.1002/cncr.28598 (Epub Feb 5, 2014)

[13] Lee S, Cho BS, Kim SY, Choi SM, Lee DG, Eom KS, et al. Allogeneic stem cell transplantation in first complete remission enhances graft-versus-leukemia effect in adults with acute lymphoblastic leukemia: Antileukemic activity of chronic graft-versus-host disease. Biology of Blood and Marrow Transplantation 2007;**13**(9):1083-1094. DOI: 10.1016/j.bbmt.2007.06.001 (Epub Jul 20, 2007)

[14] Nordlander A, Mattsson J, Ringdén O, Leblanc K, Gustafsson B, Ljungman P, et al. Graft-versus-host disease is associated with a lower relapse incidence after hematopoietic stem cell transplantation in patients with acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2004;**10**(3):195-203. DOI: 10.1016/j.bbmt.2003.11.002

[15] Bradfield SM, Radich JP, Loken MR. Graft-versus-leukemia effect in acute lymphoblastic leukemia: The importance of tumor burden and early detection. Leukemia 2004;**18**:1156-1158. DOI: 10.1038/sj.leu.2403352. Published online 25 March 2004

[16] Uzunel M, Mattsson J, Jaksch M, Remberger M, Ringdén O. The significance of graft versus host disease and pretransplantation minimal residual disease status to outcome after allogeneic stem cell transplantation in patients with acute lymphoblastic leukemia. Blood. 2001;**98**(6):1982-1984. DOI: org/10.1182/blood.V98.6.1982

[17] Gassas A, Sung L, Saunders EF, Doyle J. Graft-versus-leukemia effect in hematopoietic stem cell transplantation for pediatric acute lymphoblastic leukemia: Significantly lower relapse rate in unrelated transplantations. Bone Marrow Transplantation. 2007;**40**(10): 951-955. DOI: 10. 1038/sj. bmt. 1705853 (Epub Sep 17, 2007)

[18] Rezvani K, Yong AS, Savani BN, Mielke S, Keyvanfar K, Gostick E, et al. Graft-versus-leukemia effects associated with detectable Wilms tumor-1 specific T lymphocytes after allogeneic stem-cell transplantation for acute lymphoblastic leukemia. Blood. 2007; **110**(6):1924-1932. DOI: 10.1182/blood-2007-03-076844 (Epub May 15, 2007)

[19] Sánchez-García J, Serrano J, Gómez P, Martínez F, Martín C, Román-Gómez J, et al. The impact of acute and chronic graft-versus-host disease on normal and malignant B-lymphoid precursors after allogeneic stem cell transplantation for B-lineage acute lymphoblastic leukemia. Haematologica 2006;**91**(3):340-347

[20] Cornelissen JJ, Carston M, Kollman C, King R, Dekker AW, Löwenberg B, et al. Unrelated marrow transplantation for adult patients with poor-risk acute lymphoblastic leukemia: strong graft-versus-leukemia effect and risk factors determining outcome. Blood. 2001;**97**(6):1572-1577. DOI: org/10.1182/blood.V97.6.1572

[21] van der Velden VH, Panzer-Grümayer ER, Cazzaniga G, Flohr T, Sutton R, Schrauder A, et al. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia 2007;**21**(4):706-713. DOI: 10.1038/sj.leu.2404535 (Epub Feb 8, 2007)

[22] Brüggemann M, Schrauder A, Raff T, Pfeifer H, Dworzak M, Ottmann OG, et al. European Working Group for Adult Acute Lymphoblastic Leukemia (EWALL); International Berlin-Frankfurt-Münster Study Group (I-BFM-SG). Standardized MRD Quantification in European ALL Trials: Proceedings of the Second International Symposium on MRD Assessment in Kiel, Germany, September 18-20, 2008. Leukemia. 2010;**24**(3):521-535. DOI: 10.1038/leu.2009.268 (Epub Dec 24, 2009)

[23] Thakar MS, Talano J-AM, Tower RL, Kelly ME, Burke MJ. Indications for transplantation in childhood acute leukemia and the impact of minimal residual disease on relapse: A review. Clinical Practice. 2014;**11**(1):79-90

[24] Kotrova M, van der Velden VHJ, van Dongen JJM, Formankova R, Sedlacek P, Brüggemann M, et al. Next-generation sequencing indicates false-positive MRD results and better predicts prognosis after SCT in patients with childhood ALL. Bone Marrow Transplantation. 2017;**52**(7):962-968. DOI: 10.1038/bmt.2017.16 (Epub Feb 27, 2017)

[25] Giebel S, Stella-Holowiecka B, Krawczyk-Kulis M, Gökbuget N, Hoelzer D, Doubek M, et al. Study Group for Adult ALL of the European Leukemia Net. Status of minimal residual disease determines outcome of autologous hematopoietic SCT in adult ALL. Bone Marrow Transplantation 2010;**45**(6):1095-1101. DOI: 10.1038/bmt.2009.308 (Epub Oct 26, 2009)

[26] Ding Z, Han MZ, Chen SL, Ma QL, Wei JL, Pang AM, et al. Outcomes of adults with acute lymphoblastic leukemia after autologous hematopoietic stem cell transplantation and the significance of pretransplantation minimal residual disease: Analysis from a

single center of China. Chinese Medical Journal. 2015;**128**(15):2065-2071. DOI: 10.4103/0366-6999.161365

[27] Mannis GN, Martin III TG, Damon LE, Andreadis C, Olin RL, Kong KA, et al. Quantification of acute lymphoblastic leukemia clonotypes in leukapheresed peripheral blood progenitor cells predicts relapse risk after autologous hematopoietic stem cell transplantation. Biology of Blood and Marrow Transplantation. 2016;**22**(6):1030-1036. DOI: 10.1016/j.bbmt. 2016. 02.004 (Epub Feb 16, 2016)

[28] Dhédin N, Dombret H, Thomas X, Lhéritier V, Boiron JM, Rigal-Huguet F, et al. Autologous stem cell transplantation in adults with acute lymphoblastic leukemia in first complete remission: Analysis of the LALA-85, -87 and -94 trials. Leukemia. 2006;**20**(2):336-344. DOI: 10.1038/sj.leu. 2404065

[29] Mato AR, Luger SM. Autologous stem cell transplant in ALL: Who should we be transplanting in first remission? Bone Marrow Transplantation. 2006;**37**(11):989-995. DOI: 10.1038/sj.bmt.1705370

[30] Sirohi B, Powles R, Treleaven J, Kulkarni S, Saso R, Potter M, et al. The role of maintenance chemotherapy after autotransplantation for acute lymphoblastic leukemia in first remission: Single-center experience of 100 patients. Bone Marrow Transplantation 2008;**42**(2):105-112. DOI: 10.1038/bmt.2008.95 (Epub Apr 14, 2008)

[31] Martin TG, Linker CA. Autologous stem cell transplantation for acute lymphocytic leukemia in adults. Hematology/Oncology Clinics of North America. 2001;**15**(1):121-143

[32] Zou D, Han M, Feng S, Li C, Qiu L, Jiang R, et al. Treatment of acute lymphoblastic leukemia by autologous stem cell transplantation: An analysis of 30 cases. Zhonghua Xue Ye Xue Za Zhi. 2000;**21**(2):74-76

[33] Marks DI, Forman SJ, Blume KG, Pérez WS, Weisdorf DJ, Keating A, et al. A comparison of cyclophosphamide and total body irradiation with etoposide and total body irradiation as conditioning regimens for patients undergoing sibling allografting for acute lymphoblastic leukemia in first or second complete remission. Biology of Blood and Marrow Transplantation. 2006;**12**(4):438-453. DOI: 10.1016/j.bbmt. 2005.12.029

[34] Doney K, Gooley TA, Deeg HJ, Flowers ME, Storb R, Appelbaum FR. Allogeneic hematopoietic cell transplantation with full-intensity conditioning for adult acute lymphoblastic leukemia: Results from a single center, 1998-2006. Biology of Blood and Marrow Transplantation. 2011;**17**(8):1187-1195. DOI: 10.1016/j.bbmt.2010.12.699 (Epub Dec 21, 2010)

[35] Gassas A, Sung L, Saunders EF, Doyle JJ. Comparative outcome of hematopoietic stem cell transplantation for pediatric acute lymphoblastic leukemia following cyclophosphamide and total body irradiation or VP16 and total body irradiation conditioning regimens. Bone Marrow Transplantation. 2006;**38**(11):739-743. DOI: 10.1038/sj.bmt.1705515 (Epub Oct 2, 2006)

[36] Tracey J, Zhang M-J, Thiel E, Sobocinski KA, Eapen M. Transplantation conditioning regimens and outcomes after allogeneic hematopoietic cell transplantation in children and adolescents with acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2013;**19**(2):255-259. DOI: 10.1016/j.bbmt. 2012.09.019 (Epub Oct 3, 2012)

[37] Zohren F, Czibere A, Bruns I, Fenk R, Schroeder T, Gräf T, et al. Fludarabine, amsacrine, high-dose cytarabine and 12 Gy total body irradiation followed by allogeneic hematopoietic stem cell transplantation is effective in patients with relapsed or high-risk acute lymphoblastic leukemia. Bone Marrow Transplantation 2009;**44**(12):785-792. DOI: 10.1038/bmt. 2009.83 (Epub May 11, 2009)

[38] Daly A, Savoie ML, Geddes M, Chaudhry A, Stewart D, Duggan P, et al. Fludarabine, busulfan, antithymocyte globulin, and total body irradiation for pretransplantation conditioning in acute lymphoblastic leukemia: Excellent outcomes in all but older patients with comorbidities. Biology of Blood and Marrow Transplantation. 2012;**18**(12):1921-1926. DOI: 10.1016/j.bbmt.2012.07.017 (Epub Jul 27, 2012)

[39] Kebriaei P, Madden T, Wang X, Thall PF, Ledesma C, de Lima M, et al. Intravenous BU plus Mel: An effective, chemotherapy-only transplant conditioning regimen in patients with ALL. Bone Marrow Transplantation. 2013;**48**(1):26-31. DOI: 10.1038/bmt.2012.114 (Epub Jun 25, 2012)

[40] Kröger N, Bornhäuser M, Stelljes M, Pichlmeier U, Trenschel R, Schmid C, et al. Allogeneic stem cell transplantation after conditioning with treosulfan, etoposide and cyclophosphamide for patients with ALL: A phase II-study on behalf of the German Cooperative Transplant Study Group and ALL Study Group (GMALL). Bone Marrow Transplantation. 2015;**50**(12):1503-1507. DOI: 10.1038/bmt.2015.202 (Epub Sep 14, 2015)

[41] Santarone S, Pidala J, Di Nicola M, Field T, Alsina M, Ayala E, et al. Fludarabine and pharmacokinetic-targeted busulfan before allografting for adults with acute lymphoid leukemia. Biology of Blood and Marrow Transplantation. 2011;**17**(10):1505-1511. DOI: 10.1016/j.bbmt.2011.02.011 (Epub Mar 6, 2011)

[42] Kebriaei P, Bassett R, Lyons G, Valdez B, Ledesma C, Rondon G, et al. Clofarabine plus busulfan is an effective conditioning regimen for allogeneic hematopoietic stem cell transplantation in patients with acute lymphoblastic leukemia: Long-term study results. Biology of Blood and Marrow Transplantation. 2017;**23**(2):285-292. DOI: 10.1016/j.bbmt. 2016.v11.001 (Epub Nov 2, 2016)

[43] Kebriaei P, Basset R, Ledesma C, Ciurea S, Parmar S, Shpall EJ, et al. Clofarabine combined with busulfan provides excellent disease control in adult patients with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation. Biology of Blood and Marrow Transplantation. 2012;**18**(12):1819-1826. DOI: 10.1016/j. bbmt.2012.06.010 (Epub Jun 29, 2012)

[44] Imamura M, Shigematsu A. Allogeneic hematopoietic stem cell transplantation in adult acute lymphoblastic leukemia: Potential benefit of medium-dose etoposide conditioning. Experimental Hematology & Oncology. 2015;**4**:20. DOI: 10.1186/s40164-015-0015-0. eCollection 2015

[45] Shigematsu A, Kondo T, Yamamoto S, Sugita J, Onozawa M, Kahata K, et al. Excellent outcome of allogeneic hematopoietic stem cell transplantation using a conditioning regimen with medium-dose VP-16, cyclophosphamide and total-body irradiation for adult patients with acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2008; **14**(5):568-575. DOI: 10.1016/j.bbmt.2008.02.018

[46] Kennedy-Nasser AA, Bollard CM, Myers GD, Leung KS, Gottschalk S, Zhang Y, et al. Comparable outcome of alternative donor and matched sibling donor hematopoietic stem cell transplant for children with acute lymphoblastic leukemia in first or second remission using alemtuzumab in a myeloablative conditioning regimen. Biology of Blood and Marrow Transplantation. 2008;**14**(11):1245-1252. DOI: 10.1016/j.bbmt.2008.08.010

[47] Eder S, Canaani J, Beohou E, Labopin M, Sanz J, Arcese W, et al. Thiotepa-based conditioning versus total body irradiation as myeloablative conditioning prior to allogeneic stem cell transplantation for acute lymphoblastic leukemia: A matched-pair analysis from the acute Leukemia Working Party of the European Society for blood and marrow transplantation. American Journal of Hematology. 2017;**92**(1):18-22. DOI: 10.1002/ajh.24567

[48] Verneris MR, Eapen M, Duerst R, Carpenter PA, Burke MJ, Afanasyev BV, et al. Reduced-intensity conditioning regimens for allogeneic transplantation in children with acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation 2010;**16**(9):1237-1244. DOI: 10.1016/j.bbmt.2010.03.009 (Epub Mar 17, 2010)

[49] Tucunduva L, Ruggeri A, Sanz G, Furst S, Rio B, Socié G, et al. Impact of myeloablative and reduced intensity conditioning on outcomes after unrelated cord blood transplantation for adults with acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2013;**19**(2):S127. DOI: 10.1016/j.bbmt.2012.11.059

[50] Stein A, Forman SJ. Allogeneic transplantation for ALL in adults. Bone Marrow Transplantation. 2008;**41**(5):439-446. DOI: 10.1038/bmt.2008.1

[51] Marks DI, Aversa F, Lazarus HM. Alternative donor transplants for adult acute lymphoblastic leukaemia: A comparison of the three major options. Bone Marrow Transplantation. 2006;**38**(7):467-475. DOI: 10.1038/sj.bmt.1705464 (Epub Aug 7, 2006)

[52] Khaled SK, Thomas SH, Forman SJ. Allogeneic hematopoietic cell transplantation for acute lymphoblastic leukemia in adults. Current Opinion in Oncology. 2012;**24**(2):182-190. DOI: 10.1097/CCO.0b013e32834f5c41

[53] Lussana F, Rambaldi A. Role of allogeneic hematopoietic stem cell transplantation in adult patients with acute lymphoblastic leukemia. Mediterranean Journal of Hematology and Infectious Diseases 2014;**6**(1):e2014065. DOI: 10.4084/MJHID.2014.065. eCollection 2014

[54] Fagioli F, Quarello P, Zecca M, Lanino E, Rognoni C, Balduzzi A, et al. Hematopoietic stem cell transplantation for children with high-risk acute lymphoblastic leukemia in first complete remission: A report from the AIEOP registry. Haematologica. 2013;**98**(8):1273-1381. DOI: 10.3324/haematol.2012.079707 (Epub Feb 26, 2013)

[55] Russell LJ, Enshaei A, Jones L, Erhorn A, Masic D, Bentley H, et al. IGH@ translocations are prevalent in teenagers and young adults with acute lymphoblastic leukemia and are associated with a poor outcome. Journal of Clinical Oncology. 2014;**32**(14):1453-1462. DOI: 10.1200/JCO.2013.51.3242 (Epub Apr 7, 2014)

[56] Hoelzer D, Bassan R, Dombret H, Fielding A, Ribera JM, Buske C; ESMO Guidelines Committee. Acute lymphoblastic leukaemia in adult patients: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2016;**27**(Suppl 5): v69-v82 (Epub Apr 7, 2016). DOI: 10.1093/annonc/mdw025

[57] Larson RA. General principles of hematopoietic cell transplantation for acute lymphoblastic leukemia in adults. Edited by Negrin RS, Rosmarin AG. Up To Date 2017

[58] Ribera JM. Allogeneic stem cell transplantation for adult acute lymphoblastic leukemia: When and how. Haematologica. 2011;**96**(8):1083-1086. DOI: 10.3324/haematol.2011.048348

[59] Oyekunle A, Haferlach T, Kröger N, Klyuchnikov E, Zander AR, Schnittger S, et al. Molecular diagnostics, targeted therapy, and the indication for allogeneic stem cell transplantation in acute lymphoblastic leukemia. Advances in Hematology. 2011;**2011**: 154745. DOI: 10.1155/2011/154745

[60] Dhédin N, Huynh A, Maury S, Tabrizi R, Beldjord K, Asnafi V, et al.; GRAALL Group. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. Blood. 2015;**125**(16):2486-2496. DOI: 10.1182/blood-2014-09-599894 (Epub Jan 13, 2015)

[61] Lazarus HM, Luger S. Which patients with adult acute lymphoblastic leukemia should undergo a hematopoietic stem cell transplantation? Case-based discussion. Hematology. American Society of Hematology Education Program. 2007;**2007**(1):444-452. DOI: 10.1182/asheducation-2007.1.444

[62] Hochberg J, Khaled S, Forman SJ, Cairo MS. Criteria for and outcomes of allogeneic haematopoietic stem cell transplant in children, adolescents and young adults with acute lymphoblastic leukaemia in first complete remission. British Journal of Haematology. 2013;**161**(1):27-42. DOI: 10.1111/bjh.12239 (Epub Feb 6, 2013)

[63] Klingebiel T, Bader P. HSCT for acute lymphoblastic leukaemia in children. Chapter 36. European Group for Blood and Marrow Transplantation (EBMT). The EBMT Handbook. ALL in children. EBMT 2008_22_44: EBMT 2008 6-11-2008 9:33 Pagina 507

[64] Pulsipher MA, Peters C, Pui CH. High-risk pediatric acute lymphoblastic leukemia: To transplant or not to transplant? Biology of Blood and Marrow Transplantation. 2011;**17**(1 Suppl):S137-S348. DOI: 10.1016/j.bbmt.2010.10.005

[65] Li C-K. Current indications of bone marrow transplantation (BMT) in pediatric malignant conditions: A review Iranian Journal of Blood and Cancer. 2010;**2**(2):71-75

[66] Pidala J, Djulbegovic B, Anasetti C, Kharfan-Dabaja M, Kumar A. Allogeneic hematopoietic cell transplantation for adult acute lymphoblastic leukemia (ALL) in first complete remission. Cochrane Database of Systematic Reviews. Oct 5, 2011;**10**:CD008818. DOI: 10.1002/14651858.CD008818.pub2

[67] Gupta V, Richards S, Rowe J. On behalf of the acute Leukemia stem cell transplantation Trialists' collaborative group. Allogeneic, but not autologous, hematopoietic cell transplantation improves survival only among younger adults with acute lymphoblastic leukemia in first remission: An individual patient data meta-analysis. Blood. 2013;**121**:339-350. DOI: 10.1182/blood-201207-445098

[68] Srour SA, Milton DR, Bashey A, Karduss-Urueta A, Al Malki MM, Romee R, et al. Haploidentical transplantation with post-transplantation cyclophosphamide for high-risk acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2017; **23**(2):318-324. DOI: 10.1016/j.bbmt.2016.11.008 (Epub Nov 14, 2016)

[69] Messori A, Fadda V, Maratea D, Trippoli S. Acute lymphoblastic leukemia in first complete remission: Temporal trend of outcomes in studies comparing allogeneic transplant with autologous transplant or chemotherapy. Annals of Hematology. 2013;**92**(9):1221-1228. DOI: 10.1007/s00277-013-1766-5 (Epub May 16, 2013)

[70] Oriol A, Vives S, Hernández-Rivas JM, Tormo M, Heras I, Rivas C, et al.; Programa Español de Tratamiento en Hematologia Group. Outcome after relapse of acute lymphoblastic leukemia in adult patients included in four consecutive risk-adapted trials by the PETHEMA Study Group. Haematologica. 2010;**95**(4):589-596. DOI: 10.3324/haematol.2009.014274 (Epub Feb 9, 2010)

[71] Gökbuget N, Stanze D, Beck J, Diedrich H, Horst HA, Hüttmann A, et al.; German Multicenter study Group for Adult Acute Lymphoblastic Leukemia. Outcome of relapsed adult lymphoblastic leukemia depends on response to salvage chemotherapy, prognostic factors, and performance of stem cell transplantation. Blood. 2012;**120**(10):2032-2041 (Epub Apr 4, 2012). DOI: 10.1182/blood-2011-12-399287

[72] Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G, et al.; Medical Research Council of the United Kingdom Adult ALL Working Party; Eastern Cooperative Oncology Group. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL): An MRC UKALL12/ECOG 2993 study. Blood. 2007;**109**(3):944-950 (Epub Oct 10, 2006). DOI: 10.1182/blood-2006-05-018192

[73] Aversa F. Haploidentical haematopoietic stem cell transplantation for acute leukaemia in adults: Experience in Europe and the United States. Bone Marrow Transplantation 2008;**41**(5):473-481. DOI: 10.1038/sj.bmt.1705966 (Epub Jan 7, 2008)

[74] Santoro N, Ruggeri A, Labopin M, Bacigalupo A, Ciceri F, Gülbaş Z, et al. Unmanipulated haploidentical stem cell transplantation in adults with acute lymphoblastic leukemia: A study on behalf of the acute Leukemia working party of the EBMT. Journal of Hematology & Oncology. 2017;**10**(1):113. DOI: 10.1186/s13045-017-0480-5

[75] Liu D, Huang X, Liu K, Xu L, Chen H, Han W, et al. Haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for treatment of hematological malignancies in children. Biology of Blood and Marrow Transplantation. 2008;**14**(4):469-477. DOI: 10.1016/j.bbmt. 2008. 02.007

[76] Rowe JM, Lazarus HM. Genetically haploidentical stem cell transplantation for acute leukemia. Bone Marrow Transplantation. 2001;**27**(7):669-676. DOI: 10.1038/sj.bmt.1702856

[77] Diaz MA, Pérez-Martínez A, Herrero B, Deltoro N, Martinez I, Ramirez M, et al. Prognostic factors and outcomes for pediatric patients receiving an haploidentical relative allogeneic transplant using CD3/CD19-depleted grafts. Bone Marrow Transplantation 2016;**51**(9):1211-1216. DOI: 10.1038/bmt.2016.101 (Epub Apr 18, 2016)

[78] Ruggeri A, Labopin M, Sanz G, Piemontese S, Arcese W, Bacigalupo A, et al. Eurocord, Cord Blood Committee of Cellular Therapy and Immunobiology Working Party-EBMT; ALWP-EBMT study. Comparison of outcomes after unrelated cord blood and unmanipulated haploidentical stem cell transplantation in adults with acute leukemia. Leukemia. 2015;**29**(9):1891-1900. DOI: 10.1038/leu.2015.98 (Epub Apr 17, 2015)

[79] Mo XD, Xu LP, Zhang XH, Liu DH, Wang Y, Chen H, et al. Haploidentical hematopoietic stem cell transplantation in adults with Philadelphia-negative acute lymphoblastic leukemia: No difference in the high- and low-risk groups. International Journal of Cancer. 2015;**136**(7):1697-1707. DOI: 10.1002/ijc.29146 (Epub Sep 2, 2014)

[80] Liu Q, Han L, Fan Z, Huang F, Li X, Xu N, et al. Haploidentical hematopoietic stem cell transplant compared with matched sibling and unrelated transplants in adults with Philadelphia-negative acute lymphoblastic leukemia in first complete remission. Blood. 2016;**128**(22):2299

[81] Gao L, Zhang C, Gao L, Liu Y, Su Y, Wang S, et al. Favorable outcome of haploidentical hematopoietic stem cell transplantation in Philadelphia chromosome-positive acute lymphoblastic leukemia: A multicenter study in Southwest China. Journal of Hematology & Oncology. 2015;**8**:90. DOI: 10.1186/s13045-015-0186-5

[82] Chen H, Liu KY, Xu LP, Chen YH, Han W, Zhang XH, et al. Haploidentical hematopoietic stem cell transplantation without in vitro T cell depletion for the treatment of philadelphia chromosome-positive acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2015;**21**(6):1110-1116. DOI: 10.1016/j.bbmt.2015.02.009 (Epub Feb 16, 2015)

[83] Bonifacio M, Perbellini O, Pizzolo G. T-cell acute lymphoblastic lymphoma and T-cell lymphoblastic lymphoma: Therapy in adults. Hematol Meeting Report. 2009;**3**(1):115-122

[84] Burke MJ, Verneris MR, Le Rademacher J, He W, Abdel-Azim H, Abraham AA, et al. transplant outcomes for children with T cell acute lymphoblastic leukemia in second remission: A report from the Center for International Blood and Marrow Transplant Research. Biology of Blood and Marrow Transplantation. 2015;**21**(12):2154-2159. DOI: 10.1016/j.bbmt. 2015.08.023 (Epub Aug 29, 2015)

[85] Brammer JE, Saliba RM, Jorgensen JL, Ledesma C, Gaballa S, Poon M, et al. Multi-center analysis of the effect of T-cell acute lymphoblastic leukemia subtype and minimal residual disease on allogeneic stem cell transplantation outcomes. Bone Marrow Transplantation. 2017;**52**:20-27. DOI: 10.1038/bmt.2016.194. Published online Sep 12, 2016

[86] Hamilton BK, Rybicki L, Abounader D, Adekola K, Advani A, Aldoss I, et al. Allogeneic hematopoietic cell transplantation for adult T cell acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2017;**23**(7):1117-1121. Published online: Apr 7, 2017

[87] Brammer JE, Khouri IF, Ledesma C, Rondon G, Ciurea SO, Nieto Y, et al. T-cell acute lymphoblastic lymphoma (T-LBL) and stem cell transplantation (SCT): A comparison of outcomes with T-cell acute lymphoblastic leukemia (T-ALL). Biology of Blood and Marrow Transplantation. 2016;**22**(3):S217-S218. DOI: 10.1016/j.bbmt.2015.11.618

[88] Wei HP, Zhao XL, Huang WR, Bo J, Li HH, Zhao Y, et al. Therapeutic efficacy analysis of allogeneic peripheral blood hematopoietic stem cell transplantation for 14 adult patients with T lymphoblastic lymphoma. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2016;**24**(2):433-437. DOI: 10.7534/j.issn.1009-2137.2016.02.023

[89] Ikegame K, Tanji Y, Kitai N, Tamaki H, Kawakami M, Fujioka T, et al. Successful treatment of refractory T-cell acute lymphoblastic leukemia by unmanipulated stem cell transplantation from an HLA 3-loci mismatched (haploidentical) sibling. Bone Marrow Transplantation. 2003;**31**(6):507-510. DOI: 10.1038/sj.bmt.1703858

[90] Huo JS, Symons HJ, Robey N, Borowitz MJ, Schafer ES, Chen AR. Persistent multiyear control of relapsed T-cell acute lymphoblastic leukemia with successive donor lymphocyte infusions: A case report. Pediatric Blood & Cancer. 2016;**63**(7):1279-1282. DOI: 10.1002/pbc.25971 (Epub Mar 14, 2016)

[91] Forcade E, Leguay T, Vey N, Baruchel A, Delaunay J, Robin M, et al. Nelarabine for T cell acute lymphoblastic leukemia relapsing after allogeneic hematopoietic stem cell transplantation: An opportunity to improve survival. Biology of Blood and Marrow Transplantation. 2013;**19**(7):1124-1126. DOI: 10.1016/j.bbmt.2013.04.010 (Epub May 3, 2013)

[92] Donnellan W, Mineishi S, Wicker J, Paluri R. Gamma-delta T cell acute lymphoblastic leukemia: a single center experience. Global Journal of Cancer Therapy. 2016;**2**(1):026-029

[93] Brissot E, Labopin M, Beckers MM, Socié G, Rambaldi A, Volin L, Finke J, et al. Tyrosine kinase inhibitors improve long-term outcome of allogeneic hematopoietic stem cell transplantation for adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia. Haematologica. 2015;**100**(3):392-399. DOI: 10.3324/haematol.2014.116954 (Epub Dec 19, 2014)

[94] Tanguy-Schmidt A, Rousselot P, Chalandon Y, Cayuela JM, Hayette S, Vekemans MC, et al. Long-term follow-up of the imatinib GRAAPH-2003 study in newly diagnosed patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: A GRAALL Study. Biology of Blood and Marrow Transplantation. 2013;**19**(1):150-155. DOI: 10.1016/j.bbmt.2012.08.021 (Epub Sep 6, 2012)

[95] Sora F, Chiusolo P, Laurenti L, Soverini S, Sica S. Ponatinib before and after allogeneic stem cell transplantation for Ph+ acute lymphoblastic leukemia or lymphoid blast crisis of chronic myelogenous leukemia: A single center experience. Journal of Bone and Mineral Research. 2016;**4**:2.1000169

[96] Stirewalt DL, Guthrie KA, Beppu L, Bryant EM, Doney K, Gooley T, et al. Predictors of relapse and overall survival in Philadelphia chromosome-positive acute lymphoblastic leukemia after transplantation. Biology of Blood and Marrow Transplantation. 2003;**9**(3):206-212. DOI: 10.1053/bbmt.2003.50025

[97] Fielding AK. Philadelphia-positive acute lymphoblastic leukemia – Is bone marrow transplant still necessary? Biology of Blood and Marrow Transplantation. 2011;**17** (1 Suppl):S84-S88. DOI: 10.1016/j. bbmt.2010.11.023

[98] Couban S, Savoie L, Mourad YA, Leber B, Minden M, Turner R, et al. Evidence-based guidelines for the use of tyrosine kinase inhibitors in adults with Philadelphia chromosome-positive or BCR-ABL-positive acute lymphoblastic leukemia: A Canadian consensus. Current Oncology. 2014;**21**(2):e265-e309. DOI: 10.3747/co.21.1834

[99] Avivi I, Goldstone AH. Bone marrow transplant in Ph+ ALL patients. Bone Marrow Transplantation. 2003;**31**(8):623-632. DOI: 10.1038/sj.bmt.1703899

[100] Olsen J, Wu Q, Khera N, Adams R, Fauble V, Leis J, Noel P, Palmer J, Slack JL. Outcomes of allogeneic stem cell transplantation for Philadelphia chromosome positive acute lymphoblastic leukemia. Biol Blood Marraow Transplant. 2015;**21**(2):S12. DOI: http://dx.DOI.org/10.1016/j.bbmt.2014.11.497

[101] Egan DN, Beppu L, Radich JP. Patients with Philadelphia-positive leukemia with BCR-ABL kinase mutations before allogeneic transplantation predominantly relapse with the same mutation. Biology of Blood and Marrow Transplantation. 2015;**21**(1):184-189. DOI: 10.1016/j.bbmt.2014.09.012 (Epub Oct 6, 2014)

[102] Yanada M, Naoe T. Imatinib combined chemotherapy for Philadelphia chromosome-positive acute lymphoblastic leukemia: Major challenges in current practice. Leukemia & Lymphoma. 2006;**47**(9):1747-1753. DOI: 10.1080/10428190600634085

[103] Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, et al. improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: A children's oncology group study. Journal of Clinical Oncology 2009;**27**(31):5175-5181. DOI: 10.1200/JCO.2008.21.2514 (Epub Oct 5, 2009)

[104] Schultz KR, Carroll A, Heerema NA, Bowman WP, Aledo A, Slayton WB, et al.; Children's Oncology Group. Long-term follow-up of imatinib in pediatric Philadelphia chromosome-positive acute lymphoblastic leukemia: Children's oncology group study AALL0031. Leukemia. 2014;**28**(7):1467-1471. DOI: 10.1038/leu.2014.30 (Epub Jan 20, 2014)

[105] Aricò M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, et al. clinical outcome of children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. Journal of Clinical Oncology. 2010;**28**(31):4755-4761. DOI: 10.1200/JCO.2010.30.1325 (Epub Sep 27, 2010)

[106] Fagioli F, Zecca M, Rognoni C, Lanino E, Balduzzi A, Berger M, et al.; AIEOP-HSCT Group. Allogeneic hematopoietic stem cell transplantation for Philadelphia-positive acute lymphoblastic leukemia in children and adolescents: A retrospective multicenter study of the Italian Association of Pediatric Hematology and Oncology (AIEOP). Biology of Blood and Marrow Transplantation. 2012;**18**(6):852-860. DOI: 10.1016/j.bbmt.2011.10.015 (Epub Oct 20, 2011)

[107] Kebriaei P, Saliba R, Rondon G, Chiattone A, Luthra R, Anderlini P, et al. Long-term follow-up of allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Impact of tyrosine kinase inhibitors on treatment outcomes. Biology of Blood and Marrow Transplantation. 2012;**18**(4):584-592. DOI: 10.1016/j.bbmt.2011.08.011 (Epub Aug 23, 2011)

[108] Byun YJ, Suh JK, Lee SW, Lee D, Kim H, Choi ES, et al. Favorable outcome of allogeneic hematopoietic stem cell transplantation followed by post-transplant treatment with imatinib in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. Blood Research. 2015;**50**(3):147-153. DOI: 10.5045/br.2015.50.3.147 (Epub Sep 22, 2015)

[109] Lussana F, Intermesoli T, Gianni F, Boschini C, Masciulli A, Spinelli O, et al. Achieving molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Impact on relapse and long-term outcome. Biology of Blood and Marrow Transplantation. 2016;**22**(11):1983-1987. DOI: 10.1016/j.bbmt.2016.07.021 (Epub Aug 1, 2016)

[110] Leonard JT, Stock W. The persistence of minimal residual disease in Philadelphia chromosome-positive acute lymphoblastic leukemia: We know it's bad, now what? Biology of Blood and Marrow Transplantation. 2016;**22**(11):1913-1914. DOI: 10.1016/j.bbmt.2016.09.008 (Epub Sep 12, 2016)

[111] DeFilipp Z, Langston AA, Kota VK, Al-Kadhimi Z, Jillella AP, Waller EK, et al. Tailored post-transplant maintenance with tyrosine kinase inhibitors for high-risk Philadelphia chromosome-positive leukemia. Biology of Blood and Marrow Transplantation. 2016;**22**(3): S313. DOI: 10.1016/j.bbmt.2015.11.785

[112] Doki N, Igarashi A, Najima Y, Kobayashi T, Kakihana K, Sakamaki H, et al. The clinical outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for elderly Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) in the era of tyrosine kinase inhibitor (TKI): A single institution experience. Biol Blood Marraow Transplant. 2015;**21**(2):S279. DOI: http://dx.DOI.org/10.1016/j.bbmt. 2014.11.442

[113] Aldoss I, Stiller T, Cao TM, Palmer JM, Thomas SH, Forman SJ, et al. impact of additional cytogenetic abnormalities in adults with philadelphia chromosome-positive acute lymphoblastic leukemia undergoing allogeneic hematopoietic cell transplantation. Biology of Blood and Marrow Transplantation. 2015;**21**(7):1326-1329. DOI: 10.1016/j.bbmt.2015.03.021 (Epub Apr 1, 2015)

[114] Lee EJ, Han JY, Lee JW, Jang PS, Chung NG, Jeong DC, et al. Outcome of allogeneic hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia in second complete remission: A single institution study. Korean Journal of Pediatrics. 2012;**55**(3):100-106. DOI: 10.3345/kjp.2012.55.3.100 (Epub Mar 16, 2012)

[115] Smith AR, Baker KS, Defor TE, Verneris MR, Wagner JE, Macmillan ML. Hematopoietic cell transplantation for children with acute lymphoblastic leukemia in second complete remission: Similar outcomes in recipients of unrelated marrow and umbilical cord blood versus marrow from HLA matched sibling donors. Biology of Blood and Marrow Transplantation. 2009;**15**(9):1086-1093. DOI: 10.1016/j.bbmt.2009.05.005

[116] Kozlowski P, Åström M, Ahlberg L, Bernell P, Hulegårdh E, Hägglund H, et al. High curability via intensive reinduction chemotherapy and stem cell transplantation in young adults with relapsed acute lymphoblastic leukemia in Sweden 2003-2007. Haematologica. 2012;**97**(9):1414-1421. DOI: 10.3324/haematol.2011.057851 (Epub Apr 17, 2012)

[117] Willasch AM, Salzmann-Manrique E, Krenn T, Duerken M, Faber J, Opper J, et al. Treatment of relapse after allogeneic stem cell transplantation in children and adolescents with ALL: The Frankfurt experience. Bone Marrow Transplantation. 2017;**52**:201-208. DOI: 10.1038/bmt.2016.224. Published online Sep 19, 2016

[118] Poon LM, Hamdi A, Saliba R, Rondon G, Ledesma C, Kendrick M, et al. Outcomes of adults with acute lymphoblastic leukemia relapsing after allogeneic hematopoietic stem cell transplantation. Biology of Blood and Marrow Transplantation. 2013;**19**(7):1059-1064. DOI: 10.1016/j.bbmt.2013.04.014 (Epub Apr 30, 2013)

[119] Ge L, Ye F, Mao X, Chen J, Sun A, Zhu X, et al. Extramedullary relapse of acute leukemia after allogeneic hematopoietic stem cell transplantation: Different characteristics between acute myelogenous leukemia and acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2014;**20**(7):1040-1047. DOI: 10.1016/j.bbmt.2014.03.030 (Epub Apr 2, 2014)

[120] Chen R, Campbell JL, Chen B. Prophylaxis and treatment of acute lymphoblastic leukemia relapse after allogeneic hematopoietic stem cell transplantation. OncoTargets and Therapy. 2015;**8**:405-412. DOI: 10.2147/OTT.S78567. eCollection 2015

[121] Poon LM, Bassett R Jr, Rondon G, Hamdi A, Qazilbash M, Hosing C, et al. Outcomes of second allogeneic hematopoietic stem cell transplantation for patients with acute lymphoblastic leukemia. Bone Marrow Transplantation. 2013;**48**(5):666-670. DOI: 10.1038/bmt.2012.195 (Epub Oct 22, 2012)

[122] Yeh SP, Lin CC, Lin CH, Lo WC, Chen TT, Lo WJ, et al. Second haploidentical peripheral blood stem cell transplantation for treatment of acute leukemia with relapse after first allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplantation. 2015;**50**(7):1001-1003. DOI: 10.1038/bmt.2015.67 (Epub Apr 13, 2015)

[123] Tischer J, Engel N, Fritsch S, Prevalsek D, Hubmann M, Schulz C, et al. Second haematopoietic SCT using HLA-haploidentical donors in patients with relapse of acute leukaemia after a first allogeneic transplantation. Bone Marrow Transplantation. 2014;**49**(7):895-901. DOI: 10.1038/bmt.2014.83 (Epub May 12, 2014)

[124] Manabe A, Kawasaki H, Shimada H, Kato I, Kodama Y, Sato A, et al. Imatinib use immediately before stem cell transplantation in children with Philadelphia chromosome-positive acute lymphoblastic leukemia: Results from Japanese Pediatric Leukemia/lymphoma study group (JPLSG) study Ph(+) ALL 04. Cancer Medicine. 2015;**4**(5):682-689. DOI: 10.1002/cam4.383 (Epub Jan 31, 2015)

[125] Kebriaei P, Chiattone A, Saliba R, Jones D, Anderlini P, Andersson B, et al. Imatinib maintenance following allogeneic hematopoietic cell transplantation (HCT) for patients with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2010;**16**(Suppl. 2):S230

[126] Benjamini O, Dumlao TL, Kantarjian H, O'Brien S, Garcia-Manero G, Faderl S, et al. Phase II trial of hyper CVAD and dasatinib in patients with relapsed Philadelphia chromosome positive acute lymphoblastic leukemia or blast phase chronic myeloid leukemia. American Journal of Hematology. 2014;**89**(3):282-287. DOI: 10.1002/ajh.23624

[127] Aldoss I, Gaal K, Al Malki MM, Ali H, Nakamura R, Forman SJ, et al. Dasatinib-induced colitis after allogeneic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia. Biology of Blood and Marrow Transplantation. 2016;**22**(10):1900-1903. DOI: 10.1016/j.bbmt.2016.06.022 (Epub Jun 23, 2016)

[128] Leonard JT, Traer E, Hayes-Lattin, Tyner J, Drucker B, Chang BH. Targeting BCL-2 and BCR-Abl activity in Ph+ ALL. Biology of Blood and Marrow Transplantation. 2015;**21**(2):S190

[129] Leonard JT, Allen B, Slater S, Maziarz RT, Hayes-Lattin B. A chemotherapy-free induction followed by allogeneic HSCT for Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL). Biology of Blood and Marrow Transplantation. 2017;**23**(3):S287-S288

[130] Kang BW, Moon JH, Chae YS, Kim JG, Kim SN, Sohn SK. Pre-emptive treatment with nilotinib after second allogeneic transplantation in a Philadelphia chromosome-positive acute lymphoblastic leukemia patient with high risk of relapse. Acta Haematologica. 2010;**123**(4):242-247. DOI: 10.1159/000314538 (Epub May 12, 2010)

[131] Varda-Bloom N, Danylesko I, Shouval R, Eldror S, Lev A, Davidson J, et al. Immunological effects of nilotinib prophylaxis after allogeneic stem cell transplantation in patients with advanced chronic myeloid leukemia or philadelphia chromosome-positive acute lymphoblastic leukemia. Oncotarget. 2017;**8**(1):418-429. DOI: 10.18632/oncotarget.13439

[132] Carpenter PA, Johnston L, Fernandez H, Radich J, Mauro MJ, Flowers MED, et al. A multicenter phase I/II study of relapse prophylaxis with nilotinib after hematopoietic cell transplantation (HCT) for high-risk Philadelphia chromosome-positive (Ph+) leukemias. Biology of Blood and Marrow Transplantation. 2015;**1**(2):S266-S321

[133] Shimoni A, Volchek Y, Koren-Michowitz M, Varda-Bloom N, Somech R, Shem-Tov N, et al. Phase 1/2 study of nilotinib prophylaxis after allogeneic stem cell transplantation in patients with advanced chronic myeloid leukemia or Philadelphia chromosome–positive acute lymphoblastic leukemia. Cancer. 2015;**121**:863-873. DOI: 10.1002/cncr.29141

[134] Carpenter PA, Johnston L, Fernandez HF, Radich JP, Mauro MJ, flowers MED, et al. Posttransplant feasibility study of nilotinib prophylaxis for high-risk Philadelphia chromosome positive leukemia. Blood. 2017;**130**(9):1170-1172. DOI: 10.1182/blood-2017-03-771121 (Epub Jul 11, 2017)

[135] Lupo-Stanghellini MT, Lunghi F, Assanelli AA, Guggiari E, Greco R, Morelli M, et al. Post-transplant treatment with ponatinib for patients with high-risk Philadelphia chromosome positive leukemia. Blood. 2016;**128**:5810

[136] Nicolini FE, Basak GW, Kim DW, Olavarria E, Pinilla-Ibarz J, Apperley JF, et al. Overall survival with ponatinib versus allogeneic stem cell transplantation in Philadelphia chromosome-positive leukemias with the T315I mutation. Cancer. 2017;**123**(15):2875-2880. DOI: 10.1002/cncr.30558 (Epub Apr 7, 2017)

[137] Cooper TM. Role of nelarabine in the treatment of T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma. Therapeutics and Clinical Risk Management. 2007;**3**(6):1135-1141

[138] Hernandez-Ilizaliturri FJ, Czuczman MS. A review of nelarabine in the treatment of T-cell lymphoblastic leukemia/lymphoma. Clinical Medicine Insights: Therapeutics. 2009;**1**:505-511

[139] DeAngelo DJ. Nelarabine for the treatment of patients with relapsed or refractory T-cell acute lymphoblastic leukemia or lymphoblastic lymphoma. Hematology/Oncology Clinics of North America. 2009;**23**(5):1121-1135. vii–viii. DOI: 10.1016/j.hoc.2009.07.008

[140] Cohen MH, Johnson JR, Justice R, Pazdur R. FDA drug approval summary: Nelarabine (Arranon) for the treatment of T-cell lymphoblastic leukemia/lymphoma. The Oncologist. 2008;**3**(6):709-714. DOI: 10.1634/theoncologist.2006-0017

[141] Reilly KM, Kisor DF. Profile of nelarabine: Use in the treatment of T-cell acute lymphoblastic leukemia. OncoTargets and Therapy. 2009;**2**:219-228

[142] DeAngelo DJ, Yu D, Johnson JL, Coutre SE, stone RM, Stopeck AT, et al. Nelarabine induces complete remissions in adults with relapsed or refractory T-lineage acute lymphoblastic leukemia or lymphoblastic lymphoma: Cancer and leukemia group B study 19801. Blood. 2007;**109**(12):5136-5142 (Epub Mar 7, 2007). DOI: 10.1182/blood-2006-11-056754

[143] Gökbuget N, Basara N, Baurmann H, Beck J, Brüggemann M, Diedrich H, et al. High single-drug activity of nelarabine in relapsed T-lymphoblastic leukemia/lymphoma offers curative option with subsequent stem cell transplantation. Blood. 2011;**118**(13):3504-3511. DOI: 10.1182/blood-2011-01-329441 (Epub Jun 28, 2011)

[144] Kadia TM, Gandhi V. Nelarabine in the treatment of pediatric and adult patients with T-cell acute lymphoblastic leukemia and lymphoma. Expert Review of Hematology. 2017;**10**(1):1-8. DOI: 10.1080/17474086.2017.1262757 (Epub Dec 8, 2016)

[145] Roecker AM, Stockert A, Kisor DF. Nelarabine in the treatment of refractory T-cell malignancies. Clinical Medicine Insights: Oncology. 2010;**4**:133-141. DOI: 10.4137/CMO.S4364

[146] Winter SS, Dunsmore KP, Devidas M, Eisenberg N, Asselin BL, wood BL, et al. Safe integration of nelarabine into intensive chemotherapy in newly diagnosed T-cell acute lymphoblastic leukemia: Children's oncology group study AALL0434. Pediatric Blood & Cancer. 2015;**62**(7):1176-1183. DOI: 10.1002/pbc.25470 (Epub Mar 8, 2015)

[147] Dunsmore KP, Devidas M, Linda SB, Borowitz MJ, Winick N, Hunger SP, et al. Pilot study of nelarabine in combination with intensive chemotherapy in high-risk T-cell acute lymphoblastic leukemia: A report from the Children's oncology group. Journal of Clinical Oncology 2012;**30**(22):2753-2759. DOI: 10.1200/JCO.2011.40.8724 (Epub Jun 25, 2012)

[148] Lonetti A, Cappellini A, Bertaina A, Locatelli F, Pession A, Buontempo F, et al. Improving nelarabine efficacy in T cell acute lymphoblastic leukemia by targeting aberrant PI3K/AKT/mTOR signaling pathway. Journal of Hematology & Oncology. 2016;**9**(1):114. DOI: 10.1186/s13045-016-0344-4

[149] T1 Y, Uzui K, Nishi R, Shigemi H, Ueda T. Reduced drug incorporation into DNA and antiapoptosis as the crucial mechanisms of resistance in a novel nelarabine-resistant cell line. BMC Cancer. 2014;**14**:547. DOI: 10.1186/1471-2407-14-547

[150] Khan MW, Gul Z. Blinatumomab may induce graft versus host leukemia in patients with pre-B ALL relapsing after hematopoietic stem cell transplant. Clinical Case Reports. 2016;**4**(8):743-746. DOI: 10.1002/ccr3.604. eCollection Aug, 2016

[151] Martinelli G, Boissel N, Chevallier P, Ottmann O, Gökbuget N, Topp MS, MS, et al. complete hematologic and molecular response in adult patients with relapsed/refractory philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia following treatment with blinatumomab: Results from a phase II, single-arm, multicenter study. Journal of Clinical Oncology 2017;**35**(16):1795-1802. DOI: 10.1200/JCO.2016.69.3531 (Epub Mar 29, 2017)

[152] Pegram HJ, Smith EL, Rafiq S, Brentjens RJ. CAR therapy for hematological cancers: Can success seen in the treatment of B-cell acute lymphoblastic leukemia be applied to other hematological malignancies? Immunotherapy. 2015;**7**(5):545-561. DOI: 10.2217/imt.15.6. DOI: 10.2217/imt.15.6

[153] Wang Z, Wu Z, Liu Y, Han W. New development in CAR-T cell therapy. Journal of Hematology & Oncology. 2017;**10**(1):53. DOI: 10.1186/s13045-017-0423-1

[154] Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Science Translational Medicine. 2013;**5**(177):177ra38. DOI: 10.1126/scitranslmed.3005930

[155] Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Science Translational Medicine. 2014;**6**(224):224ra25. DOI: 10.1126/scitranslmed.3008226

[156] Davila ML, Brentjens RJ. CD19-targeted CAR T cells as novel cancer immunotherapy for relapsed or refractory B-cell acute lymphoblastic leukemia. Clinical Advances in Hematology & Oncology. 2016;**14**(10):802-808

[157] Zhu YM, Wu Z, Tan YP, Du YY, Liu Z, Ou RM, et al. Anti-CD19 chimeric antigen receptor T-cell therapy for adult Philadelphia chromosome-positive acute lymphoblastic leukemia: Two case reports. Medicine (Baltimore). 2016;**95**(51):e5676. DOI: 10.1097/MD.0000000000005676

[158] Liu J, Zhong JF, Zhang X, Zhang C. Allogeneic CD19-CAR-T cell infusion after allogeneic hematopoietic stem cell transplantation in B cell malignancies. Journal of Hematology & Oncology. 2017;**10**(1):35. DOI: 10.1186/s13045-017-0405-3

[159] Teachey DT, Lacey SF, Shaw PA, Melenhorst JJ, Maude SL, Frey N, et al. Identification of predictive biomarkers for cytokine release syndrome after chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. Cancer Discovery. 2016;**6**(6):664-679. DOI: 10.1158/2159-8290.CD-16-0040 (Epub Apr 13, 2016)

[160] Maino E, Scattolin AM, Viero P, Sancetta R, Pascarella A, Vespignani M, et al. Modern immunotherapy of adult B-lineage acute lymphoblastic leukemia with monoclonal antibodies and chimeric antigen receptor modified T cells. Mediterranean Journal of Hematology and Infectious Diseases. 2015;**7**(1):e2015001. DOI: 10.4084/MJHID.2015.001

[161] Papadantonakis N, Advani AS. Recent advances and novel treatment paradigms in acute lymphocytic leukemia. Therapeutic Advances in Hematology. 2016;**7**(5):252-269 (Epub Jun 29, 2016). DOI: 10.1177/2040620716652289

[162] Tasian SK, Gardner RA. CD19-redirected chimeric antigen receptor-modified T cells: A promising immunotherapy for children and adults with B-cell acute lymphoblastic leukemia (ALL). Therapeutic Advances in Hematology. 2015;**6**(5):228-2241. DOI: 10.1177/2040620715588916

[163] Tang XY, Sun Y, Zhang A, Hu GL, Cao W, Wang DH, et al. Third-generation CD28/4-1BB chimeric antigen receptor T cells for chemotherapy relapsed or refractory acute lymphoblastic leukaemia: A non-randomised, open-label phase I trial protocol. BMJ Open. 2016;**6**(12):e013904. DOI: 10.1136/bmjopen-2016-013904

[164] Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. The Journal of Clinical Investigation. 2016;**126**(6):2123-2138. DOI: 10.1172/JCI85309 (Epub Apr 25, 2016)

[165] Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR T-cells forward. Nature Reviews Clinical Oncology. 2016;**13**(6):370-383. Published online Mar 22, 2016. DOI: 10.1038/nrclinonc.2016.36

Chapter 9

Post-Transplantation Management Strategies

Muhammad Waqas Khan, Ahmed Elmaaz and Zartash Gul

Additional information is available at the end of the chapter

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Abstract

Relapse is an overwhelmingly difficult and tragic event for patients suffering from hematologic malignancies that have been treated with bone marrow transplantation. More often than not, treatment options are fairly limited in each disease. Selecting the appropriate maintenance therapy gives a chance to delay or avoid these recurrences entirely. Although no perfect combination of drugs has yet been established as a mainstay maintenance therapy post-transplant, the authors here discuss the most effective and safest drugs available for different diseases.

Keywords: post-transplant management, graft versus host disease, graft versus leukemia, hematopoietic stem cell transplant

1. Introduction

The transplantation of multipotent hematopoietic stem cells that are usually derived from bone marrow, umbilical cord, or peripheral blood is a process known as hematopoietic stem cell transplantation (HSCT). It may be autologous or allogeneic and although life-saving, this procedure is not without its drawbacks. Major complications associated with HSCT include graft versus host disease (GVHD), infections, and relapse. Our current discussion is based on strategies employed for prevention of relapse post-transplant in different diseases.

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2. Multiple myeloma

The use of autologous hematopoietic stem cell transplantation (A-HSCT) preceded by high-dose chemotherapy is a widely accepted modality for management of multiple myeloma (MM) in patients under the age of 65 years [1–3]. Since recurrence still remains a concern, a need for long-term regimen after transplantation to extend the response and prevent relapse is warranted. Allogenic HSCT has been associated with decreased relapse rates, in part due to its graft versus myeloma effect, but its use is limited by its complications [4]. Thalidomide [5–8], lenalidomide [9–13], proteasome inhibitors, and bortezomib [14–16] are being used as a maintenance therapy following AHSCT. They have shown to significantly decrease the relapse as well as improve the progression free survival (PFS) and overall survival (OS) rates. They have gradually replaced conventional and interferon-based therapy over the past decade due to their limited adverse effect profiles and positive impact on PFS and OS [17]. The mean survival associated with the new modalities approaches eight years post-transplant from the previous duration of three years in the past decades [18]. Several studies have been conducted to comprehensively evaluate the use of these novel agents including the assessment of their degree of response, their depth of response, and their long-term complications.

2.1. Thalidomide

Thalidomide is the first and one of the most widely studied drug following the study and evaluation of post-AHST maintenance therapies in MM. In a Phase II study conducted by Intergroupe Francophone du Myélome (IFM), 597 patients—all younger than 65 years—were divided into three groups [9]. Arm-A did not receive any maintenance therapy, while arm-B received pamidronate. Patients who received a combination of pamidronate and 400 micrograms (mcg) of Thalidomide (once daily following AHSCT) were placed in arm-C. The results showed a 36% PFS in arm-A, 37% in arm-B, and 52% in arm-C ($P < 0.009$). With a median follow-up of 39 months, the OS was 77% in arm-A, 74% in arm-B, and 87% in arm-C with a P-value of <0.04. In another study conducted by Barlogie et al., 7668 patients were divided into two groups; thalidomide versus non-thalidomide. The event-free survival (EFS) was 64% in thalidomide users versus 43% in non-thalidomide group ($P < 0.001$). The OS in the thalidomide group was 57% with 44% in the non-thalidomide group after a 72 months median follow-up ($P = 0.09$). A Phase III trial by Lokhorst et al. [6] compared the EFS between a thalidomide-based regimen and an Interferon-alpha-based regimen with a follow-up of 34 and 22 months, respectively, $P < 0.001$. The results showed no significant difference in the OS between both groups ($P = 0.77$).

The UK Medical Research Council (MRC)—Myeloma XI study divided patients into two separate groups: those with transplant and those without. The PFS was noted to be significantly higher in the transplant group following a median follow-up of 22 months and 15 months, respectively ($P < 0.0001$). No significant difference was noted in the OS between both groups ($P = 0.7$) [5–19]. This may in part be due to a difference in the cytogenetic analysis into high- and low-risk cytogenetic involving chromosome 13 [20]. A detailed analysis showed patients with a chromosome 17p deletion have decreased OS when being treated with thalidomide

indicating that thalidomide should not be used in those individuals (Hazard ratio [HR] 4.55, $P = 0.02$) [5].

A number of other studies have evaluated the use of thalidomide with a corticosteroid in comparison to using a corticosteroid alone. A study of 243 patients by Spencer et al. demonstrated a three-year survival rate; 86% with the thalidomide and corticosteroid group versus 75% in the lone steroid group ($P = 0.004$). The PFS was 42 and 23%, respectively, with a P-value of less than 0.001) [21]. In the Brazilian trial, Maiolino et al. studied 108 patients for duration of 2 years and demonstrated a PFS of 64% in the thalidomide and steroid containing group compared with 30% in dexamethasone only group ($P = 0.002$) [22]. No significant difference was found between both groups in terms of OS ($P = 0.27$). In a similar study by Stewart et al., the PFS in the thalidomide containing group was 32%, while the control group had it at 14% after a median follow-up for 4 years [8]. The survival rate in the thalidomide containing group was 68% in comparison to 60% in the lone dexamethasone group. No significant difference was observed ($P = 0.18$).

Despite the significant improvement in PFS, EFS, and the variable responses in OS rates, the most common long-term complication for thalidomide is peripheral neuropathy, in particular after long-term usage [6, 8, 9, 21]. No optimal duration of thalidomide post-transplant has been suggested by either of the trials.

2.2. Lenalidomide

Due to its higher efficacy and a lower toxicity profile the use of lenalidomide surpasses in tolerance when compared with thalidomide [7]. Although its exact mechanism is not fully understood, several hypotheses have been put forward. One such theory suggests that lenalidomide acts as a natural killer against tumor cells due to their increased cytokine production [23]. Lenalidomide is proven to decrease the production of tumor necrosis factor alpha that in turn tends to increase MM cell growth. It also inhibits the binding of MM cells to the bone marrow cells [24]. Various studies have been conducted to study the effect of lenalidomide on PFS and OS rates post AHSCT in MM. The Cancer and Leukemia Group B (CALGB) conducted a trial labeled CALGB 100104 in which 460 were randomly divided into two groups; one group receiving lenalidomide and the other a placebo with no previous consolidation therapy [12]. The time to progression (TTP) was 46 months in the lenalidomide group compared with 27 months in the placebo group ($P < 0.001$). The OS was significantly higher in patients receiving lenalidomide as compared with those receiving a placebo, 85 and 77%, respectively, ($P = 0.028$). After 36 months of follow-up, the PFS was 66% in the lenalidomide group and 39% in the placebo group. In the IFM 05-02 trial conducted by Attal et al., 614 patients received lenalidomide for a duration of 2 months after AHSCT. They were then divided into two groups: a placebo group and a lenalidomide group [10]. The lenalidomide group had a 41-month TTP versus 23 months in the placebo group with a P-value of <0.001. No difference in OS was found between both the groups, an OS of 73 and 75% in lenalidomide and placebo groups, respectively ($P = 0.7$). Following 45 months of follow-up, the PFS was 43 and 22% ($P < 0.001$), EFS of 40 and 23 months ($P < 0.001$), in the lenalidomide and placebo groups, respectively. The difference in OS in both trials was thought to be due to the difference

in the duration of follow-ups. In a study by Alisna et al., 18 months of follow-up post an AHSCT, 30 patients were started on lenalidomide at a median of 96 days [11]. The PFS was noted to be 63% (95%CI, 43–77%), the OS at 78% (95% CI, 58–90%). A similar study by Kneppers et al. showed a PFS of 60% and an OS of 93%, in 30 patients who were newly diagnosed with MM (HOVON 76 trial) [13]. Lenalidomide was started 12 weeks post an AHSCT for a duration of 24 months.

These studies concluded lenalidomide as an effective maintenance therapy following an AHSCT having significant impact on improving the PFS and OS, particularly on the elderly, patients with an increased risk of MM, and patients receiving a lenalidomide-based induction regimen [25]. Long-term usage was found to be associated with an elevated risk of hematologic complications. A grade 3–4 neutropenia was found in 51% of the patients in comparison with 18% receiving a placebo [10]. Thromboembolic complications were also noted to be markedly higher in comparison to the placebo using group (2% compared with 6%, respectively, $P = 0.01$). The risk of second primary malignancies (SPMs) as acute myeloblastic leukemias (AML) or myelodysplastic syndromes (MDS) has also been reported in various studies [10, 12]. The risk of SPM was significantly higher in patients receiving Melphalan as part of their lenalidomide treatment [27]. A few other studies noted that a lenalidomide-based maintenance therapy post AHSCT was not feasible due to the development of a GVHD [11, 13].

2.3. Bortezomib

A first of its kind, proteasome inhibitor has been used as a maintenance therapy in MM post-transplant. Trials have shown bortezomib's efficacy and its well-defined toxicity profile [14–16]. In a trial by Sonneveld et al. (HOVON 65/GMMG-HD4), 827 patients were divided into two groups; those taking Bortezomib for 2 years after induction (with bortezomib, adriamycin, dexamethasone [PAD]) and HSCT, and a non-Bortezomib group (who took vincristine, adriamycin, and dexamethasone [VAD]) followed by thalidomide [15]. A significant increase in PFS and OS was found on a multivariate analysis (35 and 28 months, respectively, $P = 0.002$), with a hazard ratio (HR) of 0.77 (95% CI, 0.6–1.0, $P = 0.048$) after a 41-month follow-up in the bortezomib group.

In a study conducted by Rosinol et al., 386 patients were divided into three groups in depending on their induction treatment regimens post-HSCT [14]. Thalidomide/dexamethasone (TD), versus bortezomib/thalidomide/dexamethasone (VTD), versus alternate chemotherapy using different regimens in patients <65 years of age who then received a maintenance therapy using bortezomib and thalidomide (VT) versus thalidomide (T) versus interferon, respectively, for 3 years. PFS in the VT group was significantly elevated in comparison with the other groups, although no difference was noted in the OS rates between the three groups. A more recent study by Scheid et al. evaluated the effect of bortezomib post-HSCT in patients with MM and renal impairment using the same management regimen used in the HONOVO 65 trial, using bortezomib for the PAD group and thalidomide for the VAD group [16]. In 746 patients, the serum creatinine was <2 mg/dL, while 81 patients had a serum creatinine of more than 2 mg/dL. The response in the VAD was 63% compared with a response of 81% in the PAD group in patients whose serum creatinine was >2 mg/dL. PFS in the VAD group was noted to

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be 16 versus 48% in the PAD group receiving bortezomib, proving the effective use of Bortezomib as a maintenance therapy in renal impaired patients suffering from MM.

We do not know the exact dose at which bortezomib may be effective during maintenance therapy; we suspect that lower doses might be effective in achieving the desired results.

In another Phase 1 trial by Abidi et al., 12 out of the 15 patients were studied to determine the safest and best-tolerated maintenance dosing of bortezomib post-HSCT [28]. The median duration of follow-up for the entire cohort was 33 months ranging from 12 to 62 months. The study concluded that bortezomib 1 mg/m^2 administered intravenously and may be subcutaneously on day(s) 1, 8, and 15 in a 28-day cycle was the best-tolerated maintenance dosing and can be safely given beginning around the hundredth day post-ASCT.

3. Chronic myeloid leukemia (CML)

Allogenic stem cells transplantation (SCT) used to be the first line in management of chronic myeloid leukemia (CML). The survival rate following SCT depends on the phase of the disease in which HSCT was performed; 80% in chronic phase (CP), 40% in accelerated phase (AP), and 20% in blast crisis phase (BP) [29]. Due to the fact that relapses had been a significant problem following SCT, donor leukocyte infusion (DLI) was used to augment the graft versus leukemia (GVL) effect [30]. The introduction of tyrosine kinase inhibitors (TKIs) has replaced the use of DLI due to its effects on bone marrow suppression and GVHD induction. TKIs have proven to greatly improve survival following SCT [31, 32]. Detection of the BCR-ABL transcript levels (the enzyme affected by tyrosine kinase) early on after SCT with the aid of reverse transcriptase polymerase chain reaction (RT-PCR), the probability of relapse after SCT can be predicted giving us an idea regarding the efficacy of TKI post-transplant. High-level positive BCR-ABL is associated with highest relapse probabilities, while negative BCR-ABL has the lowest chances of relapse. Low-level positive BCR-ABL is associated with intermediate risk (total number of BCR-ABL transcripts was <100 per mg RNA and/or the BCR-ABL/ABL ratio was <0.02%) [33].

The efficacy of TKIs following SCT depends on the phase of the disease; prognosis is better if TKIs were used for relapsed CP phase; however, substantially less if used for relapse in the AP or BP phases [31, 32, 34].

The efficacy of imatinib mesylate (IM), the first TKI to be studied has been reported by many studies [35]. Wright et al. studied the response of IM after SCT in CML relapsing patients [36]. Out of 22 patients who received IM for relapse, eight were in CP, while 14 were in AP post-transplant. A complete hematological response was observed in 19 patients, while a complete cytogenetic response was observed in 17 patients. After a 31.5-month follow-up postrelapse, the OS was at 64%. Wright concluded that a complete molecular response following relapse was a predictive factor of the OS (95% CI, 2.3–182). Similarly, Palandri et al. used IM in a total of 16 patients with CML who relapsed post-transplant either in CP or AP for average 31 months. Seventy-five percent of patients achieved a complete molecular response [37],

indicating that IM could be tolerated for longer period of time post-transplant with no major hematological drawbacks. However, peripheral blood counts should be monitored during the duration of treatment for pancytopenia, a leading side effect of TKIs [37]. A study by DeAngelo et al. evaluated 15 patients who received IM for relapse post-transplant, 10 of whom were in CP, one in AP, and four patients in BP [34]. Nine of the ten patients who received IM in CP achieved a complete cytogenetic response after six months. The OS rate was at one 100% after 25 months of follow-up. Kantarjian et al. studied 28 CML relapse patient post-SCT [32], all of whom received IM in a dose range of 400–1000 mg/day. Five patients were in CP, 15 in AP, and 8 in BP. Thirteen patients received DLI at an average of 4 months prior to the use of IM. Complete hematologic response was seen in 100% of the CP patients, 83% in AP, and 43% in BP. The OS was 74% after 1 year of follow-up. The study concluded that no difference was detected in complete response between patients who had received IM and DLI, in comparison to patients who received IM alone. A study by Hess et al. evaluated 44 patients, 37 of whom were in CP before SCT, 18 patients had molecular relapse, while 19 had cytogenetic relapse [38]. IM started post-SCT on an average of 2.1 years yielded a complete molecular response in 62% of the patients after 9 months that further improved with subsequent follow-ups. They also recommended the use of molecular end point in clinical decision making. On the other hand, a study by Olavarria et al. showed that standard PCR techniques were lacking; however, complete molecular response had been significantly high, especially in CP patients [31]. In another study by Olavarria et al., IM was administrated 35 days postrelapse in 22 patients. In a year, all patients had achieved complete molecular remission without cytogenetic relapse during the entire length of IM therapy [33]. Relapse had only been detected after the discontinuation of IM therapy. The length of IM use post-transplant is yet to be determined. These various studies have recommended IM as a feasible, effective, and well-tolerated treatment for relapsing CML patients after SCT. Currently, there is a paucity of data to determine the efficacy of using TKI after SCT for maintenance. However, given its efficacy in relapsed disease, many physicians use this strategy for maintenance.

The available data about the use of second generation TKI, such as dasatinib, is still limited [39]. A study by Klyuchnikov et al. used second-generation TKI dasatinib post HST in 11 patients; out of whom nine had CML, two were in AP, and seven in BP [40]. All the patients had received TKI prior to their SCT-IM, dasatinib, or nilotinib (sometimes in combination). Dasatinib was administrated at a median interval of 1 year post-SCT with a median duration of treatment of 8 months (it was discontinued in one patient due to gastrointestinal bleeding that was thought due to drug-related thrombocytopenia). Responses to dasatinib post-transplant were seen in four out of the nine patients. Five patients failed to respond to dasatinib, while three passed away due to disease progression. One patient had a CNS relapse. The study concluded that second-generation TKI dasatinib is effective in the treatment of relapse of CML post-transplant and is generally well tolerated despite the hematological side effects. Contrary to IM, dasatinib was able to penetrate extramedullary tissues and CNS [41, 42].

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4. Philadelphia chromosome positive acute lymphoblastic leukemia (Ph + ALL)

Before the use of allogeneic SCT for the treatment of Philadelphia chromosome positive Acute Lymphoblastic Leukemia (Ph + ALL), patients were usually treated with induction chemotherapy alone. It had poor outcomes in regard to the long-term survival with a median disease-free survival (DFS) range of 5–9 months [43]. SCT has been used as a curative treatment for Ph + ALL postinduction chemotherapy to improve the long-term survival [44]. One such retrospective study compared patients treated with chemotherapy alone, with patients being treated with chemotherapy followed by SCT [45]. The study reported significant survival improvement in patients treated with chemotherapy followed by SCT than patients treated with chemotherapy alone.

Detection of BCR-ABL post-SCT using RT-PCR was found to be a good predictor of minimal residual disease (MRD). Relapse with RT-PCR turned positive 4–6 months prior to the occurrence of relapse in patients who achieved remission after chemotherapy with and without combination with SCT [46].

In a study by Chen et al., the effects of TKI IM on DFS post-SCT in patients with Ph + ALL, 82 patients were evaluated of which 62 patients had received IM at a median of 70 days post-SCT for a median duration of 90 days. In 14 patients, BCR-ABL was positive prior to the use of IM, while 8 patients turned negative after a 1 month use of IM. Relapse rate was 10.2% in the group using IM, while it was 33.1% in non-IM group. The 5-year DFS was 81.5% in IM group in comparison to 33.5% in the non-IM group. Multivariate analysis proved the use of IM post-SCT as a prognostic factor for DFS and OS ($P = 0.000$) [47]. Twenty-two patients were studied by Carpenter et al. to assess the tolerance of IM use post-SCT [48]: 15 patients with Ph + ALL and seven patients with CML. IM was easily tolerated by 17 out of the 19 adult patients at doses of 400 mg/day. In the pediatric age group, it was tolerated by three children at doses of 260 mg/m^2/day in the first 90 days post-SCT (doses compared with those used in primary therapy for Ph + leukemia). Fourteen patients had positive BCR-ABL before SCT. After an average 1.4-year follow-up, 17 patients were alive with negative BCR-ABL transcripts. In the Ribera et al. study, 30 patients newly diagnosed Ph + ALL received IM with chemotherapy followed by SCT [49]. Transplant was then followed by IM for a median duration of 3.9 months. Twenty-seven patients achieved remission of which 21 patients underwent SCT. Twelve patients received IM after transplant. After a follow-up of 4.1 years, the DFS and the OS were 30 and 30%, respectively. Contrary to previous studies, Ribera et al. showed that adverse effects due to transplant limited the early use of IM post-SCT despite the efficacy of combined IM and chemotherapy as a primary treatment for Ph + ALL.

In another study by Teng et al., a similar TKI, dasatinib was evaluated for the same purpose [50]. Six patients with Ph + ALL were enrolled in the study and received SCT along with 100 mg/day dasatinib for 1 year. All patients achieved complete remission prior to SCT with relapse occurring in three patients only (all extramedullary). Only two patients had adverse effects from dasatinib which was improved by dividing the dose of dasatinib into two 50 mg doses. This study showed that dasatinib was effective and tolerated by Ph + ALL patients after SCT.

5. Acute myeloid leukemia (AML)

Although SCT is considered as a treatment for acute myeloid leukemia (AML) to either induce remission, or to prevent relapse, relapse is perhaps considered to be the most common complication post-SCT in patients with AML especially in the first year after transplant [51]. The duration of remission after SCT is the most important predictor of for survival after relapse [52]. Like ALL, the detection of MRD using RT-PCR could predict the post-transplant relapse in patents with AML and is much more accurate than other classic methods [53]. The 5 years OS was 79% in MRD-negative patients as detected by flow cytometry as opposed to 26% in MRD-positive patients with relapse rates of 58 and 14% after 2 years, respectively [54]. Several modalities have been studied to help control post-transplant relapse in AML patients. Jabbour et al. studied the efficacy and feasibility of Azacitidine (a DNA hypomethylating agent) in nine patients to prevent disease recurrence after SCT and in eight patients as maintenance therapy [55]. It was administrated daily for 5 days and was repeated every 4 weeks for a median duration of follow-up of 11 months. The EFS and OS rates after a year were 55 and 90%, respectively. In a paper by Cruijsen et al., 27 patients with AML who had relapsed post-SCT received Azacitidine for 3 days at a total daily dose of 100 mg which was followed by donor lymphocyte infusion on day 10 [56]. The next course started on day 22. A total of 60 courses of Azacitidine were administered. The OS from the initiation of Azacitidine was 136 days (range of 23–873), and the survival rate after 2 years was 16%. In a study by Bolanos-Meade et al., 10 patients with AML relapse post-SCT receiving Azacitidine 75 mg/m^2/day for 5–7 days were evaluated [57]. Of the 10, six achieved complete remission, one had stable disease, and three progressed after a median of six cycles. After a median follow-up of 624 days, the OS was 422.5 days (range 127–1411). These studies demonstrated that Azacitidine is effective and well tolerated by the patients without exacerbation of GVHD.

A Phase II trial was conducted by De Lima et al. to determine the maintenance dose of azacitidine postallogenic HSCT for recurrent AML or MDS [58]. Forty-five high risk patients with a median age of 60 years were treated. A combination of five different azacitidine was investigated (8, 16, 24, 32, and 40 mg/m^2), on four different schedules. Each scheduled cycle consisted of 5 days of drug administration and 25 days of rest. Reversible thrombocytopenia was found out to be the dose limiting toxicity. The optimal azacitidine combination determined from this study was 32 mg/m^2 administered for four cycles with a median follow-up of 20.5 months. The 1-year event-free survival and OS were 58 and 77%, respectively.

Decitabine (DAC) is a hypomethylating agent that irreversibly binds and inhibits DNA methyltransferase-1, resulting in loss of DNA methylation. Using DAC in maintenance therapy may help eradicate minimal residual disease and facilitate a graft versus leukemia effect by enhancing the effect of T-regulatory lymphocytes. One of the first studies to use Decitabine (DAC) as a maintenance drug postallogenic HSCT in pretreated patients with AML and MDS was conducted in 2012 by Choi et al. [59]. A total of 19 patients with a median age of 60 years were enrolled out of which 14 had AML, and five patients had MDS. All conditioning regimens were myeloablative. Three cohorts had been completed and a final fourth cohort is currently enrolling. Four doses of DAC had been investigated (5, 7.5, 10, and 15 mg/m^2/day)

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administered for 5 days in a 6 weeks cycle. The median follow-up post-HSCT was 24 months. Approximately 43% of the patients were able to receive all eight cycles. It was determined that a dose of 15 mg/m^2 administered for 5 days, every 6 weeks was safe. Although results were not statistically significant, an increasing FOXP3 expression was observed in all patients. The lack of toxicities and low GVHD incidence indicated that further studies had to be conducted to determine the exact dosage of DAC required for maintenance.

A more recent and similar study by Pusic et al. [59], DAC was assessed for its safety and efficacy as a maintenance therapy in patients with AML and MDS post-HSCT. Twenty-two patients were enrolled and divided into four cohorts. DAC was administered in four doses (5, 7.5, 10, and 15 mg/m^2/day), for 5 days every 6 weeks, for a maximum of eight cycles. Nine patients completed all eight cycles out of which eight patients remained in CR. DAC maintenance did not clearly impact the rate of chronic GVHD, although a similar trend of increased FOXP3 expression seen. DACs maintenance was associated with acceptable toxicities when given in the postallogenic HSCT setting. Although the maximum tolerated dose was not reached, the dose of 10 mg/m^2 for 5 days every 6 weeks appeared to be the optimal dose.

Sorafenib is a tyrosine kinase inhibitor (TKI) that inhibits the FLT3 tyrosine kinase receptor. The FLT3-ITD mutation is associated with a high relapse rates for patients with AML postallogenic HSCT. Chen et al. [60] conducted a Phase 1 trial using sorafenib as maintenance therapy for post-HSCT in patients with FLT3-ITD AML. The patients received an assortment of conditioning regimens (10 reduced intensity and 12 myeloablative). They were all in morphological remission with predominant donor chimerism post-HSCT before starting maintenance therapy. A dose escalation 3 + 3 cohort design was used to define the maximum tolerated dose (MTD). Ten patients were additionally treated at the MTD. Sorafenib was started between days 45 and 120 post-HSCT and given continuously for 12 cycles, each cycle consisting of 28 days. No significant flares of acute GVHD was observed after starting sorafenib. The 1 year cumulative incidence of chronic GVHD was 42% (90% CI, 23%, 60%). Serial FLT3 ligand levels were measured in seven patients. Median level at baseline and prior to any drug administration was 125 pg/ml, which significantly increased to a median level of 254 pg/ml (P = 0.016) measured on day 29 of cycle 1. The surviving patient median follow-up was 14.5 months post-HSCT. The 1 year PFS is 84% (90% CI, 63–94%) and 1 year OS is 95% (90% CI, 79–99%). Only one patient relapsed post-HSCT. The study concluded Sorafenib may reduce the rate of relapse, was safe to give as maintenance therapy after HSCT for patients with FLT3-ITD AML. The use of maintenance sorafenib and other FLT3 inhibitors post-HSCT warrants further investigation.

6. Non Hodgkins Lymphoma

The use of high-dose chemotherapy followed by ASCT has been in use for a long time as a potential treatment for diffuse large B cell lymphoma (DLBCL). However, it is now mostly restricted to chemosensitive DLBCL [61, 62]. The PARMA trial began with 216 patients who received two courses of DHAP (dexamethasone, Ara-C, cisplatin) confirmed the superiority of dose intensification with autologous bone marrow transplantation over conventional

chemotherapy in patients with relapsed diffuse NHL [62]. In different types of lymphoma, relapse is the most important cause of mortality post-transplant, particularly within the first 9 months post-SCT. This is further demonstrated in a study conducted by Hamdani et al., where patients with DLBCL were compared among autologous HCT outcomes for chemosensitive DLBCL patients between 2000 and 2011 [63]. These were divided in two cohorts based on time to relapse from diagnosis. The early rituximab failure (ERF) cohort consisted of patients with primary refractory disease or patients who had first relapse within a year of their initial diagnosis. This group was then compared with those patients who had relapses more than a year after initial diagnosis (late rituximab failure [LRF] cohort). Both the ERF and LRF cohorts included 300 and 216 patients, respectively. Nonrelapse mortality (NRM), OS, PFS values of ERF versus LRF groups at the 3 years were 9% (95% confidence interval [CI], 6–13%) versus 9% (95% CI, 5–13%, 50% (95% CI, 44–56%) versus 67% (95% CI, 60–74%), and 44% (95% CI, 38–50%) versus 52% (95% CI, 45–59%), respectively. On a multivariate analysis, the ERF was not associated with a higher NRM (relative risk [RR], 1.31; $P = 0.34$). The ERF group experienced a higher risk of treatment failure (RR, 2.08; $P < 0.001$) and overall mortality (RR, 3.75; $P < 0.001$) within the first 9 months after autologous HCT. Beyond this period, the PFS and OS were not significantly different between both groups of LRF and ERF. Several studies have evaluated different treatment approaches for relapse post-SCT. In one such study by Haioun et al., 269 patients randomized into two groups receive rituximab maintenance ($n = 139$) for four weeks, or observed without maintenance ($n = 130$) after SCT [64]. Patients were then randomized into two groups of those achieving complete response (CR) ($n = 130$), and those who achieved incomplete or partial response ($n = 139$). After a median follow-up of 4 years from the second randomization, the EFS was 80% in the rituximab arm in comparison to 70% in observation only arm with no statistically significant difference in between both groups ($P = 0.99$), though significant difference was found in both arms of patients who achieved CR post-SCT. In another study by Gisselbrecht et al., of the 477 relapsed patients enrolled, 242 responded to salvage therapy and received SCT and high-dose chemotherapy [65]. They were then assigned to receive either rituximab for 1 year ($n = 122$), or observation alone ($n = 120$). After a median 44 months of follow-up post-SCT, no significant difference was found regarding EFS, PFS, or OS between both groups. Interestingly, significant difference was found in EFS between women (63%) and men (46%) in the rituximab group. This could be explained by the higher concentration of rituximab in females due to their slower body release [66]. The quality of life of 269 patients with DLBCL randomized to receive either rituximab or observation alone post-SCT was done in a study conducted by Heutte et al. [67]. The study showed that Rituximab decreased pain and severity of symptoms, with the long-term difference in quality of life post-SCT was not dependent on rituximab maintenance. As concluded by these studies, rituximab could be used as a maintenance therapy post-SCT as being a feasible and safe option, but does not improve disease control or survival outcome and needs to be investigated further.

Though mantle cell lymphoma (MCL) is still considered a poor prognosis type of non-Hodgkin lymphoma [68], rituximab has proven efficacy in Phase III studies by prolonging disease-free survival and improving clinical response in patients with MCL undergone SCT [69]. Better response was correlated with detection of PCR undetectable markers in bone marrow and peripheral blood after SCT [70–72]. In a study by Andersen et al. [73], 74 patients showed

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complete response of 145 patients with SCT, 36 had molecular relapse after a mean of 18.5 months following SCT, and 26 patients got administrated pre-emptive rituximab which could induce a remission of 92%. Median clinical and molecular-free survival was 3.7 and 1.5 years, respectively, stating the importance of PCR analysis for patients with MCL to stratify high-risk group of patients.

7. Hodgkins Lymphoma

As in non-Hodgkin lymphoma, high-dose therapy followed by autologous stem-cell transplantation is the standard of care for relapsed patients of Hodgkin's lymphoma (HL), and for patients who did not respond to the salvage treatment [74]. Studies have shown that SCT could significantly increase the progression-free survival (PFS) [75]. Several treatment approaches have been studied to increase outcome after SCT as radiation before and after SCT and consolidation post-SCT [76, 77]. Brentuximab vedotin, an anti-CD 30 antibody linked to protease cleavage agent has been studied as a post-transplant therapy for HL. A Phase II study by Younes et al. showed that response rate to brentuximab is 75% in relapsed patients with HL and complete response rate was 34% after 2 years [78]. The AETHERA study [79] evaluated brentuximab as an early consolidation therapy post-SCT where 329 patients with relapsed or refractory HL were randomly assigned to receive 16 cycles of 1.8 mg/kg brentuximab vedotin (n = 165), or placebo (n = 164) starting 30–45 days after SCT. PFS was significantly higher in patients in the brentuximab vedotin group compared with those in the placebo group (95% CI 0.40–0.81; P = 0.0013), with median PFS of 42.5 months in brentuximab receiving patients compared with 24.1 months in patients receiving placebo. After 24 months of follow-up, 63% of brentuximab group were alive in comparison with the 51% in the placebo group. The study concluded that the administration of brentuximab early after SCT in relapsed or refractory HL patients had significantly improved EFS and OS.

8. Conclusion

IMiDs have also decreased relapse rates along with a decrease in the PFS and OS rates; increasing the mean survival from the previous 3-year survival to an 8-year post-transplant survival. The combination of thalidomide and steroid is promising and has shown significant improvement in the EFS and PF rates in comparison to a lone steroid therapy, although neuropathy is still a major concern in thalidomide-based regimen for prolonged use. Lenalidomide surpasses in tolerance when compared with thalidomide due to its unique efficacy and toxicity profile and has proven to be an effective maintenance therapy following AHSCT with a significant impact on improving the PFS and OS. Similarly, Bortezomib has its defined efficacy and toxicity profile and showed significant increase in PFS and OS in patients as a maintenance therapy in renal-impaired patients suffering from MM.

MM summary: IMiDs have decreased relapse rates and PFS OS rates. Mean survival with the new modalities is 8 years post-transplant from 3 years.

Thalidomide summary: thalidomide + dexamethasone usage has shown good improvement in EFS and PF rates compared with using dexamethasone alone. OS values are variable. Neuropathy is the major concern in thalidomide-based regimen for long-term use. No optimal duration for thalidomide has been established.

Lenalidomide summary: due to its higher efficacy and a lower toxicity profile the use of lenalidomide surpasses in tolerance when compared with thalidomide. Lenalidomide is an effective maintenance therapy following AHSCT, having and has significant impact on improving the PFS and OS. Long-term usage was found to be associated with an elevated risk of hematologic complications, neutropenia. SPM risk was significantly higher in patients receiving melphalan + lenalidomide.

Bortezomib summary: bortezomibs has a defined efficacy and toxicity profile. Significant increase in PFS and OS in patients using bortezomib post-HSCT. Bortezomib is effective as a maintenance therapy in renal impaired patients suffering from MM. Exact dose of maintenance therapy is not yet known.

CML summary: TKIs have proven to improve survival following SCT. The efficacy of TKIs following SCT depends on the phase of the disease; a good prognosis if TKIs were used for relapse CP phase, less if used for relapse in the AP or BP phases. Dasatinib is effective in the treatment of relapse of CML post-transplant, is well tolerated despite the hematological side effects. Dasatinib can penetrate extramedullary tissues and CNS. Presently, there is an insufficiency of current data required to determine the efficacy of using TKI after SCT for maintenance. Nonetheless, given its efficacy in relapsed disease, many physicians use this strategy for maintenance.

Ph + ALL summary: SCT has been used as a curative treatment for Ph + ALL postinduction chemotherapy to improve the long-term survival. Dasatinib was effective and tolerated by Ph + ALL patients after SCT.

AML summary: the duration of remission after SCT is the most important predictor of for survival after relapse. Azacitidine, decitabine, sorafenib, and other FLT3 inhibitors are effective and well tolerated by the patients without exacerbation of GVHD. Although studies of using FLT3 inhibitors as maintenance therapies are still on going, the data collected so far shows promising results and merits further trials.

NHL summary: high-dose chemotherapy followed by ASCT has been in use for a long time as a potential treatment for DLBCL. A 5-year event-free survival rate was significantly higher in patients who received ASCT and chemotherapy, than patients receiving salvage therapy alone. Rituximab could be used as a maintenance therapy post-SCT as being a feasible and safe option, but does not improve disease control or survival outcome and needs to be investigated.

HL summary: SCT could significantly increase the progression-free survival. Brentuximab early after SCT in relapsed or refractory HL patients had significantly improved EFS and OS.

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Conflict of interest

The authors have no conflict of interest, nor have received any funding.

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References

[1] Attal M, Harousseau JL, Facon T, Guilhot F, Doyen C, Fuzibet JG, Monconduit M, Hulin C, Caillot D, Bouabdallah R, Voillat L, Sotto JJ, Grosbois B, Bataille R; InterGroupe Francophone du Myélome. Single versus double autologous stem-cell transplantation for multiple myeloma. N Engl J Med. 2003 Dec 25;**349**(26):2495-502

[2] Child JA, Morgan GJ, Davies FE, Owen RG, Bell SE, Hawkins K, Brown J, Drayson MT, Selby PJ; Medical Research Council Adult Leukaemia Working Party. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med. 2003 May 8;**348**(19):1875-83

[3] Barlogie B, Tricot GJ, Van Rhee F, et al. Long-term outcome results of the first tandem autotransplant trial for multiple myeloma. Br J Haematol. 2006;**135**(2):158-164

[4] Corradini P, Voena C, Tarella C, et al. Molecular and clinical remissions in multiple myeloma: role of autologous and allogeneic transplantation of hematopoietic cells. J Clin Oncol. 1999;**17**(1):208-215

[5] Morgan GJ, Gregory WM, Davies FE, et al. The role of maintenance thalidomide therapy in multiple myeloma: MRC Myeloma IX results and meta-analysis. Blood. 2012;**119**(1):7-15

[6] Lokhorst HM, van der Holt B, Zweegman S, et al. A randomized phase 3 study on the effect of thalidomide combined with adriamycin, dexamethasone, and high-dose melphalan, followed by thalidomide maintenance in patients with multiple myeloma. Blood. 2010;**115**(6):1113-1120

[7] Barlogie B, Pineda-Roman M, van Rhee F, et al. Thalidomide arm of total therapy 2 improves complete remission duration and survival in myeloma patients with metaphase cytogenetic abnormalities. Blood. 2008;**112**(8):3115-3121

[8] Stewart AK, Chen CI, Howson-Jan K, et al. Results of a multicenter randomized phase II trial of thalidomide and prednisone maintenance therapy for multiple myeloma after autologous stem cell transplant. Clin Cancer Res. 2004;**10**(24):8170-8176

[9] Attal M, Harousseau J-L, Leyvraz S, et al. Maintenance therapy with thalidomide improves survival in patients with multiple myeloma. Blood. 2006;**108**(10):3289-3294

[10] Attal M, Lauwers-Cances V, Marit G, et al. Lenalidomide maintenance after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;**366**(19):1782-1791

[11] Alsina M, Becker PS, Zhong X, et al. Lenalidomide maintenance for high-risk multiple myeloma after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2014;**20**(8):1183-1189

[12] McCarthy PL, Owzar K, Hofmeister CC, et al. Lenalidomide after stem-cell transplantation for multiple myeloma. N Engl J Med. 2012;**366**(19):1770-1781

[13] Kneppers E, van der Holt B, Kersten M-J, et al. Lenalidomide maintenance after nonmyeloablative allogeneic stem cell transplantation in multiple myeloma is not feasible: results of the HOVON 76 Trial. Blood. 2011;**118**(9):2413-2419

[14] Rosiñol L, Oriol A, Teruel AI, et al. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/GEM study. Blood. 2012;**120**(8):1589-1596

[15] Sonneveld P, Schmidt-Wolf IGH, van der Holt B, et al. Bortezomib induction and maintenance treatment in patients with newly diagnosed multiple myeloma: results of the randomized phase III HOVON-65/ GMMG-HD4 trial. J Clin Oncol. 2012;**30**(24): 2946-2955

[16] Scheid C, Sonneveld P, Schmidt-Wolf IGH, et al. Bortezomib before and after autologous stem cell transplantation overcomes the negative prognostic impact of renal impairment in newly diagnosed multiple myeloma: a subgroup analysis from the HOVON-65/GMMG-HD4 trial. Haematologica. 2014;**99**(1):148-154

[17] Fritz E, Ludwig H. Interferon-alpha treatment in multiple myeloma: meta-analysis of 30 randomised trials among 3948 patients. Ann Oncol. 2000;**11**(11):1427-1436

[18] Kumar SK, Rajkumar SV, Dispenzieri A, et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;**111**(5):2516-2520

[19] Morgan GJ, Davies FE, Gregory WM, et al. Long-term follow-up of MRC myeloma IX trial: Survival outcomes with bisphosphonate and thalidomide treatment. Clin Cancer Res. 2013;**19**(21):6030-6038

[20] Fonseca R, Harrington D, Oken MM, et al. Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities

((Delta)13) in multiple myeloma: an Eastern Cooperative Oncology Group study. Cancer Res. 2002;**62**(3):715-720

[21] Spencer A, Prince HM, Roberts AW, et al. Consolidation therapy with low-dose thalidomide and prednisolone prolongs the survival of multiple myeloma patients undergoing a single autologous stem-cell transplantation procedure. J Clin Oncol. 2009;**27**(11):1788-1793

[22] Maiolino A, Hungria VT, Garnica M, et al. Thalidomide plus dexamethasone as a maintenance therapy after autologous hematopoietic stem cell transplantation improves progression-free survival in multiple myeloma. Am J Hematol. 2012;**87**(10): 948-952

[23] Dredge K, Marriott JB, Todryk SM, et al. Protective antitumor immunity induced by a costimulatory thalidomide analog in conjunction with whole tumor cell vaccination is mediated by increased Th1-type immunity. J Immunol. 2002;**168**(10):4914-4919

[24] Richardson PG, Schlossman RL, Weller E, et al. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. Blood. 2002;**100**(9):3063-3067

[25] Attal M, Roussel M. Maintenance therapy for myeloma: how much, how long, and at what cost? Am Soc Clin Oncol Educ Book. 2012:515-522

[26] McCarthy PL, Hahn T. Strategies for induction, autologous hematopoietic stem cell transplantation, consolidation, and maintenance for transplantation-eligible multiple myeloma patients. Hematology Am Soc Hematol Educ Program. 2013;**2013**:496-503. doi:10.1182/asheducation-2013.1.496

[27] Palumbo A, Bringhen S, Kumar SK, et al. Second primary malignancies with lenalidomide therapy for newly diagnosed myeloma: a meta-analysis of individual patient data. Lancet Oncol. 2014;**15**(3):333-342

[28] Abidi MH, Gul Z, Abrams J, et al. Phase I trial of bortezomib during maintenance phase after high dose melphalan and autologous stem cell transplantation in patients with multiple myeloma. J Chemother. 2012 Jun;**24**(3)

[29] Horowitz MM, Rowlings PA, Passweg JR. Allogeneic bone marrow transplantation for CML: a report from the International Bone Marrow Transplant Registry. Bone Marrow Transplant. 1996;**17**(Suppl 3):S5-S6

[30] Dazzi F, Szydlo RM, Cross NC, et al. Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood. 2000;**96**(8):2712-2716

[31] Olavarria E, Ottmann OG, Deininger M, et al. Response to imatinib in patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Leukemia. 2003;**17**(9):1707-1712

[32] Kantarjian HM, O'Brien S, Cortes JE, et al. Imatinib mesylate therapy for relapse after allogeneic stem cell transplantation for chronic myelogenous leukemia. Blood. 2002;**100**(5):1590-1595

[33] Olavarria E, Kanfer E, Szydlo R, et al. Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood. 2001;**97**(6): 1560-1565

[34] DeAngelo DJ, Hochberg EP, Alyea EP, et al. Extended follow-up of patients treated with imatinib mesylate (Gleevec) for chronic myelogenous leukemia relapse after allogeneic transplantation: Durable cytogenetic remission and conversion to complete donor chimerism without graft-versus-host disease. Clin Cancer Res. 2004;**10**(15):5065-5071

[35] Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;**2**(5):561-566

[36] Wright MP, Shepherd JD, Barnett MJ, et al. Response to tyrosine kinase inhibitor therapy in patients with chronic myelogenous leukemia relapsing in chronic and advanced phase following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2010;**16**(5):639-646

[37] Palandri F, Amabile M, Rosti G, et al. Imatinib therapy for chronic myeloid leukemia patients who relapse after allogeneic stem cell transplantation: a molecular analysis. Bone Marrow Transplant. 2007;**39**(3):189-191

[38] Hess G, Bunjes D, Siegert W, et al. Sustained complete molecular remissions after treatment with imatinib-mesylate in patients with failure after allogeneic stem cell transplantation for chronic myelogenous leukemia: results of a prospective phase II open-label multicenter study. J Clin Oncol. 2005;**23**(30):7583-7593

[39] Reddiconto G, Chiusolo P, Fiorina A, et al. Dasatinib restores full donor chimerism in a patient with imatinib-resistant Ph + ALL relapsing after unrelated cord blood transplantation. Leuk Lymphoma. 2015;**48**(10):2054-2057

[40] Klyuchnikov E, Schafhausen P, Kröger N, et al. Second-generation tyrosine kinase inhibitors in the post-transplant period in patients with chronic myeloid leukemia or Philadelphia-positive acute lymphoblastic leukemia. Acta Haematol. 2009;**122**(1):6-10

[41] Altintas A, Cil T, Kilinc I, Kaplan MA, Ayyildiz O. Central nervous system blastic crisis in chronic myeloid leukemia on imatinib mesylate therapy: a case report. J Neurooncol. 2007;**84**(1):103-105

[42] Aichberger KJ, Herndlhofer S, Agis H, et al. Liposomal cytarabine for treatment of myeloid central nervous system relapse in chronic myeloid leukaemia occurring during imatinib therapy. Eur J Clin Invest. 2007;**37**(10):808-813

[43] Faderl S, Kantarjian HM, Thomas DA, et al. Outcome of Philadelphia chromosome-positive adult acute lymphoblastic leukemia. Leuk Lymphoma. 2000;**36**:263-273

[44] Snyder DS. Allogeneic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2000;**6**(1083–8791 SB–IM):597-603

[45] Arico M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000;**342**(14):998-1006

[46] Preudhomme C, Henic N, Cazin B, et al. Good correlation between RT-PCR analysis and relapse in Philadelphia (Ph1)-positive acute lymphoblastic leukemia (ALL). Leukemia. 1997;**11**(0887–6924 (Print)):294-298

[47] Chen H, Liu K, Xu L, et al. Administration of imatinib after allogeneic hematopoietic stem cell transplantation may improve disease-free survival for patients with Philadelphia chromosome-positive acute lymphobla stic leukemia. J Hematol Oncol. 2012;**5**:29

[48] Carpenter PA, Snyder DS, Flowers MED, et al. Prophylactic administration of imatinib after hematopoietic cell transplantation for high-risk Philadelphia chromosome-positive leukemia. Blood. 2007;**109**(7):2791-2793

[49] Ribera J-M, Oriol A, González M, et al. Concurrent intensive chemotherapy and imatinib before and after stem cell transplantation in newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia. Final results of the CSTIBES02 trial. Haematologica. 2010;**95**(1):87-95

[50] Teng C-LJ, Yu J-T, Chen H-C, Hwang W-L. Maintenance therapy with dasatinib after allogeneic hematopoietic stem cell transplantation in Philadelphia chromosome-positive acute lymphoblastic leukemia. Ann Hematol. 2013;**92**(8):1137-1139

[51] Meijer E, Cornelissen JJ. Allogeneic stem cell transplantation in acute myeloid leukemia in first or subsequent remission: weighing prognostic markers predicting relapse and risk factors for non-relapse mortality. Semin Oncol. 2008;**35**(4):449-457

[52] Oran B, de Lima M. Prevention and treatment of acute myeloid leukemia relapse after allogeneic stem cell transplantation. Curr Opin Hematol. 2011;**18**(6):388-394

[53] Voskova D, Schoch C, Schnittger S, Hiddemann W, Haferlach T, Kern W. Stability of leukemia-associated aberrant immunophenotypes in patients with acute myeloid leukemia between diagnosis and relapse: comparison with cytomorphologic, cytogenetic, and molecular genetic findings. Cytom Part B—Clin Cytom. 2004;**62**(1):25-38

[54] Walter RB, Gooley TA, Wood BL, et al. Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol. 2011;**29**(9):1190-1197

[55] Jabbour E, Giralt S, Kantarjian H, et al. Low-dose azacitidine after allogeneic stem cell transplantation for acute leukemia. Cancer. 2009;**115**(9):1899-1905

[56] Cruijsen M, Lübbert M, Wijermans P, et al. Clinical results of hypomethylating agents in AML treatment. J Clin Med. 2015 Jan;**4**(1):1-17

[57] Bolaños-Meade J, Smith BD, Gore SD, et al. 5-azacytidine as salvage treatment in relapsed myeloid tumors after allogeneic bone marrow transplantation. Biol Blood Marrow Transplant. 2011;**17**(5):754-758

[58] Choi J, Bernabe N, Abboud CN, et al. Maintenance therapy with decitabine after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia and high-risk myelodysplastic syndrome. Blood. 2013;**122**:4638

[59] Pusic I, Choi J, Fiala MA, et al. Maintenance therapy with decitabine after allogeneic stem cell transplantation for acute myelogenous leukemia and myelodysplastic syndrome. Biol Blood Marrow Transplant. 2015 Oct;**21**(10):1761-1769

[60] Chen YB, Shuli L, Andrew LA, et al. Phase I trial of maintenance Sorafenib after allogeneic hematopoietic stem cell transplantation for patients with FLT3-ITD AML. Blood. 2014;**124**:671

[61] Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. N Engl J Med. 1997;**336**(18):1290-1297

[62] Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995;**333**(23):1540-1545

[63] Hamdani M, Hari PN, Zhang Y, et al. Early failure of frontline rituximab-containing chemo-immunotherapy in diffuse large B cell lymphoma does not predict futility of autologous hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2014;**20**(11):1729-1736

[64] Haioun C, Mounier N, Emile JF, et al. Rituximab compared to observation after high-dose consolidative first-line chemotherapy (HDC) with autologous stem cell transplantation in poor-risk diffuse large B-cell lymphoma: updated results of the LNH98-B3 GELA study. J Clin Oncol (Meeting Abstr). 2007;**25**(18_suppl)

[65] Gisselbrecht C, Schmitz N, Mounier N, et al. Rituximab maintenance therapy after autologous stem-cell transplantation in patients with relapsed CD20+ diffuse large B-cell lymphoma: final analysis of the collaborative trial in relapsed aggressive lymphoma. J Clin Oncol. 2012;**30**(36):4462-4469

[66] Pfreundschuh M, Poeschel V, Zeynalova S, et al. Optimization of rituximab for the treatment of diffuse large B-cell lymphoma (II): extended rituximab exposure time in the SMARTE-R-CHOP-14 trial of the German high-grade non-Hodgkin lymphoma study group. J Clin Oncol. 2014;**32**(36):4127-4133

[67] Heutte N, Haioun C, Feugier P, et al. Quality of life in 269 patients with poor-risk diffuse large B-cell lymphoma treated with rituximab versus observation after autologous stem cell transplant. Leuk Lymphoma. 2011;**52**(7):1239-1248

[68] Epperla N, Fenske TS, Hari PN, Hamadani M. Recent advances in post autologous transplantation maintenance therapies in B-cell non-Hodgkin lymphomas. World J Transplant. 2015;**5**(3):81-88

[69] Andersen NS, Jensen MK, de Nully Brown P, Geisler CH. A Danish population-based analysis of 105 mantle cell lymphoma patients: incidences, clinical features, response, survival and prognostic factors. Eur J Cancer. 2002;**38**(3):401-408. doi:10.1016/S0959-8049(01)00366-5

[70] Magni M, Di Nicola M, Devizzi L, et al. Successful in vivo purging of CD34-containing peripheral blood harvests in mantle cell and indolent lymphoma: evidence for a role of both chemotherapy and rituximab infusion. Blood. 2000;**96**(3):864-869. doi:10.1016/s0889-8588(05)70470-6

[71] Geisler CH, Kolstad A, Laurell A, et al. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. Blood. 2008;**112**(7):2687-2693. doi:10.1182/blood-2008-03-147025

[72] Corradini P, Astolfi M, Cherasco C, et al. Molecular monitoring of minimal residual disease in follicular and mantle cell non-Hodgkin's lymphomas treated with high-dose chemotherapy and peripheral blood progenitor cell autografting. Blood. 1997;**89**(2):724-731

[73] Andersen NS, Pedersen LB, Laurell A, et al. Pre-emptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. J Clin Oncol. 2009;**27**(26):4365-4370

[74] Moskowitz CH, Nademanee A, Masszi T, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2015;**385**(9980):1853-1862

[75] Schmitz N, Pfistner B, Sextro M, et al. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haemopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: A randomised trial. Lancet. 2002;**359**(9323):2065-2071

[76] Moskowitz AJ, Perales MA, Kewalramani T, et al. Outcomes for patients who fail high dose chemoradiotherapy and autologous stem cell rescue for relapsed and primary refractory Hodgkin lymphoma. Br J Haematol. 2009;**146**(2):158-163

[77] Rapoport AP, Guo C, Badros A, et al. Autologous stem cell transplantation followed by consolidation chemotherapy for relapsed or refractory Hodgkin's lymphoma. Bone Marrow Transplant. 2004;**34**(10):883-890

[78] Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. J Clin Oncol. 2012;**30**(18):2183-2189

[79] Moskowitz C, Nadamanee A, Masszi T, et al. The AETHERA trial: an ongoing phase 3 study of brentuximab vedotin in the treatment of patients at high risk of residual Hodgkin lymphom a following autologous stem cell transplant. Biol Blood and Bone Marrow. Feb 2015;**21**(2):S28

Chapter 10

New Horizons in Regenerative Medicine in Organ Repair

Paramjit Singh Dhot and Mayurika S. Tyagi

Additional information is available at the end of the chapter

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Abstract

Regenerative medicine is a scientific field that focuses on new approaches in the autologous repair and/or replacement of cells, tissues and/or organs. With time and technical advancements, urethral regeneration, corneal and retinal regeneration, genetically modified skin transplantation has become routine clinical and tissue reconstructive art only due to successful clinical use of stem cells and engineered tissue grafting at defined locations in respective organs in order to bring back the natural or improved physiological functions with enhanced capacity. The tissue engineering and reconstructive art are becoming integral part of the regenerative medicine. This chapter highlights the importance of regenerative medicine in successful tissue reconstruction for organ transplantation.

Keywords: regenerative medicine, urethral defects, bronchomalacia, limbal stem cells, skin transplants, amniotic stem cells, corneal and retinal stem cells

1. Introduction

There have been exponential advances in the field of tissue engineering and regenerative medicine (TERM). There is an active and progressive focus of research to study the mechanisms of injury and how they interface and activate endogenous progenitor cell populations and with a particular focus on elucidating how progenitor cells interact with cells of the immune system. A lot of work has focused on identifying precisely which pool of stem cells actively participates in endogenous repair/regeneration processes.

Human embryonic stem cells have an endless capacity to divide, offer an unlimited source of cells, are capable of becoming any type of cell, and can be differentiated in the laboratory.

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The use of hESC-derived cells is an attractive treatment approach, in fact, for many different diseases because transplanted cells may be able to react to changing conditions in the microenvironment, which is an important biological process.

In the biomaterial area of regenerative medicine, much attention has been paid to the advancement of material design through the promotion of endogenous stem cell differentiation toward a specific phenotype of interest by modulating physical attributes of the scaffold (including stiffness, topography and porosity). Advanced materials that select refined populations and direct their differentiation may provide a mechanism to achieve improved *in vivo* regeneration.

This chapter presents the current state of the art on identifying organ defects, applying feasibility testing of regenerative host-progenitor cell interactions and finally, assessment of endogenous repair or reconstructed urethra, cornea, bronchi, retina, skin, foetal tissues with a possibility of engineered tissue transplants widely used in routine clinical practice.

2. Clinical success in tissue reconstruction

2.1. Urethral reconstruction for large ureteral defects

In an interesting research area, a tissue engineering approach was used to restore function in a small group of boys with large urethral defects. Biopsies of bladder tissue were obtained, and smooth muscle and epithelial cells were isolated and cultured. The cells were then seeded onto tubular poly(glycolic acid)-poly(lactic-co-glycolic acid) scaffolds. Following culture for 1 week to ensure cell viability and matrix production, grafts were used for urethral reconstruction. The authors report that all five patients maintained functional flow rates at 36–72 months post-implantation, and biopsies confirmed that tissue organisation is similar to the native tissue.

2.2. Autologous limbal stem cells in corneal damage

Autologous stem cell therapy has been used to reverse corneal destruction due to burns. In one study, autologous limbal stem cells were cultured on fibrin to treat corneal damage in over 100 patients. At 10 years of follow-up, more than 75% of patients had a restored corneal epithelium layer. Clinical success was correlated to the percentage of functional stem cells (holoclone-forming) observed in the culture. Specifically, if cultures contained more than 3% holoclone-forming cells, clinical success was found in 78% of patients. On the other hand, if less than 3% holoclone-forming cells were found, success was seen in only 11% of the patients. This chapter elucidates the potential for limbal stem cells for corneal repair.

2.3. Platelet-rich plasma in orthopaedic sports injuries

Currently, use of platelet-rich plasma (PRP) to treat orthopaedic sports injuries is showing promise in this area. Several randomised control trials are underway, and early results have been recently published. Peerbooms *et al.* first reported the beneficial effects of PRP over corticosteroid injections for the treatment of lateral epicondylitis in a double-blind RCT. At 1 year, marked improvements were seen in both patient-reported outcomes as well as functional scores, and more recent data suggest these results persist up to 2 years.

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2.4. Tissue engineered airway

A 30-year-old woman with end-stage bronchomalacia was transplanted with a tissue-engineered airway. Cells and MHC antigens were removed from human donor trachea and then colonised by epithelial cells, and mesenchymal stem cell-derived chondrocytes were taken from the recipient. This graft was then used to replace the recipient's left main bronchus. The graft immediately provided the recipient with a functional airway, improved her quality of life and had a normal appearance and mechanical properties at 4 months. The patient had no anti-donor antibodies and was not on immunosuppressive drugs. This case suggested that autologous cells combined with appropriate biomaterials might provide successful treatment for patient with serious clinical disorders (**Figure 1**) [1].

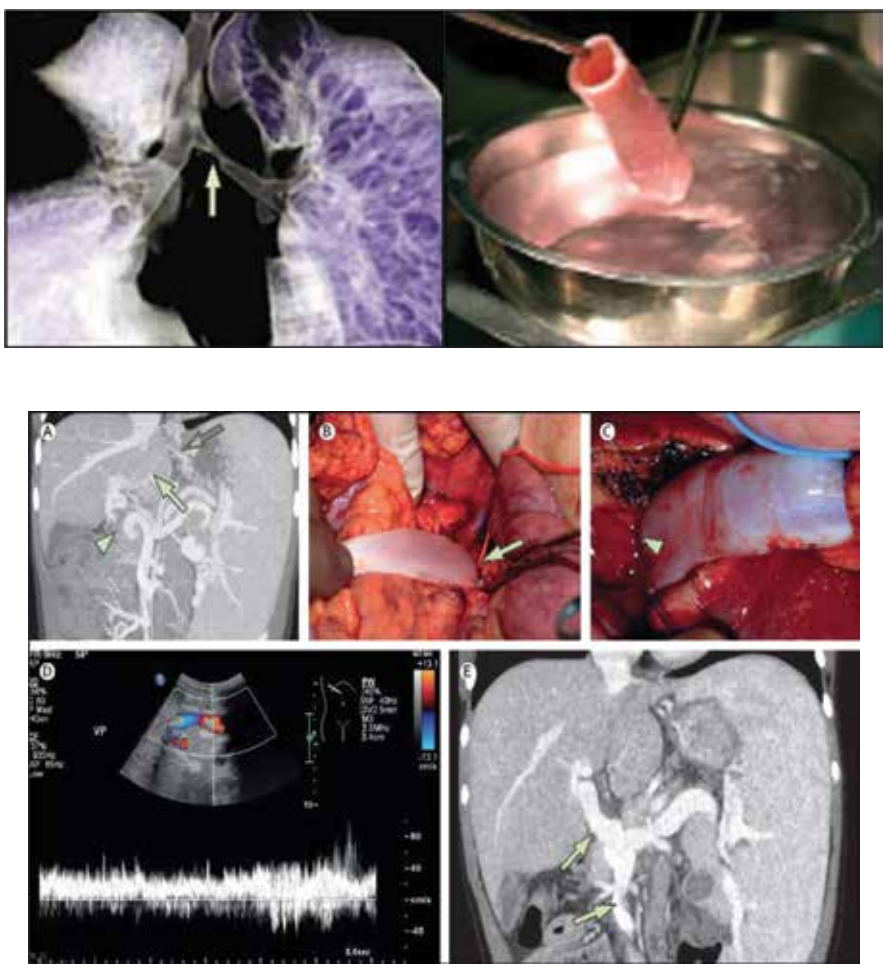

Figure 1. (A) Diagnostic CT angiography of intrahepatic portal flow (arrow), collaterals feeding the portal vein but no external portal vein in continuity (arrowhead) and enlarged spleen and collaterals around the oesophagus and in the liver hilum (open arrow). (B and C) Surgical correction showing graft attachment to the superior-mesenteric vein (arrow) and left portal vein (arrowhead). (D) Perioperative ultrasound with blood flows of 25–40 cm/s in the graft and intrahepatic portal vein. (E) Angiography showing a patent graft (arrows) 1 week after the surgery (reconstruction of 3–4 CT scans).

2.5. Transplant of genetically modified skin

Recently, Dr Michele De Luca, MD and his colleagues in Italy saved the life of a boy who had lost most of his epidermis by life-saving regeneration of virtually the entire epidermis. Patient's own epidermal stem cells were genetically modified to have functional copies of the gene that was mutant [2].

The boy was presented with blistered skin, which is the characteristic of junctional epidermolysis bullosa (JEB), and associated bacterial skin infections. Within days, about 60% of his epidermis had vanished. LAMB3 is one of three genes that encode a laminin protein that links the epidermis to the dermis.

The researchers cultured primary keratinocytes from a 4-cm^2 biopsy specimen from an unblistered area in the boy's left inguinal region. Then, they used retroviral vectors to introduce LAMB3 genes. The grafts grew. The genetic modification of those cells by introducing extra copies of the LAMB3 gene restored the epidermal machinery.

Three types of cultures grew into grafts from the boy's cells: holoclones, which are all stem cells; paraclones, which are specialised cells and meroclones, which are partly differentiated cells. The transgenic grafts harbour all three types of clones, but only the holoclones persist.

Procedures were done to cover the affected areas with genetically modified and regenerated grafts. The patches were up to several inches in diameter and were applied on a properly prepared wound bed. After engraftment, the epidermis looks basically normal, and that is also true at the molecular level in terms of the adhesion machinery that has been replaced. Within 5 weeks, the cells had covered about 80% of the boy's body. Even hairs grew, which usually does not happen with the conventional skin grafts. This case suggested that transgenic epidermal stem cells can regenerate a fully functional epidermis virtually indistinguishable from a normal epidermis, in the absence of related adverse events [2].

2.6. Amniotic-derived tissue grafts for enhanced skin regeneration

Amniotic tissues contain many regenerative cytokines, growth factors and extracellular matrix molecules, including proteoglycans, hyaluronic acid and collagens I, III and IV. Dehydrated amnion/chorion grafts are currently used to treat a variety of wounds such as diabetic foot ulcers and burns. In a recent study, Mowry et al. [3] found that all amniotic-derived tissue grafts appeared to stimulate improved healing over sham wounds (ungrafted wounds), evidenced by more normal appearing dermal matrix architecture, epidermal structure and maturity. In addition, the hypothermically stored amniotic membrane (HSAM) grafts promoted greater tissue regeneration than the dehydrated amnion/chorion (dHACM) meshed grafts, as measured by the presence of basket-weave collagen matrix and formation of follicles and glands (**Figures 2** and **3**).

Current studies point to several critical factors that may contribute to enhanced wound repair with amniotic-derived tissues including ECM, cytokines and growth factors, stem cells and

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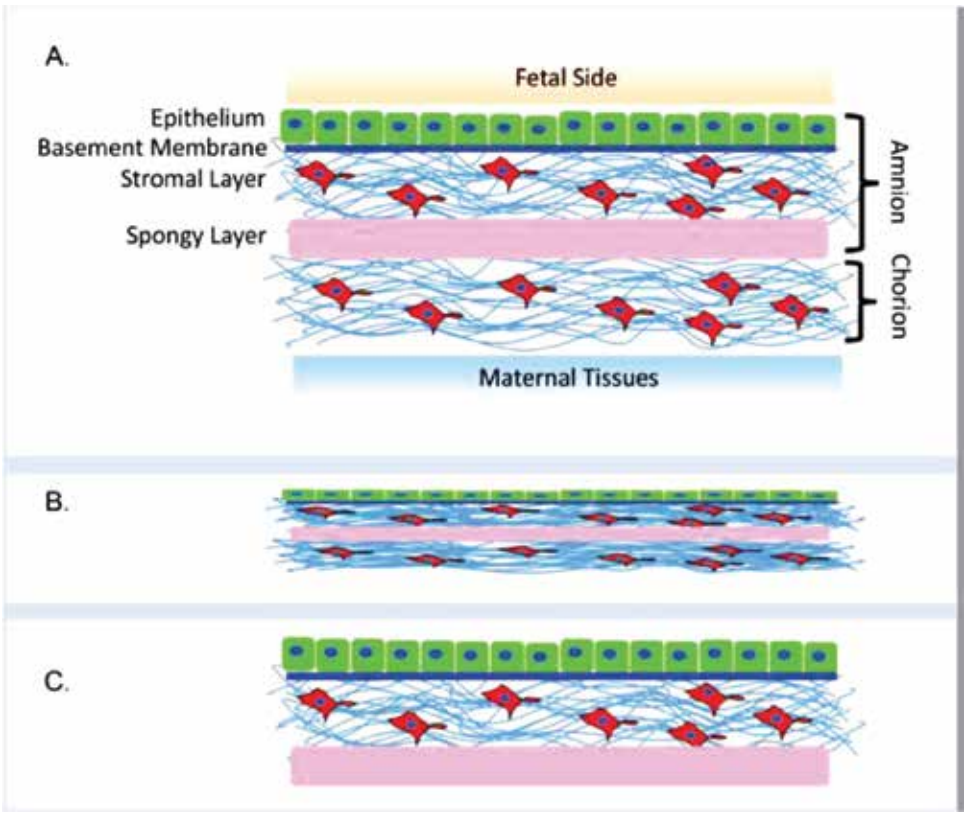

Figure 2. Schematic of the composition of amniotic-derived products. (A) Basic structure of native placental tissues outlining amniotic membrane, which interfaces with the foetus and major components such as epithelium, basement membrane, stromal layer and the spongy layer and the chorion which interfaces maternal tissues. Schematic of (B) dehydrated amnion/chorion and (C) hypothermically stored amniotic membrane.

immunomodulation of the wound environment [4]. This study highlights the importance of processing techniques and how they influence the quality of wound healing.

2.7. Stem cell therapy in retinal degeneration

Retinal pigment epithelial (RPE) cells derived from human embryonic stem cells can be safely transplanted into the eyes of the patients with retinal degeneration, with early signs of vision gain, according to pioneers in the field.

Two teams of researchers (Dr Eyal Banin from Israel and Dr. Ninel Z. Gregori from Florida) reported preliminary findings from phase 1 and phase 2 trials at the American Academy of Ophthalmology (AAO) 2017 Annual Meeting [5]. Patients had the dry form of age-related macular degeneration(AMD) or Stargardt disease and received injections of human embryonic stem cell (hESC)-derived RPE cells. Results of the studies are optimistic even though the

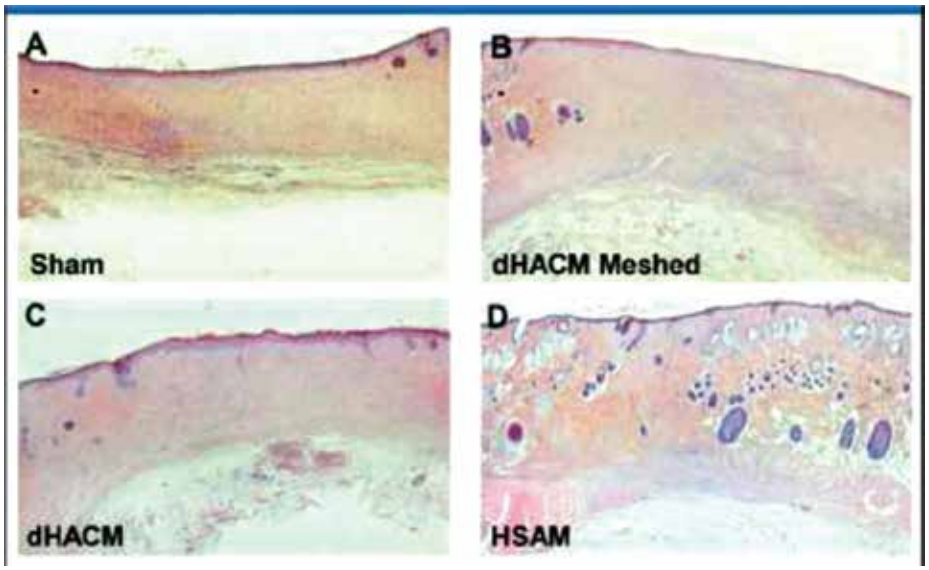

Figure 3. Twenty-one day representative histological sections: (A) sham; (B) dehydrated amnion-chorion (dHACM) meshed; (C) dHACM; and (D) hypothermically stored amniotic membrane (HSAM). Histologic summary: High-magnification images were used to assess the wounds qualitatively, and representative images are shown in this figure. Amniotic-derived grafts stimulated more robust healing and wound repair than sham wounds. Interestingly, HSAM-treated wounds displayed early epidermal formation, reconstitution of dermal appendages and a high degree of the basket-weave matrix, thus producing regenerated skin tissue that closely mimics unwounded skin at 21 days.

studies are very early and very small. This is just a first step in the long road toward making regenerative cell therapy a reality in macular and retinal degeneration.

Dysfunction and degeneration of RPE cells contribute to vision loss in AMD. In both studies, human embryonic stem cells were turned into RPE cells and injected into the sub-retinal space of the patients with retinal degeneration at a dose of 50,000–200,000 cells. The expectation is that, once in place, the new RPE cells will support or replace the patient's own failing RPE cells and boost the survival of photoreceptors.

3. Conclusion

With science and technical advancement in the regenerative medicine and tissue engineering, recent research data suggest detailed investigative studies on the mechanisms of endogenous injury, interactions at organ or tissue cell interface with activated endogenous progenitor cell populations, with a particular focus on mechanisms of how progenitor cells behave with cells of the immune system. Several reports suggest success in identifying pool of stem cells transplanted and actively participated in endogenous corneal, retinal, epicondyle, skin, bronchi and foetal organ repair/regeneration processes.

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Conflict of interest

Authors have no conflict of interest.

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References

[1] Macchiarini P et al. Clinical transplantation of a tissue-engineered airway. The Lancet. 2008;**372**(9655):2023-2030

[2] Stem Cell Gene Therapy Replaces Boy's Entire Epidermis. Medscape. Nov 8, 2017

[3] Mowry KC, Bonvallet PP, Bellis SL. Enhanced skin regeneration using a novel amniotic-derived tissue graft. Wounds: A Compendium of Clinical Research and Practice. 2017;**29**(9):277

[4] Litwiniuk M, Grzela T. Amniotic membrane: New concepts for an old dressing. Wound Repair and Regeneration. 2014;**22**(4):451-456

[5] Stem Cell Therapy Shows Promise for Retinal Degeneration. Medscape. Nov 16, 2017

Section 4

Tissue Engineering Mechanisms and Stem Cell Based Product Manufacturing

Chapter 11

Optimal Delivery Strategy for Stem Cell Therapy in Patients with Ischemic Heart Disease

Andrei Cismaru and Gabriel Cismaru

Additional information is available at the end of the chapter

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Abstract

Stem cell therapy is a new strategy for patients with ischemic heart disease. However, no consensus exists on the most optimal delivery strategy, but an important factor that determines the success of stem cell therapy is the choice of cell delivery route to the heart. Delivery strategy affects the fate of cells and subsequently influences outcome of procedure. Our review summarizes current approaches for administration of stem cells to the heart. Three most used approaches are intracoronary, intramyocardial, and epicardial injection. They have been widely used for delivery of different types of cells. There are several advantages of these stem cell administration approaches, but stem cell retention and stem cell survival rates are quite low using these methods, which might limit their therapeutic effects. Alternative attempts to improve current stem cell therapy methods are reviewed along with emerging new stem cell delivery approaches. The present chapter displays the current status on stem cell delivery techniques, their efficacy, and clinical success in different trials.

Keywords: stem cell therapy, delivery method, ischemic heart disease, intramyocardial injection

1. Introduction

Regenerative medicine with stem cell therapy has been tested in clinical trials in patients with ischemic heart disease [1]. The aim of this method is to induce growth of new blood vessels in the myocardium or replacement of damaged myocardial cells either directly by differentiation of stem cells or by a paracrine effect of cytokines secreted from the stem cells.

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When applied to the heart stem cell therapy, it has several important factors that might influence therapeutic success, including the properties of stem cells and type of the disease that affect the heart of host.

The choice of the delivery methods is also very important because this will affect the retention rate, survival, integration in the host, and functionality of stem cells. Therefore, delivery method influences the subsequent outcome of this new emerging treatment [2].

The aim of this review is to discuss methods of delivery in regenerative stem cell therapy in patients with ischemic heart disease. We will focus on current issues derived from conducted clinical trials and emerging new approaches.

2. Routes to the heart: advantages and disadvantages

Different approaches for delivering cells to the heart were developed and are utilized in preclinical and clinical current studies: intramyocardial (IM), intracoronary (IC), and intravenous (IV) (**Figure 1**) approaches were widely used, but no method currently meets the criteria of a perfect delivery method [3]. A stepwise approach of optimal delivery would consider if the patient needs open chest surgery. Surgical intramyocardial delivery is the most direct but

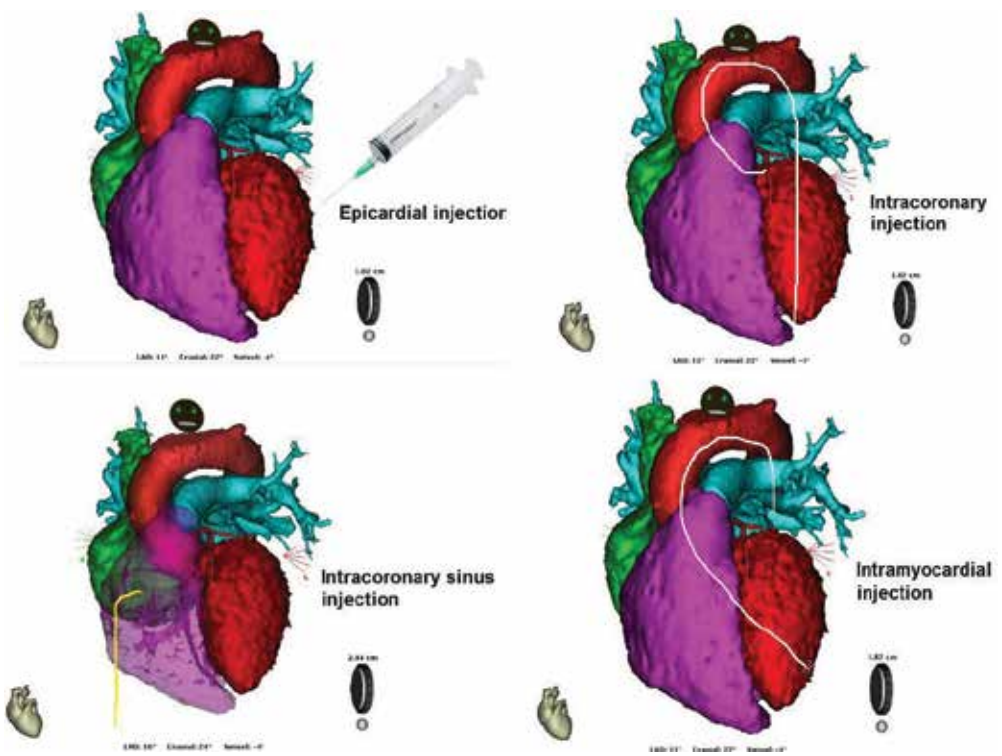

Figure 1. Major routes for delivering stem cells to the heart.

reserved for patients necessitating coronary artery bypass grafting (CABG) with direct thoracotomy. Catheter-based intramyocardial delivery is limited by the technology of catheters and mapping systems. In patients with recent myocardial ischemia and injury due to a significant stenosis or occlusion of a coronary artery, intracoronary artery delivery may not be the optimal route regardless of experimental results with the technique. In this case, intravenous or intracoronary venous injection is preferred.

Each of the techniques has its own advantages and disadvantages. The optimal method is still unclear. Studies have used either intravenous, intracoronary, or intramyocardial injection.

2.1. Chronology of optimal delivery developments

In 2001, the first rodent study with stem cells was published by Orlic et al., showing improvement in the heart function by regeneration [4]. Six months later, the first clinical trial on humans reported positive results for intracoronary injection of bone marrow stem cells after acute myocardial infarction [5].

Starting with the preclinical pioneering work of Orlic et al., intramyocardial and intravenous deliveries of BMC have been shown to improve left ventricular function in ischemic heart disease (**Table 1**).

- The transcoronary catheter-based procedure was first performed by Thompson et al. using a catheter in combination with an intravascular ultrasound imaging and demonstrated in swines that are feasible and safe [6]. Few years later, Siminiak et al. finished the first phase I clinical trial with his method confirming the feasibility and safety of the procedure in humans [7].
- The transendocardial technique was first used in a swine model by Fuchs et al. who demonstrated improvement of the cardiac function [8]. Since then, clinical studies have been published with positive results.
- The first trial of bone marrow stem cells in chronic ischemic cardiomyopathy was performed by Perin et al. [9]. He studied percutaneous transendocardial injection of stem cells and provided encouraging results.
- For the intravenous infusion, the safety and feasibility have been confirmed using a swine model [10] as well as later in a phase I clinical study on humans [11].
- In a swine model of myocardial injury, Vicario et al. [12] and Yokoyama et al. [13] demonstrated that retrograde coronary sinus injection does not produce significant hemodynamic changes and reported presence of autologous bone marrow stem cells in the myocardium.

2.1.1. Intravenous delivery

The systemic route of delivery is simple, not so invasive, but retention to the heart of stem cells is very low. Higher rates of retention were seen with mesenchymal cells because of their homing capacity. This route needs to be associated with methods of enhancing homing to the

Clinical trial	Administration	Reference
2002 TOPCARE-AMI	Intracoronary	[14]
2004 Perin et al.	Intracoronary	[47]
2004 BOOST	Intracoronary	[16]
2004 Chen et al.	Intracoronary	[38]
2004 Siminiak et al.	Epicardial	[48]
2005 Katritsis et al.	Intracoronary	[36]
2005 Erbs et al.	Intracoronary	[49]
2006 REPAIR-AMI	Intracoronary	[50]
2006 Assmus et al.	Intracoronary	[51]
2006 ASTAMI	Intracoronary	[19]
2006 Chen et al.	Intracoronary	[39]
2006 Fuchs et al.	Intracoronary	[34, 35]
2006 Beeres et al.	Intracoronary	[52]
2006 Hendrikx	Epicardial	[53]
2006 Kang et al.	Intracoronary	[54]
2007 Losordo et al.	i.m.	[30]
2007 Katritsis et al.	Intracoronary	[36]
2007 Mohyeddin-Bonab et al.	i.m.	[55]
2007 Beeres et al.	Intracoronary	[56]
2007 Ahmadi et al.	i.m.	[57]
2008 Diederichsen et al.	Intracoronary	[58]
2008 FINCELL	Intracoronary	[59]
2008 Menasche et al.	Epicardial	[60]
2008 HEBE	Intracoronary	[61]
2008 Beeres et al.	Intracoronary	[62]
2009 Hare et al.	i.v.	[11]
2009 Van Ramshorst et al.	i.m.	[63]
2009 BALANCE	Intracoronary	[64]
2009 MYSTAR	Intracoronary/i.m.	[65]
2009 REGENT	Intracoronary	[66]
2010 Kastrup et al.	i.m.	
2010 Strauer et al.	Intracoronary	[67]
2011 Yerebakan et al.	Epicardial	[68]

Clinical trial	Administration	Reference
2011 Williams et al.	i.m.	[69]
2011 Perin et al.	i.m.	[70]
2011 Povsic et al.	i.m.	[71]
2011 Duckers et al.	i.m..	[72]
2011 Hirsch et al. HEBE	Intracoronary	[73]
2011 Roncali et al. BONAMI	Intracoronary	[74]
2011 Traverse et al. Late TIME	Intracoronary	[75]
2011 Quyyumi	Intracoronary	[76]
2011 Tuma	Retrograde coronary	[45]
2011 Moreira	Retrograde coronary	[46]
2012 Makkar et al. CADUCEUS	Intracoronary	[77]
2013 Bolli et al. SCIPIO	Intracoronary	[78]
2013 Vrtovec	Intracoronary	[79]
2013 Huang	Intracoronary	[80]
2013 Kurbonov et al.	Intracoronary	[81]
2013 Forcillo et al	Via CABG + i.m.	[82]
2014 Assmann et al.	Via CABG+epicardial	[83]
2014 Nasseri et al	i.m.	[84]
2014 Brickwedel et al.	Via CABG	[85]
2014 Hong	Intracoronary + retrograde coronary sinus	[86]
2015 Hao	Intracoronary	[87]
2015 Chang	Intracoronary	[88]
2015 Gao	Intracoronary	[89]
2015 Fiarresga	Intracoronary	[90]
2015 Helseth	Intracoronary	[91]
2015 Eirin	Intrarenal	[92]
2015 Lee	Intracoronary	[93]
2016 Tseliou	Intracoronary	[94]
2017 Xiao	Intracoronary	[95]

i.m., intramyocardial.

Table 1. Cell delivery in studies and publications on myocardial infarction and chronic ischemic heart failure.

ischemic tissue because most of the stem cells show localization in other tissues with only a small part of injected cells engrafted at the level of the heart [14–17]. This method may be limited to acute myocardial infarction and not be suitable for chronic ischemic heart disease because it relies on physiologic homing signals present few days after an acute myocardial infarction.

2.1.2. Intracoronary delivery

An attractive method is intracoronary infusion because it can disseminate relatively uniformly cells to the entire region infused [18]. It is also widely available, less invasive than intramyocardial method, and it is used in numerous clinical trials [14–22]. Intracoronary infusion implies a percutaneous approach typically through the femoral artery with a standard balloon catheter. The catheter used for delivery infuses cells to the myocardial regions in which blood supply is preserved. For injection, balloon occlusion is needed in order to reduce the washout into the systemic circulation and increase adhesion of cells and transmigration of the infused cells to the myocardium [19–24].

One reason to use this method is the familiarity between interventional cardiologists. The method is less invasive than injection directly in the myocardium and requires the equipment standardly found in a catheterization laboratory. The method enables a relatively homogeneous dissemination of stem cells to the target area.

The disadvantage of this method is that some adult stem cells such as autologous cardiosphere-derived cells [25] or mesenchymal stem cells (MSCs) [26, 27], produced microvascular occlusion after intracoronary delivery, raising concerns over the use of this method delivery in patients with ischemic heart disease. Actually, the diameter of autologous cardiosphere-derived cells and mesenchymal stem cells is around 20 μm, which could exceed the diameter of some arterioles [28]. The great majority of clinical studies use this approach to inject smaller cells such as bone marrow mononuclear cells. Another disadvantage could be the poor retention rate following intracoronary injection. This is caused by the loss of a high proportion of stem cells in the systemic circulation during several minutes.

2.1.3. Intramyocardial-transendocardial injection

Transendocardial injection is performed percutaneously and is less invasive than epicardial injection [29, 30] but more invasive than intracoronary injection. The access is made by puncture of the femoral artery or vein (transseptal approach) and then the catheter is passed in the left ventricle. An electroanatomical map of the left ventricle is realized in order to navigate inside the cavity and position the injection catheter to specific areas (**Figure 2**).

Electroanatomical mapping system permits left ventricular mapping and guides injection to the border zone between healthy and necrosed endocardium [26, 31–35]. Intramyocardial injection of bone marrow-derived stem cells and also angiogenic genes has been reported to be safe in terms of arrhythmia or death [26, 30–35]. On the other hand, intramyocardial injection of skeletal myoblasts has been shown to have a pro-arrhythmogenic effect [26, 31–35].

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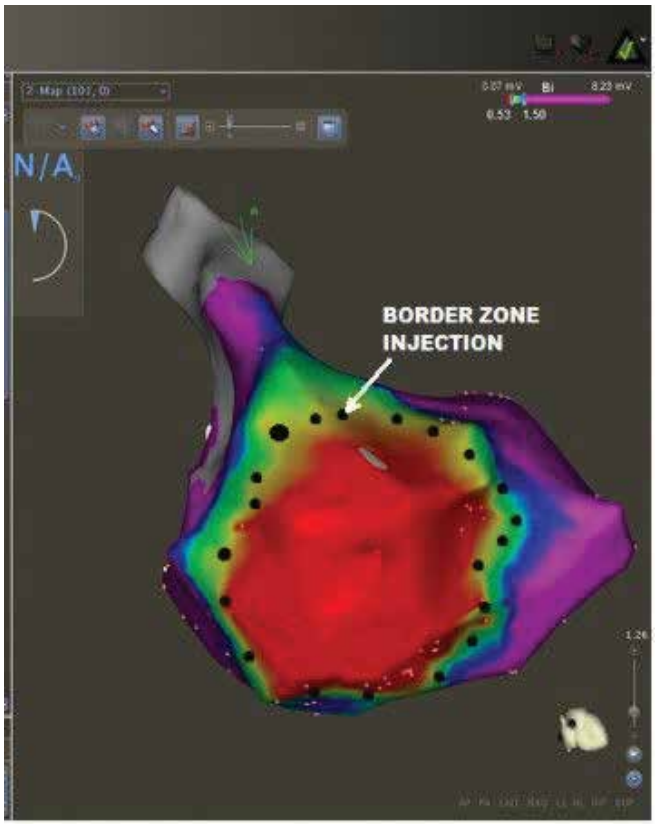

Figure 2. Intramyocardial injection at the border zone between healthy myocardium and dense necrosis. The border zone is probably hibernating but viable myocardium.

In a comparative study [26], intramyocardial technique was compared to intravenous delivery and intracoronary method. Intramyocardial injection of MSCs was better than intracoronarian delivery in terms of blood flow to the myocardium. In dogs and also in pigs micro-infarctions were seen probably due to cell micro-thrombi which created obstruction to the blood flow when injected through intracoronary path. In humans, this complication was not seen [36–39].

One disadvantage of intramyocardial injection is the formation of islet-like clusters of stem cells at this level. *Another disadvantage of intramyocardial injection is association with a higher risk of ventricular arrhythmias than with other methods of deliver* [26].

2.1.4. Intramyocardial-transepicardial injection

Epicardial injection is performed using a needle-syringe system under direct visualization of the operated heart. This method is the most used in preclinical research using animal models.

The intramyocardial delivery of stem cells can be achieved by direct injection after open thoracotomy (sternotomy or left thoracotomy). Most of the time this method is used in conjunction

with cardiac surgery such as coronary artery bypass grafting (CABG) or left ventricular assist device (LVAD) [40–42]. Myocardial retention rates have been similar to those of a transendocardial approach [1, 3, 43]. The advantage is that in some types of necrosis: intramyocardial, epicardial, or combined, targeted tissue can be reached only through a direct epicardial access.

Other options not so invasive like open chest thoracotomy have been tried: minimally invasive lateral thoracotomy using thoracoscopic injection to the epicardium and minimally invasive subxifoidian technique using robotic devices [44].

2.1.5. Intracoronary sinus injection

Another technique to inject stem cells to the heart of animals or humans [45–47] is through the coronary sinus or tributary veins. The percutaneous approach is made through the femoral vein. With the use of a catheter that passes in the right atrium, one can cannulate the coronary sinus and access the middle cardiac vein, the great cardiac vein, or other tributaries of the coronary sinus. For injection, balloon occlusion is needed in order to reduce the washout into the systemic circulation. Comparing with the intracoronary injection, this method has the advantage of lower risk of coronary embolism and injection can be made even in areas with low arterial supply.

3. Electroanatomical mapping using the NOGA system

Intramyocardial injection is the method by which stem cell suspension is directly injected in the myocardium using a needle. This method needs electroanatomical mapping in order to identify the zone of necrosis. Intramyocardial injection enables stem cells to be targeted into this localized area. In patients with new or old myocardial infarction, stem cells are usually injected at the border zone of the infarct with the healthy tissue. This area has a relatively good blood supply to ensure stem cell survival compared to the infarcted area with no blood flow. Intramyocardial injection permits to target zones even with low blood flow. Intracoronary injection instead requires a normal flow through a coronary artery. Intramyocardial injection enables cells to be delivered to areas with a limited vascularity. Because this method has no risk of coronary embolism, larger cells can be used, like skeletal myoblasts, mesenchymal stem cells, and others.

The current system for intramyocardial delivery is the NOGA® XP Cardiac Navigation System (Biologics Delivery Systems Group of Cordis Corporation, a Johnson & Johnson Company). This system is able to perform electromechanical mapping of both left ventricle and right ventricle. Electromechanical mapping permits clear delineation of the targeted area and precise deployment of the therapeutic product [3]. This delivery method has proved to be feasible in the presence of chronic ischemic heart disease and acute myocardial infarction (within 10 days after infarction). The system incorporates an injection catheter and the real-time reconstruction of the heart's endocardial surface in three dimensions using collection of points with spatial, electrophysiologic, and mechanical information.

By this, a left ventricular endocardial map is obtained with electromechanical information that characterizes the underlying tissue and permits to navigate into the heart. This real map helps to precisely localize the injection catheter at the level of necrosis, border zone of necrosis or healthy tissue. The map is constructed by acquiring multiple points at different locations in the left ventricle from base to apex, from inferior to anterior, and from septal to lateral. These anatomical points with electrical value are gated to a surface electrocardiogram. Ultra-low magnetic fields are generated by the system using a triangular magnetic pad placed under the patient and other three patches positioned on the thorax of the patient. Each point sample contains electrical information about local activity such as unipolar voltage and local contractility such as linear local shortening. The resulting tridimensional map of the left ventricle gives information about electromechanical properties of the myocardium and is able to distinguish between ischemic areas (which have low lineal local shortening and preserved unipolar voltage) from infarcted areas (low linear local shortening and low unipolar voltage) [1].

3.1. Transfemoral approach with the NOGA system

Most of the studies using NOGA tridimensional system for intramyocardial delivery used the conventional right femoral approach in order to reach the endocardium. However there are cases with tortuous, angled right iliac artery, it implies difficulty in advancing the mapping catheter to the left ventricle and manipulating it inside the cavity. When the right femoral artery cannot be used, then left artery can be tried, and in case of failure, other arteries (like radial) or veins (femoral vein with transseptal approach) can be accessed.

3.2. Transradial approach with the NOGA system

The NOGA mapping system was designed for the transfemoral approach. This precludes its use in patients who have peripheral vascular disease with intense calcified and tortuous iliac or femoral arteries. Because NOGA catheters are advanced into the LV without using a guidewire, manipulation can be difficult inside the heart especially when arteries are tortuous and do not permit free rotation and angulation of the catheter. Manipulation can be even more challenging when using a stiffer injection catheter. Although there has been no formal recommendation concerning alternative approaches in patients with peripheral arterial tortuosity, there are reports showing a benefit of using radial artery or femoral vein and transseptal approach in this category of patients. In the case of tortuous iliac or femoral arteries, the brachial access could be taken in order to avoid procedural complications.

4. Perspectives for 2017–2020

To date, there are still many unanswered questions regarding delivery methods in stem cell therapy. Some of these questions will be answered in the ongoing trials. Larger double-blinded placebo-controlled clinical trials are needed to elucidate whether it is trans-aortic or transseptal approach. It is the best method to reach different zones of endocardial necrosis. In

cases of intramyocardial or epicardial necrosis, epicardial approach should be compared with endocardial one. Brachial can be an option for patients who have peripheral vascular disease with impossible femoral approach. Novel biomedical *engineering* is used in several emerging technologies for delivering stem cells to the heart. These include transplantation of stem cells as tissue-engineered constructs [80]. All these delivery options will permit a more individual and personalized stem cell treatment strategy in patients with ischemic heart disease.

5. Conclusions

There are several methods of cell delivery to the heart. However, none of these are perfect for every type of ischemic disease or every stem cell type. Advantages and disadvantages of each technique will help in tailoring the treatment protocol for every individual patient and will aid in planning future clinical trials. Combining these techniques (e.g. intracoronary artery + intracoronary sinus injection) could reduce washout and increase adhesion to the necrosed area. Emerging new approaches need to be also developed for the future of clinical success using stem cell therapy administered for ischemic heart disease.

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References

[1] Perin EC, Lopez J. Methods of stem cell delivery in cardiac diseases. Nature Clinical Practice Cardiovascular Medicine. 2006;**3**(Suppl 1):S110-113

[2] Perin EC, Silva GV, Assad JA, Vela D, Buja LM, Sousa AL, Litovsky S, Lin J, Vaughn WK, Coulter S, Fernandes MR, Willerson JT. Comparison of intracoronary and transendocardial delivery of allogeneic mesenchymal cells in a canine model of acute myocardial infarction. Journal of Molecular and Cellular Cardiology. 2008;**44**:486-495

[3] Psaltis PJ, Zannettino AC, Gronthos S, Worthley SG. Intramyocardial navigation and mapping for stem cell delivery. Journal of Cardiovascular Translational Research. 2010;**3**(2):135-146

[4] Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. Nature. 2001;**410**:701-705

[5] Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002;**106**:1913-1918

[6] Thompson CA, Nasseri BA, Makower J, et al. Percutaneous transvenous cellular cardiomyoplasty: A novel nonsurgical approach for myocardial cell transplantation. Journal of the American College of Cardiology. 2003;**41**(11):1964-1971

[7] Siminiak T, Fiszer D, Jerzykowska O, et al. Percutaneous trans-coronary-venous transplantation of autologous skeletal myoblasts in the treatment of post-infarction myocardial contractility impairment: The POZNAN trial. European Heart Journal. 2005;**26**(12):1188-1195

[8] Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, Weissman NJ, Leon MB, Epstein SE, Kornowski R. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. Journal of the American College of Cardiology. 2001:**37**(6):1726-1732

[9] Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation. 2003; **107**:2294-2302

[10] Halkos ME, Zhao ZQ, Kerendi F, et al. Intravenous infusion of mesenchymal stem cells enhances regional perfusion and improves ventricular function in a porcine model of myocardial infarction. Basic Research in Cardiology. 2008;**103**(6):525-536

[11] Hare JM, Traverse JH, Henry TD, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. Journal of the American College of Cardiology. 2009;**54**(24):2277-2286

[12] Vicario J, Piva J, Pierini A, et al. Transcoronary sinus delivery of autologous bone marrow and angiogenesis in pig models with myocardial injury. Cardiovascular Radiation Medicine. 2002;**3**(2):91-94

[13] Yokoyama SI, Fukuda N, Li Y, et al. A strategy of retrograde injection of bone marrow mononuclear cells into the myocardium for the treatment of ischemic heart disease. Journal of Molecular and Cellular Cardiology. 2006;**40**(1):24-34

[14] Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Döbert N, Grünwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation. 2002;**106**:3009-3017

[15] Fernández-Avilés F, San Román JA, García-Frade J, Fernández ME, Peñarrubia MJ, de la Fuente L, Gómez-Bueno M, Cantalapiedra A, Fernández J, Gutierrez O, Sánchez PL,

Hernández C, Sanz R, García- Sancho J, Sánchez A. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circulation Research. 2004;**95**:742-748

[16] Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone marrow cell transfer after myocardial infarction: the BOOST randomized controlled clinical trial. Lancet. 2004;**364**:141-148

[17] Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, Kalantzi M, Herbots L, Sinnaeve P, Dens J, Maertens J, Rademakers F, Dymarkowski S, Gheysens O, Van Cleemput J, Bormans G, Nuyts J, Belmans A, Mortelmans L, Boogaerts M, Van de Werf F. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: Double-blind, randomised controlled trial. Lancet. 2006;**367**:113-121

[18] Li Q, Guo Y, Ou Q, Chen N, Wu WJ, Yuan F, O'Brien E, Wang T, Luo L, Hunt GN, Zhu X, Bolli R. Intracoronary administration of cardiac stem cells in mice: A new, improved technique for cell therapy in murine models. Basic Research in Cardiology. 2011;**106**:849-864

[19] Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grøgaard HK, Bjørnerheim R, Brekke M, Müller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. The New England Journal of Medicine. 2006;**355**:1199-1209

[20] Erbs S, Linke A, Schuler G, Hambrecht R. Intracoronary administration of circulating blood-derived progenitor cells after recanalization of chronic coronary artery occlusion improves endothelial function. Circulation Research. 2006;**98**:e48

[21] Abdel-Latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, Zuba-Surma EK, Al-Mallah M, Dawn B. Adult bone marrow-derived cells for cardiac repair: A systematic review and meta-analysis. Archives of Internal Medicine. 2007;**167**:989-997

[22] Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary bone marrow cell transfer after myocardial infarction: Eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrow transfer to enhance ST-elevation infarct regeneration) trial. Circulation. 2006;**113**:1287-1294

[23] Schachinger V, Erbs S, Elsasser A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. The New England Journal of Medicine. 2006;**355**:1210-1221

[24] Kastrup J. Gene therapy and angiogenesis in patients with coronary artery disease. Expert Review of Cardiovascular Therapy. 2010;**8**(8):1127-1138

[25] Johnston PV, Sasano T, Mills K, Evers R, Lee ST, Smith RR, Lardo AC, Lai S, Steenbergen C, Gerstenblith G, Lange R, Marbán E. Engraftment, differentiation, and functional

benefits of autologous cardiosphere-derived cells in porcine ischemic cardiomyopathy. Circulation. 2009;**120**:1075-1083

[26] Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, Palasis M, Wilensky RL. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. European Heart Journal. 2006;**27**:1114-1122

[27] Vulliet PR, Greeley M, Halloran SM, MacDonald KA, Kittleson MD. Intra-coronary arterial injection of mesenchymal stromal cells and microinfarction in dogs. Lancet. 2004;**363**:783-784

[28] Kassab GS, Fung YC. Topology and dimensions of pig coronary capillary network. American Journal of Physiology. 1994;**267**(1 pt 2):H319-H325

[29] Krause K, Jaquet K, Schneider C, Haupt S, Lioznov MV, Otte KM, Kuck KH. Percutaneous intramyocardial stem cell injection in patients with acute myocardial in farction: First-inman study. Heart. 2009;**95**(14):1145-1152

[30] Losordo DW, Schatz RA, White CJ, Udelson JE, Veereshwarayya V, Durgin M, et al. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angin a. Circ ulation. 2007;**115**(25):3165-3172

[31] Fernandes S, Amirault JC, Lande G, et al. Autologous myoblast transplantation after myocardial infarction increases the inducibility of ventricular arrhythmias. Cardiovascular Research. 2006;**69**:348-358

[32] Smits PC, van Geuns RJ, Poldermans D, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: Clinical experience with six-month follow-up. Journal of the American College of Cardiology. 2003;**42**:2063-2069

[33] Veltman CE, Soliman OI, Geleijnse ML, et al. Four-year followup of treatment with intramyocardial skeletal myoblasts injection in patients with ischaemic cardiomyopathy. European Heart Journal. 2008;**29**:1386-1396

[34] Fuchs S, Kornowski R, Weisz G, et al. Safety and feasibility of transendocardial autologous bone marrow cell transplantation in patients with advanced heart disease. American Journal of Cardiology. 2006;**97**:823-829

[35] Baldazzi F, Jorgensen E, Ripa RS, et al. Release of biomarkers of myocardial damage after direct intramyocardial injection of genes and stem cells via the percutaneous transluminal route. European Heart Journal. 2008;**29**:1819-1826

[36] Katritsis DG, Sotiropoulou PA, Karvouni E, et al. Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium. Catheterization and Cardiovascular Interventions. 2005;**65**:321-329

[37] Katritsis DG, Sotiropoulou P, Giazitzoglou E, et al. Electrophysiological effects of intracoronary transplantation of autologous mesenchymal and endothelial progenitor cells. Europace. 2007;**9**:167-171

[38] Chen SL, Fang WW, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. American Journal of Cardiology. 2004;**94**:92-95

[39] Chen S, Liu Z, Tian N, et al. Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery. Journal of Invasive Cardiology. 2006;**8**:552-556

[40] Zhao Q, Sun Y, Xia L, Chen A, Wang Z. Ran- domized study of mononuclear bone marrow cell transplantation in patients with coronary surgery. The Annals of Thoracic Surgery. 2008;**86**(6):1833-1840

[41] Dib N, Michler RE, Pagani FD, Wright S, Kereiakes DJ, Lengerich R, et al. Safety and feasibility of autolo- gous myoblast transplantation in patients with ischemic cardiomyopathy. Circulation. 2005;**112**(12):1748-1755

[42] Ota T, Patronik NA, Schwartzman D, Riviere CN, Zenati MA. Minimally invasive epicardial injections using a novel semiautonomous robotic device. Circulation. 2008;**118**(14 suppl 1):S115-S120

[43] Dib N, Dinsmore J, Lababidi Z, White B, Moravec S, Campbell A, et al. One-year follow-up of feasibility and safety of the first U.S., randomized, controlled study using 3-dimensional guided catheter-based delivery of autologous skeletal myoblasts for ischemic cardiomyopathy (CAuSMIC study). JACC Cardiovascular Interventions. 2009;**2**(1):9-16

[44] Arom KV, Ruengsakulrach P, Jotisakulratana V. Intramyocardial angiogenic cell precursor injection for cardiomyopathy. Asian Cardiovascular and Thoracic Annals. 2008;**16**(2):143-148

[45] Tuma J, Fernanadez-Vina R, Carrasco A, Castillo J, Cruz C, Carrillo A, et al. Safety and feasibility of percutane- ous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina. Journal of Translational Medicine. 2011;**9**(1):183

[46] Moreira Rde C, Haddad AF, Silva SA, Souza AL, Tuche FA, Oliveira MA, et al. Intracoronary stem-cell injection after myocardial infarction: Microcirculation substudy. Arquivos Brasileiros de Cardiologia. 2011;**97**(5):420-426

[47] Perin EC, Dohmann HF, Borojevic R, et al. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. Circulation. 2004;**110**:II213-218

[48] Siminiak T, Kalawski R, Fiszer D, Jerzykowska O, Rzezniczak J, Rozwadowska N, et al. Autologous skeletal myoblast transplantation for the treatment of postinfarction myocardial injury: Phase I clinical study with 12 months of follow-up. American Heart Journal. 2004;**148**(3):531-537

[49] Erbs S, Linke A, Adams V, et al. Transplantation of blood-derived progenitor cells after recanalization of chronic coronary artery occlusion: First randomized and placebo-controlled study. Circulation Research. 2005;**97**(8):756-762

[50] Schachinger V, Erbs S, Elsasser A, et al. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: Final 1-year results of the REPAIR-AMI trial. European Heart Journal. 2006;**27**(23): 2775-2783

[51] Assmus B, Honold B, Schächinger V, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. The New England Journal of Medicine. 2006;**355**:1222-1232

[52] Beeres SLMA, Bax JJ, Kaandorp TAM, et al. Usefulness of intramyocardial injection of autologous bone marrow-derived mononuclear cells in patients with severe angina pectoris and stressinduced myocardial ischemia. American Journal of Cardiology. 2006;**97**:1326-1331

[53] Hendrikx M, Hensen K, Clijsters C, et al. Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation: Results from a randomized controlled clinical trial. Circulation. 2006;**114**(1Suppl):I101-107

[54] Kang HJ, Lee HY, Na SH, et al. Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony–stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction. The MAGIC Cell-3-DES randomized, controlled trial. Circulation. 2006;**114**:I-145-I-151

[55] Mohyeddin-Bonab M, Mohamad-Hassani MR, Alimoghaddam K, et al. Autologous in vitro expanded mesenchymal stem cell therapy for human old myocardial infarction. Archives of Iranian Medicine. 2007;**10**(4):467-473

[56] Beeres SLMA, Bax JJ, Dibbets-Schneider P, et al. Intramyocardial injection of autologous bone marrow mononuclear cells in patients with chronic myocardial infarction and severe leftventricular dysfunction. American Journal of Cardiology. 2007;**100**:1094-1098

[57] Ahmadi H, Baharvand H, Ashtiani SK, et al. Safety analysis and improved cardiac function following local autologous transplantation of CD133(+) enriched bone marrow cells after myocardial infarction. Current Neurovascular Research. 2007;**4**(3):153-160

[58] Diederichsen ACP, Møller JE, Thayssen P, et al. Effect of intracoronary injection of bene marrow cells in patients with ischaemic heart failure. The Danish Stem Cell study—Congestive Heart Failure trial (DanCell-CHF). European Journal of Heart Failure. 2008;**10**:661-667

[59] Huikuri HV, Kervinen K, Niemela M, et al. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, and restenosis after thrombolytic therapy of acute myocardial infarction. European Heart Journal. 2008;**29**:2723-2732

[60] Menasche P, Alfieri O, Janssens S, et al. The myoblastAutologous grafting in ischemic cardiomyopathy (MAGIC) trial: First randomized placebo-controlled study of myoblast transplantation. Circulation. 2008;**117**(9):1189-1200

[61] Hirsch A, Nijveldt R, van der Vleuten PA, et al. Intracoronary infusion of autologous mononuclear bone marrow cells in patients with acute myocardial infarction treated with primary PCI: Pilot study of the multicenter HEBE trial. Catheterization and Cardiovascular Interventions. 2008;**71**:273-281

[62] Beeres SLMA, Lamb HJ, Roes SD, et al. Effect of intramyocardial bone marrow cell injection on diastolic function in patients with chronic myocardial isc hemia. Journal of Magnetic Resonance Imaging. 2008;**27**:992-997

[63] Van Ramshorst J, Bax JJ, Beeres SLMA, et al. Intramyocardial bone marrow cell injection for chronic myocardial ischemia: A randomized controlled trial. The Journal of the American Medical Association. 2009;**301**(19):1997-2004

[64] Yousef M, Schannwell CM, Kostering M, et al. The BALANCE study: Clinical benefit and long-term outcome after intracoronary autologous bone marrow cell transplantation in patients with acute myocardial infarction. Journal of the American College of Cardiology. 2009;**53**:2262-2269

[65] Gyongyosi M, Lang I, Dettke M, et al. Combined delivery approach of bone marrow mononuclear stem cells early and late after myocardial infarction: The MYSTAR prospective, randomized study. Nature Clinical Practice Cardiovascular Medicine. 2009;**6**:70-81

[66] Tendera M, Wojakowski W, Ruzyłło W, et al. Intracoronary infusion of bone marrow-derived selected CD34+CXCR4+ cells and non-selected mononuclear cells in patients with acute STEMI and reduced left ventricular ejection fraction: Results of randomized, multicentre Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENT) Trial. European Heart Journal. 2009;**30**:1313-1321

[67] Strauer BE, Yousef M, Schannwell CM. The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic HeSRt failure: The STAR-heart study. European Journal of Heart Failure. 2010;**12**:721-729

[68] Yerebakan C, Kaminski A, Westphal B, Donndorf P, Glass A, Liebold A, et al. Impact of preoperative left ventric- ular function and time from infarction on the long-term benefits after intramyocardial CD133+ bone marrow stem cell transplant. The Journal of Thoracic and Cardiovascular Surgery. 2011;**142**(6):1530.e3-1539.e3

[69] Williams AR, Trachtenberg B, Velazquez DL, McNiece I, Altman P, Rouy D, et al. Intramyocardial stem cell injection in patients with ischemic cardiomyopathy/novelty and s i gnifi cance. Circulation Research. 2011;**108**(7):792-796

[70] Perin EC, Silva GV, Henry TD, Cabreira-Hansen MG, Moore WH, Coulter SA, et al. A randomized study of transendocardial injection of autologous bone marrow mononuclear cells and cell function analysis in ischemic heart. American Heart Journal. 2011;**161**(6):1078-1087

[71] Povsic TJ, O'ConnorCM, Henry T, Taussig A, Kereiakes DJ, Fortuin FD, et al. A double-blind, randomized, controlled, multicenter study to assess the safety and cardiovas- cular

effects of skeletal myoblast implantation by catheter deliv- ery in patients with chronic heart failure after myocardial infarction. American Heart Journal. 2011;**162**(4):654. e1-662.e1

[72] Duckers HJ, Houtgraaf J, Hehrlein C, Schofer J, Waltenberger J, Gershlick A, et al. Final results of a phase IIa, rando- mised, open-label trial to evaluate the percutaneous intra-myocardial transplantation of autologous skeletal myoblasts in congestive heart failure patients: The SEISMIC trial. EuroIntervention. 2011;**6**(7):805-812

[73] Hirsch A, Nijveldt R, van der Vleuten PA, Tijssen JGP, van der Giessen WJ, Tio RA, et al. Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myo- cardial infarction treated by primary percutaneous coronary inter- vention: Results of the randomized controlled HEBE trial. European Heart Journal. 2011;**32**(14):1736-1747

[74] Roncalli J, Mouquet F, Piot C, Trochu J-N, Le Corvoisier P, Neuder Y, et al. Intracoronary autologous mononu- cleated bone marrow cell infusion for acute myocardial infarction: Results of the randomized multicenter BONAMI trial. European Heart Journal. 2011;**32**(14):1748-1757

[75] Traverse JH, Henry TD, Ellis SG, Pepine CJ, Willerson JT, Zhao DXM, et al. Effect of intracoronary delivery of autologous bone marrow mononuclear cells 2 to 3 weeks following acute myocardial infarction on left ventricular function. JAMA: The Journal of the American Medical Association. 2011;**306**(19):2110-2119

[76] Quyyumi AA, Waller EK, Murrow J, Esteves F, Galt J, Oshinski J, et al. CD34(+) cell infusion after ST elevation myocardial infarction is associated with improved perfusion and is dose dependent. American Heart Journal. 2011;**161**(1):98-105

[77] Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LEJ, Berman D, et al. Intracoronary cardiosphere- derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. The Lancet. 2012;**379**(9819):895-904

[78] Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, et al. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): Initial results of a randomised phase 1 trial. The Lancet. 2011;**378**(9806):1847-1857

[79] Chiu LL, Iyer RK, Reis LA, Nunes SS, Radisic M. Cardiac tissue engineering: Current state and perspectives. Frontiers in Bioscience: A Journal and Virtual Library. 2012;**17**: 1533-1535

[80] Zhao X, Huang L. Cardiac stem cells. A promising treatment option for heart failure. Experimental and Therapeutic Medicine. 2013;**5**:379-383

[81] Kurbonov U, Dustov A, Barotov A, et al. Intracoronary infision of autologous CD133 cells in myocardial infarction and tracing TC99MIBI scintigraphy of the heart areas involved in cell homing. Stem Cells International. 2013;**2013**:ID 582527. http://dx.doi.org/10.1155/2013/582527

[82] Forcillo J, Stevens L-M, Mansour S, et al. Implantation of CD133+stem cells in patients undergoing coronary bypass surgery: IMPACT-CABG pilot trial. Canadian Journal of Cardiology. 2013;**29**:441-447. ID 582527. http://dx.doi.org/10.1155/2013/582527

[83] Assmann A, Heke M, Kropil P, et al. Laser-supported CD133+ cell therapy in patients with ischemic cardiomyopathy: Initial results from a prospective phase I multicenter trial. PLoS ONE. 2014;**9**:Ide101449

[84] Nasseri BA, Ebell W, Dandel M, et al. Autologous CD133+bone marrow cells and bypass grafting for regeneration of ischaemic myocardium: The Cardio133 trial. European Heart Journal. 2014;**35**:1263-1274

[85] Brickwedel J, Gulbins H, Reichenspurner H. Long-term follow-up after autologous skeletal myoblast transplantation in ischaemic heart disease. Interactive Cardiovascular and Thoracic Surgery. 2014;**18**:61-66

[86] Hong SJ, Hou D, Brinton TJ, Johnstone B, Feng D, et al. Intracoronary and retrograde coronary venous myocardial delivery of adipose-derived stem cells in swine infarction lead to transient myocardil trapping with predominant pulmonary distribution. Catheterization and Cardiovascular Interventions. 2014;**83**:E17-E25

[87] Hao L, Hao J, Fang W, Han C, Zhang W, Wang X. Dual isotope simultaneous imaging to evaluate the effects of intracoronary bone marrow derived mesenchymal stem cells on perfusion and metabolism in canines with acute myocardial infarction. Biomedical Reports. 2015;**3**:447-452

[88] Chung ES, Miller L, Patel AN, et al. Changes in ventricular remodeling and clinical status during the year following a single administration of stromal cell-derived factor 1 non-viral gene therapy in chronic heart failure patients: the STOP-HF randomized Phase II trial. European Heart Journal. 2015;**36**:2228-2238

[89] Gao LR, Chen Y, Zhang NK, Yang XL, et al. Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: double-blind, randomized controlled trial. BMC Medicine. 2015;**13**:162. doi: 10.1186/s12916-015-0399-z

[90] Fiarresga A, Mata MF, Cavaco-Goncalyes S, et al. Intracoronary delivery of human mesenchymal/stromal stem cells: Insight from coronary microcirculation invasive assessment in a swine model. PLoS ONE. 2015;**10**:e139870. 2015 Jul 10;13:162. doi: 10.1186/s12916-015-0399-z

[91] Helseth R, Opstad T, Solheim S, et al. the effect of intracoronary stem cells injection on markers of leukocyte activation in acute myocardial infarction. Cardiology Research. 2015;**6**:209-215

[92] Eirin A, Zhu XY, Ferguson CM, et al. Intra-renal delivery of mesenchymal stem cells attenuate myocardial injury after reversal of hypertension in porcine renovascular disease. Stem Cell Research & Therapy. 2015;**6**:7

[93] Lee HW, Lee HC, Park JH, et al. Effects of intracoronary administration of autologous asipose tissue-derived stem cells on acute myocardial infarction in porcine model. Yonsei Medical Journal. 2015;**56**:1522-1529

[94] Tseliou E, Kanazawa H, Dawkins J, et al. Widespread myocardial delivery of heart-derived stem cells by nonoclusive triple-vessel intracoronary infusion in porcine ischemic cardiomyopathy: Superior attenuation of adverse remodeling documented by magnetc reasonnce imaging and histology. PLoS ONE. 2016;**11**:e0144523

[95] Xiao W, Guo S, Gao C, Dai G, Gao Y, et al. A randomized comparative studyon the efficacy of intracoronary infusion of autologous bone marrow mononuclear cells and mesenchymal stem cells in patients with dilated cardiomyopathy. International Heart Journal. Int Heart J 2017;**58**:238-244. doi: 10.1536/ihj.16-328

Chapter 12

Landscape of Manufacturing Process of ATMP Cell Therapy Products for Unmet Clinical Needs

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Additional information is available at the end of the chapter

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Abstract

Immune cell therapies have been studied in numerous clinical trials using Advanced Therapy Medicinal Products (ATMP) against a number of diseases having no or inadequate alternative therapies available, for example, various cancer types, cerebral stroke, cardiac infarction, severe autoimmune disorders, or chronic infections. Despite the enormous number of positive observation in *ex vivo* or animal studies, convincing results in clinical studies remain scanty. The chapter presents a survey and reveals that the manufacturing of immune cells especially for clinical trials is until today primarily performed using archaic, scarcely controlled, and incomparable processes and methods. A deeper characterization of *ex vivo* expanded immune cells is urgently needed not only on the level of a few receptors and ligands on the cell surface but also with respect to the ever-contained subtypes in an expanded immune cell population, the pattern of secreted effector molecules, and their amounts over time and influences from *in vivo* components on them.

Keywords: immune cells, cell therapy, expansion technologies, T cells, TIL, NK cells, MSC, GMP production, ATMP

1. Introduction

Immune cells have been the key players as well as glamor of active clinical research of the current decade. T-lymphocytes (T cells), tumor-infiltrating lymphocytes (TIL), chimeric antigen receptor T cells (CAR-T cells), natural killer cells (NK cells), mesenchymal stem/stromal cells (MSC) from bone marrow, umbilical cord blood, umbilical cord tissue layers, placenta, and adipose tissues are the main objects studied in immune cell therapies for various diseases. Publications from numerous preclinical studies and developments on isolation, expansion,

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activation, and phenotyping of the different immune cells are increasing exponentially over the last years. Numerous clinical trials have been conducted or are running, evaluating immune cells as novel Advanced Therapy Medicinal Products (ATMP) therapy against number of diseases having no or inadequate alternative therapies available (see **Table 1**) [2]. There is a strong belief that progress for many severe, life-threatening diseases with bad prognosis that happens in various fulminant cancer types, cerebral stroke, cardiac infarction, severe autoimmune disorders, or chronic infections can either be effectively treated or even cured by immune cell therapy when applied optimally in combinations with other conventional therapies. It is not clear if this belief is justified at this juncture with adequate evidence. It is crucial to critically analyze this question for an insight to address the stumbling blocks to make significant advances in this field.

Isolated immune cells consistently display effectiveness against cancer cells, micro-organisms, inhibition of inflammation parameters, etc., in *ex vivo* test systems and often also in *in vivo* mouse models. The enormous number of positive observations are very encouraging and propel the immune cell research field and drives growing numbers of clinical trials. However, convincing results in clinical studies remain scanty over decades despite the wider engagement and R&D investments [3–6]. Several tumor-infiltrating lymphocytes (TIL) as well as treating therapy-resistant hematopoietic and solid tumor cancers with specific activated CAR-T cells show long-lasting benefits in otherwise grim cases [7, 8]. In cancer of the hematopoietic system, immune cell therapy has demonstrated its real potency and has become an effective standard therapy [9]. Leukemia and similar forms of cancer of the hematopoietic system can be cured with disease-free survival or without progression in a high percentage through the transplantation of bone marrow cells from healthy and genetically compatible (allogeneic) donors.

Immune cell type	Clinical trials in total	Open clinical trials	Clinical trials		
			Europe total	US total	China total
T-lymphocytes	2343	723	116	401	184
TIL	77	27	4	17	0
CAR-T-lymphocytes	170	123	7	40	78
NK cells	407	140	10	49	62
MSC	339	134	34	29	39
BM-MSC	104	46	12	12	7
UC-MSC	54	24	2	0	16
UCB-MSC	7	3	0	0	2
AT-MSC	20	6	1	1	2

Total number of clinical trials worldwide and in several geographical regions as registered on www.clinicaltrials.gov/, accessed on Feb 15, 2017 [1]. TIL: tumor-infiltrating lymphocytes; CAR-T-lymphocytes: chimeric antigen receptor lymphocytes; MSC: mesenchymal stromal cells; BM-MSC: bone marrow MSC; UC-MSC: umbilical cord MSC; AT-MSC: adipose tissue MSC.

Table 1. Current ongoing clinical studies with selected immune cells.

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It is important to reflect into the realities of the limited clinical success in contrary to the promising *in vitro* or *ex vivo* findings. All the inadequate, inconsistent clinical outcomes observed using immune cells *in vivo* following *ex vivo* expansion and all the knowledge gained with immune cells in the numerous clinical trials and individual or small clinical studies using hospital exemptions might have to do with underestimating the key issues with respect to identity, quality, potency, and functionality of the used cells. All the characteristics of the cells as well as influences from production technology, characterization methods, etc. are seldom taken into broader account in robust ways before cells are infused as therapeutics. This will be highlighted in the following sections.

2. Requirements and existent challenges in producing immune cells for cell therapy

Looking on the methods, technologies, specific equipment, and analytical tools, it is obvious that cell expansion technologies suited for producing specific immune cells for individual immune cell therapy are the weak side of the story. The actual knowledge on the potentials of immune cells is so far only scarcely translated into practical technical solutions, broadly available methods, congruent with the capabilities. That is true for standardized and reproducible expansion of defined immune cell preparations as well as for estimates and methods of measuring specific functionalities of an expanded immune cell population against tumor cells, infections, or inflammations. Moreover, there are also missing generally available methods and techniques for fast and precise measurement of homogeneity of a cell population, of characteristics of sub populations, and single cells [10–13]. All these aspects appear to be widely responsible for the limited progress by these very promising new therapies. Several key issues are critical determinants for ATMP cell therapy which are enumerated below.

- Isolation of specific immune cells from blood or tissues and initial expansion steps are performed with broadly differing methods. The procedures need to be better standardized and harmonized with the validation of the composition of the starting populations of immune cells.
- Ideally, expansion of immune cells should be started with a pure population of specific immune cells. When a mixture of cells (like PBMC) or a piece of tissue are taken, isolation of cells should be performed in a defined procedure consistently.
- Immune cells for individual cell therapies must be produced in standardized, reproducible, and GMP conform processes. Amounts of 10^9 to more than 10^{10} cells of a pure immune cell population are assumed as a single optimal therapeutic dose. The manufacturing process with newer available technologies for each cell type is yet to be established coherently for the clinical trials.
- Standardized and consistent production processes need to be dynamically controlled and documented during the entire culturing process. Only a few production technologies are currently on the market fulfilling all of the abovementioned requirements and thus are capable of manufacturing individual cell therapeutics as ATMP.

- To ensure reproducibility of immune cell production for cell therapies, process conditions have to be controlled, evaluated, documented, and validated. Continuous dynamic control of temperature, pH, and pO_2 in the medium during the immune cell expansion process is indispensable. Glucose and lactate concentration as lead substances for substrates and metabolites should also be under steady control during processing.
- All immune cells can be expanded in a common basic medium. However, until now supplementing with serum or thrombocyte lysate is still indispensable. For optimized growth over a prolonged period of time and mass production of the different immune cell specimen, supply with nutrients, gases, and supplements should normally take place in a dynamic, homogenous, and stress-free process to maintain differentiation status, phenotype, and function of those cells within physiological ranges.
- Irradiated feeder cells are used to achieve an initial expansion with low numbers of isolated effector cells and to increase cell expansion when using insufficient technical equipment (e.g., wells or flasks with uncontrolled processing). Appropriate novel technologies can possibly eliminate the use of feeder cells.
- Important criteria of immune cells for therapies are a deeper marker profiling and standard estimates for chemokine and cytokine production efficiency (paracrine factors). Both together are decisive measures for the potency and functional power of immune cell populations/subpopulations for the intended effects. Standardized and comparable values as correlate measures should be mandatory for immune cell therapeutics which need to be established.
- Subpopulations or even monoclonal immune cells can be produced by guiding an *ex vivo* expansion process through specific activation/inhibition/triggering/priming the cells. Respective coating/fixed antibodies at bioreactor surface, O_2 concentration in culture conditions (hypoxic/normoxic/hyperoxic), flow dynamics of medium have to be explored, compared, and optimized for each condition.
- Immune cells for therapies have to fulfill all the conditions as well as national regulatory requirements and international standards for approval for clinical trial use as investigational medicinal product (IMP) and/or market authorization as ATMP. All the manufacturing steps of an individual immune cell preparation must still be performed in a Clean Room A containment for most processes used for production that consist of more or less open steps.
- Current international regulatory standards are in process of evolution as ATMP cannot simply follow the standard of a chemical compound which is a fixed inert molecule, whereas a cell is a living dynamic entity with too many variables and dynamic potentials. It is being considered that regulations on individual cell therapeutics might be registered as distinct category and might be oriented on indications. This needs to mature in course of time to bring ATMP cell therapy for real clinical use in routine practice.
- Faster scientific progress, earlier availability, access, and affordable prices of immune cell ATMP can only be achieved when advanced production technologies can be utilized or further developed to realize these desired objectives.

3. Techniques and methods used for isolation and expansion of immune cells

Blood from patients or donors is a convenient starting material for cell purification and expansion. **Table 2** shows the number of different immune cells contained in a 200 ml sample of peripheral blood. A simple gradient centrifugation of a blood sample is normally taken to obtain "peripheral blood mononuclear cells" (PBMC). *Ex vivo* expansion of the total fraction of T cells, NK, and other cell types within PBMC is possible and is the most commonly used technique to provide starting material for subsequent *ex vivo* expansion of the different immune cell types.

Apheresis is a standard practice to obtain a larger number of CD3+ cells as a starting material for CAR-T cell production with the goal of obtaining a minimum of 0.6×10^9 to a target of 2×10^9 CD3+ T cells [24]. Apheresis has the advantage and choice of extracting one or more components of the whole blood with help of the appropriate device and return the rest of the donation to the donor. Standard leukapheresis and use of anti-CD25 magnetic bead resulted in a yield of 130×10^6 CD4+ CD25+ T regulator (T_{reg}) cells that could be expanded 8.3-fold over three weeks before a dose of 1×10^9 can be used for clinical trials designed to control HLA-mismatched GvHD or organ transplantation rejection [18]. Although the apheresis technology is available in clinical research, no advantages have been comparatively established to that of peripheral blood as a source. Routine production of pure immune cell specimen by apheresis is much more expensive (due to the costly procedure and the infrastructure required to carry out the intervention as well as subsequent large amounts of antibodies

Immune cell types#	Number of cells in 200 ml of human blood sample ($\times 10^6$)	Clones of differentiation commonly determined for identification	Reference
Neutrophils	400–1300	CD11b+CD16+CD66b+	[14, 15]
Lymphocytes	200–600	T cells, B cells (CD3−CD19+)	[16]
T cells, total	100–400	CD3+CD4+CD8+CD56−	[17]
Naive T cells	40–160	CD3+CD25+CD45RA+CD45RO−CD127+CCR7+CD62L	[17]
Memory T cells	40–160	CD3+CD56−CD45RA−CD45RO+CCR7+CD62L+	[17]
T regulatory cells	5–20	CD3+CD4+CD25+CD127−	[18, 19]
NK cells	16–80	CD3−CD16+CD56+	[20]
NKT cells	0.4–5	CD1d+CD4+CD161+	[21]
Dendritic cells	4–12	CD11c+HLA−DR+CD3−CD19−CD80+CD86+	[22]
Monocytes	40–180	CD14+CD16+CD64+	[23]

Table 2. Prevalence of immune cells in blood of healthy donors and surface marker normally used for their identification.

needed for purification of specific cell types from the large number of cells). Another study from Germany reported that ~10^{10} PBMC from leukapheresis result in about 8% of NK cell yield using Clinimacs (Miltenyi) for further GMP therapeutic expansion [20]. Difference between *ex vivo* expanded cells for cytokine-induced cell therapy for hepatocarcinoma from apheresis and PBMC-derived cells has been reported [25], but this needs further evaluation for a comparative conclusion for various cell types. The initial procedure of cell collection may have an influence on the biological effects of the final cell product.

The whole PBMC fraction consists mainly of T cells (naive and memory CD3+/CD56− T cells, mostly of central T memory type). A minor part of the PBMC contained NK cells identified as CD3−/CD56+ cells. Isolation and *ex vivo* expansion of CD3+/CD56− T cells or CD3−/CD56+ NK cells (and even other specific cell types in patients' PBMC) to bigger numbers does not pose a big problem. It is crucial that T cells are only effective against a cancer cell type or an infectious agent when specifically activated prior to infusion. Hence, T cells, NK cells, and other PBMC-derived cells have to be separated in the first instance and then specifically activated/primed during the expansion process. Presentation of peptides or epitopes from tumor cells or micro-organisms to T cells *ex vivo*, directly or mediated by dendritic cells, and subsequent *ex vivo* expansion was evaluated over prolonged period of time with no or only limited success in *in vivo* efficiency. *Ex vivo* expanded NK cells showed similar negative results, even when applied to patients in greater numbers [26]. It was recognized that (in blood of patients contained) T regulatory cells do strongly inhibit lytic power of T cells and NK cells. However, this seems not the only reason for disappointing results with only T cell or NK cell fractions.

It is being increasingly recognized that particularly in cancer treatment, breakthrough can be achieved with specific activation and expansion of sub-clones of T cells and/or defined NK cells *ex vivo*. The PBMC-derived cells must be specifically stimulated during expansion which can only be realized by sophisticated integration of steps in the process (either positive or negative selection of unwanted cells through antibodies; enhanced growth of cells of interest through speeding up their mitotic division). In case of T cells as much as possible, effector T cell clones should be present at least in the expanded population with capabilities to persist and execute its desired functions [27]. Expanded NK cells should express high cytotoxicity against patient's tumor cells or infected cells. Specific activation has to be induced during the initiation steps by specific coatings of the surface of the culture flasks/container/bioreactor as well as by addition of different cytokines and growth factors in the medium during the subsequent expansion phase [6, 28, 29]. Cultivation of larger numbers of immune cells under those conditions is often challenging and can scarcely be realized in culture flasks.

A general belief is that a basic prerequisite for a therapeutic application of immune cell types is the provision of adequate number of cells. It is assumed that about 10^{10} of effective immune cells must be used for treating cancer or infections [1]. A tumor or metastasis of 1 cm^3 size yields around 10^9 cells. In the common cytotoxicity assays using effector versus target cells, five to tenfold more effector cells are often needed to destroy one tumor or infected cell. However, such *ex vivo* results might lead into wrong direction. It is crucial that the cells should have strong binding ability and be armed with specific cytotoxicity against the target cells. Meanwhile, it is apparent that it is important to know how to select, activate,

trigger, and/or prime and expand immune cells *ex vivo*. Single T cell clones being trained already *in vivo* against mutated patients' tumor cells or infected cells are normally present in PBMC only in very low numbers, but they can destroy tumor or infected cells manifold [30]. Concerning T cells, identifying and isolation of specific sub-clones and *ex vivo* expansion of those clones should get more attention in the future research. Besides there are strong hints that treatment with immune cells can be successful only when a systemic response of the total immune system in a patient is achieved along with the local response in the lesions [31]. These are extremely important aspects in the context of production technology enabling to manufacture adequate effective immune cell type for optimal treatment of a patient and a holistic analytical follow up of systemic profiling of the patient in order to gain further insights to iteratively make the process more efficient.

In recent times, the isolation of TIL from tissue and/or microenvironment has proven a much more promising way to get access to T cells being already specifically activated by antigens/epitopes shed from/presented by cells of solid tumors or metastases they are originating from. Generation of TIL seems no longer a problem. Resected parts of tumor tissues taken by tumor biopsies or from the microenvironment of a tumor is the method of choice, and different suited techniques have been published [32, 33]. It is yet not evident whether devices for standardized processing for cell suspension from the initial tumor tissue by mechanical dispersion and/or additional treatment with enzymes are of advantage [34, 35]. Expansion of TIL even in bigger numbers is possible; up to 10^{10} can be grown in common media supplemented with serum or thrombocyte lysate and specific cytokines (see **Table 4**). Long time expanded TIL often contain CD3+/CD4+/CD8+TIL. Deeper FACS analyses make likely that sub-clones are contained which are primed and directed against some single mutated clones [30].

Currently, there has been a major interest in genetically manipulated T cells, which can be transduced with chimeric antigen receptors (CAR). These CAR-T cells express this single chain antigen-binding domain (scFv), which ideally binds to a tumor-associated antigen (TAA). The CAR-T cell/tumor-binding reaction induces an activation signal in the T cells strong enough to destroy tumor cells completely, and contrary to normal T cells, the cytotoxic power of CAR-T cells is not suppressed when CAR-T cells are administered *in vivo*. However, CAR-T cells often elicit a dangerous cytokine storm. This potential adverse effect and the difficult complex and costly construction of efficient CAR-T cells seems to inhibit fast progress with this promising cell therapy [41–43].

Natural killer (NK) cells are getting more and more attention in the ATMP field, fighting cancer and infections since these innate immune cells can be successfully expanded not only in greater numbers but also in high degree of purity [41–43]. In contrast to T cells, NK cells do not feature immunological incompatibility when administered in haploidentical or even allogenic clinical trials. It is, however, important that NK cells in such settings are totally free from T cells. Advanced production technology makes NK cells attractive to use them in broader clinical perspective. Pure NK cells have shown nearly no unwanted side effects in clinical trials even when administered in high doses. The modern production processes deliver NK cells with enhanced functionalities (high cytotoxicity against many cancer cells in *ex vivo* tests, enhanced paracrine production). Pure NK cells can be manufactured in an

easy-to-handle closed system as ATMP in clinical settings near to patients [44, 45]. A particular advantage is that mass amounts of individual NK cells can be produced in a relatively inexpensive way due to low costs for selection, medium, activation, compared with other functionalized immune cells. NK cells can be expanded 2000–50,000-fold in designated perfusion bioreactors, whereas that has by far not been achieved in culture flasks. Adjuvant treatment of stem cell-transplanted patients with pure NK cells becomes a common clinical practice. NK cells isolated from donor blood and expanded effectively avoid infections and GvHD when applied immediately following transplantation.

Common sources for isolation of MSC are bone marrow aspirate, cord blood, and pieces of umbilical cord/placenta tissue/adipose tissue. MSC were originally identified in the 1970s from cellular suspensions from spleen and bone marrow by their capacity to adhere to plastic—which is still the standard form to culture MSCs and also by their ability to form colonies from single cells (explanted *ex vivo*), their fibroblast-like appearance and their capacity to differentiate into fat, cartilage, and bone. MSC are defined by surface markers of CD105, CD90, and CD73 expression, yet not CD45, CD34, and CD14 as of the consensus of ICSCT working group [46]. Recently, ICSCT also have defined a broad consensus of the international standards for harmonized potency assays to boost the clinical development of ATMP MSC therapy for many unmet clinical needs despite different tissue sources and disparate culture expansion protocols. Three preferred analytic methods in a matrix assay approach, namely, quantitative RNA analysis of selected gene products; flow cytometry analysis of functionally relevant surface markers, and protein-based assay of secretome have been proposed to reflect on the immunomodulatory potential of the ATMP cells for different clinical therapeutics as well as to evolve the regulatory landscape for the sake of the progress in the field [47].

Several techniques are employed for liposuction used for adipose tissue-derived stromal cell collections [48]. Processed lipoaspirate (LPA) contains multipotent cells that can be an alternate stem cell source to bone-marrow-derived MSCs. LPA contains stromal vascular fraction (SVF) containing a number of different cell types such as adipose stromal cells (ASC), pericytes, endothelial cells, fibroblasts, preadipocytes, and hematopoietic stem cells. ASC have differentiation potential to myogenic, osteogenic, chondrogenic, or adipogenic on culturing with specific induction media [49]. SVF contains a lot of vascular cells and hematopoetic cells that have to be eliminated before expansion of remaining MSC.

For the isolation of MSC adherence of these cells, plastic surfaces are used. The usual procedure is to put the starting material into culture flasks or discs. After 10–20 days, nonadherent cells are washed out, and the adhered MSC colonies are passaged, suspended in a fresh medium, and seeded in new flasks for further expansion. Thus, expanded MSC have been used in all clinical trials (see **Table 7**). The unavoidable detaching procedures of MSC at passaging influence the receptor quality of MSC. Long-term cultivation of big numbers of MSC in bioreactors is possible and the provision of large seeding areas avoids unwanted differentiation of expanded MSC [54–57]. Procedures have been worked out to proceed directly into the expansion phase of MSC. Outgrowth and isolation of MSC can be successfully performed by giving BM aspirate into the sterile plastic vessels of perfusion bioreactors. MSC from BM aspirate are not only diverse tumor tissue preparations but can also be placed directly in ZRP meander perfusion bioreactors. MSC colonies or outgrown TIL from tumor tissue pieces can then further be

expanded to big numbers of both cell types in the same bioreactor system under totally closed conditions (unpublished results with ZRP meander type bioreactors, Zellwerk, Germany).

The reports about the ever-used methods in the clinical trials do not always contain complete descriptions of the processes and show inconsistencies. However, in general, it can be seen that high-fold expansion as well as big numbers of cells have only been achieved when irradiated feeder cells are used. This might be tolerable during a developmental phase of immune cell therapeutics but should be overcome in case of ATMP manufacturing. In ZRP perfusion bioreactors it was shown that individual immune cell specimen can be expanded to therapeutic amounts without irradiated feeder cells.

4. Quantity and quality of T cells, TIL, and CAR-T used in acknowledged clinical trials

T cells are the most powerful immune cells. Despite the immense research on these cells enriching our deeper knowledge into T cell biology as well as their kinetics in health and diseases, the different T cell subsets are yet not routinely used as cell therapeutics. This is at least partly due to the complex nature of immune cells and many unsolved technical problems to produce and handle them. In **Tables 3–5**, some parameters have been compiled giving insight into methods and technologies as well as expansion success and purity of the different T cell types being used in clinical trials. The chosen examples in the tables are representative of the field and enlighten the diversity of production processes and produced cell specimen, and it explains probably in part the inconsistent and predominantly unsatisfying clinical results with more or less identical cell specimens.

Vaccination with dendritic cells being *ex vivo* treated with proteins or peptides from tumor cells or micro-organisms as well as present target-specific peptides to T cells *ex vivo*, directly

Cell source; supplements; activation	Expansion device used	Cultivation time (days)	Expansion by x-fold	Cell harvest	Cell purity (%)	Reference
PBMC/apheresis isolated by αCD3+ coated dynabeads, exp with IL2/αCD28	Wave system	13	101	1.37×10^{11} T cells	98.5% CD3+	[27]
PBMC	Flask	14	169	33×10^{8}	62%	[58]
PBMCs/apheresis CD25+Tregs depl, Stim with autol DC pulsed MART peptides, suppl IL2, IL7, IL21, CD8+ CTL sorted, exp Rapid Exp Prot	Flask	42	n.a.	n.a.	n.a.	[59]

Table 3. Manufacturing processes of T cells in clinical trials (n.a.= not available).

Cell source; supplements; activation	Expansion device used	Cultivation time (days)	Expansion by x-fold	Cell harvest	Cell purity (%)	Reference
TIL, IL2, serum, feeder cells	Bag	>14	1041	5×10^{10}	n.a.	[36]
TIL, IL2, serum, feeder cells, anti-CD3	Flask Wave	24	72 228	0.4×10^{10} 1.5×10^{10}	35 + 52 CD4 + CD8 35 + 63	[37]
TIL (6x GRex10) TIL, IL2,	GRex Flask	29	180 170	NA	62–72 CD3+ CD8+ 62	[38]
TIL, IL2, feeder cells, anti CD3	Bag Wave	>21	1259 1130	4.5×10^{10} 1.5×10^{10}	CD3+: F = W CD4+: F > W CD8+: F < W	[39]
TIL, IL2, feeder cells, anti CD3	Flask Wave	>14	1433 5576	NA	>97	[40]
TIL, serum, feeder cells, anti CD3, IL2, IL 15, IL21	ZRP Meander bioreactor	>20	5000	2×10^{9}	CD8: ~60% CD4: 38% (High% Tem)	Own data, not published

Table 4. Manufacturing processes of TIL in clinical trials (n.a.= not available).

Cell source; supplements, activation	Expansion device used	Cultivation time (days)	Expansion by x-fold	Cell harvest	Cell purity (%)	Reference
PBMC, anti-CD3, IL-2, feeder cells, serum	Well plates	14–24	n.a.	n.a. (infusion: $\leq 10^{10}$)	n.a.	[41]
PBMC, anti-CD3, anti-CD28	Bag	12	10.6	n.a.	CD3+: >95	[43]
PBMC, anti-CD3, IL-2, IL-15	WAVE	35	n.a.	10^{9}	n.a.	[33]
PBMC, serum, feeder PBMCs, IL-2	Bag	≤48	15.000	~3×10^{11}	CD4+: ≤82 CD8+: ≤85	[60]

Table 5. Manufacturing processes of CAR-T-cells in clinical trials (n.a.= not available).

or mediated by dendritic cells, and subsequent *ex vivo* expansion is used over a long time, and there are even a few cell therapeutics on the market. However, adjuvant treatment in different conditions of cancer in clinics show only limited success. In recent times, the isolation of TILs from tissue and/or microenvironment of solid tumors and expansion of TILs *ex vivo* has proven a much more promising way to get specific activated T cell in larger amounts.

From **Table 3**, it can be observed that the fold expansion in flasks and Wave bioreactor is similar, although the total number of T cells harvested differs enormously. Whether the (apheresis

derived) large number of generally stimulated T cells was of advantage for the treated patients could not be evidently established from published literatures. The example of CTL expansion and therapeutic use did not state details of the expansion process with missing information.

To get effective, functional T cells out of PBMC expansion process have to be "conditional" (activation of sub-clones by specific cancer/micro-organism-derived structures; naive cell/cell and cell/matrix contact; preferred or suppressed growth of a cell population/sub population by specific cytokine/growth factor/antibody stimulation; controlled gas and nutrient supply).

It is obvious from **Table 4** that the expansion of TIL even in bigger numbers is possible, up to more than 10^{10} TIL might be grown in flasks as well as in bags, G-rex flasks, Wave bioreactors, or ZRP perfusion bioreactors. Media and supplements are qualitatively similar. In all examples, use of feeder cells seem to be essential. Looking closer into **Table 4**, the fold expansion differs over a wide range. This implies that strongly differing numbers of TIL must have been isolated. G-Rex flasks is of advantage for TIL expansion by providing high O_2 concentration near to the sedimented TIL that enhances growth. In own studies (not published) of ZRP meander bioreactors, O_2 (Zellwerk, Germany) concentration can be up-regulated to find an optimal O_2 value in the medium for TIL growth.

Although a lot of clinical trials with TIL have been performed so far, there is not much information about clinical efficiency of TIL (with an exception of late-stage metastatic melanoma). Expanded TIL over a prolonged period often contains CD3+/CD4+/CD8+CD25+cells in different ratios. Deeper FACS analyses make likely that subclones that are contained are primed and directed against some single mutated clones. Again, it must be emphasized that even with TIL stronger standardized cell production processes, better characterization of contained clones, more comparable preclinical data as well as professional designed clinical trials are needed.

The expansion of several CAR-T cell specimen seems not to be a problem (**Table 5**). Blood-derived natural T cells were used for CAR transduction. Expansion to large numbers of the CAR-T cells was achieved by using the same interleukin activation and culturing procedures as in the case of normal T cells. Some of the advanced centers are engaged in optimizing newer technologies including Miltenyi's CliniMACS Prodigy as well as Zellwerk's ZRP technology system.

5. State-of-the-art with *ex vivo* expanded NK cells utilized in clinical trials

NK cells are getting more and more attention in fighting cancer and infections since it succeeded to expand these immune cells not only in huge amounts but also in pure quality [4–6, 61]. In contrast to T cells, NK cells do not show immunological incompatibility when administered in haploidentical or even allogenic clinical trials. It is, however, important that NK cells in such settings are totally free from T cells. In **Table 6**, a selection of clinical studies is listed, among them are those that are performed by known clinical research groups. It can be deduced that bigger numbers of NK cells can be produced in culture flaks as well as in some cell culture equipment consisting of plastic bags or vessels (VueLife static culturing in bags; wave system using different bags, mixing of medium by slow shaking movement of the bag). The bag systems are closed

systems, and some of the equipment are provided with regulating and/or steering elements for gasing, measurement of pH, pO_2, and medium temperature is obligatory. However, changing bag volumes and upscaling within a cultivation run is not easy and possible with these systems. One main advantage of NK cells is the good compatibility of this immune cell specimen. NK cells can be expanded up to 50,000-fold in suited perfusion bioreactors (**Table 6**), whereas this is by far not achieved in culture flasks. Adjuvant treatment of stem cell-transplanted patients with pure NK cells becomes common. NK cells isolated from donor blood and expanded avoids effectively GvHD when applied during the first phase after transplantation.

Advanced production technology makes NK cells attractive for use in broader bases: Pure NK cells have shown nearly no unwanted side effects in clinical trials even when administered in high doses. The modern production processes deliver NK cells with enhanced functionalities (high cytotoxicity against many cancer cells in *ex vivo* tests and enhanced paracrine production). Pure NK cells can be manufactured in an easy-to-handle closed system as ATMP in clinical settings near to patients. A particular advantage is that mass amounts of individual NK cells can be produced economically (due to low costs of selection, medium, and activation).

Cell source; supplements, activation	Expansion device used	Cultivation time (days)	Expansion by x-fold	Cell purity	Reference
PBMC fraction, serum; IL 2,	Stirred bioreactor (750 ml)	33	352	96%	[62]
PBMC fraction; serum; IL 2;IL 15; PHA	VueLife bag system (800 ml)	14	80–200	85–91%	[63]
PBMC fraction; serum; IL2/ IL12/IL15; feeder cell line	T-flaks	10	40	62–95%	[64]
PBMC fraction; serum; IL 2; IL 15; feeder cell line K529; 4 BBL1	VueLife bag system	21	277	97%	[65]
PBMC fraction; medium; serum; IL 2	Wave bioreactor	21	12–354	37–54%	[66]
PBMC fraction; medium; serum; IL 2; feeder cell line K529;	G-Rex flask	8–10	442	54–79	[67]
PBMC fraction; medium; serum; IL 2;	ZRP type M single-use bioreactor	30	1000–2000	95–99%	Own data, not published
PBMC fraction; serum; IL 2/IL 21; coating with CD 16	ZRP typ M single-use bioreactor	30–35	1000–50,000	99%	Own data, not published

Table 6. NK cells for cell therapies. Different production methods.

6. State-of-the-art with *ex vivo* expanded MSCs utilized in clinical trials

With numbers of 10^9 MSC, therapeutic effects were reported in the treatment of autoimmune and infectious diseases [68, 69]. The issue on reliabilities and logistically practical methods for

production of MSC for adequate amounts of immune cells needs specific attention. Most of the current clinical trials use open culture system in flasks even though there have been ongoing efforts with newer technologies (compare **Table 7**).

Consistent and logistically practical methods for production of MSC for adequate numbers of immune cells need specific attention. In addition, there are many unresolved issues relating to the isolation, expansion technique, phenotyping characterization, mechanisms of action, and incomparability of study results due to different protocols and definitions. In most of the current clinical trials, open culture system in flasks is in use (compare **Table 7**) even though there have been ongoing efforts with newer technologies [56, 70, 71]. In contrast, MSC, in particular those derived from bone marrow, lead candidates to fight many important disease entities. It is believed that modulation of immune responses, remodeling of impaired tissues, pro-regenerative as well as antifibrotic effects can be attained with these immune cells. With numbers of 10^9 MSC, effects were reported in treatment of some autoimmune and infectious diseases [68, 69]. A lot of clinical trials have been performed or are ongoing on treating cardiovascular diseases (myocardial infarction, cardiomyopathy, critical limb ischemia, stroke [72–74]). Single or repeated doses of 10^7 to more than 10^9 are injected systemically or topically. Until now, some positive influences on cardiovascular disease could be ensured through cytokines and chemokines secreted from MSC. Avoiding GvHD is also under intense investigation due to increasing stem cell transplantation in cancer and organ transplant patients. In all these indications, autologous or allogenic MSC are being infused that are not really manufactured under controlled conditions.

Cell source; supplements	Expansion device used	Cultivation time (days)	Expansion by x-fold	Cell harvest	Cell purity	Reference
BM-MNC, serum	Flasks	30	n.a.	n.a.	99%	[50]
BM-MNC, serum	Flasks	30–45	6–52	2.4–5.7 x 10^7	>60%	[51]
BM-E serum	Five layer flasks	10–28	n.a.	6–27 x 10^7	n.a.	[52]
BM-W, serum 59 donors	Five layer flasks	28	5–145	2 x 10^7 to 5 x 10^9	n.a.	[53]
UC blood, serum	FIve layer flasks	22–28	n.a.	1–8 x 10^8	n.a.	[52]

Table 7. Manufacturing processes of MSC in clinical trials (n.a.= not available).

7. Newer techniques for characterization and production of immune cells therapeutics

Identification of different cell types, subpopulations, and even single subclones within a final cell therapeutics product can be a challenging exercise due to many constraints including the limited number of cells available. Immune cells are usually identified from its displayed surface receptors that also gives a hint on its characters to fight infections or cancer. Cell sorting by flow cytometry or magnetic beads are modern techniques allowing isolation and separation

of immune cell subtypes. These methods are still time-consuming and costly exercises due to the quantity of antibodies and reagents required in the manufacturing process when dealing with larger starting material such as cells derived through apheresis. GMP-grade antibodies are particularly costly making the ATMP cell therapy an unaffordable range.

However, it is pertinent to analyze and to predict the potency of the cells that give a prior indication of the anticipated possible effects of the immune cells on the intended outcome. In case of T cells, it has been recently accomplished by tracking the fate and origin of clinically relevant adoptively transferred CD8+ T cells *in vivo* to identify and track single subclones specifically activated against few tumor cell mutants specific T memory cells [30]. By using high-throughput T cell receptor sequencing, the group has worked out a strategy to identify and track those very low frequency monoclonal T cells among the total bulk of polyclonal T cell pool with varying cancer-killing and fighting capabilities that have been given as adoptive cell therapy to 10 metastatic malignant melanoma patients being specifically activated against melanoma and correlated with the treatment response in patients. They were then also able to decipher the specific clonal population of extremely low density of T cells that were persisting and effective *in vivo* among two patients out of ten demonstrating complete remission. It is worth proving whether this new approach can be applied effectively in clinical practice and to prove its rationale in other cancer types. Only around 0.001% of all T cells in blood consisted of these active monoclonal T cells. This study also indicated that the younger T cells nearly in the phase of development had a better ability to fight tumors than older ones.

Further progress in the identification of subpopulations, primed monoclonal T cells, and information on functionality of cell preparations may be obtained by the spectra of single cells, their typical receptors/ligands/paracrine production. Raman microscopy has long been used in cell and metabolites analysis. However, the combination of this method with sophisticated software programs with in-depth analyses tools can lead to sharper, high-resolution Raman spectra enabling differentiating looks onto cells enabling subtype identification, quantification, analysis of functional status, etc. [10–13].

CyTOF, the latest novel format of flow cytometry combined with mass spectrometry, often referred as mass cytometry provides a measurement of >40 simultaneous cellular parameters at single-cell resolution, significantly augmenting the ability of cytometry to evaluate complex cellular systems and processes at any given point. This has been a greatest tool to unravel the mechanism of immune cells by studying kinetics before and after infusion. Currently, research on solid cancers has a strong focus on immune cells infiltrations in the microenvironment of tumors. In a mouse model of triple negative breast carcinoma, the new methods using CyTOF assessing immune cells at single cell level within the tumor in the microenvironment of the tumor as well systemically in different organs over time demonstrated a striking difference of the sustained response at systemic level in the effective treatment responsive group in comparison to the nonresponsive treatment group [31]. Local carcinoma treatment was followed by an infiltration of CD8+/CD4+ T cells into the tumor leading to death of tumor cells. Different immune cell specimens were expanded during the rejection phase not only in the microenvironment of the carcinoma but also in many lymphatic organs and blood to reflect on the changes taking place systemically. A systemic coordinated immune response of

CD4+ as well as CD8+ T cells, NK cells, DC cells, and activated B cells seems to be essential for achieving sustainable effects to eradicate tumors with immune cell therapy. This has much more connotation for the ATMP cell therapy to reach its full potential.

The ZRP platform of Zellwerk and the belonging bioreactor types provide sophisticated features not only for mass production of different immune cells but also for realizing isolation and culturing the cells in closed processes [54–57, 70, 75]. It enables controlled phases of selection/priming/activation of seeded cells by regulated medium flow, suited coating of seeding surfaces and/or fixed antibodies, followed by rapid expansion, all in a single run. This is due to the technical attributes:

Bioreactors of the ZRP system can be operated in the GMP breeder (**Figure 1**). The breeder combines a laminar flow sterile bench and an incubator. Controlling of the essential bioreactor and breeder functions is by the control unit (automatic regulation of pO_2, pH, medium temperature, medium feeding, mixing, and flow of gasses over a touch screen).

The cell cultivation platform and the belonging bioreactors enable the manufacturing of large quantities of individual immune cell preparations under GMP conditions. A series of ZRP systems can be driven in parallel in one clean room equipment due to the closed steps of the perfusion bioreactor processes. During a period of 1 year, several immune cell preparations from individuals amounting to ~100 can be undertaken in one B clean room thus reducing expenditures for production of immune cell therapeutics massive. Manufacturing of immune cells as ATMP is authorized by the national and regional German authorities.

Important features of the meander type bioreactors are as follows: A directed laminar flow of medium, which can be chosen over a wide range, makes an undisturbed cell/cell- and cell/surface-contact possible and minimizes cell stress. The ratio of medium circulation and fresh medium flow is automatically regulated over time by a chosen algorithm guaranteeing ananan consistent homogenous supply with nutrients and gasses as well as precise regulated pO_2, pH, and temperature in the medium. T cells, TILs, and NK cells can be expanded to more than a 10^{10} cells in one closed cultivation run. In parallel cultivation run with pure NK

Figure 1. Zellwerk's GMP Z®RP cell breeder with M type bioreactor for NK/T/TIL cells.

cells using the same medium and density of seeded cells, expansion in static flasks was not more than 50–100-fold, whereas in meander type bioreactor 5000–50,000-fold was achieved. Coating of seeding areas with specific matrices/antibodies, i.e., can be exploited to promote suppression or expansion of several immune cell specimen (e.g., Treg; $CD56^{bright}$ NK cells).

8. Current advances and future prospects

Despite many clinical trials with immune cell therapy and much progresses in the field, the manufacturing of cells as ATMP for routine clinical use has not been realized in its full potential. Here we attempt to give an overview of different advanced technologies and devices currently available for cell expansion. These different devices need to be validated and compared in head-to-head comparison for different cell type to exploit the opportunities for unmet clinical needs.

Sterility, purity, identity, and potency are four cardinal requirements. Initially, cell expansions have been carried out in the conventional process of open flask culture system with gradual incremental innovations to meet the growing needs of regulatory requirements as well as better quality products in terms of safety (phenotype), consistency (reproducibility and controlled robust process), numbers, quality (structural and functional), and efficacy (functionality and potency) to qualify as ATMP to be used in patients/human subjects. From simple culture flasks, multilayered flask was made for ease of handling as well as scaling up with the numbers (such as Millicell, Millipore or BD Multiflask).

Different microcarrier system came into place to increase surface area to expand cells in 3D than in flasks in 2D [76–78]. G-Rex system is a gas permeable rapid expansion device with a silicone membrane at the base, allowing gas exchange to occur uninhibited by the depth of the medium above with high O_2 concentration stimulating cell expansion and simplify handling [99]. WAVE-type bioreactor systems [79] make use of sterile, transparent, and disposable culture bags with provision for media perfusion, harvest, sampling, and gas exchange, which greatly reduces the cross-contamination problem, one of the biggest challenge in conventional culture system [37, 38]. The culture bag is put on the device's temperature-controlled tray inside an incubator having the option of controlled rocking movement. Optional perfusion modules enable controlled addition and removal of culture media for optimized nutrient concentrations while disposing spent media resulting in higher cell densities with involvement of less time and effort for media exchange. Magnetic beads are used in the bag for various priming and activation of cells [80]. However, the chaotic medium flow causes cell stress [78, 79]. These factors have been well addressed in ZRP cell breeder that has controlled and directed medium flow and fulfills most of the regulatory requirements (see Section 7).

MILTENYI's CliniMACS Prodigy® [81] is an automated integrated sensor-controlled closed system device that uses single-use sterile disposable tubing set and can perform fractionation of cells, cell washing, cell separation, cell culture, and final product formulation in the workflow connected through sterile docking devices. TERUMO quantum system is a functionally

closed automated hollow fiber bioreactor system for GMP cell manufacturing that has been tried in MSC expansion [82]. Huge efforts are ongoing in Japan to build modular robotics-manipulated, automated high-throughput cell-processing machine to make off-the-shelf cell therapy products for wider application [83].

Product dictates the process development and optimization to ensure cost-effectiveness, well-controlled steps, high reliability, high cell density and viability, high product quality, easy recovery, high yield, and high safety for personnel. Perfusion system takes care of controlled dynamic gas exchange with a homogenous environment allowing high cell density with controllable and flexible process control, but demands complicated validation procedure.

Evolving regulatory landscape for ATMPs (cell therapy) [84–87] has the following two mandates to be fulfilled (quoted from EMA document [88]):

- Cells or tissues that have been subjected to substantial manipulation so that biological characteristics, physiological functions, or structural properties relevant for the intended clinical use have been altered, or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor.
- Have properties for, or are used in or administered to human beings with a view to treating, preventing, or diagnosing a disease through the pharmacological, immunological, or metabolic action of their cells or tissues.

Regulatory landscape for ATMP cell therapy at this juncture is quite diverse in various regions of the world and is evolving though in a gradient [89–95]. It is a daunting task, but is an absolute necessity to harmonize all the harmonizers (regulatory bodies) across the globe with an unified coherent process to make ATMP cell therapy possible for unmet clinical needs. The regulatory bodies have the responsibilities to assure the safety and rights of patients and to ensure quality of the nonclinical and clinical evidences to allow appropriate evaluation of the safety and effectiveness of the cell therapy product through clinical trials for market authorization. FDA and EMA have distinct guidelines, whereas there are distinct guidelines in other parts such as Australia, Brazil, Japan, Korea, Singapore, and Taiwan just to give a flavor of the unique differences that pose constraints in the current clinical development of ATMP cell therapy. While such efforts to converge on an unified regulatory process are underway through engagements, two critical issues have been identified to be addressed, the concept of potency assessment as well as *in vivo* tumorigenicity studies [96].

FDA has developed a number of expedited programs to facilitate ATMP cell therapy use in patients when no satisfactory alternative therapies are available while ensuring the standards of the products for safety and efficacy. EMA made a new regulation in 2009 for all 28 member countries in EU with obligatory centralized market authorization process. Eight cell therapy ATMPs have been authorized in EU [97], namely ChondroCelect (withdrawn in January 2017) and Maci (suspended from July 2014 due to closure of European manufacturing unit), both the products are autologous cartilage cells grown *ex vivo* for cartilage repair; Provenge (autologous PBMC activated with fusion protein of prostatic acid phosphatase attached to GM-CSF *ex vivo*) for treatment of advanced prostate cancer, withdrawn in May

2015; Holoclar, autologous limbal stem cells to repair damaged corneal epithelium, this has orphan drug status due to rare condition. A great deal of effort are underway in EMA to refine and execute adaptive regulatory pathway to foster rapid development and accelerated assessment for innovative cell therapies. Korea has 14 cell products authorized including four stem cell products with 46 ongoing clinical trials with other cell therapy products. New adaptive regulatory framework has been enacted under the Pharmaceuticals, Medical Devices and Other Therapeutic Products Act (PMD Act) in Japan in late 2014 to facilitate access of promising ATMP cell therapy to the patients with limited treatment options as well as creating conducive regulatory environment to give accelerated conditional and time-limited authorization to stimulate further clinical development. Two products namely, MSC for GvHD second-line therapy and skeletal myoblast sheets for ischaemic heart failure have been authorized under the new scheme in 2015 and 2016.

Since cell culture uses animal-derived serum or growth factors, the sources need to be certified and proven free of any adventitious agents, consistent in quality and free of risk of any possible infections.

First ATMP cell therapy product to get market approval was in Canada for Prochymal (Remestemcel-L), adult MSC for IV infusion for acute GvHD in May 2012. Allogeneic adipose tissue-derived MSC expanded *ex vivo* have been shown efficacious in Phase 3 clinical trial when given intra-lesionally in complex perianal fistulas in Crohn's disease patients and a decision is awaited in 2017 for market authorization by EMA. This product named as Cx601 by Takeda and TiGenix has received orphan status by the Swiss Agency for Therapeutic Products (Swissmedic) for the rare disease. While these innovations are taking place at immense pace, there is growing requirement of policymakers to be engaged along with patients' community to see how best a value-based frameworks and be drawn as rational approaches to use these expensive novel therapeutic modalities in patient care [98].

9. Conclusion

The manufacturing of immune cells is until today primarily performed using archaic, scarcely controlled, incomparable processes and methods. These issues need to be better harmonized and put into standard practice. When looking into the processes of immune cell production used in clinical studies, it is obvious that the cells in most settings are expanded totally or partly in conventional culture flasks or similar vessels. That is due to the fact, that all immune cell types can be grown in this simple and cheap way without special skills. However, deeper characterization of *ex vivo* expanded immune cells is urgently needed not only on the level of a few receptors and ligands on the cell surface but also with respect to the ever-contained subtypes in an expanded immune cell population, the pattern of secreted effector molecules, their amounts over time and influences from *in vivo* components on them.

More research on aspects of modern cell therapy might be qualified as too costly, but will be more targeted and will at least avoid expensive and unjustified clinical studies maximizing the best use of the available R&D resources for better outcomes.

Conflict of Interest

All authors RP, SKP and CS do not have any conflict of interest. HH is the CEO of Zellwerk GmbH.

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References

[1] ClinicalTrials Registry. USA: NIH; 2017

[2] Hanna E, Remuzat C, Auquier P, Toumi M. Advanced therapy medicinal products: Current and future perspectives. Journal of Market Access & Health Policy. 2016;**4**: 31036. DOI: 10.3402/jmahp.v4.31036

[3] Galluzzi L, Zitvogel L, Kroemer G. Immunological mechanisms underneath the efficacy of cancer therapy. Cancer Immunology Research. 2016;**4**:895-902. DOI: 10.1158/2326-6066.CIR-16-0197

[4] Koepsell SA, Miller JS, McKenna DH. Natural killer cells: A review of manufacturing and clinical utility. Transfusion. 2013;**53**:404-410. DOI: 10.1155/2011/676198

[5] Levy EM, Roberti MP, Mordoh J. Natural killer cells in human cancer: From biological functions to clinical applications. Journal of Biomedicine & Biotechnology. 2011;**676198**. DOI: 10.1155/2011/676198

[6] Miller JS. Therapeutic applications: Natural killer cells in the clinic. Hematology. ASH Education Book. 2013;**1**:247-253

[7] Yang JC, Rosenberg SA. Adoptive T-cell therapy for cancer. Advances in Immunology. 2016;**130**:279-294

[8] Wang Z, Wu Z, Liu Y, Han W. New development in CAR-T cell therapy. Journal of Hematology & Oncology. 2017;**10**:53

[9] Universal CAR T cells treat leukemia. Cancer Discovery. 2017;**7**(4):342. DOI: 10.1158/2159-8290.CD-NB2017-023

[10] Leelatian N, et al. Single cell analysis of human tissues and solid tumours with mass cytometry. Cytometry. Part B, Clinical Cytometry. 2017;**92**:68-78

[11] McReynolds N, Cooke FG, Chen M, Powis SJ, Dholakia K. Multimodal discrimination of immune cells using a combination of Raman spectroscopy and digital holographic microscopy. Scientific Reports. 2017;**7**:43631

[12] Ichimura T, et al. Non-label immune cell state prediction using Raman spectroscopy. Scientific Reports. 2016;**6**:37562

[13] Next Generation Cell Analysis. In: www.horizon2020projects.com, editor; 2016

[14] Oh H, Siano B, Diamond S. Neutrophil isolation protocol. Journal of Visualized Experiments. 2008;**17**:745

[15] Lakschevitz FS, et al. Identification of neutrophil surface marker changes in health and inflammation using high-throughput screening flow cytometry. Experimental Cell Research. 2016;**342**:200-209

[16] Boyum A. Isolation of lymphocytes, granulocytes and macrophages. Scandinavian Journal of Immunology. Supplement 1976;**5**:9-15

[17] Golubovskaya V, Wu L. Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. Cancers (Basel) 2016;**15**:8(3). pii: E36. DOI: 10.3390/cancers8030036

[18] Cherai M et al. Generation of human alloantigen-specific regulatory t cells under good manufacturing practice-compliant conditions for cell therapy. Cell Transplantation. 2015;**24**:2527-2540

[19] Haase D, et al. Large-scale isolation of highly pure "untouched" regulatory T cells in a GMP environment for adoptive cell therapy. Journal of Immunotherapy. 2015;**38**:250-258

[20] Torelli GF, et al. A good manufacturing practice method to *ex vivo* expand natural killer cells for clinical use. Blood Transfusion. 2015;**13**:464-471

[21] Vivier E, Ugolini S, Blaise D, Chabannon C, Brossay L. Targeting natural killer cells and natural killer T cells in cancer. Nature Reviews. Immunology. 2012;**12**:239-252

[22] Reardon DA, Mitchell DA. The development of dendritic cell vaccine-based immunotherapies for glioblastoma. Seminars in Immunopathology. 2017;**39**:225-239

[23] Krenkel O, Tacke F. Liver macrophages in tissue homeostasis and disease. Nature Reviews. Immunology. 2017;**17**(5):306-321. doi: 10.1038/nri.2017.11

[24] Allen ES, Stroncek DF, Ren J, Eder AF, West KA, Fry TJ, Lee DW, Mackall CL, Conry-Cantilena C. Autologous lymphapheresis for the production of chimeric antigen receptor T cells. Transfusion 2017;**57**:1133-1141

[25] Liu H, et al. Comparative study of different procedures for the separation of peripheral blood mononuclear cells in cytokine-induced killer cell immunotherapy for hepatocarcinoma. Tumour Biology. 2015;**36**:2299-2307

[26] Parkhurst MR, Riley JP, Dudley ME, Rosenberg SA. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumour regression. Clinical Cancer Research. 2011;**17**:6287-6297

[27] Hami LS, et al. GMP production and testing of Xcellerated T cells for the treatment of patients with CLL. Cytotherapy. 2004;**6**:554-562

[28] Konjevic G, Vuletic A, Mirjacic MK. Natural killer cell receptors. Alterations and therapeutic targeting in malignancies. Immunologic Research. 2016;**64**:25-35

[29] Huber CM, Doisne JM, Colucci F. IL-12/15/18-pre activated NK cells suppress GvHD in a mouse model of mismatched hematopoietic cell transplantation. European Journal of Immunology. 2015;**45**(6):1727-1735

[30] Chapuis AG, et al. Tracking the fate and origin of clinically relevant adoptively transferred CD8+ T cells *in vivo*. Science Immunology. 2017;**2**(8):eaal2568

[31] Spitzer MH, et al. Systemic immunity is required for effective cancer immunotherapy. Cell. 2017;**168**:487-502, e415

[32] Ellebaek E, et al. Adoptive cell therapy with autologous tumour infiltrating lymphocytes and low-dose Interleukin-2 in metastatic melanoma patients. Journal of Translational Medicine. 2012;**10**:169. DOI: 10.1186/1479-5876-10-169

[33] Wang X, Riviere I. Manufacture of tumour- and virus-specific T lymphocytes for adoptive cell therapies. Cancer Gene Therapy. 2015;**22**:85-94

[34] Donia M, et al. Characterization and comparison of 'standard' and 'young' tumour-infiltrating lymphocytes for adoptive cell therapy at a Danish translational research institution. Scandinavian Journal of Immunology. 2012;**75**:157-167

[35] Topalian SL, Muul LM, Solomon D, Rosenberg SA. Expansion of human tumour infiltrating lymphocytes for use in immunotherapy trials. Journal of Immunological Methods. 1987;**102**:127-141

[36] Itzhaki O, et al. Establishment and large-scale expansion of minimally cultured "young" tumour infiltrating lymphocytes for adoptive transfer therapy. Journal of Immunotherapy. 2011;**34**:212-220

[37] Sadeghi A, et al. Large-scale bioreactor expansion of tumour-infiltrating lymphocytes. Journal of Immunological Methods. 2011;**364**:94-100

[38] Jin J, et al. Simplified method of the growth of human tumour infiltrating lymphocytes in gas-permeable flasks to numbers needed for patient treatment. Journal of Immunotherapy. 2012;**35**:283-292

[39] Somerville RP, Devillier L, Parkhurst MR, Rosenberg SA, Dudley ME. Clinical scale rapid expansion of lymphocytes for adoptive cell transfer therapy in the WAVE(R) bioreactor. Journal of Translational Medicine. 2012;**10**:69

[40] Donia M, Larsen SM, Met O, Svane IM. Simplified protocol for clinical-grade tumour-infiltrating lymphocyte manufacturing with use of the Wave bioreactor. Cytotherapy. 2014;**16**:1117-1120

[41] Morgan RA, et al. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. Molecular Therapy. 2010;**18**:843-851

[42] Wang X, Riviere I. Clinical manufacturing of CAR T cells: Foundation of a promising therapy. Molecular Therapy Oncolytics. 2016;**3**:16015

[43] Tumaini B, et al. Simplified process for the production of anti-CD19-CAR-engineered T cells. Cytotherapy. 2013;**15**:1406-1415

[44] Bouwmans EE, et al. NK cells expanded with the ZRP cultivation system keep cytotoxic properties and remain alloreactive. NK Cell Meeting. 2013. Heidelberg, Germany

[45] Friedrichs B, et al. Expansion of CD3-ve CD56+ve NK Cells for Repeated Application in a GMP Compliant Process Using a Novel Bioreactor System. Geneva, Switzerland: EMBT; 2012

[46] Krampera M, et al. Immunological characterization of multipotent mesenchymal stromal cells—The International Society for Cellular Therapy (ISCT) working proposal. Cytotherapy. 2013;**15**:1054-1061

[47] Galipeau J, et al. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. Cytotherapy. 2016;**18**:151-159

[48] Heymans O, Castus P, Grandjean FX, Van Zele D. Liposuction: Review of the techniques, innovations and applications. Acta Chirurgica Belgica. 2006;**106**:647-653

[49] Zuk PA, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Engineering. 2001;**7**:211-228

[50] Baron F, et al. Cotransplantation of mesenchymal stem cells might prevent death from graft-versus-host disease (GvHD) without abrogating graft-versus-tumour effects after HLA-mismatched allogeneic transplantation following nonmyeloablative conditioning. Biology of Blood and Marrow Transplantation. 2010;**16**:838-847

[51] Emadedin M, et al. Long-term follow-up of intra-articular injection of autologous mesenchymal stem cells in patients with knee, ankle, or hip osteoarthritis. Archives of Iranian Medicine. 2015;**18**(6):336-344

[52] Capelli C, Pedrini O, Valgardsdottir R, Da Roit F, Golay J, Introna M. Clinical grade expansion of MSCs. Immunology Letters. 2015;**168**:222-227

[53] Lechanteur C, et al. Clinical-scale expansion of mesenchymal stromal cells: a large banking experience. Journal of Translational Medicine. 2016;**14**(1):145. DOI: 10.1186/s12967-016-0892-y

[54] Diederichs S, et al. Dynamic cultivation of human mesenchymal stem cells in a rotating bed bioreactor system based on the Z RP platform. Biotechnology Progress. 2009; **25**:1762-1771

[55] Lavrentieva A, Hatlapatka T, Neumann A, Weyand B, Kasper C. Potential for osteogenic and chondrogenic differentiation of MSC. Advances in Biochemical Engineering and Biotechnology. 2013;**129**:73-88

[56] Neumann A, Lavrentieva A, Heilkenbrinker A, Loenne M, Kasper C. Characterization and application of a disposable rotating bed bioreactor for mesenchymal stem cell expansion. Bioengineering. 2014;**1**:231-245

[57] Suck K, et al. A rotating bed system bioreactor enables cultivation of primary osteoblasts on well-characterized Sponceram regarding structural and flow properties. Biotechnology Progress. 2010;**6**:671-678

[58] Subramani B, et al. Autologous immune enhancement therapy: A case report of a stage IV colonic cancer. Oncology Letters. 2013;**5**:1611-1614

[59] Chapuis AG, et al. T-cell therapy using interleukin-21-primed cytotoxic T-cell lymphocytes combined with cytotoxic T-cell lymphocyte antigen-4 blockade results in long-term cell persistence and durable tumour regression. Journal of Clinical Oncology 2016,**34**,31:3787-3795. DOI: 10.1200/JCO.2015.65.5142

[60] Kershaw MH, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clinical Cancer Research. 2006;**12**:6106-6115

[61] Koehl U, Brehm C, Huenecke S, et al. Clinical grade purification and expansion of NK cell products for an optimized manufacturing protocol. Frontiers in Oncology. 2013;**3**:118. DOI: 10.3389/fonc.2013.00118

[62] Pierson BA, Europa AF, Hu WS, et al. Production of human natural killer cells for adoptive immunotherapy using a computer-controlled stirred-tank bioreactor. Journal of Hematotherapy. 1996;**5**(5):475-483

[63] Luhm J, Brand JM, Koritke P, Hoppner M, Kirchner H, Frohn C. Large-scale generation of natural killer lymphocytes for clinical application. Journal of Hematotherapy & Stem Cell Research. 2002;**11**:651-657

[64] Torelli GF, Guarini A, Maggio R,et al. Expansion of natural killer cells with lytic activity against autologous blasts from adult and pediatric acute lymphoid leukemia patients in complete hematologic remission. Haematologica. 2005;**90**(6):785-792

[65] Fujisaki H, Kakuda H, Shimasaki N, et al. Expansion of highly cytotoxic human natural killer cells for cancer cell therapy. Cancer Research. 2009;**69**(9):4010-4017. DOI: 10.1158/0008-5472.CAN-08-3712

[66] GE Healthcare. Application note 28-9936-25 AA. Perfusion culture of human natural killer cells in the WAVE Bioreactor 2/10 system. 2011. http://www.cellgenix.com/fileadmin/published_content/3_downloads/4.1_application_notes/GE_paper_NK_cells.pdf

[67] Lapteva N, Durett AG, Sun J, et al. Large-scale *ex vivo* expansion and characterization of natural killer cells for clinical applications. Cytotherapy. 2012;**14**(9):1131-1143. DOI: 10.3109/14653249.2012.700767

[68] Skrahin A, et al. Autologous mesenchymal stromal cell infusion as adjunct treatment in patients with multidrug and extensively drug-resistant tuberculosis: An open-label phase 1 safety trial. The Lancet Respiratory Medicine. 2014;**2**:108-122

[69] Panes J, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: A phase 3 randomised, double-blind controlled trial. Lancet. 2016;**388**:1281-1290

[70] Reichardt A, et al. Large scale expansion of human umbilical cord cells in a rotating bed system bioreactor for cardiovascular tissue engineering applications. Open Biomedical Engineering Journal. 2013;**7**:50-61

[71] Gnecchi M, editor. Mesenchymal Stem Cells. Methods and Protocols. 2nd ed. Humana Press, Springer Protocols; 2016

[72] Skorska A, Müller P, Steinhoff G, et al. GMP-conformant on-site manufacturing of a CD133+ stem cell product for cardiovascular regeneration. Stem Cell Research & Therapy. 2017;**8**(1):33

[73] Karantalis V, Hare JM. Use of mesenchymal stem cells for therapy of cardiac diseases. Circulation Research. 2015;1413-1430

[74] Heldman AW, DiFede DL, et al. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy. The TAC-HFT randomized trial. JAMA. 2014;**311**(1):62-73

[75] Egger D, Spitz S, Fischer M, Handschuh S, Glösmann M, Friemert B, Egerbacher M, Kasper C. Application of a Parallelizable Perfusion Bioreactor for Physiologic 3D Cell Culture. Cells Tissues Organs. 2017;**203**(5):316-326. doi: 10.1159/000457792

[76] Cierpka K, Elseberg CL, Niss K, Kassem M, Salzig D, Czermak. hMSC production in disposable bioreactors with regards to GMP and PAT. Chemie Ingenieur Technik. 2013;**85**:1-2, 67-75. DOI: 10.1002/cite.201200151

[77] Schirmaier C, Jossen V, Kaiser SC, Jüngerkes F, Brill S, Safavi-Nab A, et al. Scale-up of adipose tissue-derived mesenchymal stem cell production in stirred single-use bioreactors under low-serum conditions. Engineering in Life Sciences. 2014;**14**(3):292-303. DOI: 10.1002/elsc.201300134

[78] Jossen V, Schirmer C, Mostafa Sindi D, Eibl R, Kraume M, Pörtner R, Eibl D. Theoretical and practical issues that are relevant when scaling up hMSC microcarrier production processes. Stem Cells International. 2016;**2**:1-15. DOI: 10.1155/2016/4760414

[79] Jossen V, Pörtner R, Kaiser SC, Kraume M, Eibl D, Eibl R. Mass production of mesenchymal stem cells—Impact of bioreactor design and flow conditions on proliferation and differentiation. In: Eberli D, editor. Cells and Biomaterials in Regenerative Medicine. InTech, Rijeka, Croatia; 2014. DOI: 10.5772/59385

[80] Neurauter AA, Bonyhadi M, Lien E, Nokleby L, Ruud E, Camacho S, Aarvak T. Cell isolation and expansion using Dynabeads. Advances in Biochemical Engineering/Biotechnology. 2007;**106**:41-73. DOI: 10.1007/10_2007_072

[81] Apel M, Brüning M, Granzin M, Essl M, Stuth, J, Blaschke J, et al. Integrated clinical scale manufacturing system for cellular products derived by magnetic cell separation, centrifugation and cell culture. Chemie Ingenieur Technik. 2013;**85**:1-2, 103-110. DOI: 10.1002/cite.201200175

[82] Barckhausen C, Rice B, Baila S, Sensebe L, Schrezenmeier H, Nold P, et al. GMP-compliant expansion of clinical-grade human mesenchymal stromal/stem cells using a closed hollow fiber bioreactor. Methods in Molecular Biology (Clifton, N.J.). 2016;**1416**:389-412. DOI: 10.1007/978-1-4939-3584-0_23

[83] Kami D, Watakabe K, Yamazaki-Inoue M, Minami K, Kitani T, Itakura Y, et al. Large-scale cell production of stem cells for clinical application using the automated cell processing machine. BMC Biotechnology. 2013;**13**:102. DOI: 10.1186/1472-6750-13-102

[84] Parolini O, Alviano F, Bergwerf I, Boraschi D, De Bari C, De Waele P, Dominici M, Evangelista M, Falk W, Hennerbichler S, Hess DC, Lanzoni G, Liu B, Marongiu F, McGuckin C, Mohr S, Nolli ML, Ofir R, Ponsaerts P, Romagnoli L, Solomon A, Soncini M, Strom S, Surbek D, Venkatachalam S, Wolbank S, Zeisberger S, Zeitlin A, Zisch A, Borlongan CV. Toward cell therapy using placenta-derived cells: Disease mechanisms, cell biology, preclinical studies, and regulatory aspects at the round table. Stem Cells and Development. 2010;**19**(2):143-154. DOI: 10.1089/scd.2009.0404

[85] Culme-Seymour EJ, Davie NL, Brindley DA, Edwards-Parton S, Mason C. A decade of cell therapy clinical trials (2000-2010). Regenerative Medicine. 2012;**7**(4):455-462. DOI: 10.2217/rme.12.45

[86] Maciulaitis R, D'Apote L, Buchanan A, Pioppo L, Schneider CK. Clinical development of advanced therapy medicinal products in Europe: evidence that regulators must be proactive. Molecular Therapy. 2012;**20**(3):479-482. DOI: 10.1038/mt.2012.13

[87] Ancans J. Cell therapy medicinal product regulatory framework in Europe and its application for MSC-based therapy development. Frontiers in Immunology. 2012;**3**:253. DOI: 10.3389/fimmu.2012.00253

[88] www.ema.europa.eu/docs/en_GB?document_library/Scientific-guideline/2015/06/WC500187744.pdf/)

[89] European Medicines Agency. Advanced Therapies. 2017. http://www.ema.europa.eu/ema/

[90] Advanced Therapy Medicinal Products (ATMPs)—ICH: 2017

[91] http://www.ich.org/fileadmin/Public_Web_Site/Training/ASEAN_Q5C_workshop_May_2011/SESSION_IVb_ATMPs.pdf

[92] ATMP Manufacturing Community. 2017. https://atmpmanufacture.org/

[93] Advanced Therapy Medicinal Products - Parenteral Drug Association. 2017. https://www.pda.org/global-event-calendar/event-detail/advanced-therapy-medicinal-products

[94] ATMP Regulation - LFB CellForCure. 2017. www.cellforcure.com/atmp-regulation/

[95] Advanced therapy medicinal products: Regulation and licensing—Gov.uk.2017. https://www.gov.uk/guidance/advanced-therapy-medicinal-products-regulation-and-licensing

[96] Hayakawa Y, Kawada M, Nishikawa H, Ochiya T, Saya H, Seimiya H, Yao R, Hayashi M, Kai C, Matsuda A, Naoe T, Ohtsu A, Okazaki T, Saji H, Sata M, Sugimura H, Sugiyama Y, Toi M, Irimura T. Report on the use of non-clinical studies in the regulatory evaluation of oncology drugs. Cancer Science. 2016;**107**(2):189-202. DOI: 10.1111/cas.12857

[97] CAT monthly report of application procedures, guidelines and related documents on advanced therapies. March 2017 Meeting. http://www.ema.europa.eu/docs/en_GB/document_library/Committee_meeting_report/2017/03/WC500224413.pdf

[98] Lawler M, French D, Henderson R, Aggarwal A, Sullivan R. Shooting for the moon or flying too near the sun? Crossing the value rubicon in precision cancer care. Public Health Genomics. 2016;**19**(3):132-136. DOI: 10.1159/000446530

[99] Vera JF, Brenner LJ, Gerdemann U, Ngo MC, Sili U, Liu H, Wilson J, Dotti G, Heslop HE, Leen AM, Rooney CM. Accelerated production of antigen-specific T cells for preclinical and clinical applications using gas-permeable rapid expansion cultureware (G-Rex). Journal of Immunotherapy. 2010;**33**:305-315

Chapter 13

Tissue Engineering Applications in Maxillofacial Surgery

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Additional information is available at the end of the chapter

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Abstract

Nowadays, oral and maxillofacial surgeons face serious difficulties in reconstruction of large defects caused by trauma, cancer, or congenital deformities. Considering each part of oral and maxillofacial region consisting of several tissues, it is necessary to reconstruct these architectures layer by layer. Through years surgeons use different forms of grafts to reconstruct these defects. As these grafts and techniques are well described and used routinely, it should have been noticed that they are not without complications. This is where idea behind tissue engineering steps in. "Tissue engineering" due to its multi-aspect properties can be defined as application of methods and science of engineering toward the understanding of structure-function relationships of mammalian tissues in both normal and pathological forms to improve and develop biologic substitutes to reach the main goal of restoring, maintaining, and stabilization of tissue function. From standpoint of surgery, tissue engineering is not considered as a potential step anymore, but as an available approach to reach the ultimate goal of reconstruction procedures. The aim of this chapter is to defne concepts and advances in tissue engineering (TE). Also, review TE applications in the field of oral and maxillofacial surgery with bolding its clinical applications and complications based on novel and high-quality published researches.

Keywords: tissue engineering, scaffolds, bioprinting, stem cells, regenerative medicine, oral surgery, maxillofacial surgery

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1. Introduction

For the first time, Langer and Vacanti introduced the definition of tissue engineering [1] to explain the basics of functional substitutes for tissue damage and how to reconstruct and regenerate these tissues based on principles of biology and medical engineering. This new field in contrast to the former biomaterial thoughts presents incredible disciplines which diverse the goal of regeneration induction of traumatized or damaged tissue rather than substitution with inert parts. In recent decades, a number of articles were being published about the tissue engineering and regenerative medicine (TERM) field over 360 yearly just at the beginning of the twenty-first century. Just in 2010, the number of original articles in this field reaches 4000. This over-increasing attraction to this field—involving almost all tissues even whole organs—leads to researches across the world [2].

Herein, we review latest scientific researches and recent advances of tissue engineering in major field of oral and maxillofacial surgery by subtopics categorized by facial complex parts.

2. Basic principles of tissue engineering

Tissue engineering is composed of three pillars: the cells, scaffolds, and growth factors. The combination of cells in a suitable scaffold was designed by the appropriate biochemical signals that can facilitate and make possible growth, so it could be a treatment option that is very suitable for clinical application. Various studies have shown that one of the important issues is proper design of scaffolds and associated mechanical signals to regulate tissue that is engineered. Scaffold that can be temporarily or permanently used for three-dimensional porous can also be natural or artificial, which in any case must be biocompatible [3]. A biocompatible environmental issue is crucial importance because it facilitates progenitor cells for migration and differentiation [4]. Some of the important issues that include the physical properties of the scaffold such as biodegradability, porosity, hardness and strength to be as much in excess of migration, cell adhesion, and proliferation (such as osteoconduction), which reflects the influence of signals on the cell is followed by the clinical efficacy of chemical signals and ultimately success factor for the link to be followed. Perhaps the problem for surgeons and maxillofacial surgery is more important than other counterparts, being careful scaffold designing on human anatomy for the repair of any defects in the face. Various studies designed to use the computer in the exact scaffold have shown promising results and have built a biomimetic scaffold that has special significance [5].

To complement these three pillars, tissue, cell lines that require ease of access and availability, differentiation capacity, and lack of stimulation of the immune system or have tumor genesis [6]. Choosing the right cell lines in tissue engineering is still under discussion. New research hopes to use stem cells and gene therapy with viral vectors to express growth factors in cultured cell lines successfully, but stem cell research is outside the scope of this topic [7]. Today, the laboratory of tissue engineering that leads clinicians to living tissue is a concept

that has the potential to create a great impact on the future treatments. One of the major obstacles to the proper functioning of the tissue outside the body is to understand the way in which cells can be set in niches under certain physical and chemical conditions which would be difficult [8, 9]. In this case, bioreactors can control the situation and imitate the natural environment. Bioreactor devices can control and adjust the physiological conditions. With advances in tissue engineering, scaffold design could put several layers of cells onto scaffolds for three-dimensional position. That purpose requires a microenvironment for growth in vitro. The mathematical model was able to calculate the fluid flow rate for scaffold to provide nutrients and remove wastes and release oxygen used. As well as other variables such as external mechanical force needed to stimulate the proliferation of osteoblasts and then followed it can also be provided. Another alternative method that involves the cultivation of graft in vivo using animal models or humans as a bioreactor to simulate the growth of cells is provided. The remainder of this chapter presents various examples discussed regarding various tissues of the jaw and face [9].

3. Oral and maxillofacial bone defects

3.1. Mandibular defects

The atrophic mandible presents its own unique set of challenges in reconstructive maxillofacial surgery. A mandibular vertical height of less than 2 cm (20 mm) is universally considered atrophic and presents with characteristic anatomic and physiologic features, such as hypovascularity, which might contribute to tooth and alveolar process loss. The atrophic resorption patterns also contribute to the consistent anatomic changes, such as prominent mylohyoid and internal oblique ridges, which are covered with a thin mucosal lining, contributing to an increased risk of soft tissue breakdown and dehiscence. These anatomic changes happen secondary to a deficiency in blood supply from the lack of muscle attachments in those areas, whereas the areas that have a healthy musculature show an increased blood supply, making it more resistant to postdental extraction resorption. An important concept that reconstructive surgeons need to understand is that atrophic mandibles depend heavily on periosteal blood supply because of the narrowing of the inferior alveolar artery [10–12].

Cawood and his group from the United Kingdom found that alveolar bone resorption seemed to have a predictable pattern:

- Class I, dentate
- Class II, immediately post-extraction
- Class III, well-rounded ridge form, adequate in height and width
- Class IV, knife-edge ridge form, adequate in height and inadequate in width
- Class V, flat ridge form, inadequate in height and width
- Class VI, depressed ridge form, with some basilar bone loss evident [13]

This classification has more relevance to implant dentistry because it gives the operator an idea of whether an adjunctive bone graft would be necessary (classes IV and V).

Marx and colleagues [14] published a novel soft tissue matrix expansion also known as the "tent pole," where the dental implants effectively "tent" the soft tissue envelope up to maintain the bone graft volume and prevent soft tissue collapse. The original description used an extraoral submental approach, and the bone graft material of choice was the anterior iliac crest bone graft, with four to five implants placed (each one 15 mm in height), with a 1-cm interimplant distance. Primary stability was obtained by engaging the inferior border of the mandible with the implants. Autogenous corticocancellous bone graft is then packed around the implants.

Patel et al. reported that the addition of rhBMP in the tent pole technique had a favorable impact on bone healing and allowed substitution of the posterior iliac crest as a donor site with the anterior iliac crest bone graft because of the enhanced osteoinduction that happens with rhBMP. Furthermore, the authors rarely use the classical anterior iliac crest bone grafting approach, instead opting for the trephine to harvest the bone from the anterior iliac crest, with excellent increase in vertical bone height and final implant placement. This translated to less donor-site morbidity and earlier mobilization [15].

Many surgeons have modified Marx's original tent pole technique, and some have replaced dental implants with bone screws; this modification seemed to improve the buccolingual orientation of the final implant placement, because the dental implants would be placed at a second procedure, when all of the bones have been consolidated, and the position of the implants is more ideal. A second advantage of this modification is that it allows the use of surgical implant guides, especially if a maxillary prosthesis exists. Another commonly used method is the use of a titanium mesh to tent the soft tissue and maintain the bone graft and the contour of the ridge. However, the main disadvantage of this technique is that the surgical site must be reentered to remove the titanium mesh before implant placement. This has presented its own set of challenges, especially when the graft grows over the mesh, and the procedure requires excessive soft tissue reflection.

Another bioactive agent that has been studied in maxillofacial reconstructive surgery is recombinant human platelet-derived growth factor. This is a product of platelets and functions as a chemotactic and mitogenic factor for osteoblasts and is critical for angiogenesis and thus can be applied to treating ridge defects [16]. This growth factor has been combined with several different types of grafting materials and carriers, such as mineralized and demineralized FDBA [17], xenograft (specifically deproteinized bovine block graft), equine block graft [18], and bTCP [19], in multiple case series and has been shown to help produce intact woven and lamellar bone contributing to an increase in vertical ridge height in humans, which was of appropriate quality to accommodate the placement of dental implants at a second stage. The concept of engineered heterotopic bone formation has also been studied; however, this has not yet gained much notoriety. In 2004, it was studied in the reconstruction of large segmental mandibular defects by way of an engineered growth of a mandibular transplant within a muscular environment (in this case the latissimus dorsi muscle) with the help of BMPs, with subsequent free tissue transfer of the bone-muscle flap approximately 7 weeks later [20].

A prefabricated titanium mesh was filled with bone mineral blocks, BMPs, and the patient's own bone marrow. Although a clinically successful result was obtained, this procedure may not be as cost-effective as some of the more traditional and established methods of free tissue transfer for mandibular reconstruction and does carry with it significant morbidity related to the surgery itself and potential complications, such as brachial plexus injury and shoulder drop [21]. Nonetheless, it certainly does open up a different aspect of tissue engineering and strategies for maxillofacial reconstruction [22, 23].

3.2. Maxillary sinus augmentation

It is challenging to reconstruct the edentulous posterior maxilla with dental implants due to insufficient bone height after crestal bone resorption and also maxillary sinus pneumatization [24, 25]. In recent years, with aid of existing space in the maxillary sinus, clinicians introduced techniques for surgical augmentation that use to restore bone height and also create a sufficient implant bed area which seems to resolve patient's treatment difficulties [26, 27]. Researchers suggested a variety of modifications in original sinus augmentation technique to ease different difficulties for clinicians and also patients [28, 29], but the basic principle of each technique remained unchanged which is to increase maxillary bone height with aid of placing graft material in the maxillary sinus after attending to detach the sinus membrane [25, 28, 29]. Nowadays, for rehabilitation of the posterior maxilla with dental implants, the use of maxillary sinus augmentation (MSA) is considered as a standard procedure [24, 25].

In original technique, before dental implant insertion, MSA was performed with the autogenous bone [25, 30]. Autogenous bone has usually been cited as the most eligible material to achieve predictable and favorable results in MSA. It is due to the fact that autogenous bone contains living cells and growth factors which cause osteogenic ability [25, 30]. In contrast, it should have been noticed that available supplies for autogenous bone are limited. Also, as disadvantage, harvesting autogenous bone is painful and includes procedures with risk of infection. With these in mind, it is necessary to investigate and develop alternative techniques to overcome these drawbacks [24, 25, 31–37].

Introduction of different osteoconductive biomaterials such as allogeneic bone [31, 32], xenogeneic bone [32–34], or alloplastic or composite materials [34, 35] which are cell-free and due to that require more time for bone healing. This is a disadvantage that none of mentioned materials have biological and structural properties similar to the native bone [24, 25, 34–37].

The modern science of bone tissue engineering, a fusion of recent discoveries in the field of molecular cell biology with the most innovative methods of reconstructive surgery, aims to overcome these boundaries [38].

In **Table 1**, studies of stem cell approach for tissue engineering dealing with sinus augmentation were illustrated.

A major disadvantage of potential bone substitutes is their inherent slow ability to induce new bone at a foreseeable rate. By advances and innovation in technology in tissue engineering, introduced alternative materials which are used as bone show significant advantages in

Study	Control	Type SC	Scaffold	Evaluation	Follow-up	Complications
Gonshor et al.	Allograft	MSC (bone marrow)	Allograft (Osteocel)	Histology after 3–4 months		None
Voss et al.	ABG	MSC (periosteum)	Polymer fleece	Radiography, histology on 18 patients	24 months	A
Shayesteh et al.	None	MSC (bone marrow)	HA/TCP	Histology after 3 months	12 months	None
Yamada et al.	None	MSC (bone marrow)	PRP	Radiography	2- to 6.3-year	None
Rickert et al.	30% ABG and 70% BBM	MSC (bone marrow)	BBM	Histology after 13–16 weeks		None
Schimming et al.	None	MSC (periosteum)	Polymer fleece	Histology after 3 months	6 months in 9 patients	B
MacAllister et al.	None	MSC (bone marrow)	Allograft (Osteocel)	Histology after 4 months		None
Mangano et al.	Calcium phosphate	MSC (bone marrow)	Polymer fleece	Histology after 6 months, CT scan		C
Zizelmann et al.	ABG	MSC (periosteum)	Polymer fleece	Radiography after 3 months		D
Ueda et al.	None	MSC (bone marrow)	PRP	Radiography	2–5 years	None
Sauerbier et al.	MSC (bone marrow) vs. BMAC	MSC (bone marrow)	BBM	Histology after 3 months	12 months	None
Fuerst et al.	None	Autogenous culture-expanded bone cells	BBM	Histology after 6 months		None
Trautvetter et al.	None	MSC (periosteum)	Polymer fleece	Histology after 6 months	5 years	None
Schmelziesen et al.	None	MSC (periosteum)	Polymer fleece	Histology after 4 months		None
Hermund et al.	50% ABG + 50% BBM	Autogenous culture-expanded bone cells	50% ABG + 50% BBM	Histology after 4 months	8 months	None
Sauerbier et al.	30% ABG + 70% BBM	MSC (bone marrow)	BBM	Histology after 3–4 months		None
Springer et al.	BBM	MSC (periosteum) (l) Autogenous culture-expanded bone cells (2a)	Collagen [1] BBM (2a)	Histology after 6–8 months		None

Study	Control	Type SC	Scaffold	Evaluation	Follow-up	Complications
Beaumont et al.	None	MSC (periosteum)	BBM + polymer fleece	Histology after 4 and 6 months	18 months	None

Adopted from Jakobsen et al. [39]

BBM, bovine bone mineral; HA/TCP, hydroxyapatite/tricalcium phosphate; NA, data not available; MSCs, mesenchymal stem cells; PRP, platelet-rich plasma; RCT, randomized clinical trial

A: 11 augmentations failed in test group, none in control group

B: Histology shows little or no bone formation in 8 of 15 patients, when a two-stage protocol was used.

C: Less bone and more medullar spaces were found in test group compared with control group.

D: 13 of 14 sinuses showed insufficient bone formation. Resorption 90% in test group after 3 months, 29% in control group

Table 1. Studies regarding cell-based sinus lift procedure.

their bone-inductive capabilities. These results are similar to the result of study conducted by Neiva et al., which revealed favorable outcomes with the use of PepGen P-15 Putty. They conclude this result from initial osteogenesis of intervened group which is guided by the putty and achieved an additional mature trabecular pattern in shorter period of time comparing to the control group. Earlier bone formation is evaluated and revealed in 3D radiographic assessment as early as 8 weeks [40].

A variety of study designs used in cellular studies on sinus grafting techniques outcome the square measure constituent. Briefly, most studies comparing cell therapy with a traditional grafting technique showed similar results [41–44].

3.3. Dorsal augmentation in rhinoplasty

As application of allografts for dorsal augmentation seems to have serious disadvantages [45], it appears that we might tend to observe the appliance of tissue engineering in rhinoplasty; Kim et al. in 2014 within the article made a case for the chondrocytes and porcine cartilage substance (PCS) construct as an attainable dorsal augmentation material in rhinoplasty cultured with a porcine cartilage-derived substance (PCS) scaffold as a potential substitute for normal tissue use for augmentation in rhinoplasty. A scaffold is derived from decellularized and fine-grained porcine articular cartilage prepared. The use of the rabbit articular cartilage was due to ability to supply homologous chondrocytes, which for 7 weeks were enlarged and polite with the PCS scaffold. The chondrocyte-PCS constructs were then surgically implanted on the nasal dorsum of six rabbits. Four and 8 weeks after implantation, complete evaluations such as the gross morphology, radiologic pictures, and microscopic anatomy options of the location of implant were analyzed. The rabbits showed no signs of surgical inflammation and infection. The degree of dorsal augmentation was maintained throughout the 8-week surgical observation amount. Surgical examinations showed chondrocyte proliferation while there was no inflammatory response. However, neo-cartilage formation from the constructs was not confirmed. The biocompatibility and structural options of tissue-engineered chondrocyte-PCS constructs indicate their potential as candidate dorsal augmentation material to be used in rhinoplasty [46].

Cultured chondrocytes and porcine cartilage substance (PCS) constructs as an attainable dorsal augmentation material in rhinoplasty: preliminary animal study and, additionally Mendelson et al. in 2014 conferred this concept than designed nasal cartilage by cell homing: a model for augmentative and rehabilitative rhinoplasty. Bioactive scaffolds were developed that not solely recruited cells within the nasal dorsum in vivo, however, additionally induced chondrogenesis of the recruited cells. Bilayered scaffolds were fictional with alginate-containing gelatin microspheres encapsulating cytokines atop a porous poly(lactic-co-glycolic acid) base. Microspheres were fictional to contain recombinant human remodeling growth factor β3 at doses of 200, 500, or 1000 ng, with phosphate-buffered saline-loaded microspheres used as a bearing. A rat model of augmentation facelift was created by implanting scaffolds atop the native nasal cartilage surface that was scored to induce cell migration. Tissue formation and chondrogenesis within the scaffolds were evaluated by image analysis and microscopic anatomy staining with hematoxylin and eosin, toluidine blue, Verhoeff elastic-van Gieson, and aggrecan immunohistochemistry. Sustained release of increasing doses of remodeling growth factor β3 for up to the tested 10 weeks promoted orthotopic cartilage-like tissue formation in an exceedingly dose-dependent manner. It appears that these findings represent the primary commitment to engineer cartilage tissue by the cell orientating for facelift and will doubtlessly function as an alternate material for augmentative and rehabilitative rhinoplasty [46].

An important feature for rehabilitative and augmentative rhinoplasty is the ability of the graft to be tailored to the individual patient. Autologous graft area unit is stacked and sutured along in a very bundle before implantation. The bioactive poly(lactic-co-glycolic acid) scaffolds are simply changed to larger augmentations by varied the mold diameter wont to create the poly(lactic-co-glycolic acid) scaffold base. For associate degree off-the-shelf product, three totally different scaffolds can be generated with a variety of forms and sizes and simply cut for precise adjustments. Thus, the bioactive scaffolds might probably be used as completely unique various implant styles to current rhinoplasty treatment [46].

4. Skin

It seems that one of the important years for tissue engineering is 1975; in this year, some occurrence about skin engineering was evolved in this field even though the Washington National Science Foundation applied science panel meeting to formally adopt the term "tissue engineering" for this field a decade later in 1987 [47] and Langer and Vacanti explained the definition of this field later in 1993 [1]. The first step is ascribed to the actions of two teams in the United States 40 years ago. Rheinwatd and Green were the first team who are unskilled and ignored cultivation of human epidermal keratinocytes in vitro [48]; they also created potentially the enlargement of those cultivation of cells into numerous epithelial cells for graft in 1975 [49] from a little skin diagnostic assay. Today, the work that was done in those days is called "skin epidermis tissue engineering." At the same time, Yannas et al. worked on the features of scleroprotein and degradation mechanism [50] in 1975, which now

we believe that these efforts facilitate the way for the new generation of artificial dermal substitute, resulting in the "skin dermis tissue engineering" [51]. Fortunately, after 6 years, both of these groups independently revealed the clinical effect of tissue-engineered substitutes for the treatment of different grade burns, albeit in different approaches. The first graft of extensive burns with sheets of cultured epithelium (produced from epidermal cells which are autologous) was reported by O'Connor et al., two adult patients were experienced at the Peter Bent Brigham Hospital [52, 53]. Cultured epidermal autografts (CEA) were the next generation of cultured sheets which are autologous and also successively revealed to prepare cover of full-thickness burns in pediatric patients [54]. Meantime, only a short time after O'Connor et al.'s study, Burke et al. revealed that artificial dermis had experienced functional and physiological acceptable dermis in the treatment of extensive full-thickness burns on several patients [55]. These evidences resulted in randomized clinical trial for extensive burn injuries led by Heimbach et al. [56] about the application of artificial dermis; now new generation of artificial dermis is known as IntegraTM Dermal Regeneration Template. This study was done successfully by collaborating 11 centers, and other studies [57, 58] could inevitably demonstrate this dermal substitute a "gold standard" material treatment of full-thickness burns [59].

But there are still challenges, and those two groups are still far from reaching the final goal of replacing autologous skin for coverage of permanent deep dermal or full-thickness injuries in extensive burns.

Table 2 demonstrates the current status of available tissue-engineered materials and techniques for skin substitution.

	Skin substitute/ surgical technique	**Structure**	**Advantage**	**Disadvantage**
Epidermal	Cultured epithelial autograft (CEA)	Confluent autologous keratinocytes	In vitro expansion for large bum area, permanent	Fragility, infection, high cost, and variable take rate
	CUONO's method		Extensive bums	Two-stage procedure, precise grafting time coordination
	CEA with meshed split-thickness skin autograft		Expansion 1:4, no rejection	Beyond 1:4 expansion: poor cosmetic and functional results, delayed reepithelialization
	CEA with microskin autograft		Expansion 1:9–15, no rejection, high take rate, shorter epithelization time	Time-consuming, labor-intensive, hypertrophic scarring

		Skin substitute/ surgical technique	Structure	Advantage	Disadvantage
Dermal	Artificial biological materials	Integra™	Cross-linked bovine tendon collagen-based dermal matrix linked with glycosaminoglycan (GAG)		Two-stage procedure, infection, hematomas, seromas
		Integra™ with CEA		Good long-term esthetic and functional outcome	High cost, poor adhesion
		Integra™ with Meek			
		MatriDerm®	Bovine non-cross-linked lyophilized dermis, coated with alpha-elastin hydrolysate		
		Composite skin substitute	MatriDerm as a template, seeded with expanded autologous skin fibroblast and keratinocytes	Full wound closure	
		Blobrane®	Silicone membrane and nylon mesh impregnated with porcine dermal collagen	One-stage procedure, coverage of partial-thickness bums	Intolerant to contaminated wound bed
	Natural biological	AlloDerm®	Human acellular lyophilized dermis	Acellular, immunologically inert, provide natural dermal porosities for regeneration and vascularization on the wound bed	High cost, risk of transmitting disease, two-stage procedure
		AlloDerm® with CEA			Multiple applications
		Permacol™	Porcine acellular lyophilized dermis	Good esthetic and functional	Infection, hematomas, seromas
	Synthetic materials	TransCyte®	Porcine collagen-coated nylon mesh seeded with allogeneic neonatal human foreskin fibroblasts	Immediate availability, ease of storage	Temporary
		Dermagraft®	Bioabsorbable polyglactin mesh scaffold seeded with cryopreserved allogeneic neonatal human foreskin fibroblasts	Ease of handling, no rejection, chronic wounds—diabetic ulcers	Poor ECM structure, infections, cellulitis,

	Skin substitute/ surgical technique	Structure	Advantage	Disadvantage
Dermo-epidermal	PermaDerm™	Collagen-glycosaminoglycan substrates containing autologous fibroblasts and keratinocytes	Permanent replacement of both dermal and epidermal layers, one-step procedure	No clinical trial reported yet
	DenovoSkin	Plastically compressed collagen type 1 hydrogels engineered with human keratinocytes and fibroblasts	Near-normal skin architecture	Long culture time, no clinical series reported yet

Adopted from Chua et al. [60]

Table 2. Tissue-engineered materials and current surgical techniques for skin substitution.

The happen which facilitates efforts is that combination between a skin allograft bank and professional laboratory which culture autologous epithelial cell sheet could be an important step, and also we should gather many scientists and engineers and absorb finance in this field. The only way that can create the demand of engineered tissue for patients is through working and collaborating with clinicians, and also this expert team brings us innovation, novel technologies, and cost management and realizes the challenges in advancement of skin tissue engineering [61–65].

5. Oral mucosa

There is a recognized lot to reconstruct and restore advanced craniomaxillofacial (CMF) soft tissues that are broken and/or disfigured as a consequence of automobile accident, trauma, burn injury, or tumor surgery. In trauma, injuries usually produce extraordinarily advanced geometric and avulsion defects, and also the anatomic and purposeful intricacies of CMF composite soft tissue structures like the lips, eyelids, and nasal advanced create the reconstruction significantly difficult for maxillofacial surgeons (**Table 3**).

Kenji Izumi et al. in 2013 within the article evaluated the appliance of tissue engineering in oral mucosa [67]; the first objective of this study was to gauge the security of a tissue-engineered human ex vivo produced oral mucosa equivalent (EVPOME) in intraoral graft procedures. The secondary objective was to assess the efficacy of the grafted EVPOME in manufacturing a keratinized mucosal surface epithelial tissue. Five patients World Health Organization based on inclusion criteria that defects in mucogingival region or an absence of gingiva which is keratinized on incisors and premolars teeth, together with radiographies of adequate bone height in interdental region, were used to expand the amount of keratinized gingiva in the defect site. A specimen was taken by a punch biopsy from hard palate to accumulate oral keratinocytes, which were enlarged, associate degreed cultured on associate degree noncellular matrix of the dermis for make of an EVPOME.

Approach	Advantages	Disadvantages
Free grafts (full-thickness skin grafts, split-thickness skin grafts, etc.)	Simply harvested, vital tissue accessibility	Poor color match, donor-site morbidity, lack of bulk, no performance
Local advancement and motility flaps	Sensible color match, functional	Restricted quantity, might need staged surgeries, will result in microstomia and associated purposeful deficits in speech and swallowing
Free vascularized tissue transfer	Wonderful tube peduncle, applicable tissue thickness, and a technique to suspend the lip with the incorporated tendon	Long recovery, donor-site morbidity, lack of performance, poor color match/esthetics, needs specialized surgical skills
Allogeneic tissue transfer/ face transplant	Sensible color match, purposeful tissue, esthetics	Needs long immunosuppression, facet effects of immunosuppression, long and troublesome recovery, donor accessibility, needs specialized surgical skills and facility

Table 3. The advantages and downsides of the contemporary approach to soft tissue reconstruction [66].

EVPOME grafts have special features which are used directly over associate degree healthy periosteal bed and preserved in situ. At 1 and at 7, 14, 30, 90, and 180 days postsurgery, Plaque Index and Gingival Index were recorded for every subject. Additionally, inquisitory depths, keratinized animal tissue dimension, and keratinized animal tissue thickness were recorded at baseline, 30, 90, and 180 days. Fortunately, there were no adverse outcomes or complications to EVPOME ascertained in all cases throughout the research. But the mean increased in keratinized animal tissue dimension was 3 mm (range, 3–4 mm), and no vital changes in depths were ascertained. According to our findings, we can terminate that EVPOME is useful for oral application and has the flexibility to reinforce keratinized gingiva. More randomized clinical trials in this field should be performed to demonstrate other dimensions of tissue engineering [67].

The maintenance of associate degree sufficient strip of attached gingiva includes a minimum of 2 mm of keratinized gingiva and has revealed that it could be necessary for preservation of periodontal tissue [68, 69]. Historically, FGGs' associate degreed grafts which were taken from connective tissue are applied to gain sufficient strip of attached gingiva [70]. Unfortunately, clinical evidence about the use of autologous and allogenic products confronts to some problems about FGGs, i.e., problems about morbidity around donor region and also amount of tissue for graft which is restricted [71]. Nevins [72] applied treatment by bilayered cell for mucogingival region, and this research revealed that adequate keratinized tissue was gained; however, the amount was not more than it gained with FGGs. Interestingly, each research tried to prove that clinical evidence such as texture and color have better results when compared with FGGs. Nevertheless, application of products that have allogenic cells could have important effect on the wound bed; the impact of treatment could be as a completely unique "biologic dressing" to motivate encompassing cells. One of the reliable materials was a scaffold which is biodegradable with gingival autologous fibroblasts which are cultured [73]. Another research in this field used oral mucosa cells to transplant directly to the cornea [74].

Another researchers believed that the long time (more than 22 months) existence of the cultured keratinocytes from oral cavity, expressed markers of stem cell. Also evidence tried to by pharmacologic approach produce cultured oral keratinocytes to produce stem cell population; and now all efforts focus on clinical application of this technology that has a lot of simply for the event of a lot of strong EVPOME for intraoral graft procedures [75]. As a result of oral mucosa keratinocyte area unit simply getable and expand quicker in vitro than skin keratinocytes [76], they will be a lot of efficacious to be used in future clinical applications in regenerative medicine. This platform technique might produce other potential extraoral uses, like repair of facial skin [77], reconstruction of eyelids and nose, or in situ tissue layer substitutes for the urethra and conjunctiva [78].

6. Temporomandibular joint disorders

The temporomandibular joint (TMJ) may be a synovial joint that has for articulator motion relative to the os base and distributes the traditional stresses of perform (chewing and speaking) and parafunction (clenching and bruxism). It is usually noted as ginglymoarthrodial joint attributable to its slippy performance and hinging. The temporomandibular joint links the condyloid process (mandibular bone) to the temporal bone. The cartilage disc is the middle of mandibular condyle and the glenoid fossa eminence of the temporal bone and separates the joint area into inferior and superior compartments, each of that area unit crammed with synovial fluid [79].

Because of the advanced loading patterns that designed tissues can expertise within the TMJ, acquisition of complete style parameters from the native tissue is important. Significantly, TMJ disc, condyle, and condylar cartilage replacement area unit in nice demand attributable to these tissues' poor regenerative capability and high rate of involvement in TMD. In response, many studies characterizing the properties of those elements are performed [79]. Though glenoid fossa and articular eminence are the concern in TMD, they need not be absolutely characterized. Within the following section, structural characteristics of the TMJ tissue area unit are summarized. Application of tissue engineering in the treatment of temporomandibular joint defects is rising as a progressive choice to substitute and remove the pathological defects automatically in the near future. Historically, basics of regenerative medicine are three necessary elements, cells, stimulator factors, and scaffolds. But new technology introduced novel methodologies and recommends how to manage TMJ disk and stimulate relevant cartilage [80]. This approach includes the scaffold-free and cell-based methodology and cells and stimulators that work together. But another approach is to construct a structure and render appropriate form of engineered tissues, permitting well-designed structure and simple handling [81]. Another mechanical feature may be appropriate for designed tissue. In the best manner the chemistry of scaffold that could be degradation with matrix synthesis. But the rate of scaffold degradation depends on the nature of scaffold and might be modified by manipulation. For induction of the mass of matrix synthesis, growth factors are added to the scaffolds. Today, all engineered tissue materials are used to regenerate condyle and TMJ disc; however, similar strategies for regeneration of mandibular fossa are not successful [82].

7. Conclusion

Unfortunately, limiting factors still existed; most of them could be the differences of lab environment and human body such as unknown exact dose of BMP and applicate high dose of this material to creation of bony scaffold [83]. On the other hand, unpredictable effects of BMP and complications about application of BMP together with oncogenesis. Based on these evidences, Food and Drug Administration (FDA) restricted application of BMP to sinus alveolar process augmentation in the United States; other substitute materials in maxillofacial region should be considered moral issues even if these materials reveal high level of evidence in several experimentations [84].

Another problem like tissue transfer decreases the chance of high-quality and priced experimentations [85], and also Ripamonti et al. study revealed that growth factor and signaling systems in animal and human are totally different and huge variation between them is observed. Unfortunately, there are few clinical trials in the maxillofacial region, but the question is what the obstacles are? The main obstacles are how to predict regenerate cells to not become oncogenesis and produce our wanted cells, how to manage signaling factors to facilitate the procedure of regeneration, and how to create the scaffold that permits cell growth in the best way. More clinical trials are needed to remove the obstacles [86].

Tissue engineering is the field that is surrounded by other fields like histology, medical engineering, and pathology that every progress in these fields could change principles of tissue engineering. Our goals are simple which are to know how to regenerate human tissues from host cells and somehow that these regenerates have desirable function and esthetics. To reach to this goal, we have long way, but today engineers progressed biocompatible scaffolds, increase the flexibility to 3D tissue constructs, and designed complex tissue for the different facial areas. The latest progress guarantees that tissue engineering is the trustworthy choice for the treatment of maxillofacial defects. In future, the role of tissue engineering will increase and become routine in surgeries.

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Conflict of interest

Authors declare that there is no conflict of interest that may damage the integrity and validity of this research.

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References

[1] Lanza R, Langer R, Vacanti JP. Principles of Tissue Engineering. Academic Press; Elsevier Academic Press: MA, USA. 2011

[2] Fisher MB, Mauck RL. Tissue engineering and regenerative medicine: Recent innovations and the transition to translation. Tissue Engineering Part B: Reviews. 2013;**19**(1):1-13

[3] Salih V. Biodegradable scaffolds for tissue engineering. Cellular response to biomaterials. Boca Raton: CRC Press; 2008. p. 185-211

[4] Mihaylova Z, Mitev V, Stanimirov P, Isaeva A, Gateva N, Ishkitiev N. Use of platelet concentrates in oral and maxillofacial surgery: An overview. Acta Odontologica Scandinavica. 2017;**75**(1):1-11

[5] Y-f L, Zhu F-d, Dong X-t, Peng W. Digital design of scaffold for mandibular defect repair based on tissue engineering. Journal of Zhejiang University Science B. 2011;**12**(9):769

[6] Seo S, Na K. Mesenchymal stem cell-based tissue engineering for chondrogenesis. BioMed Research International. 2011;**2011**:163-170

[7] Betz VM, Betz OB, Harris MB, Vrahas MS, Evans CH. Bone tissue engineering and repair by gene therapy. Frontiers in Bioscience. 2008;**13**(13):833-841

[8] Depprich R, Handschel J, Wiesmann H-P, Jäsche-Meyer J, Meyer U. Use of bioreactors in maxillofacial tissue engineering. British Journal of Oral and Maxillofacial Surgery. 2008;**46**(5):349-354

[9] Deb S, Mandegaran R, Di Silvio L. A porous scaffold for bone tissue engineering/45S5 bioglass® derived porous scaffolds for co-culturing osteoblasts and endothelial cells. Journal of Materials Science: Materials in Medicine. 2010;**21**(3):893-905

[10] Misch CE. Dental Implant Prosthetics-E-Book. Elsevier Health Sciences; 2014

[11] Aziz SR, Najjar T. Management of the edentulous/atrophic mandibular fracture. Atlas of the Oral and Maxillofacial Surgery Clinics. 2009;**17**(1):75-79

[12] Madsen MJ, Haug RH, Christensen BS, Aldridge E. Management of atrophic mandible fractures. Oral and Maxillofacial Surgery Clinics of North America. 2009;**21**(2):175-183

[13] Cawood J, Howell R. A classification of the edentulous jaws. International Journal of Oral and Maxillofacial Surgery. 1988;**17**(4):232-236

[14] Marx RE, Shellenberger T, Wimsatt J, Correa P. Severely resorbed mandible: Predictable reconstruction with soft tissue matrix expansion (tent pole) grafts. Journal of Oral and Maxillofacial Surgery. 2002;**60**(8):878-888

[15] Patel N, Kim B, Zaid W, Spagnoli D. Tissue engineering for vertical ridge reconstruction. Oral and Maxillofacial Surgery Clinics. 2017;**29**(1):27-49

[16] Giannobile W. Periodontal tissue regeneration by polypeptide growth factors and gene transfer. Tissue Engineering: Applications in Maxillofacial Surgery and Periodontics. 1999;**1**:231-243

[17] Guze KA, Arguello E, Kim D, Nevins M, Karimbux NY. Growth factor-mediated vertical mandibular ridge augmentation: A case report. International Journal of Periodontics & Restorative Dentistry. 2013;**33**(5):611-617

[18] Simion M, Rocchietta I, Kim D, Nevins M, Fiorellini J. Vertical ridge augmentation by means of deproteinized bovine bone block and recombinant human platelet-derived growth factor-BB: A histologic study in a dog model. International Journal of Periodontics & Restorative Dentistry. 2006;**26**(5):415-423

[19] Stephan EB, Renjen R, Lynch SE, Dziak R. Platelet-derived growth factor enhancement of a mineral-collagen bone substitute. Journal of Periodontology. 2000;**71**(12):1887-1892

[20] Warnke P, Springer I, Wiltfang J, Acil Y, Eufinger H, Wehmöller M, et al. Growth and transplantation of a custom vascularised bone graft in a man. The Lancet. 2004;**364**(9436): 766-770

[21] Meijer GJ, de Bruijn JD, Koole R, van Blitterswijk CA. Cell-based bone tissue engineering. PLoS Medicine. 2007;**4**(2):e9

[22] Patel N, Kim B, Zaid W, Spagnoli D. Tissue engineering for vertical ridge reconstruction. Oral and Maxillofacial Surgery Clinics of North America. 2017;**29**(1):27-49

[23] Melek LN. Tissue engineering in oral and maxillofacial reconstruction. Tanta Dental Journal. 2015;**12**(3):211-223

[24] Park J-B. Use of cell-based approaches in maxillary sinus augmentation procedures. Journal of Craniofacial Surgery. 2010;**21**(2):557-560

[25] Browaeys H, Bouvry P, De Bruyn H. A literature review on biomaterials in sinus augmentation procedures. Clinical Implant Dentistry and Related Research. 2007;**9**(3):166-177

[26] Tatum H Jr. Maxillary and sinus implant reconstructions. Dental Clinics of North America. 1986;**30**(2):207-229

[27] Boyne PJ. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of Oral Surgery. 1980;**38**:613-616

[28] Summers RB. A new concept in maxillary implant surgery: The osteotome technique. Compendium (Newtown, Pa). 1994;**15**(2):152, 4-6, 8 passim; quiz 62

[29] Davarpanah M, Martinez H, Tecucianu J-F, Hage G, Lazzara R. The modified osteotome technique. International Journal of Periodontics & Restorative Dentistry. 2001;**21**(6): 599-607

[30] Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. International Journal of Oral and Maxillofacial Implants. 2002;**17**(5):635-643

[31] Cammack GV, Nevins M, Clem DR, Hatch JP, Mellonig JT. Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations. The International Journal of Periodontics & Restorative Dentistry. 2005;**25**(3):231-237

[32] Froum SJ, Wallace SS, Elian N, Cho SC, Tarnow DP. Comparison of mineralized cancellous bone allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: Histomorphometry at 26 to 32 weeks after grafting. International Journal of Periodontics & Restorative Dentistry. 2006;**26**(6):543-551

[33] Pettinicchio M, Traini T, Murmura G, Caputi S, Degidi M, Mangano C, et al. Histologic and histomorphometric results of three bone graft substitutes after sinus augmentation in humans. Clinical Oral Investigations. 2012;**16**(1):45-53

[34] Mangano C, Scarano A, Perrotti V, Iezzi G, Piattelli A. Maxillary sinus augmentation with a porous synthetic hydroxyapatite and bovine-derived hydroxyapatite: A comparative clinical and histologic study. International Journal of Oral & Maxillofacial Implants. 2007;**22**(6):980-986

[35] Scarano A, Degidi M, Iezzi G, Pecora G, Piattelli M, Orsini G, et al. Maxillary sinus augmentation with different biomaterials: A comparative histologic and histomorphometric study in man. Implant Dentistry. 2006;**15**(2):197-207

[36] Schimming R, Schmelzeisen R. Tissue-engineered bone for maxillary sinus augmentation. Journal of Oral and Maxillofacial Surgery. 2004;**62**(6):724-729

[37] Schmelzeisen R, Schimming R, Sittinger M. Making bone: Implant insertion into tissue-engineered bone for maxillary sinus floor augmentation—A preliminary report. Journal of Cranio-Maxillofacial Surgery. 2003;**31**(1):34-39

[38] Parveen S, Krishnakumar K, Sahoo S. New era in health care: Tissue engineering. Journal of Stem Cells & Regenerative Medicine. 2006;**1**(1):8

[39] Jakobsen C, Sørensen J, Kassem M, Thygesen T. Mesenchymal stem cells in oral reconstructive surgery: A systematic review of the literature. Journal of Oral Rehabilitation. 2013;**40**(9):693-706

[40] Neiva RF, Tsao Y-P, Eber R, Shotwell J, Billy E, Wang H-L. Effects of a putty-form hydroxyapatite matrix combined with the synthetic cell-binding peptide P-15 on alveolar ridge preservation. Journal of Periodontology. 2008;**79**(2):291-299

[41] Gonshor A, McAllister BS, Wallace SS, Prasad H. Histologic and histomorphometric evaluation of an allograft stem cell-based matrix sinus augmentation procedure. International Journal of Oral & Maxillofacial Implants. 2011;**26**(1):123-131

[42] Hermund NU, Stavropoulos A, Donatsky O, Nielsen H, Clausen C, Reibel J, et al. Reimplantation of cultivated human bone cells from the posterior maxilla for sinus floor augmentation. Histological results from a randomized controlled clinical trial. Clinical Oral Implants Research. 2012;**23**(9):1031-1037

[43] McAllister BS, Haghighat K, Gonshor A. Histologic evaluation of a stem cell-based sinus-augmentation procedure. Journal of Periodontology. 2009;**80**(4):679-686

[44] Kramer IR, Pindborg JJ, Shear M. Histological Typing of Odontogenic Tumours. Springer Science & Business Media. Springer-Varlag: Berlin Heidelberg; 1992

[45] Mohammad Hosein Amirzade-Iranaq SMRM, Seied Omid Keyhan. Effectiveness of alloplastic materials in rhinoplasty surgery: A systematic review and meta-analysis. 15th International Congress of Iranian Society of Oral and Maxillofacial Surgeans (OMFS); Tehran, Iran. Tehran, Iran: Iranian Society of Oral and Maxillofacial Surgeans; 2017

[46] Kim YS, Park DY, Cho YH, Chang JW, Choi JW, Park JK, et al. Cultured chondrocyte and porcine cartilage-derived substance (PCS) construct as a possible dorsal augmentation

material in rhinoplasty: A preliminary animal study. Journal of Plastic Reconstructive and Aesthetic Surgery. 2015;**68**(5):659-666

[47] Nerem R. Tissue engineering in the USA. Medical and Biological Engineering and Computing. 1992;**30**(4):CE8-CE12

[48] Rheinwatd JG, Green H. Seria cultivation of strains of human epidermal keratinocytes: The formation keratinizing colonies from single cell is. Cell. 1975;**6**(3):331-343

[49] Green H, Kehinde O, Thomas J. Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. Proceedings of the National Academy of Sciences. 1979;**76**(11):5665-5668

[50] Yannas I, Burke J, Huang C, Gordon P. Correlation of in vivo collagen degradation rate with in vitro measurements. Journal of Biomedical Materials Research. 1975;**9**(6):623-628

[51] Yannas I, Burke JF. Design of an artificial skin. I. Basic design principles. Journal of Biomedical Materials Research. 1980;**14**(1):65-81

[52] O'Connor N, Mulliken J, Banks-Schlegel S, Kehinde O, Green H. Grafting of burns with cultured epithelium prepared from autologous epidermal cells. The Lancet. 1981;**317**(8211):75-78

[53] Green H. The birth of therapy with cultured cells. BioEssays. 2008;**30**(9):897-903

[54] Gallico GG III, O'Connor NE, Compton CC, Kehinde O, Green H. Permanent coverage of large burn wounds with autologous cultured human epithelium. New England Journal of Medicine. 1984;**311**(7):448-451

[55] Burke JF, Yannas IV, Quinby WC Jr, Bondoc CC, Jung WK. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Annals of Surgery. 1981;**194**(4):413

[56] Heimbach D, Luterman A, Burke J, Cram A, Herndon D, Hunt J, et al. Artificial dermis for major burns. A multi-center randomized clinical trial. Annals of Surgery. 1988;**208**(3):313

[57] Heimbach DM, Warden GD, Luterman A, Jordan MH, Ozobia N, Ryan CM, et al. Multicenter postapproval clinical trial of Integra® dermal regeneration template for burn treatment. Journal of Burn Care & Research. 2003;**24**(1):42-48

[58] Heitland A, Piatkowski A, Noah E, Pallua N. Update on the use of collagen/glycosaminoglycate skin substitute—Six years of experiences with artificial skin in 15 German burn centers. Burns. 2004;**30**(5):471-475

[59] Shevchenko RV, James SL, James SE. A review of tissue-engineered skin bioconstructs available for skin reconstruction. Journal of the Royal Society Interface. 2009;**7**(43):229-258

[60] Chua AWC, Khoo YC, Tan BK, Tan KC, Foo CL, Chong SJ. Skin tissue engineering advances in severe burns: Review and therapeutic applications. Burns & Trauma. 2016;**4**(1):3

[61] MacNeil S. Progress and opportunities for tissue-engineered skin. Nature. 2007;**445**(7130): 874-880

[62] Conconi MT, Coppi PD, Liddo RD, Vigolo S, Zanon GF, Parnigotto PP, et al. Tracheal matrices, obtained by a detergent-enzymatic method, support in vitro the adhesion of chondrocytes and tracheal epithelial cells. Transplant International. 2005;**18**(6):727-734

[63] Van der Veen VC, van der Wal MB, van Leeuwen MC, Ulrich MM, Middelkoop E. Biological background of dermal substitutes. Burns. 2010;**36**(3):305-321

[64] Wainwright D. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. Burns. 1995;**21**(4):243-248

[65] Yu P, Qi Z. Prospective randomized comparison of scar appearances between Cograft of Acellular dermal matrix with autologous split-thickness skin and autologous split-thickness skin graft alone for full-thickness skin defects of the extremities. Plastic and Reconstructive Surgery. 2016;**137**(5):906e

[66] Kim RY, Bae SS, Feinberg SE. Soft tissue engineering. Oral and Maxillofacial Surgery Clinics of North America. 2017;**29**(1):89-104

[67] Izumi K, Neiva RF, Feinberg SE. Intraoral grafting of tissue-engineered human oral mucosa. The International Journal Of Oral & Maxillofacial Implants. 2013;**28**(5):e295

[68] Crisciani S. Efficacia Clinica Degli Innesti di Collagene Xenogeno Nella Terapia Chirurgica delle Recessioni Gengivali multiple: Clinical Trial Randomizzato. Graduate thesis LC5. Universita Di Pisa; 2012

[69] Nevins M. Attached gingiva-mucogingival therapy and restorative dentistry. The International Journal of Periodontics & Restorative Dentistry. 1985;**6**(4):9-27

[70] Wennström JL, Zucchelli G. Increased gingival dimensions. A significant factor for successful outcome of root coverage procedures? Journal of Clinical Periodontology. 1996;**23**(8):770-777

[71] McGuire MK, Scheyer ET, Nunn ME, Lavin PT. A pilot study to evaluate a tissue-engineered bilayered cell therapy as an alternative to tissue from the palate. Journal of Periodontology. 2008;**79**(10):1847-1856

[72] Nevins ML. Tissue-engineered bilayered cell therapy for the treatment of oral mucosal defects: A case series. International Journal of Periodontics & Restorative Dentistry. 2010;**30**(1):31-39

[73] Prato GPP, Rotundo R, Mognoni C, Soranzo C. Tissue engineering technology for gingival augmentation procedures: A case report. International Journal of Periodontics & Restorative Dentistry. 2000;**20**(6):552-559

[74] Chen H-CJ, Chen H-L, Lai J-Y, Chen C-C, Tsai Y-J, Kuo M-T, et al. Persistence of transplanted oral mucosal epithelial cells in human cornea. Investigative Ophthalmology & Visual Science. 2009;**50**(10):4660-4668

[75] Izumi K, Inoki K, Fujimori Y, Marcelo C, Feinberg S. Pharmacological retention of oral mucosa progenitor/stem cells. Journal of Dental Research. 2009;**88**(12):1113-1118

[76] Pomahač B, Svensjö T, Yao F, Brown H, Eriksson E. Tissue engineering of skin. Critical Reviews in Oral Biology & Medicine. 1998;**9**(3):333-344

[77] C-y T, Ueda M, K-i H, Horie K, Hibino Y, Sugimura Y, et al. Clinical results of cultured epithelial cell grafting in the oral and maxillofacial region. Journal of Cranio-Maxillofacial Surgery. 1997;**25**(1):4-8

[78] Yoshizawa M, Feinberg SE, Marcelo CL, Elner VM. Ex vivo produced human conjunctiva and oral mucosa equivalents grown in a serum-free culture system. Journal of Oral and Maxillofacial Surgery. 2004;**62**(8):980-988

[79] Aryaei A, Vapniarsky N, JC H, Athanasiou KA. Recent tissue engineering advances for the treatment of temporomandibular joint disorders. Current Osteoporosis Reports. 2016;**14**(6):269-279

[80] Hu JC, Athanasiou KA. A self-assembling process in articular cartilage tissue engineering. Tissue Engineering. 2006;**12**(4):969-979

[81] Vacanti JP, Morse MA, Saltzman WM, Domb AJ, Perez-Atayde A, Langer R. Selective cell transplantation using bioabsorbable artificial polymers as matrices. Journal of Pediatric Surgery. 1988;**23**(1):3-9

[82] Murphy MK, MacBarb RF, Wong ME, Athanasiou KA. Temporomandibular joint disorders: A review of etiology, clinical management, and tissue engineering strategies. The International Journal of Oral & Maxillofacial Implants. 2013;**28**(6):e393

[83] Boyne PJ, Lilly LC, Marx RE, Moy PK, Nevins M, Spagnoli DB, et al. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. Journal of Oral and Maxillofacial Surgery. 2005;**63**(12):1693-1707

[84] Woo EJ. Adverse events reported after the use of recombinant human bone morphogenetic protein 2. Journal of Oral and Maxillofacial Surgery. 2012;**70**(4):765-767

[85] Zhang Z. Bone regeneration by stem cell and tissue engineering in oral and maxillofacial region. Frontiers of Medicine. 2011;**5**(4):401-413

[86] Ripamonti U, Tsiridis E, Ferretti C, Kerawala CJ, Mantalaris A, Heliotis M, Ripamonti U, Tsiridis E, Ferretti C, Kerawala CJ, Mantalaris A, Heliotis M. Perspectives in regenerative medicine and tissue engineering of bone. British Journal of Oral and Maxillofacial Surgery. 2011;**49**(7):507-509

Chapter 14

Human Adipose-Derived Stem Cells for Tissue Engineering Approaches: Current Challenges and Perspectives

Sorina Dinescu, Anca Hermenean and Marieta Costache

Additional information is available at the end of the chapter

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Abstract

Human adipose-derived stem cells (hASCs) currently represent a viable source of mesenchymal-*like* stem cells, with similar properties and differentiation potential to bone-marrow-derived mesenchymal stem cells (BM-MSCs) but with a different and more accessible source—the adipose tissue. hASCs are able to produce almost all of the factors that contribute to normal wound healing, and therefore, they are preferred for all types of tissue engineering (TE) and regenerative medical applications. This chapter will review hASCs regeneration potential and the most modern approaches in TE for bone, cartilage and adipose tissue regeneration using hASCs. Furthermore, an overview of novel and original hASCs-scaffold constructs studied in our group completes an up-to-date presentation of hASCs multiple uses. Additionally, this chapter will highlight the relevance of ultimate advances in regenerative medicine and the need for this evolution to increase the quality of life in patients with tissue defects.

Keywords: human adipose-derived stem cells, tissue engineering, regenerative medicine, stem cell differentiation

1. Introduction

Current tissue engineering (TE) approaches registered an evolution over the past few years in the area of regenerative medicine. If the last decade was dedicated to repair and reconstructive procedures involving the implantation of inert materials to solve tissue defects, the modern trend uses regenerative strategies frequently based on advanced biomaterials and the differentiation potential of stem cells.

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The basic principle underlying this modern tissue engineering concept is that an equilibrium should be established simultaneously between the elements required to obtain a functional engineered tissue—suitable cells, appropriate scaffolds and the adequate signalling molecules. The goal of tissue engineering is to recreate a microenvironment as similar as possible to the *in vivo* natural tissue.

Human adipose-derived stem cells (hASCs) represent a viable source of mesenchymal-*like* stem cells, with similar properties to bone-marrow-derived mesenchymal stem cells (BM-MSCs) but with a different and more accessible source—the adipose tissue. A particular population of stem cells with self-renewal properties and multilineage differentiation potential, isolated from the adipose stromal-vascular compartment, was first reported by Zuk et al. [1]. In further studies, it has been proved that these cells display (i) a hypoimmunogenic profile [2], (ii) can be easily harvested from subcutaneous adipose tissue during liposuction procedures [3] and (iii) since they can be isolated from autologous fat, there is no ethical issue involved in case of transplantation for regenerative purposes. All these properties that hASCs display make them a more viable solution for regenerative medicine approaches then MSCs or other adult stem cells.

Apart from the fact they can be more easily harvested than MSCs, hASCs are able to produce almost all of the factors that contribute to normal wound healing [4]. Consequently, at the injury site, implanted cells that undergo differentiation generate not only an inert filling tissue but also are able to stimulate cell recruitment from stem cell niches in order to aesthetically restore the site of injury in a paracrine manner (by secretion of growth factors and cytokines) [5]. These observations suggest that hASCs could be better candidates for TE applications than other traditional cell sources.

hASCs' clinical and TE applications: Modern regenerative therapies use hASCs, based on their abundance, distribution and multilineage differentiation ability. There are certain studies that put, under a question mark, the safety issues related to hASCs use for regeneration purposes, since several genetic abnormalities after their *in vitro* expansion or differentiation have been reported by several groups [6–8]. However, no interdiction of hASCs in clinical practice for TE has been yet announced.

Regarding the scaffolds appropriate for TE applications, there has been an evolution in the composition and properties of biomaterials in the last decade. Nowadays, biomaterials tend to be made of natural and biodegradable compounds, thus favouring their biocompatibility. In addition, a 3D scaffold displays a significantly increase in capacity to closely mimic *in vivo* cellular microenvironments [9, 10].

Different biomaterials have been used in studies to favour hASCs growth in 3D scaffolds. hASCs displayed potential to attach, proliferate and differentiate in contact with favourable biomaterial compositions. Ideally, biofabricated scaffolds should offer hASCs proper environments to facilitate their proliferation and maintain their differentiation potentials [3]. Besides the biocompatibility condition, these materials should also be synthesized to have highly porous structures with interconnected architecture to mimic the native tissue niche [3]. Science in the field of scaffold engineering has evolved towards biofabrication using modern approaches

such as bioprinting, patterning, self-assembling and organ-on-a-chip [10]. According to Dai et al., most of these approaches have been employed to encapsulate hASCs in 3D structures (resulting in 3D culture systems) specifically designed for a TE application.

Related to *in vivo* experiments using hASCs, it was both experimentally and clinically shown that the topical administration of hASCs to full-thickness radiated wounds increases the healing rate of the wound [4]. It was also shown that hASCs stimulate fibroblast proliferation and migration and type I collagen secretion in an *in vivo* wound model. These findings suggest that hASCs may promote *in vivo* wound healing.

hASCs are currently recognized as an attractive and efficient adult stem cell type for regenerative medicine. Still, there are problems that need to be clarified including the mechanisms of the interactions among hASCs and their long-term safety. Only a small number of clinical trials have been performed by now [3].

The majority of clinical trials involving hASCs or hASCs-enriched fat grafts are incipient phase clinical trials (phase I or II), while only one trial reached phase IV in human subjects (NCT00616135). Several directions were approached by clinicians in order to test the efficiency of hASCs in tissue regeneration. Breast reconstruction is one of the major applications approached by clinicians, not only for breast augmentation purposes (NCT01771913) but also for breast reconstruction after tumour resection (NCT00616135). Special care should be given for implantation of hASCs in a former tumour microenvironment, since little is known about hASCs stability at genomic level. Both clinical trials resulted in a favourable primary outcome. However, this direction is considered to have limitations since the implanted fat graft suffered resorption over time and thus the implant shape and dimension altered after 6–12 months. Additional successful applications where hASCs were involved are liver tissue reconstruction (NCT01062750), cardiovascular disease (NCT01449032; NCT00442806; NCT00426868; NCT01216995), osteoarthritis (NCT01585857) or brain injury (NCT01649700). All results showed hASCs display high therapeutic potential and generally display safety for *in vivo* implantation.

Considering all the abovementioned, the chapter will further present a few biomaterial compositions that we used in conjunction with hASCs and stimulating factors. In particular, we have studied the potential of hASCs differentiation towards the adipose, cartilage and bone lineages during *in vitro* studies in different 3D original scaffolds. For adipose tissue engineering (ATE), we have identified novel biomaterials based on gelatin-alginate-polyacrylamide (GAPAA) [11] and collagen-sericin (CollSS) [12] which proved to be efficient for soft tissue reconstruction. In parallel, the addition of hyaluronic acid and chondroitin sulphate proved to increase the quality of the CollSS hydrogel and to transform it in a scaffold designed for cartilage tissue engineering (CTE) [13]. We have also tested and validated different scaffolds based on chitosan, polyvinyl alcohol, polysulphone, etc. each improved by the addition of graphene oxide (GO), giving stability to the structure. An appropriate concentration of GO in scaffolds composition resulted in significantly better cell differentiation towards bone tissue.

Both chitosan/GO biomaterials and improved collagen scaffolds for cartilage repair were tested for response during *in vivo* studies on mouse models. Results supported the conclusions

obtained *in vitro* and confirmed the efficiency of these differentiation-specific cell-scaffold systems. However, only a small number of studies have addressed *in vivo* applications involving ASCs. More studies need to be developed in this direction in order to evaluate the good and the bad potential of hASCs.

2. Human adipose-derived stem cells: source, properties and differentiation potential

2.1. hASCs isolation and characterization

hASCs can be reproducibly isolated from liposuction aspirates through a procedure involving collagenase digestion, differential centrifugation and expansion in culture [5, 14]. Undifferentiated hASCs express a distinct immunophenotype (hASCs express the MSC markers CD10, CD13, CD29, CD34, CD44, CD54, CD71, CD90, CD105, CD106, CD117 and STRO-1 and are negative for hematopoietic lineage and endothelial cell markers) detectable by flow cytometry and produce additional adipocyte-specific proteins upon induction [15, 16]. The hASCs immunophenotype was also assessed by flow cytometry in our team and the results were successfully reported [14].

The protocol for the isolation of hASCs involves the removal of subcutaneous fat by liposuction and treatment with collagenase, a hydrolytic enzyme [1]. hASCs are found in a homogeneous mixture called stromal vascular fraction (SVF), together with endothelial cells, stromal and hematopoietic cells; due to their tendency to adhere to the substrate in the culture medium, hASCs can be isolated easily [17].

Mainly distributed at subcutaneous and visceral fat, adipose tissue is an excellent source of stem cells, providing approximately 300,000 cells/ml, 5 times more than in the bone marrow [1]. Similar to BM-MSCs, hASCs may differentiate towards osteogenic, adipogenic, myogenic, neurogenic and chondrogenic pathways, depending on the experimental conditions [16]. Similarities between hASCs and BM-MSCs are found in the secretory profile and CD surface markers [16, 18].

Adipose tissue has an endocrine auxiliary function, secreting cytokines and growth factors. Thus, it was found that hASCs produce increased amounts of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), the keratinocytes growth factor (KGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor-β (TGF-β), insulin-*like* growth factor (IGF) and brain-derived neurotrophic factor (BDNF) [2, 19]. There are also products and cytokines such as Flt-3-ligand, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), bone morphogenetic proteins (BMP), interleukins-6,-7,-8,-11,-12, leukaemia inhibitory factor (LIF) and tumour necrosis factor alpha (TNF-α) [2, 19]. Based on hASCs properties to secrete molecules that modulate all stages of healing and to differentiate into multiple cell types, stem cells are actively involved in wound healing when administered in the vicinity of the affected

tissues [20]. In many experiments, hASCs administered at a lesion site have stimulated the development of granulation tissue and increased cell density and traumatic wound re-epithelialization [20, 21]. Recent research developments have shown that hASCs produce a volume of cytokines and growth factors superior to BM-MSCs and to human dermal fibroblasts, thus being ideal tools in tissue regeneration [22].

Since fat tissue actively intervenes in the endocrine processes, paracrine action of transplanted hASCs can negatively influence peripheral tissues. For example, the secretion of active hormones such as leptin, certain cytokines such as IL-6 and TNF-α or of certain growth factors can lead to a disturbance of the peripheral metabolic pathways [23].

2.2. hASCs regenerative potential

Most organs display "reservoirs" of adult stem cells that are activated in case of trauma, infection or disease [24]. In many of these cases, the endogenous stem cell populations are insufficient to cope with compromised tissue regeneration process, and therefore, modern strategies in the field of regenerative medicine involve the use of exogenous stem cells. Restoration of damaged structures, as well as the resumption of the restored tissue functionality, was associated with stem cells ability to adopt a specific phenotype through differentiation and to paracrine actions of stem cells [25].

Numerous studies showed that hASCs have the potential to differentiate into bone, cartilage and muscle, as well as adipose and neural tissue [15, 26, 27]. This ability to differentiate towards different mesenchymal lineages has stimulated interest in their clinical use. hASCs have also been used for breast augmentation and to treat congenital deformities and other defects as well as for reconstruction after mastectomy [28]. Immunomodulatory molecules, growth factors, angiogenic and antifibrotic factors released by hASCs, matrix metalloproteinases and collagen stimulate the regeneration and remodelling of altered structures [29], whereas secreted anti-apoptotic molecules and antioxidants protect cells in proximity [30].

The most important differentiation pathways reported for hASCs will be further described:

2.2.1. Adipogenic differentiation and adipose tissue engineering involving hASCs

Adipogenic differentiation is induced *in vitro* by treatment with a cocktail based on dexamethasone, indomethacin, isobutyl-methyl-xanthine [31] and insulin [32]. The mixture stimulates expression of the receptor peroxisome proliferation-activated receptor ɣ2 (PPAR-ɣ2), key inducer of adipogenic differentiation, which, once activated, triggers the transcription of a set of genes involved in differentiation of terminal adipocytes [33]. These include genes encoding the synthase fatty acid (FAS), the protein binding of fatty acids P2 (aP2), perilipin marker of adipogenic differentiation, lipoprotein lipase (LPL), the carrier protein fatty acid-1 (FATP-1) and adipocytokines (adiponectin, leptin and resistin). All of these events stimulate the lipid metabolism, leading in the end to the formation of intracellular lipid vesicles [34].

Paracrine action of MSCs plays a key role in the modulation of adipogenic differentiation. Thus, BMP, with cytokine function, stimulates *in vivo* adipogenesis. Bone morphogenetic

proteins receptor type 1A (BMPR-1A) can direct the differentiation of mesenchymal stem cells either to adipogenic lineage or osteogenic direction [35]. Insulin, glucocorticoids and FGF factors promote adipogenic differentiation, while molecules such as TNF-α and Wnt stop this process [36].

Eljaafari et al. [37] have recently found that hASCs isolated from obese individuals are able to induce a pro-inflammatory response by monocyte activation and stimulation of T helper 17 cells (Th17), which inhibit adipogenesis and response of adipocytes to insulin. Vascularization of *de novo* generated tissue is promoted by molecules such as bFGF, VEGF, TGF-β, PDGF, angiopoietin-1, monocyte chemoattractant protein-1 (MCP-1) and even extracellular vesicles (exosomes) that transfer genetic material and pro-angiogenic molecules from stem cells to cells in proximity [38].

In many experiments, hASCs were combined with biodegradable polymer-based scaffolds in order to validate efficient systems for adipogenesis. Adipogenic differentiation is exploited most often for breast reconstruction, either in normal conditions or after a breast tumour removal [17]. In contrast to the classical inert materials used as breast implants, the tissue generated *de novo* after differentiation from hASCs exhibits an optimal biocompatibility, is well vascularized, does not shrink, is not absorbed over time and does not trigger allergic reactions. In conclusion, autologous fat implants with active biomolecules and synthetic substitutes appear to be more efficient for adipose tissue regeneration than other methods in the field and ensure better quality of life in patients with fat tissue defects.

2.2.2. Osteogenic differentiation potential and bone tissue engineering involving hASCs

hASCs secretory profile includes many molecules that promote bone regeneration. Cytokines IGF-1, VEGF, HGF, BMP-1, IL-6, IL-3, MCP-1 and MCP-3 modulate the most important step in the process of bone formation: angiogenesis, cell migration and proliferation and differentiation of osteoblastic precursors [39]. IGF-1 is the main factor that stimulates cell proliferation and migration, VEGF promotes angiogenesis, while the other factors promote bone formation, favouring the recruitment of osteoprogenitor cells, their proliferation and differentiation into osteocytes [40]. Secretory profile and osteogenic differentiation capacity of hASCs sites are ideal resources for bone regeneration.

It was observed that hASCs exposed to a cocktail of pro-osteogenic inducers are able to initiate and develop osteogenic differentiation process. In a study, Halvorsen et al. [41] used a medium containing ascorbic acid, BMP-2, dexamethasone and 1, 25 dihydroxyvitamin D3 to induce osteogenesis. Results indicated that osteoblast-*like* cells were obtained.

The first attempts of bone regeneration were carried out on animal experimental models in order to determine the ideal conditions for mineralization of the new tissue. Then, in a study involving mice infected with severe combined immunodeficiency (SCID), the formation of osteoid in 80% of mice under investigation due to an implant of hydroxyapatite-tricalcium phosphate scaffold seeded with hASCs was revealed [42].

Microenvironment conditions affect the ability of hASCs to commit to osteogenesis *in vitro*. This was shown, for example, when comparing hASCs cultured in a medium supplemented

with human serum (better differentiation) with cells exposed to a serum-free medium [43]. Gender differences between donors can also affect the ability of hASCs to differentiate, probably because of differences in steroid hormones [44]. Osteogenic potential decreases with increasing age [45].

Using hASCs in bone tissue engineering (BTE) applications is an alternative strategy to replace or restore bone function, where the tissue was traumatized, damaged or lost. Typically, when hASCs differentiate towards bone-*like* cells, three phases can be observed: cell proliferation, extracellular matrix synthesis and mineralization of the matrix [16]. After 2–3 weeks of differentiation, the induction of alkaline phosphatase activity is observed, an enzyme involved in calcification of the matrix. In parallel, synthesis of calcium phosphate in the extracellular matrix is activated. Extracellular matrix mineralization can be detected by Alizarin red staining or von Kossa staining [16, 41].

Mesimäki et al. [46] reported a method to reconstruct a defect in the jaw in an adult patient using hASCs exposed to BMP-2 treatment. Another study developed on 23 patients with craniofacial defects [47] revealed that bone-*like* tissue could be obtained by a synergistic effect of hASCs and a tricalcium phosphate scaffold (β-TCP) in the presence of growth factors (BMP-2). Similarly, skull defects were successfully reconstructed or their healing was accelerated by using hASCs and pro-osteogenic conditions [46, 47].

A more recent study revealed that hASCs were able to differentiate towards the osteogenic lineage also in contact with synthetic polymers, such as polyethylene glycol diacrylate co-N-acryloyl-6-aminocaproic acid [48]. In this case, cells were allowed to infiltrate in this gel and then to differentiate, leading to the increase in the osteoblast cell differentiation. Differentiation was confirmed by analysis of calcium deposits, by quantification of alkaline phosphatase and by specific determination of molecular markers of bone formation, such as osteocalcin, osteopontin and the transcription factor correlated with Runt-2 (Runx2).

Another experiment involved osteogenesis of MSCs encapsulated in microspheres made of chitosan and collagen in a media supplemented with dexamethasone, β-glycerophosphate and ascorbic acid 2-phosphate. During differentiation, the level of expression found in genes encoding for collagen I, bone sialoprotein and osterix osteogenic markers demonstrated hASCs capacity to differentiate the osteogenic lineage [49].

2.2.3. Chondrogenic differentiation potential and cartilage tissue engineering involving hASCs

A crucial condition for chondrogenic differentiation is that hASCs need to be cultured in a pellet system to form spheroids. These systems are suitable because they allow cell condensation that occurs during embryonic development, increasing the interactions between cells and eventually forming a cartilaginous matrix [50]. Thus, 3D biomaterials with certain architectural and mechanical properties would be very suitable microenvironments to allow hASCs condensation and differentiation.

It was generally shown that there are several growth factors which stimulate cell proliferation and differentiation towards the chondrogenic lineage. Adding these growth factors to the scaffold structure or in the culture media greatly favours chondrogenesis. They maintain

homeostasis, integrity and influence the degree of development of hyaline cartilage [51]. The most used factors are TGF-β1-which stimulates collagen II and aggrecan overexpression—molecules present in the extracellular cartilage matrix [52], transforming growth factor-β3 (TGF-β3) induces the production of glycosaminoglycans in the extracellular matrix [53], bone morphogenetic protein-7 (BMP-7) is synthesized *in vivo* where the cartilage is damaged [54] and *in vitro* decreases the rate of proliferation of MSC and increases the rate of differentiation [55], growth and differentiation factor-5 (GDF-5) increases the expression of Sox9, the main inducer of chondrogenesis [56], IGF stimulates the proliferation of chondrocytes and induces the expression of specific genes [57] and fibroblast growth factor (FGF-2) is present in the extracellular matrix and plays a role in the prevention of native cartilage degradation [58].

Numerous studies in CTE concluded that collagen-based materials display the highest biocompatibility among the tested materials and an increased rate of biodegradation [59, 60]. Moreover, it was found that the addition of active biomolecules (e.g. sericin) in the structure of scaffolds seeded with stem cells can actually improve their performance, stimulating adhesion and proliferation and even synthesis of extracellular matrix [61, 62].

Surprisingly, it was observed that most studies aiming to regenerate elastic or hyaline cartilage using hASCs failed. Consequently, it was concluded that hASCs can only regenerate fibrocartilage [63].

In 2004, Awad et al. [64] conducted an experiment that involved seeding hASCs in a scaffold made of alginate, agarose and gelatin in a medium and differentiation towards the chondrogenic pathway. Biomaterials favoured adhesion, cell proliferation and differentiation, and at the end of the experiment, the ability of hASC to differentiate into chondrocytes was proved.

Im et al. [65] used a synthetic scaffold based on poly-lactic-co-glycolic acid seeded with hASCs transfected with a plasmid vector expressing Sox5, Sox6 and Sox9 genes. A culture medium supplemented with insulin-transferrin-selenium (ITS), dexamethasone, ascorbate-2-phosphate, proline and sodium pyruvate was used. Results indicated the presence of DNA coding for chondrogenesis inducers of Sox9, Sox5 and Sox6, showing a positive influence on the rate of cell differentiation.

Mardani et al. [66] has shown that hASCs cultivated in the presence of an inducing chondrogenic cocktail (culture medium supplemented with sodium pyruvate, transforming growth factor-β (TGF-β1), dexamethasone, insulin-transferrin-selenium (ITS), proline, ascorbic acid 2-phosphate) are able to differentiate into chondrocyte-*like* cells. Platelet-rich plasma (PRP) contains many growth factors, such as TGF-β, insulin growth factor (IGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF). An experiment investigated hASCs chondrogenic differentiation when the culture medium was enriched with 10% of TGF-β1 from PRP; in this case, the cells differentiated and expressed Sox9 and ColIIα1 in similar levels to the control, represented by cells cultured in a classic medium [51].

Chondrogenic differentiation was also studied *in vivo* [67]; hASCs cultured at high cell density on surfaces coated with fibrin and maintained in a culture media supplemented with TGF-β and FGF-2 generated a chondrogenic matrix; these structures were implanted in areas of joint damage in rabbits, and the cells adopted a chondrocyte phenotype [67].

2.2.4. Neural regeneration using mesenchymal-like stem cells

Nervous tissue reconstruction involves injecting mesenchymal-*like* stem cells by the intrathecal, intracerebral or by the intranasal route, in the form of infusion [68]. Stem cells migrate to the lesion site, secrete neurotrophic factors and thus the survival and functioning of the affected nerve tissue can be supported [69].

However, there are opinions arguing that the generated neuronal extensions are non-authentic and without signalling capacity [70]. These assumptions are due to the fact that MSCs have the ability to carry membrane proteins by exosome transport, trans-endocytosis or even as a result of cell-cell contact. Despite this theory, neuroregenerative and neuroprotective capabilities of stem cells cannot be questioned.

Current research directions in the field of neurodegenerative diseases (degenerative myopathies, Parkinson's disease, amyotrophic lateral sclerosis, Huntington disease, etc.) target to exploit the neuroprotective effects of stem cells by investigating the mechanisms of production and action of neurotrophic factors. Analysis of the secretory profiles of human MSCs showed that all types of stem cells have the ability to secrete compounds that exert a protective action in the central nervous system (CNS), of which the most important are tissue glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factors (BDNF [71]. Experiments conducted on animal models revealed that genetically modified hMSCs overexpressing GDNF were able to induce novel neuromuscular junctions and stop motor neuron degeneration, thus offering new perspectives in the treatment of amyotrophic lateral sclerosis [72].

2.2.5. The regeneration of the optic nerve and retinal cells

hASCs also display the ability to regenerate optic nerves responsible for visual function. Optical nerve damage occurs frequently in the population; it is associated with local inflammation, infection or brain trauma.

For this application, murine experimental models received therapy with BM-MSCs; based on their paracrine action, these mesenchymal stem cells were actively involved in the repair and replacement of the epithelial cells of the retina (RPE), glial cells of the retina (RGC) and neurons of the optic nerve [73]. Another experiment revealed an increase in survival of RGC-treated retina and increased production of ciliary neurotrophic factor after injection of hASCs at lesion site [74]. With these results, it becomes increasingly clear that the paracrine secretion of MSCs enhances their therapeutic effect.

Johnson and his collaborators [75] investigated which biomolecules secreted by MSCs are essential to protect nerve structures of the eye. For this purpose, a co-culture system using retinal cells and BM-MSCs was established. Retinal cells expressed an increased survival rate in the presence of BM-MSCs possibly because of the paracrine action of MSCs.

2.2.6. Cardiac regeneration and cardioprotective effect of hASCs

Stem cell-based therapies have proven effective in heart regeneration, in particular in the post-myocardial infarction. Heart attack triggers irreversible alteration of the cardiac function

by the permanent loss of cardiomyocytes and myocardial tissue scarring [76]. Even if traditional medications containing aspirin, beta blockers, and statins and angiotensin-converting enzyme (ACE) inhibitors of angiotensin prevent possible damage to the affected tissue and reduce the risk of heart attacks, it is necessary to replace altered cardiomyocytes; mesenchymal stem cells were successfully used in this case. Furthermore, recent *in vivo* studies demonstrated that injected BM-MSCs survive and differentiate to form the complex junction structure of the damaged cardiac myocytes [77].

Chen et al. conducted a clinical trial involving 69 patients who suffered heart attacks. Following transplantation of BM-MSCs at the lesion sites, the cells infiltrated within the damaged myocardial areas and improved cardiac activity without risks of morbidity, mortality, arrhythmias or malignant processes [78]. Subsequent studies have shown that hASCs have the ability to form functional cardiomyocyte-*like* cells and exert protective effects on the heart. One such experiment, conducted by Bai et al. [79], showed that hASCs moved to the altered regions in close proximity to cardiomyocytes and adopted similar phenotypes, regenerating the damaged structure and stimulating its functioning.

Both *in vitro* and *in vivo* studies have shown that BM-MSCs and hASCs secrete molecules such as VEGF, PDGF, IL-1β, IL-10, stem cell-derived factor (SDF-1), IGF-1 and HGF proteins angiopoietin-1 and-2 (Ang-1 and-2) that act synergistically to regenerate and protect cardiac tissue [80].

2.2.7. Liver regeneration using hASCs

Mesenchymal-*like* stem cells, including hASCs, can regenerate the liver tissue. This tissue is composed mostly of hepatocytes, polarized epithelial cells representing approximately 80% of liver mass [81].

In 2005, Seo and co-workers reported, for the first time, to obtain a population of cells structurally and functionally similar to hepatocytes by inducing hASCs differentiation using a culture medium supplemented with growth factors, cytokines and dimethyl sulfoxide (DMSO) [82]. Continuing to investigate the regenerative potential of hASCs, the researchers transplanted cells in nude mice with acute liver lesions and found repopulation of the damaged tissue with newly generated hepatocyte-*like* cells embedded in liver parenchyma [82]. Microarray analysis showed that there were considerable similarities between sets of genes expressed in normal hepatocytes and the cells generated from hASCs. Thus, mesenchymal differentiation-specific genes (vimentin and N-cadherin-2) are downregulated, while genes that are overexpressed are direct epithelial differentiation [83]. These results suggest that hASCs are able to differentiate into hepatocyte-*like* cells through a mesenchymal-epithelial transition process [84]. However, it is obvious that liver regeneration would not be possible in the absence of stem-cell paracrine action.

In a more recent study [85], revealed that the factors released by MSCs and hASCs have immunosuppressive properties (IL-6, IL-8, IL-1RA and VEGF), can inhibit fibrosis and apoptosis of liver cells, can promote angiogenesis or they can stimulate progenitor cells to divide and differentiate to regenerate damaged tissue (G-CSF, TNF-α and IL-6). Currently, very few

details are known about the mechanisms by which these biomolecules modulate an integrated dynamic response to mitigate damage and scarring of the liver tissue *in vivo*. New clinical strategies in the field are oriented towards the use of MSCs Akt-modified Frizzled protein 2, which can produce large amounts of VEGF, HGF, FGF2 and insulin-*like* growth factors [86]. Increased production of these molecules ensures a viable regeneration; for example, an increased amount of HGF ensures suppression of the immune response of the graft (transplanted tissue) versus host which has disastrous consequences for the host organism, whereas the other molecules maintain angiogenesis, cell growth and proliferation [86].

3. Original biocompatible scaffolds validated for hASCs differentiation in the context of tissue engineering applications

Advanced research in tissue engineering promotes the use of bioconstructs as an effective solution to perform regeneration of damaged tissues. Using 3D microenvironments brings benefits for tissue engineering applications, since 3D microenvironment recreates better *in vivo* conditions and mimics closely the natural tissue. A number of cell-scaffold bioconstructs with structure and properties adapted to the nature of the tissue in need of reconstruction have been shown to be useful in the production of functional *de novo* tissue. These bioconstructs can function as 3D (i) transport systems ("shuttle") to deliver the cells to the injured site, encouraging the self-healing ability of the tissue and (ii) biocompatible and biodegradable bioconstructs, supporting the cellular component during tissue reconstruction.

In this context, a group of researchers from the Department of Biochemistry and Molecular Biology, University of Bucharest, partially in collaboration with the Department of Histology from Western University Vasile Goldis of Arad, investigated hASCs potential to differentiate in contact with several original recipes of materials designed for adipose, cartilage and bone tissue engineering.

For adipose tissue engineering (ATE), the optimal conditions required for successful differentiation of hASCs in case of implantations at a wound healing site were studied. Cells should first proliferate and then receive the optimal amount of pro-adipogenic signals to induce the differentiation process with a certain rate. Therefore, a modulation of the adipogenic conditions would be required, aiming to ensure the long-term proliferation of the precursor cells and to control the kinetics of the differentiation process [87].

One novel scaffold validated for ATE by *in vitro* studies was a spongious 60% collagen and 40% sericin hydrogel preceded with hASCs (CollSS) [12]. In this case, the novel CollSS composition was compared in terms of biocompatibility and ability to support adipogenic differentiation of hASCs to a pure collagen hydrogel (Coll). The addition of the sticky protein sericin in the composition of a classical collagen sponge enhanced the adhesion and also the proliferation rate of hASCs. CollSS proved to be more biocompatible than pure Coll. hASCs-CollSS bioconstruct proved to efficiently support the adipogenic differentiation process, as confirmed by the expression levels of PPARγ2, fatty acid synthase (FAS), adipocyte protein 2 (aP2) and perilipin adipogenic markers [12].

hASCs ability to differentiate towards mature adipocytes in 3D environment was also confirmed when using a gelatin- alginate- polyacril amide (GAPAA) scaffold. This type of material proved to be at least as efficient as the gelatin-alginate biomaterial (GA) for adipogenic differentiation, hASCs generating mature adipocytes in this system in 21 days of induced differentiation [88].

With regard to bone tissue engineering (BTE) direction, original research validated a composite material based on chitosan (CHT) and graphene oxides (GO) (hASCs/CHT/GO 3 wt.%) as most suitable for hASCs osteogenesis [paper under review]. The study of the osteogenic differentiation potential of hASCs in contact with chitosan-based scaffolds enriched with 0.5–3 wt.% GO showed the highest rate of differentiation in cells cultured in the presence of 3 wt.% GO, suggesting that hASCs/CHT/GO 3 wt.% may be a candidate for future bone regeneration applications and BTE. Currently, the potential of graphene and its derivatives is exploited increasingly in tissue engineering because of their positive influence on cellular interaction with the material and on the bioactivity of the material. Graphene oxide (GO) in particular was shown to improve the biological properties of materials and to promote adhesion, proliferation and osteogenic differentiation of mesenchymal stem cells. Experiments demonstrated good biocompatibility of CHT/GO materials, where the degree of biocompatibility depends on the GO content. Similar to previous studies, GO favoured hASCs contact with the materials and influenced the proliferation rate.

For cartilage tissue engineering (CTE), two distinct bioconstructs were studied: (i) a three component inter-polymeric network (IPN) hydrogel based on gelatin, alginate and polyacrylamide (GAPAA) was evaluated for potential to support hASCs chondrogenesis (hASCs/GAPAA) and (ii) a hydrogel based on collagen (Coll), improved with sericin (SS), hyaluronic acid (HA) and chondroitin sulphate (CS) (hASCs/CollSSHACS), was tested for hASCs proliferation, adhesion and chondrogenic differentiation [11, 13].

Chondrogenic differentiation studies conducted in GAPAA IPN have provided important information about the essential characteristics of a 3D scaffold to be used effectively in cartilage reconstruction. In this case, the adequate porosity of the system and the control over scaffold's 3D architecture for hASCs chondrogenic differentiation were achieved by adding the synthetic component polyacrylamide in the composition. Normally, acrylamide (AA) would exert a toxic effect on cells, but the polymerized form of PAA has a lower toxicity when used in very low concentration in the composition of GAPAA to control pore size [11].

Similarly, in hASCs/CollSSHACS bioconstruct exposed to pro-chondrogenic differentiation conditions, the results indicated a more efficient chondrogenesis of hASCs in the collagen hydrogel enriched with hyaluronic acid and chondroitin sulphate (CollSSHACS), two cartilage markers present in the extracellular matrix, as compared to the results obtained for the reference system (hASCs/Coll). The simultaneous presence of both molecules in HA and CS biomaterial composition favoured the initiation and controlled hASC chondrogenesis for 28 days *in vitro* [paper under review]. Therefore, hASCs/CollSSHACS bioconstruct would be a good candidate with high potential for use in CTE applications.

4. Most recent advances and future perspectives

The optimization of biomaterials composition, structure and properties to mimic as well as possibilities in the *in vivo* architecture has led to the development of the advanced technology of bioprinting. Recently, highly advanced bioprinted 3D constructs comprising of material, stem cells and additives have been reported to successfully support differentiation and regeneration processes.

In order to combine as efficiently as possible this modern technology with hASCs properties, several groups have conducted studies to observe hASCs behaviour in these systems and to assess their utility for tissue engineering purposes.

One of these studies explored the behaviour of hASCs when printed in a free-scalable 3D grid pattern by means of laser-assisted bioprinting (LaBP). Cells in this system managed to differentiate towards the adipogenic lineage, thus proving that such 3D bioprints could be used successfully in tissue regeneration or the biofabrication of living grafts [89].

In order to be efficient, the bioprinted constructs should resemble the native tissue microenvironment as close as possible. For example, muscular soft tissue constructs can benefit from bioinks that mimic their nanofibrous matrix constitution [90]. Thus, it is critical to maximize the biocompatibility between cells and the type of fibre materials used to create the constructs. Narayanan et al. have used hASCs with a fibrous bioink composed of alginate hydrogel and polylactic acid nanofibers to obtain bioprinted constructs as replacement for knee meniscus. Results indicated favourable behaviour of hASCs, high levels of cell proliferation as well as positive chondrogenic differentiation.

For bone reconstruction purposes, hASCs were included in a mixture together with nano-hydroxyapatite and bioprinted in a 3D construct, further evaluated for cell proliferation and osteogenesis ability [91]. In this case, it was concluded that nano-hydroxyapatite could increase osteogenic differentiation of the hASCs mixture after bioprinting, in which the cells still have a good proliferation.

Similarly, Wang et al. [92] aimed to investigate osteogenic differentiation of hASCs in 3D bioprinted tissue constructs, both *in vitro* and *in vivo*. After performing tests to assess osteogenic markers expression both at gene and protein levels, researchers reported significantly increased expression levels of RUNX2, OSX and OCN after 7 and 14 days of osteogenesis. *In vivo* studies demonstrated obvious bone matrix formation in the 3D bioprinted constructs. Based on this study, it was concluded that hASCs could be used in 3D bioprinted constructs for the repair of large bone tissue defects.

In cardiac regeneration, a great interest is directed to obtaining 3D cocultures of stem cells and endothelial cells to closely mimic the native tissue conditions. In this context, a team of researchers [93] used laser-induced-forward-transfer (LIFT) cell printing technique to prepare a polyester urethane urea (PEUU) cardiac patch seeded with human umbilical vein endothelial cells (HUVEC) and human MSCs. Results showed an increased production of blood vessels, which reflected an improvement of functionality in infarcted tissue.

4.1. Risks associated with hASCs use for tissue engineering and regenerative medicine

According to good manufacturing practices (GMP) rules, mesenchymal stem cells including hASCs are considered as advanced therapy medical products and are validated safe for medical practice. Thus, these cells are widely used in tissue engineering and regenerative medicine applications based on the fact that they are adult stem cells and that they have a relatively limited potential for proliferation, differentiation and an extremely low risk of transformation.

Most clinical applications involving hASCs, however, require the use of a large number of cells at the implant site for regenerative therapy success and therefore most often, the *in vitro* expansion of these cells is necessary. Risks associated with hASCs use in regenerative medicine are mainly associated with the *in vitro* handling of cells and, thus, most studies are directed towards assessing changes that may occur in the genome of these cells during *ex vivo* cultivation.

Furthermore, a series of contradictory studies discussed the transformation potential of hASCs in different circumstances since the implantation of these cells in the body for regenerative purposes can have long-term consequences if they have tumorigenic potential. Although the accumulation of chromosomal aberrations and mutations was repeatedly reported in the genome of hASCs during *in vitro* culture and expansion, most of these mutations are proven to have a transient nature and can be normally removed by DNA repair systems. Although no case of malignant transformation of hASCs implanted in humans was reported so far, caution is necessary. The interaction of hASCs with the *in vivo* existing microenvironment is specific to each organism and could have risks. In this context, hASCs genomic instability when in contact with tumour microenvironment should be further studied, considering the implications for tissue engineering.

5. Conclusions

Human adipose-derived stem cells are a versatile source of mesenchymal-*like* stem cells, with enormous potential for tissue engineering and regenerative medicine. Regardless of the application and tissue that require restoration, hASCs proved to be efficient in a great number of cases. However, the stem-ness status of these cells and their unexplored risks of transformation should not be forgotten and thus more cautiousness should be taken before recommending therapy with hASCs. If correctly controlled, the potential of these cells could become a very powerful tool to increase the quality of life in patients with tissue defects.

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Authors' contribution

Sorina Dinescu, Anca Hermenean and Marieta Costache contributed to designing the chapter, reviewing the literature and writing the chapter.

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References

[1] Zuk PA, Zhu M, Mizuno H, Huang JI, Futrell WJ, Katz AJ, Benhaim P, Lorenz HP, Hedrick, MH. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Engineering. 2001;**7**:211-228

[2] Tobita M, Orbay H, Mizuno H. Adipose-derived stem cells: Current findings and future perspectives. Discovery Medicine. 2011;**11**(57):160-170

[3] Dai R, Wang Z, Samanipour R, Koo KI, Kim K. Adipose-derived stem cells for tissue engineering and regenerative medicine applications. Stem Cells International. 2016;**2016**:6737345

[4] Ebrahimian TG, Pouzoulet F, Squiban C, Buard V, André M, Cousin B, Gourmelon P, Benderitter M, Casteilla L, Tamarat R. Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing. Arteriosclerosis Thrombosis and Vascular Biology. 2009;**29**:503-510

[5] Gimble JM, Kate AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circulation Research. 2007;**100**(9):1249-1260

[6] Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC. Spontaneous human adult stem cell transformation. Cancer Research. 2005;**65**:3035-3039

[7] Rosland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. Cancer Research. 2009;**69**:5331-5339

[8] Ren Z, Wang J, Zhu W, Guan Y, Zou C, Chen Z. Spontaneous transformation of adult mesenchymal stem cells from cynomolgus macaques *in vitro*. Experimental Cell Research. 2011;**317**:2950-2957

[9] Landers R, Pfister A, Hubner U, John H, Schmelzeisen R, Mulhaupt R. Fabrication of soft tissue engineering scaffolds by means of rapid prototyping techniques. Journal of Materials Science. 2002;**37**(15):3107-3116

[10] Wang Z, Samanipour R, Kim K. Organ-on a-chip platforms for drug screening and tissue engineering. Biomedical Engineering: Frontier Research and Converging Technologies. 2016;**9**:209-233

[11] Dinescu S, Galateanu B, Radu E, Hermenean A, Lungu A, Stancu IC, Jianu D, Tumbar T, Costache M. A 3D porous gelatin-alginate-based-IPN acts as an efficient promoter of chondrogenesis from human adipose-derived stem cells. Stem Cells International. 2015;**2015**:1-19

[12] Dinescu S, Galateanu B, Albu M, Cimpean A, Dinischiotu A, Costache M. Sericin enhances the bioperformance of collagen-based matrices preseeded with hASCs. International Journal of Molecular Sciences. 2013;**14**(1):1870-1889

[13] Dinescu S, Galateanu B, Albu M, Lungu A, Radu E, Hermenean A, Costache M. Biocompatibility assessment of novel collagen-sericin scaffolds improved with hyaluronic acid and chondroitin sulfate for cartilage regeneration. BioMed Research International. 2013;**2013**:1-11

[14] Galateanu B, Dimonie D, Vasile E, Nae S, Cimpean A, Costache M. Layer-shaped alginate hydrogels enhance the biological performance of human adipose-derived stem cells. BMC Biotechnology. 2012;**12**:35

[15] Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. Journal of Cellular Physiology. 2001;**189**:54-63

[16] Zuk P, Zhu M, Ashjian P, De Ugarte D, Huang H, Mizuno H, Alfonso Z, Fraser J, Benhaim P, Hedrick M. Human adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell. 2002;**13**:4279-4295

[17] Wankhade U, Shen M, Kolhe R, Fulzele S. Advances in adipose-derived stem cells isolation, characterization, and application in regenerative tissue engineering. Stem Cells International. 2016;**2016**:3206807

[18] Katz AJ, Tholpady A, Tholpady SS, Shang H, Ogle RC. Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells. Stem Cells. 2005;**23**:412-423

[19] Kilroy GE, Foster SJ, Wu X, Ruiz J, Sherwood S, Heifetz A, Ludlow JW, Stricker DM, Potiny S, Green P, Halvorsen YD, Cheatham B, Storms RW, Gimble JM. Cytokine profile of human adipose-derived stem cells: Expression of angiogenic, hematopoietic, and proinflammatory factors. Journal of Cellular Physiology. 2007;**212**(3):702-709

[20] Nie C, Yang D, Morris SF. Local delivery of adipose-derived stem cells via acellular dermal matrix as a scaffold: A new promising strategy to accelerate wound healing. Medical Hypotheses. 2009;**72**(6):679-682

[21] Nambu M, Kishimoto S, Nakamura S, Mizuno H, Yanagibayashi S, Yamamoto N, Azuma R, Nakamura S, Kiyosawa T, Ishihara M, Kanatani Y. Accelerated wound healing in healing-impaired db/db mice by autologous adipose tissue-derived stromal cells combined with atelocollagen matrix. Annals of Plastic Surgery. 2009;**62**(3):317-321

[22] Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita M, Osaki M, Kawamata M, Kato T, Okochi H, Ochiya T. *In vivo* therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. Stem Cells. 2008;**26**(10):2705-2712

[23] Pearl RA, Leedham SJ, Pacifico MD. The safety of autologous fat transfer in breast cancer: Lessons from stem cell biology. Journal of Plastic, Reconstructive and Aesthetic Surgery. 2012;**65**(3):283-288

[24] Lau K, Paus R, Tiede S, Day P, Bayat A. Exploring the role of stem cells in cutaneous wound healing. Experimental Dermatology. 2009;**18**(11):921-933

[25] Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circulation Research. 2008;**103**:1204-1219

[26] Badillo AT, Redden RA, Zhang L, Doolin EJ, Liechty KW. Treatment of diabetic wounds with fetal murine mesenchymal stromal cells enhances wound closure. Cell and Tissue Research. 2007;**329**:301-311

[27] Safford KM, Safford SD, Gimble JM, Shetty AK, Rice HE. Characterization of neural/glial differentiation of murine adult adipose-derived stromal cells. Experimental Neurology. 2004;**187**:319-328

[28] Khouri R, Del Vecchio D. Breast reconstruction and augmentation using pre-expansion and autologous fat transplantation. Clinics in Plastic Surgery. 2009;**36**:269-280

[29] Scadden DT. The stem-cell niche as an entity of action. Nature. 2006;**441**(7097):1075-1079

[30] Schinkothe T, Bloch W, Schmidt A. In vitro secreting profile of human mesenchymal stem cells. Stem Cells and Development. 2008;**17**(1):199-206

[31] Dennis J, Charbord P. Origin and differentiation of human and murine stroma. Stem Cells. 2002;**20**(3):205-214

[32] Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;**25**(11):2739-2749

[33] Totonoz P, Hu E, Graves RA, Budvari AI, Speigelman BM. mPPAR 2: Tissue-specific regulator of an adipocyte enhancer. Genes & Development. 1994;**8**:1224-1234

[34] Ailhaud G, Grimaldi P, Negrel R. Cellular and molecular aspects of adipose tissue development. Annual Review of Nutrition. 1992;**12**:207-233

[35] Bobis S, Jarocha D, Majka M. Mesenchymal stem cells: Characteristics and clinical applications. Folia Histochemica et Cytobiologica. 2006;**44**(4):215-230

[36] Peirce V, Carobbio S, Vidal-Puig A. The different shades of fat. Nature. 2014;**510**:76-83

[37] Eljaafari A, Robert M, Chehimi M, Chanon S, Durand C, Vial G, Bendridi N, Madec AM, Disse E, Laville M, Rieusset J, Lefai E, Vidal H, Pirola L. Adipose tissue-derived stem cells from obese subjects contribute to inflammation and reduced insulin response in adipocytes through differential regulation of the Th1/Th17 balance and monocyte activation. Diabetes. 2015;**64**(7):2477-2488

[38] Meissburger B, Perdikari A, Moest H, Müller S, Geiger M, Wolfrum C. Regulation of adipogenesis by paracrine factors from adipose stromal-vascular fraction: A link to fat depot-specific differences. Biochimica et Biophysica Acta. 2016;**1861**(9A):1121-1131

[39] Osugi M, Katagiri W, Yoshimi R, Inukai T, Hibi H, Ueda M. Conditioned media from mesenchymal stem cells enhanced bone regeneration in rat calvarial bone defects. Tissue Engineering Part A. 2012;**18**:1479-1489

[40] Linero I, Chaparro O. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. PLoS One. 2014;**9**(9):e107001

[41] Halvorsen YD, Franklin D, Bond AL, Hitt DC, Auchter C, Boskey AL, Paschalis EP, Wilkison WO, Gimble JM. Extracellular matrix mineralization and osteoblast gene expression by human adipose tissue-derived stromal cells. Tissue Engineering. 2001;**7**(6):729-741

[42] Hicok KC, Du Laney TV, Zhou YS, Halvorsen YDC, Hitt DC, Cooper LF, Gimble JM. Human adipose-derived adult stem cells produce osteoid in vivo. Tissue Engineering. 2004;**10**:371-380

[43] Lindroos B, Aho KL, Kuokkanen H. Differential gene expression in adipose stem cells cultured in allogeneic human serum versus fetal bovine serum. Tissue Engineering Part A. 2010;**16**:2281-2294

[44] Aksu AE, Rubin JP, Dudas JR. Role of gender and anatomical region on induction of osteogenic differentiation of human adipose-derived stem cells. Annals of Plastic Surgery. 2008;**60**:306-322

[45] Zhu M, Kohan E, Bradley J. The effect of age on osteogenic, adipogenic and proliferative potential of female adipose-derived stem cells. Journal of Tissue Engineering and Regenerative Medicine. 2009;**3**:290-301

[46] Mesimäki K, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. International Journal of Oral and Maxillofacial Surgery. 2009;**38**: 201-209

[47] Sándor GK, Tuovinen VJ, Wolff J, Patrikoski M, Jokinen J, Nieminen E, Mannerström B, Lappalainen OP, Seppänen R, Miettinen S. Adipose stem cell tissue-engineered construct used to treat large anterior mandibular defect: A case report and review of the clinical application of good manufacturing practice-level adipose stem cells for bone regeneration. Journal of Oral and Maxillofacial Surgery. 2013;**71**:938-950

[48] Phadke A, Hwang YS, Kim SH, Kim SH, Yamaguchi T, Masuda K, Varghese S. Effect of scaffold microarchitecture on osteogenic differentiation of human mesenchymal stem cells. European Cells & Materials. 2013;**25**:114-129

[49] Wang L, Rao RR, Stegemann JP. Delivery of mesenchymal stem cells in chitosan/collagen microbeads for orthopaedic tissue repair. Cells Tissues Organs. 2013;**197**(5):333-343

[50] Wei Y, Sun X, Wang W. Adipose-derived stem cells and chondrogenesis. Cytotherapy. 2007;**9**:712-716

[51] Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clinical Orthopaedics and Related Research. 2011;**469**:2706-2715

[52] Danisovic L, Lesny P, Havla V, Teyssler P, Syrova Z, Kopani M, Fujerikova G, Trc T, Sykova E, Jendelova P. Chondrogenic differentiation of human bone marrow and adipose tissue-derived mesenchymal stem cells. Journal of Applied Biomedicine. 2007;**5**:139-150

[53] Thorpe SD, Buckley CT, Vinardell T, O'Brien FJ, Campbell VA, Kelly DJ. The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. Annals of Biomedical Engineering. 2010;**38**:2896-2909

[54] Chubinskaya S, Hurtig M, Rueger DC. OP-1/BMP-7 in cartilage repair. International Orthopaedics. 2007;**31**:773-781

[55] An C, Cheng Y, Yuan Q, Li J. IGF-1 and BMP-2 induces differentiation of adipose-derived mesenchymal stem cells into chondrocytes-like cells. Annals of Biomedical Engineering. 2010;**38**:1647-1654

[56] Kuo Y, Leou SN. Chondrogenesis of articular chondrocytes in hydroxyapatite/chitin/chitosan scaffolds supplemented with pituitary extract. Engineering in Life Sciences. 2010;**10**:65-74

[57] Longobardi L, O'Rear L, Aakula S, Johnstone B, Shimer K, Chytil A, Horton WA, Moses HL, Spagnoli A. Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. Journal of Bone and Mineral Research. 1006;**21**:626-636

[58] Ge Z, Hu Y, Heng BC, Yang Z, Ouyang H, Lee EH, Cao T. Osteoarthritis and therapy. Arthritis & Rheumatology. 2006;**55**:493-500

[59] Glowacki J, Mizuno S. Collagen scaffolds for tissue engineering. Biopolymers. 2008;**89**: 338-344

[60] Dong C, Yonggang L. Application of collagen scaffold in tissue engineering: Recent advances and new perspectives. Polymers. 2016;**8**(2):42

[61] Tsubouchi K, Igarashi Y, Takasu Y, Yamada H. Sericin enhances attachment of cultured human skin fibroblasts. Bioscience, Biotechnology, and Biochemistry. 2005;**69**:403-405

[62] Mandal BB, Priya AS, Kundu SC. Novel silk sericin/gelatin 3-D scaffolds and 2-D films: Fabrication and characterization for potential tissue engineering applications. Acta Biomaterialia. 2009;**5**:3007-3020

[63] Ogawa R, Mizuno S. Cartilage regeneration using adipose-derived stem cells. Current Stem Cell Research & Therapy. 2010;**5**(2):129-132

[64] Awad HA, Wickham MQ, Leddy HA. Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. Biomaterials. 2004;**25**:3211-3222

[65] Im GI, Kim HI, Lee JH. Chondrogenesis of adipose stem cells in a porous PLGA scaffold impregnated with plasmid DNA containing SOX trio (SOX-5,-6 and-9) genes. Biomaterials. 2011;**32**(19):4385-4392

[66] Mardani M, Kabiri A, Esfandiari E, Esmaeili A, Pourazar A, Ansar M, Hashemibeni B. The effect of platelet rich plasma on chondrogenic differentiation of human adipose derived stem cells in transwell culture. Iranian Journal of Basic Medical Sciences. 2013;**16**(11):1163-1169

[67] Dragoo JL, Carlson G, McCormick F, Khan-Farooqi H, Zhu M, Zuk PA, Benhaim P. Healing full-thickness cartilage defects using adipose derived stem cells. Tissue Engineering. 2007;**13**:1615-1621

[68] Joyce N, Annett G, Wirthlin L, Olson S, Bauer G, Nolta JA. Mesenchymal stem cells for the treatment of neurodegenerative disease. Regenerative Medicine. 2010;**5**(6):933-946

[69] Crigler L, Robey RC, Asawachaicharn A, Gaupp D, Phinney DG. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Experimental Neurology. 2006;**198**(1):54-64

[70] Hardy SA, Maltman DJ, Przyborski SA. Mesenchymal stem cells as mediators of neural differentiation. Current Stem Cell Research and Therapy. 2008;**3**:43-52

[71] Vercelli A, Mereuta OM, Garbossa D, Muraca G, Mareschi K, Rustichelli D, Ferrero I, Mazzini L, Madon E, Fagioli F. Human mesenchymal stem cell transplantation extends survival, improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis. Neurobiology of Disease. 2008;**31**:395-405

[72] Kim SU, de Vellis J. Stem cell-based cell therapy in neurological diseases: A review. Journal of Neuroscience Research. 2009;**87**:2183-2200

[73] Mesentier-Louro L, Zaverucha-do-Valle C, Rosado-de-Castro P, Silva-Junior A Pimentel-Coelho P, Mendez-Otero R, Santiago M. Bone marrow-derived cells as a therapeutic approach to optic nerve diseases. Stem Cells International. 2016;**2016**:5078619

[74] Yu S, Tanabe T, Dezawa M, Ishikawa H, Yoshimura N. Effects of bone marrow stromal cell injection in an experimental glaucoma model. Biochemical and Biophysical Research Communications. 2006;**344**(4):1071-1079

[75] Johnson TV, Dekorver NW, Levasseur VA, Osborne A, Tassoni A, Lorber B, Heller JP, Villasmil R, Bull ND, Martin KR, Tomarev SI. Identification of retinal ganglion cell neuroprotection conferred by platelet-derived growth factor through analysis of the mesenchymal stem cell secretome. Brain. 2014;**137**(2):503-519

[76] Krishna KA, Krishna S, Berrocal R, Rao KS, Sambasiva Rao KRS. Myocardial infarction and stem cells. Journal of Pharmacy and Bioallied Sciences. 2011;**3**(2):182-188

[77] Nygren JM, Jovinge S, Breitbach M, Säwén P, Röll W, Hescheler J, Taneera J, Fleischmann BK, Jacobsen SE. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nature Medicine. 2004;**10**(5):494-501

[78] Chen SL, Fang WW, Qian J, Ye F, Liu YH, Shan SJ, Zhang JJ, Lin S, Liao LM, Zhao RC. Improvement of cardiac function after transplantation of autologous bone marrow mesenchymal stem cells in patients with acute myocardial infarction. Chinese Medical Journal. 2004;**117**(10):1443-1448

[79] Bai X, Yan Y, Song YH, Seidensticker M, Rabinovich B, Metzele R, Bankson JA, Vykoukal D, Alt E. Both cultured and freshly isolated adipose tissue-derived stem cells enhance cardiac function after acute myocardial infarction. European Heart Journal. 2010;**31**:489-501

[80] Takahashi M, Li TS, Suzuki R, Kobayashi T, Ito H, Ikeda Y, Matsuzaki M, Hamano K. Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. American Journal of Physiology. Heart and Circulatory Physiology. 2006;**291**(2):H886-H893

[81] Esrefoglu M. Role of stem cells in repair of liver injury: Experimental and clinical benefit of transferred stem cells on liver failure. World Journal of Gastroenterology. 2013;**19**(40):6757-6773

[82] Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochemical and Biophysical Research Communications. 2005;**328**:258-264

[83] Ochiya T. A comparative analysis of the transcriptome and signal pathways in hepatic differentiation of human adipose mesenchymal stem cells. The FEBS Journal. 2008;**275**:1260-1273

[84] al Battah F, de Kock J, Vanhaecke T, Rogiers V. Current status of human adipose–derived stem cells: Differentiation into hepatocyte-like cells. The Scientific World Journal. 2011;**11**: 1568-1581

[85] Puglisi MA, Tesori V, Lattanzi W, Piscaglia AC, Gasbarrini GB, D'Ugo DM, Gasbarrini A. Therapeutic implications of mesenchymal stem cells in liver injury. Journal of Biomedicine and Biotechnology. 2011;**2011**:860578

[86] Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified

mesenchymal stem cell-mediated cardiac protection and functional improvement. The FASEB Journal. 2006;**20**:661-669

[87] Galateanu B, Dinescu S, Cimpean A, Dinischiotu A, Costache M. Modulation of adipogenic conditions for prospective use of hASCss in adipose TE. International Journal of Molecular Sciences. 2012;**13**(12):15881-15900

[88] Dinescu S, Galateanu B, Lungu A, Radu E, Nae S, Iovu H, Costache M. Perilipin expression reveals adipogenic potential of hASCss inside superporous polymeric cellular delivery systems. BioMed Research International. 2014;**2014**:830791

[89] Gruene M, Pflaum M, Deiwick A, Koch L, Schlie S, Unger C, Wilhelmi M, Haverich A, Chichkov BN. Adipogenic differentiation of laser-printed 3D tissue grafts consisting of human adipose-derived stem cells. Biofabrication. 2011;**3**(1):015005

[90] Narayanan LK, Huebner P, Fisher MB, Spang JT, Starly B, Shirwaiker RA. 3D-bioprinting of polylactic acid (PLA) nanofiber–alginate hydrogel bioink containing human adipose-derived stem cells, ACS. Biomaterials Science & Engineering. 2016;**2**(10):1732-1742

[91] Song Y, Wang XF, Wang YG, Dong F, Lv PJ. A preliminary study for the effect of nano hydroxyapatite on human adipose-derived mesenchymal stem cells mixture 3D bioprinting. Beijing Da Xue Xue Bao. 2016;**48**(5):894-899

[92] Wang X-F, Song Y, Liu Y-S, Sun Y-c, Wang Y-g, Wang Y, Lyu PJ. Osteogenic differentiation of three-dimensional bioprinted constructs consisting of human adipose-derived stem cells in vitro and in vivo. PLoS One. 2016;**11**(6):e0157214

[93] Gaebel R, Ma N, Liu J, Guan J, Koch L, Klopsch C, Gruene M, Toelk A, Wang W, Mark P, Wang F, Chichkov B, Li W, Steinhoff G. Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. Biomaterials. 2011;**32**(35):9218-9130

Chapter 15

Tissue Engineering for Skin Replacement Methods

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Additional information is available at the end of the chapter

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Abstract

The skin is the biggest structure of the body, and it plays a significant role in maintaining the unity of the body environment. The skin is important for the endurance of the organism as an outer coat for the thermal regulation and hydration preservation. With the intention of helping these significant utilities, the skin continually experiences regeneration and holds the capability to overhaul wound by repair and regeneration of several kinds of skin stem cells. Noteworthy, development has been accomplished throughout the recent times in the generation of engineered skin alternates which imitate human skin cells in vitro for replacement or modeling. Conversely, existing new skin alternatives do not reinstate completely the healthy skin anatomy and suffer from deficiency of natural supplements in skin covering, sebaceous glands, hair follicles, and sweat glands. Improvements in stem cell biology and skin morphogenesis show significant potentials to evidently advance the engineering of skin replacements which would preferably be vague from normal skin. This chapter reviews these developments in the in vivo and in vitro techniques of engineered and manufactured skin scaffold biomaterials.

Keywords: skin tissue engineering, scaffolds, skin, skin alternates, in vitro skin models, in vivo skin models, biomaterials, skin regeneration, skin renewal

1. Introduction

Tissue engineering is growing as a novel area in biomedical engineering which purposes to redevelop newfangled material for substituting problematic or injured tissues. In order to accomplish this, not only is a basis of cells necessary but also a simulated extracellular matrix (ECM) that the cells which may be reinforced should exist. Human skin signifies about one-tenth of the body form, and injuries like physical distress, infection, burn, disease, or operation to a portion of this main organ carry intense penalties. Tissue engineering

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of skin substitutes signifies a potential foundation of improved treatment in fighting acute and chronic skin wounds. Currently, there are no significant prototypes of engineered skin which entirely duplicate the composition, structure, organic constancy, or visual environment of healthy skin. Skin alternates should carry some important physiognomies that comprise being simple to use and implement to the wound location; deliver vital blockade utility with suitable aquatic fluidity; be willingly adherent; have fitting corporeal and mechanical possessions; experience regulated deprivation; be disinfected, nontoxic, and nonantigenic; and induce negligible inflammatory effect. Moreover, they should join to the congregation with nominal damaging and agony and ease angiogenesis, whereas yet being cost operative. The eventual aim of tissue engineering is to gratify most if not all of these standards when creating original, clever skin replacement [1].

2. Structural and progressive provisions

The skin covers epidermal and dermal sheets pervaded via a multifaceted vascular and nervous system. The hypodermis is located underneath, made by moveable linking tissue and fat. Epidermal basal cells and stem cells existing in the basal layer and hair follicles are in control of an unremitting progression of epidermal regeneration. Additional cell varieties that exist in the epidermis comprise melanocytes, Langerhans cells, and Merkel cells [2].

The dermis carries two stratums: a superior papillary layer carrying a reedy organization of collagen fibers and a dense inferior reticular layer with profuse collagen fibers similar to the superficial of the skin. The dermal extracellular matrix is made mostly of collagen, elastin, and reticular fibers. The key constituent of the dermis is the fibroblasts that deliver continuous excretion of the collagen and proteoglycan matrix [2].

Fetal wound repair shows a lack of scarring and fibrosis. This progression is categorized via negligible irritation and renewal of healthy collagen deposition and skin adnexa. The development dynamic outline in fetal renewing skin is significantly diverse from the adult one, being described via advanced intensities of transforming growth factor (TGF)-β3 and minor stages of platelet-derived growth factor (PDGF), TGF-β1, and TGF-β2 [2].

3. Aims of skin tissue engineering

Tissue-contrived skin is a noteworthy improvement in the arena of wound healing. It has primarily been advanced related to the limits linked with the utilization of autografts and allografts where the contributor site agonizes from aching, contamination, and blemishing. Lately, engineered skin substitutes have been covering extensive submission, particularly in the circumstance of injuries, where the main preventive issue is the obtainability of autologous skin. The expansion of an imitated skin enables the action of patients with burns and several skin-associated disorders [2]. The existing review contributes an inclusive outline of the improvements and upcoming forecasts of skin alternates for tissue overhaul and renewal.

4. Current skin substitutes

Autologous keratinocytes may be obtained and cultivated into interconnected layers of the epithelium which may be displaced onto big skin deficiencies on the suffering individual. Clonogenic keratinocytes, defined as holoclones, may be obtained from the skin and consecutively proliferated in culture for more than 140 replications and have revealed to be bona fide multipotent stem cells founded on their aptitude to renovate manifold lines in the skin [3, 4].

These embedded stem cells inside of these epithelial expanses provide repair and regeneration of the epidermis. Developing the epidermal stem cells over fibrin environments or allogeneic dermis has established to be beneficial. The funding materials have significantly enhanced the receiving amounts of the implants, advanced the affluence of managing and operation of the implants, and reduced the wound refutation and scarring. Cultivation of autologous epidermal stem cells simplifies to obtain large epithelial areas for transfer from a minor skin biopsy; therefore, this method needs more than a few weeks [5].

Mounting the stem cells on a substance drops the period necessary to brand outsized epithelial layers from a minor skin biopsy for the epithelia on the material which does not necessitate to attain full confluence of the previous replacement. Furthermore, epidermal stem cells on fibrin environments or allogeneic dermis converse the capability to renew the usual rolled dermal/epidermal connection and the artificial ration of the dermis, named the papillary dermis. Nevertheless, these epidermal stem cell implants lack renovation of a complete practical skin. Epidermal adjuncts, containing hair follicles, sebaceous glands, or sweat glands, are not redeveloped after transferring these implants of epidermal stem cells, signifying that multifaceted epithelial and mesenchymal connections are essential to generate additions. Additionally, the implantations do not reinstate the automated possessions or visual form of the novel skin. Improvements in stem cell biology and skin morphogenesis have the prospective to expand the manufacturing of the skin that may interchange the typical utility and esthetics of healthy skin [5].

5. Skin stem cells

Up to now, scientists have recognized numerous diverse sorts of skin stem cell covering epidermal stem cells, hair follicle stem cells, melanocyte stem cells, mesenchymal stem cells, and recently identified human newborn foreskin stem cells. Epidermal stem cells are in charge for routine regeneration of the dissimilar stratums of the epidermis. These stem cells exist in the basal layer of the epidermis [6]. Hair follicle stem cells safeguard continuous renewal of the hair follicles. They can also restore the epidermis and sebaceous glands in case of injury. Hair follicle stem cells originated through the hair follicles [7].

Melanocyte stem cells are in control of melanocyte revival which is a kind of pigment cell. Melanocytes generate the pigment melanin and so carry a significant part in skin and hair follicle pigmentation. It is not yet clear where these stem cells are located [8]. Studies also indicate

another type of stem cell, known as mesenchymal stem cells, which can be established in the dermis and hypodermis. Mesenchymal stem cells conquer lymphocyte production in vitro and extend skin graft endurance in vivo [9].

Another stem cell that resides in the skin is recently established and named as human newborn foreskin stem cells. They carry pluripotency, and they are capable to turn into different cell types. They show fibroblastic shape; however, they express both mesenchymal stem cell markers and some of the hematopoietic stem cell markers [10].

6. In vivo applications

Autologous skin transfer is now the scientific main protocol for full-breadth skin injuries covering burn damages. Before grafting, primary editing is a significant portion of the handling of burn wounds, as skin temperature-denatured proteins have to be detached to avoid numerous difficulties like contamination, manifold organ impairment condition, hypertrophic mutilation development, unrestrained inflammatory reaction, or infection with pathogenic microorganisms. Microbes could utilize the eschar as a basis of nutriments and are particularly damaging to seriously burnt individual, as this damage also triggers a provisional destruction of cell-related and humoral immunity [11].

Autologous split skin grafts (SSGs) are reaped with a dermatome which separates the epidermis and an artificial portion of the dermis. Residual epidermal cells in the enduring dermis of the SSG giver site will recreate an epidermis. Subsequent to the submission of an SSG to a full-width wound, its vessels unite with the capillary system in the removed wound. This "graft take" is vital for a correct source of nourishment and brings implant endurance. The divided skin contributor place patches up in 1 week and may be utilized for SSG collecting up to four times; though, continual reaping is linked with blemishing at the contributor sites in addition to long hospital visits. Furthermore, in the situation of a wider damage, contributor sites are tremendously restricted and might leave the individual with very small unharmed skin to produce sufficient autologous SSGs. An initial and enduring wound healing is wanted, as it outcomes in negligible or no scarring difficulties, poorer impermanence, and improved practical extended duration outcomes [12]. Oppositely, wound healing postponement is straightly relative to vigorous hypertrophic scarring. In order to indicate the difficulty of restricted SSG reaping sites, an interconnecting method is applied that expanses the implant and so may cope a superior wounded region at the expenditure of cosmetic and practical result [13].

Additional option is the usage of allografts, for a provisional deterrence of liquid loss or infection of the wound. Allografts integrate into serious injuries and deliver ache relief. Therefore, moral as well as protection matters endure, as the severe broadcast for virus-related illnesses and consistent disinfection methods cannot entirely eradicate the probability of infectious mediator conduction. When compared to autologous SSGs, a leading trouble of allografts is that they consent the patients for a long time with wounds likely to problems. Ultimately, allografts experience immunogenic refusal, and the location of wound requires to be enclosed with an autologous SSG. Deferred refusal may happen in people with wide injuries because

of their clinically repressed immune reaction and, nonetheless, finally may be activated via the extremely immunogenic epithelial cells of the allograft throughout its vascularization. Consequently, there is a countless requirement for a substitute that may deliver a more enduring clarification [13].

7. Tissue-engineered skin alternates

Manufactured cell free along with allogeneic cell comprising skin alternates delivers a conceivable resolution to the difficulty of donor implant scarcity. The engineered skin alternatives propose defense from liquid loss and infection while transporting dermal matrix constituents, cytokines, and evolution elements to the wound bed, increasing usual host wound therapeutic answers. Bioengineered skin alternatives may be utilized as impermanent covers once wound damaged tissue up to there is an autograft accessible. Subsequent to assimilation, these assemblies persevere in the wound throughout healing or even afterward. Cell-free biomaterial-related skin alternates may be utilized in integration with autografts as a defensive cover over interconnected autografts to fund their income in addition to arouse the wound bed in the spaces or to expand implant engraftment in parts of pressure. Nevertheless, in contradiction of autografts, tissue-engineered allogeneic skin implants may tolerate the danger of conveying like hepatitis B virus (HBV) or human immunodeficiency virus (HIV). One benefit over autologous in vitro engineered skin alternates is that they have decreased industrial prices [14].

In order to manufacture epidermal replacements, a skin biopsy of 2–5 cm^2 must be picked up from the individual. This may be joined with the first debridement of the injured person. Consequently, the epidermis is detached from the dermis, and solitary keratinocytes are chemically discharged and cultivated on mitotically incapacitated mouse fibroblasts (**Figure 1**). The utilized development media cover fetal calf serum and other essential additions; conversely, it is also likely to enlarge these cells in xenogeneic-free situations. There are numerous revisions analyzing epithelial allografts such as Celaderm; conversely, the efficiency and protection of these harvests have to be established in organized scientific trainings. Along with these custom-built concepts, there have been several laboratories manufacturing cultivated epithelial allografts. Allogeneic crops carry the benefit of abridged industrial charges equated to autologous crops. Yet, an inadequacy of both harvests is that they demonstrate deprived attachment levels that may bring the creation of wounds [15].

On behalf of the management of full-width burns, mutually the epidermal and the dermal layers of the skin require to be substituted, as the action with expanded epidermal (keratinocyte) layers would end in a mediocre conclusion. In contradiction of cultured epidermal layers, engineered dermal concepts can inhibit wound shrinkage, and they deliver a better constancy. The dermal and epidermal counterparts should be submitted successively, as decent dermal vascularization via the debrided wound bed requires to be attained previously to submission of the epidermal stratum. There is an extensive diversity of advertised dermal concepts, both natural and artificial. Some of these alternates are chemical-treated allografts

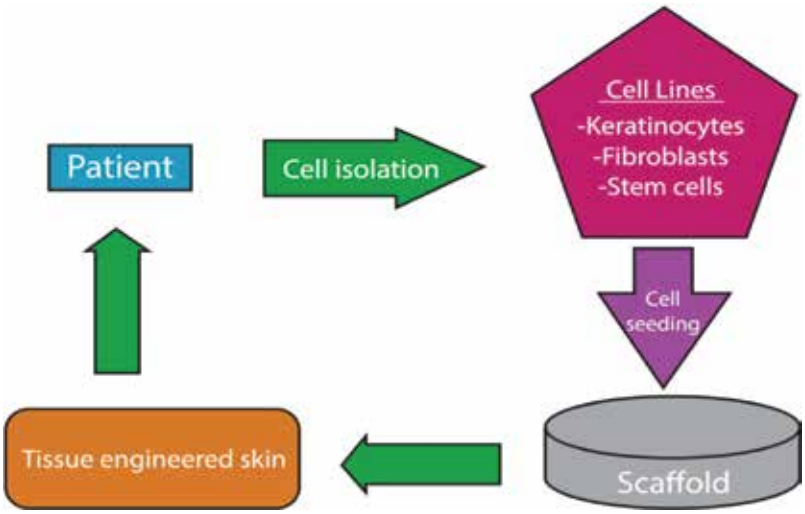

Figure 1. Representation of perspectives of skin tissue engineering. Primary keratinocytes, fibroblasts, and stem cells are obtained from human contributor tissues that are afterward in vitro expanded previously to seeding onto appropriate scaffold materials.

such as Alloderm, deficient for cellular rudiments that are important for the immunogenic refusal [16]. Besides, Dermagraft contains human foreskin fibroblasts, expanded in a fissionable polyglactin network. In these alternates, cells secrete extracellular matrix (ECM) proteins, a variation of growth factors and cytokines into the wound till they experience usual programmed cell death 1 or 2 weeks post-embedding [17].

The most progressive and refined concepts that are accessible for scientific utilization are alternates that imitate epidermal along with dermal sheets of the skin. Even though imitating the histo-architecture of healthy skin, the epidermal/dermal skin alternates would be thought as provisional structurally effective wound layers. Skin replacements deliver growth factors, cytokines, and ECM for host cells; control wound remedial; and may consequence in active pain relief. Main drawbacks are the elevated industrial charges and their insufficiency to heal the wound enduringly regarding to tissue refusal. The immunogenic acceptance of a host in the direction of allogeneic fibroblasts is controversially deliberated. There are different revisions sustaining the theories that allogeneic fibroblasts are individual autologous keratinocytes and are passable for the creation of a perpetual epidermal-dermal skin alternate [18].

8. In vitro applications

Tissue-engineered human skin has been technologically advanced to replicate the main fundamental and practical features of normal skin. In this background, they allow not only the examination of essential procedures in the skin but also the risk valuation of several chemicals which are locally presented to the skin deprived of the necessity to utilize animal models. Outcomes obtained from experimentations showed in animal models are mostly restricted regarding the alterations in the metabolism and the functional architecture. In vitro tests in two-flat monolayer

cultivation of human cells are also of nominal significance because of the absence of multifaceted cell-cell and cell-ECM connections [18]. Conversely, manufactured skin alternates may eliminate these difficulties via utilizing human cells which are organized in a 3D physical background, letting the interface of the dissimilar cell sorts with one additional and nearby matrix [12].

Presently, skin alternatives are utilized in pharmaceutical investigation and in rudimentary examination. In these experimentations, skin alternatives attend as consistent archetypal schemes to classify irrigative, toxic, or destructive possessions of chemicals that arise into communication with the human skin. In basic investigation, skin replacements may assist to explain essential progressions in the skin like the inducements that bring the creation of the epidermis, the cross talk among dissimilar cell sorts, the conservation of the stem cells, the development of wound healing, and the contamination with diverse types of pathogens. One excessive benefit of skin replacements is that the cellular arrangement is entirely manageable via the scientist. Therefore, a specific cell sort may be precisely combined or misplaced to regulate the significance of the cell kind in the organic progression under investigation [12]. Skin tissue engineering may be examined with several in vitro models shown in **Table 1**.

Up to the present time, several sorts of skin alternatives have been established via dissimilar scientific groups. These skin alternatives may be categorized in two kinds. The primary one contains keratinocytes applied on an artificial or collagen transporter faking only the human epidermis. The subsequent one contains a dermal sheet of human fibroblasts entrenched in numerous types of scaffolds [12].

In vitro skin models	Cell foundation	References
Melanoma model	Melanoma mesenchymal cells, fibroblasts, keratinocytes	[19]
UV radiation and phototype	Keratinocytes, melanocytes, and fibroblasts	[20]
Wound healing model	Fibroblasts and keratinocytes	[21]
Psoriasis model	Keratinocytes and fibroblasts	[22]
Full-thickness model	Epithelial sheath, fibroblasts, and keratinocytes	[23]
Ex vivo model	No requirement of cells	[24]

Table 1. In vitro skin tissue engineering models.

9. Full-thickness in vitro models

Although the excessive mainstream of the skin alternates utilized in pharmaceutical investigation is made of an epidermal sheet, these skin replacements could then be developed via the totaling of a dermal stratum covering fibroblasts. In this perspective, fibroblasts have only lately started to have more consideration. It was revealed that skin fibroblasts are distant from being uniform, and it was guessed that a few of the chronic wounds are related to an

alteration in the arrangement of the fibroblasts. It was displayed that fibroblasts clearly effect keratinocyte development in vitro, most probably because of the circumstance that these cells discharge solvable growth factors. In normal skin, the communication among fibroblasts and keratinocytes covers a key role in progressions like wound healing and the creation of the base membrane [1].

Utilizing skin alternatives, it was established that fibroblasts carry a vital part in the normal epidermal histology (**Figure 2**). In the absence of fibroblasts, the keratinocyte differentiation harshly changes and outcomes only in some sheets of extremely distinguished epithelial cells. Fascinatingly, keratinocytes carry a significant influence on the expansion of fibroblasts. This interface of epidermal and dermal cells is theorized because of a double paracrine tool that controls the development of keratinocytes and fibroblasts. Due to the theory, keratinocytes discharge IL-1 which rouses the skin fibroblasts to secrete keratinocyte growth factor (KGF) and granulocyte-monocyte colony-stimulating factor (GM-CSF) that sequentially effect the production of the keratinocytes. Moreover, dermal fibroblasts carry an important part in the renovation of the skin and in the tightening of acute wounds, and they can upturn the struggles of keratinocytes to toxic chemicals. Regarding the outcomes, one could determine that for obtaining significant data from toxicological in vitro experiments. Oppositely, epidermal replacements might be more appropriate for the determination of the diffusion constant through the skin. In monotonous in vitro diffusion studies, a specific part of skin alternates

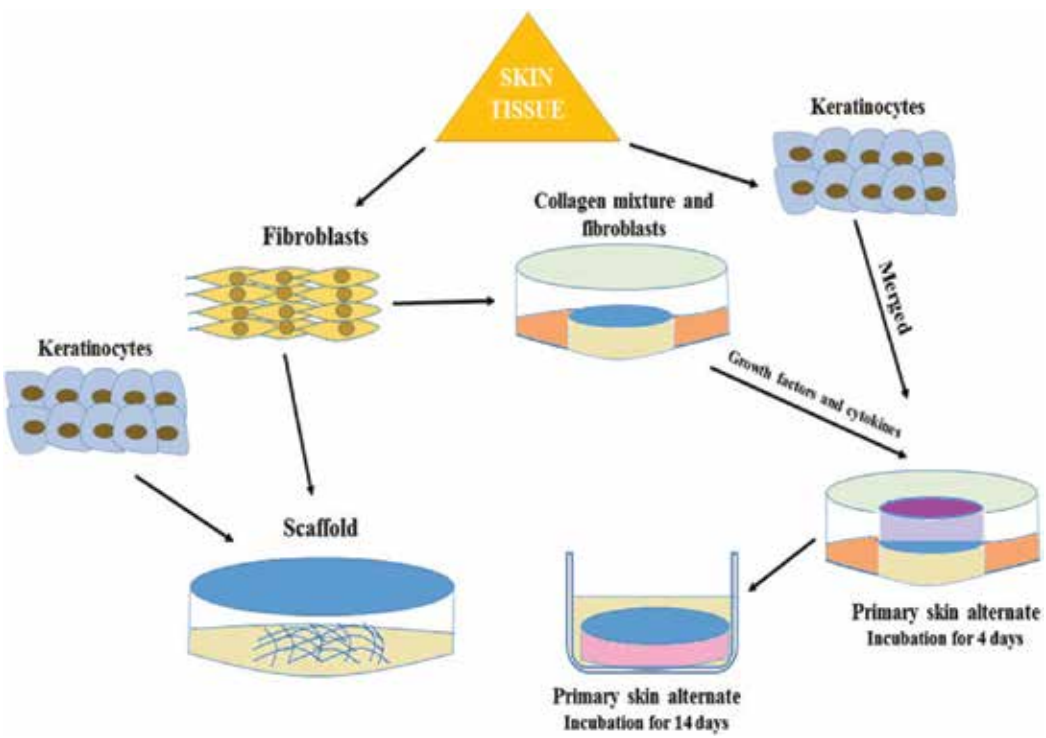

Figure 2. Utilization of skin fibroblast and keratinocyte for skin tissue engineering.

divorces a contributor from an acceptor compartment. Collagen-related full-breadth skin alternates are not ultimate for that kind of tests since they do not cover the entire external part regarding to a low mechanic pliability, consequently finishing in open superiorities, over that the constituent under study may flexibly diffuse [12].

10. Skin gene therapy

The aptitude to hereditarily alter cells utilized to organize skin alternates allows ex vivo gene treatment methods to treat cutaneous illnesses and damages. Nonetheless, gene therapy stays as an unsatisfied potential of cell treatments with skin alternatives. Still, designated analysis of gene therapy in skin wounds has been accomplished recently. Initial models utilizing overexpression of angiogenic growth factors with duplication-incompetent retroviral vectors established viability for constructive distribution of physically dynamic composites such as VEGF and PDGF, with a capacity for prompt wound healing in diminished wounds. Therefore, usage of retroviral gene transmission for the action of hematopoietic syndromes was linked with expansion of leukemia regarding the addition of mutagenesis, an outcome which has fundamentally banned the method from potential deliberation [25].

Parallel threats have been recognized in lentiviruses which have also decreased their forthcoming custom in therapeutics. Replacements to viral tools have been industrialized that comprise plasmid transfection for expression of endogenous antimicrobial peptides like cathelicidin. Additional lately advanced methods such as gathered frequently interspaced short palindromic repeat (CRISPR) arrangement enable site-specific genome editing, greatly reducing the risk of insertional mutagenesis. In addition if gene therapies are managed in allogenic cells that are eradicated immunologically after a restricted period of time, then threats can be minimalized [25]. Nonviral skin gene transfer techniques are listed in **Table 2** individually.

Transfer techniques	Therapeutic mediator	Submission reasons	References
Direct injection	Cytosine-phosphate-guanine class C/ immunestimulatory sequence oligodeoxynucleotides	Tumor treatment	[26]
Electroporation	Antisense oligodeoxynucleotides	Wound healing	[27]
Electroporation	Chimeric RNA/DNA oligodeoxynucleotides	Hair follicle manipulation	[28]
Topical	Liposome-coated DNA	Expressions of growth factors, cytokines, and hormones	[26]
Biolistic	Naked DNA	Immunization	[29]

Table 2. Nonviral skin gene therapy methods.

Induced pluripotent stem (iPS) cells may progress the competence of genome excision. The application of iPS cells includes deterioration of donor cells to a pluripotent state in vitro, growth of cell quantities, and adjustment of cell populace in the direction of a distinguished phenotype of concern. The arrangement of genetic adjustment methods, permitting accumulation of healthy genes or alteration of mutated genes, with iPS knowledge delivers the aptitude to fix hereditary illnesses [25].

Skin alternates consequent to skin stem cells also carry a potential for practicable gene therapy for inactivating inherited illnesses of the skin, like epidermolysis bullosa. The epidermis is systematized into epidermal multiplying components which are self-renovated via at least one epidermal stem cell an propose that transport of epidermal stem cells from the basal stratum of the epidermis for gene treatment might consequence in enduring expression of the transgene [26]. Ex vivo transduced keratinocytes of holoclones have been revealed to have transgene expression which continues for more than 150 cell productions in culture and, more significantly, have been publicized to express the transgene protein once implanted in epidermal stratums in vivo. Autologous epidermal stem cells obtained in culture via development of holoclones were retrovirally interacted with laminin 5 and were effectively relocated in people with junctional epidermolysis bullosa. The implants renewed a healthy epidermis at day 8, and the usual epidermis was preserved during 1 year of continuation [30].

11. Scaffold biomaterials in tissue engineering of the skin

In order to manufacture a body-compatible scaffold, it is important that the scaffold does not cause any acute or chronic response in the body. The scaffold must have a surface that is suitable for cell attachment so that it can replace the damaged tissue and help creating new tissue. If the biomaterial used in the making of the scaffold is biodegradable, newly regenerated tissue can replace the scaffold. Therefore, it is crucial that the scaffold is compatible with the skin tissue. For a scaffold to hold, it must have certain physical and mechanical properties and have a certain chemical structure in the surface. Researchers may use different biomaterials such as collagen, chitosan, hyaluronic acid, and poly(lactic acid) (PLA) in tissue engineering to build scaffolds [31].

11.1. Chitosan

Chitosan is one of the materials that is used in tissue engineering, which is used in wound healing. It is biodegradable, biocompatible, and nontoxic. In addition, it has hemostatic activity. It is also advantageous that chitosan is antibacterial. Chitosan can be used in stimulating collagen synthesis, and its electrostatic function can speed up the healing process. Sponges and gels that are made from chitosan are utilized in the healing process of full-width burn wounds. Chitosan loses its effect in acidic environments, and since wound healing is an acidic incident, cross-link agents may be used to stabilize chitosan structures [32].

11.2. Hyaluronic acid

Hyaluronic acid is a lineal polysaccharide made of repeating disaccharide elements of N-acetyl-glucosamine and n-glucuronic acid. Hyaluronic acid can be found in human skin and is known to be speeding up the healing process. Apart from these, it is observed that hyaluronic acid amount is increased in fetal skin and wounds in case of scar-free healing. Hyaluronic acid is a material with so much perks in scaffolding such as expanding the possibilities of cross-linking, delaying the biodegrading of materials, and more control over mechanical aspects of the process. Also, hyaluronic acid offers more incorporation of cell adhesion ligands and growth factors in the making of scaffolds. Aquatic uptake ability, flexibility, and biocompatibility of the scaffold are some of the properties that are made possible and enhanced by HA [32].

11.3. Collagen

Collagen is a naturally found protein that can enhance the structural integrity. Collagen can be found in human skin tissue and mostly created by fibroblasts and myofibroblasts. In the body parts that are under stress and used often, for example, the skin, tendons and bones, collagen can be found in fibrils. One of the most common types of collagen which is also seen widely in scar tissues as well as the dermis, fasciae, and tendons is type I collagen. There are 20 variations of collagens, and only types II, III, V, and XI can make up fibrils. Collagen is one of the most used materials that have been utilized in skin tissue engineering, and only recently it has been possible to create a model that can promote human capillary-like network [33].

It is an excellent material for scaffolding because of its ability to boost cell attachment, migration, proliferation, and differentiation. It is preferred in medical applications as a primary material since it is excellent in biocompatibility, biodegradability, and weak in antigenicity. Scientific researches provided recombinant human collagen, and it proves to be a more dependable foundation for collagen which is not animal based. Human-based collagens are used in scaffolding, and they show promising results in efficiency for manufacturing skin, cartilage, and periodontal ligaments. Permeable collagen matrices with specific structural, biochemical, and biotic characteristics are interesting materials for tissue engineering, and introducing glycosaminoglycans may add to these characteristics because they are constituents of ECM proteins [34].

11.4. Silk

Silk is a biopolymer that is found in nature and has been used in medical applications for centuries. It contains filament core protein, named fibroin, and a glue-similar coating with sericin proteins. Silk can be composed into many forms such as films, fibers, meshes, and sponges, and these forms have been used in many incidents, show great promise in supporting stem cell union, multiplying, and distinction in vitro, and are known to be boosting tissue repair in vivo. Skeletal structures such as bone, ligament, cartilage, and connective tissues such as the skin have been engineered using 3D silk fibroin scaffolds in stem cell-related tissue manufacturing [35].

11.5. Fibrin glue

For some time now, fibrin glue has been used for medical applications such as plastic surgery and reconstructions as an adhesive compound. It is antibacterial, as well as it boosts hemostasis. Apart from these, fibrin helps grow keratinocyte and fibroblast in vitro and in vivo and therefore promotes cellular movement in the wound. It has been observed that endogenous fibrin clots to create a temporary matrix in a purpose of promoting angiogenesis in the primary stage of wound healing. It is recognized that some growth factors are increased during the wound healing process to promote angiogenesis. Vascular endothelial growth factor (VEGF) is one of these. Furthermore, if fibrin is preferred as a dermal substrate for an alternative of cultivated skin, it upsurges the discharge of VEGF, thus indorsing angiogenesis [34].

11.6. Artificial fragmental polymers

A few of the artificial fragmentable polymers utilized as permeable scaffolding constituents cover polyethylene glycol (PEG), poly(lactic acid) (PLA), polyglycolide (PGA), poly(lactic-co-glycolic acid) (PLGA) [34], polycaprolactone (PCL), poly(D,L-lactic acid or D,L-lactide) (PDLLA), polyester elastomer (PEE) founded on polyethylene oxide (PEO), and polybutylene terephthalate (PBT). There are some synthetic polymers with biodegradable properties that are highly preferred as permeable scaffolding constituents such as polyethylene glycol (PEG), poly(lactic acid) (PLA), polyglycolide (PGA), poly(lactic-co-glycolic acid) (PLGA) [35], polycaprolactone (PCL) [17], poly(D,L-lactic acid or D,L-lactide) (PDLLA), polyester elastomer (PEE) based on polyethylene oxide (PEO), and polybutylene terephthalate (PBT) [37].

A superlative absorbent scaffold in skin tissue engineering should be the one which imitates the normal surroundings for skin development over suitable cell penetration, propagation, and differentiation. It should be biodegradable and penetrable to oxygen, aqua, and nutrition interchange and must be defensive contrary to contamination and injury [38]. Up to the present time, there have been numerous kinds of absorbent scaffolds defined for skin tissue renewal, and most of them may be branded as fibrous permeable scaffolds. Nevertheless, there are some spongy or foamy scaffold sorts with advanced absorbency that may be utilized as operative concepts for skin renewal. Supreme of these scaffolds has collagen as a foundation, and then keratinocytes or fibroblasts are planted into the scaffolds [39].

When choosing a porous scaffold to be used in skin tissue engineering, one must look for some properties and characteristics to create optimum conditions that resemble the usual background for skin development of the most over suitable cell permeation, creation, and distinction. The ideal scaffold also should safeguard against contamination and injury. There have been plenty of porous scaffolds with various forms to this day that are described for the regeneration of skin tissue, and most of these may be seen as fibrous absorbent scaffolds. Other than these scaffolds, there are also various types of spongy or foamy scaffolds which have higher porosity and can be used in skin regeneration. Collagen is the main ingredient in most of these porous scaffolds with keratinocytes or fibroblasts that are seeded into the scaffolds [36].

Scaffolds are designated in severe burns and skin deficiencies persuaded because of the elimination of tumors or skin implanting in patients experiencing necrotizing fasciitis owing to

bacterial contaminations [40]. Meanwhile, some original sponge scaffolds in arrangement with biomaterials like human keratin and polyvinyl alcohol/chitosan have also been described for their utilization as operative skin alternatives. Scaffolds have been used in medical applications such as acute burns and skin defects. Apart from these, some novel sponge scaffolds when used with biomaterials like human keratin and polyvinyl alcohol/chitosan have also been used since they are so effective as skin substitutes [1].

Nanofibrous scaffolds are extensively utilized for firming along with lenient tissue engineering submissions, and they also perform as tools for the regulated distribution of drugs and numerous biological particles in the arrangement of proteins and DNA [41]. Numerous usual and synthetic polymers have been applied for nanofiber constructions to generate fibrous scaffolds for biomedical applications [42]. These nanofibers are occasionally precisely functionalized via a basic interference or coating method or with superficial implanting polymerization by adding ligands and adhesive proteins on the nanofiber shallow. Combination of drugs, development factors, and genes straightly into the polymer elucidation throughout electrospinning is also a training for precise discharge possessions. Current methods for integrating therapeutic mediators or bioactive particles comprise coaxial electrospinning, suspension electrospinning, and alterations by external absorption or chemical conjugation [43]. In lieu of soft and hard tissue engineering and its submissions, nanofibrous scaffolds are widely used as well as deliver drugs in a controlled manner. Synthetic and natural polymers have been used for nanofiber manufactures to harvest rubbery scaffolds for biomedical presentations [44].

12. Discussion

The largest part of the human body is the skin. One of its key responsibilities is to protect the body from the environmental influences. When this shield is lost both acutely and chronically, it must repair itself to avoid termination of the life of its carrier. When the skin is wounded, re-epithelialization of the wounded surface occurs [1, 2, 15–17]. Another important subject is that extensive wounds require an additional protection layer. This layer helps preventing desiccation and infections. It also guides cells to repair the wound and improve healing rates. This requirement led to the evolution of biologic and synthetic dressings and skin substitutes [14]. Wounds that occur due to massive burning made inventing different kinds of temporary and permanent skin alternatives necessary. This was needed because the patient could not repair its skin by itself. To be successful in long-term recovery, certain properties from both dermal and epidermal skin should be considered [9]. There is no example where an artificial skin is better than the original. Because of this, tissue engineers must focus their efforts on producing a universal skin that would be the best alternative in the shortest amount of time [13, 19].

A scaffold is used to create the three-dimensional structure for cell interactions and ECM production. It carries proper cytokines and growth factors to the target area [18]. It also supports the structure and functions of the newly formed tissue. There are certain criteria for a scaffold to provide all of the above [34, 36, 39, 41]: it should have a proper internal structure and surface that can support cellular behaviors such as adhesion, differentiation, and proliferation [30]; it should have mechanical properties alike to the ones in the repair site [30]; and

it should be biocompatible. Its properties rely on the modifications that were applied to it and the nature of the biomaterial. Sponginess and pore scope of polymeric scaffolds display a critical role in tissue regeneration [38]. These considerations have been comprehensively deliberated and covered in different publications. Porous organization of scaffolds is important with regard to their allowance of migration, adhesion, and multiplication and also diffusion of wastes, oxygen, and nutrients [31]. It is certain that larger pores are used for supplying nutrients and removal of wastes. Small pores on the other hand provide surface area for cell adhesion. In order to generate dissimilar pore sizes and porosities, used techniques vary from salt discharge, modified lyophilization, phase separation, application of numerous freezing temperatures, application of various acoustic heaviness amplitudes, and application of different electrospinning rates [36].

Reports show several types of scaffolds used in skin tissue regeneration. Even though there are some setbacks, they have been notably successful for repairing tissue and wound healing. They are also successful for delivering regular supply of nutrients and oxygen to cells, due to their variable porosities [36]. Mechanical strength and biocompatibility may be a subject of concern for fabrication of scaffolds, but composite and ceramic types have a promising future. Various biomaterials, both natural and synthetic, are used separately or in combination to create scaffolds. Combinations of these materials overcome the issues with biocompatibility, biodegradability, and mechanical strength [33]. Collagen, cellulose, and chitosan are some of the examples for natural biomaterials [34]. They are found as either polysaccharide or proteins. These are highly biocompatible and easily degraded due to their similarities to the natural ECM. This makes them highly suitable for skin cell growth. If we look at synthetic biomaterials, some nanomaterial-type examples like polyvinylpyrrolidone (PVP), polycaprolactone (PCL) [41], poly-ethylene-glycol (PEG), poly lactic acid (PLA) [31, 35], and others are good for enhancing the strength of the scaffold. This requires more research and efforts toward the direction of composite scaffolds. An extensive knowledge of all the facts listed above will lead to producing highly effective and suitable scaffolds for skin tissue regeneration. There are many subjects in tissue engineering that should be overcome though such as scaffold interaction with cells, cellular proliferation and differentiation speed, and vascularization of the engineered tissues [37–39]. Rapid advancement of organ-on-chip technology, which led to "skin-on-chip" technology, has cleared the way for generating engineered skin for wound healing and drug testing [12]. The development of perfused chip-based bioreactors offers improvement of culturing conditions for skin organ cultures, as well as variable mechanical stress. Another advancement to note is microfluidic technologies, which are developed to create perfused skin-equivalent cultures and show a promise in the field of applying various drug molecules that are associated with skin tissue and wound healing [14, 17, 32, 33].

In vitro skin prototypes are used for recognizing skin destructive or poisonous materials and have been established to be beneficial implements for the examination of rudimentary evolving progressions as well as for the documentation of compulsive circumstances. Even though the enterprise of the epidermal and dermal stratum imitating in vitro skin alternatives is almost ultramodern, there are obviously alterations among these replicas and natural in vivo skin [45]. One drawback of in vitro replacements is that they are not simply shaped, organized, or deposited. Practically, all in vitro skin is tailored and factory-made manually. A mechanized method would expressively decrease industrial charges and would propose

the comprehensive regulation of the procedure with a consistent result. The utilization of human keratinocyte derivative cell lines that are capable to cornify would support to lessen the charge of a skin replica even further and recover the expectedness of the last construct. Inopportunely, the mainstream of the presently obtainable cell lines is derivative of carcinoma cells that lack the capacity to arrange a corneous layer; only lately, a novel cornifying cell line was presented [46]. Additional factor that is restraining the accomplishment of skin alternatives is their short lifetime. One method to accomplish a prolonged usability is the expansion of appropriate skin substitute conservation procedures as it had been done for dermal skin grafts [47].

The sustained development of iPS cell reprogramming widens a perspective to patient with precise stem cell foundations for tissue substitution. The competence of iPS production was lately established to be enriched with the utilization of keratinocytes [48]. Moreover, iPS can be produced from keratinocytes obtained from hair. Therefore, pulling a single hair from a person delivers only the initial substantial required to make iPS cells that may afterward be differentiated to custom tissue-related stem cells [49]. In different words, it can be thinkable for keratinocytes isolated from hair to be unswervingly reprogrammed to many skin stem cells deprived of an in-between iPS state. The aptitude to generate different skin stem cells which can be integrated into an engineered skin tissue will allow renewal of all of the complex cell types within the skin.

Recently identified human newborn foreskin stem cells also carry an enormous potential to differentiate into different cell types which may also eliminate the risk factors of gene transfections in order to obtain stem cell properties. These cells have found to carry mesenchymal stem cell markers and some of the hematopoietic stem cell makers. Specific study of ours proved that they may be differentiated into chondrogenic, adipogenic, osteogenic, neurogenic, epithelial, and myogenic cells. In future prospects, they carry a potential to be used in tissue engineering models [10].

13. Future prospects

Future advances in in vitro skin alternatives should cover skin additions. The combination of sweat glands and hair follicles will assist to imitate a more accurate in vivo state and consequently involve a more precise experimental format. Many developments have been completed in the last decades for creating vascularized full-thickness in vitro skin models; nevertheless, the vascular-like structures could not be combined with an external vascular system wherein physiological shear circumstances are continued; thus, the growth of the in vitro vasculature was disallowed. The usage of more progressive biological scaffolds or synthetic vasculature imitating structures might assist to eliminate these problems.

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References

[1] Böttcher-Haberzeth S, Biedermann T, Reichmann E. Tissue engineering of skin. Burns. 2010;**36**(4):450-460

[2] Kamel RA, Ong JF, Eriksson E, Junker JP, Caterson EJ. Tissue engineering of skin. Journal of American College of Surgeons. 2013;**217**(3):533-555

[3] Claudinot S, Nicolas M, Oshima H, Rochat A, Barrandon Y. Long-term renewal of hair follicles from clonogenic multipotent stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(41):14677-14682

[4] Dickson MA, Hahn WC, Ino Y, Ronfard V, Wu JY, Weinberg RA, Rheinwald JG. Human keratinocytes that express hTERT and also bypass a p16INK4a-enforced mechanism that limits life span become immortal yet retain normal growth and differentiation characteristics. Molecular and Cellular Biology. 2000;**20**(4):1436-1447

[5] Lavker RM, Sun TT. Epidermal stem cells. Journal of Investigative Dermatology. 1983; **81**(1):S121-S127

[6] Lavker RM, Sun TT, Oshima H, Barrandon Y, Akiyama M, Ferraris C, Panteleyev AA. Hair follicle stem cells. In Journal of Investigative Dermatology Symposium Proceedings. Elsevier. 2003;**8**(1):28-38

[7] Nishimura EK, Granter SR, Fisher DE. Mechanisms of hair graying: incomplete melanocyte stem cell maintenance in the niche. Science. 2005;**307**(5710):720-724

[8] Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Moseley A. Mesenchymal stem cells suppress lymphocyte proliferation *in vitro* and prolong skin graft survival *in vivo*. Experimental Hematology. 2002;**30**(1):42-48

[9] Somuncu ÖS, Taşlı PN, Şişli HB, Somuncu S, Şahin F. Characterization and differentiation of stem cells isolated from human newborn foreskin tissue. Applied Biochemistry and Biotechnology. 2015;**177**(5):1040-1054

[10] Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Jaenisch R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science. 2007;**318**(5858):1920-1923

[11] Groeber F, Holeiter M, Hampel M, Hinderer S, Schenke-Layland K. Skin tissue engineering—*in vivo* and *in vitro* applications. Advanced Drug Delivery Reviews. 2011; **63**(4):352-366

[12] Shevchenko RV, James SL, James SE. A review of tissue-engineered skin bioconstructs available for skin reconstruction. Journal of the Royal Society Interface. 2009;**7**(43):229-258

[13] Culver DH, Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG, Hughes JM. Surgical wound infection rates by wound class, operative procedure, and patient risk index. The American Journal of Medicine. 1991;**91**(3):S152-S157

[14] Gielen V, Faure M, Mauduit G, Thivolet J. Progressive replacement of human cultured epithelial allografts by recipient cells as evidenced by HLA class I antigens expression. Dermatology. 1987;**175**(4):166-170

[15] Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. Burns. 1995;**21**(4):243-248

[16] Kolokol'chikova EG, Budkevich LI, Bobrovnikov AE, Badikova AK, Tumanov VP. Morphological changes in burn wounds after transplantation of allogenic fibroblasts. Bulletin of Experimental Biology and Medicine. 2001;**131**(1):89-93

[17] Sun T, Jackson S, Haycock JW, MacNeil S, Culture of skin cells in 3D rather than 2D improves their ability to survive exposure to cytotoxic agents. Journal of Biotechnology. 2006;**122**(3):372-381

[18] Vörsmann H, Groeber F, Walles H, Busch S, Beissert S, Walczak H, Kulms D. Development of a human three-dimensional organotypic skin-melanoma spheroid model for *in vitro* drug testing. Cell Death & Disease. 2013;**4**(7):e719

[19] Astner S, Anderson RR. Skin phototypes. Journal of Investigative Dermatology. 2004;**122**(2): 1523-1747

[20] Gonzalez-Andrades M, Alonso-Pastor L, Mauris J, Cruzat A, Dohlman CH, Argüeso P. Establishment of a novel *in vitro* model of stratified epithelial wound healing with barrier function. Scientific Reports. 2016;**6**:19395

[21] Saiag PH, Coulomb B, Lebreton C, Bell E, Dubertret L. Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model *in vitro*. Science. 1985;**230**(4726):669-672

[22] Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T. Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness. Science. 1981;**211**(4486):1052-1054

[23] Andrade TA, Aguiar AF, Guedes FA, Leite MN, Caetano GF, Coelho EB, Frade MA. *Ex vivo* model of human skin (hOSEC) as alternative to animal use for cosmetic tests. Procedia Engineering. 2015;**110**:67-73

[24] Ennis WJ, Hill D. Skin Tissue Engineering and Regenerative Medicine. Vol. 239. In Elsevier Inc.; 2016

[25] Niidome T, Huang L. Gene therapy progress and prospects: Nonviral vectors. Gene Therapy. 2002;**9**(24):1647

[26] Regnier V, Préat V. Localization of a FITC-labeled phosphorothioate oligodeoxynucleotide in the skin after topical delivery by iontophoresis and electroporation. Pharmaceutical Research. 1998;**15**(10):1596-1602

[27] Vogel JC. Nonviral skin gene therapy. Human Gene Therapy. 2000;**11**(16):2253-2259

[28] Zhang L, Nolan E, Kreitschitz S, Rabussay DP. Enhanced delivery of naked DNA to the skin by non-invasive *in vivo* electroporation. Biochimica et Biophysica Acta (BBA)-General Subjects. 2002;**1572**(1):1-9

[29] Mathor MB, Ferrari G, Dellambra E, Cilli M, Mavilio F, Cancedda R, De Luca M. Clonal analysis of stably transduced human epidermal stem cells in culture. Proceedings of the National Academy of Sciences. 1996;**93**(19):10371-10376

[30] Riva R, Schmeits S, Croisier F, Lecomte P, Jérôme C. Poly (ethylene glycol) grafted polylactide based copolymers for the preparation of PLA-based nanocarriers and hybrid hydrogels. Clinical Hemorheology and Microcirculation. 2015;**60**(1):65-75

[31] Zhong SP, Zhang YZ, Lim CT. Tissue scaffolds for skin wound healing and dermal reconstruction. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. 2010;**2**(5):510-525

[32] Metcalfe AD, Ferguson MW. Tissue engineering of replacement skin: The crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. Journal of the Royal Society Interface. 2007;**4**(14):413-437

[33] Priya SG, Jungvid H, Kumar A. Skin tissue engineering for tissue repair and regeneration. Tissue Engineering Part B: Reviews. 2008;**14**(1);105-118

[34] Gentile P, Chiono V, Carmagnola I, Hatton PV. An overview of poly (lactic-co-glycolic) acid (PLGA)-based biomaterials for bone tissue engineering. International Journal of Molecular Sciences. 2014;**15**(3):3640-3659

[35] Hollister SJ. Porous scaffold design for tissue engineering. Nature Materials. 2005;**4**(7): 518-524

[36] Kramschuster A, Turng LS. Fabrication of tissue engineering scaffolds. Handbook of Biopolymers and Biodegradable Plastics: Properties, Processing and Applications. 2012;**427**

[37] Ma PX, Elisseeff J. Scaffolding in Tissue Engineering. Vol. 5. CRC Press; 2005. pp. 52

[38] Chen G, Ushida T, Tateishi T. Scaffold design for tissue engineering. Macromolecular Bioscience. 2002;**2**(2):67-77

[39] Saravanan S, Nethala S, Pattnaik S, Tripathi A, Moorthi A, Selvamurugan N. Preparation, characterization and antimicrobial activity of a bio-composite scaffold containing chitosan/nano-hydroxyapatite/nano-silver for bone tissue engineering. International Journal of Biological Macromolecules. 2011;**49**(2):188-193

[40] Xu CY, Inai R, Kotaki M, Ramakrishna S. Aligned biodegradable nanofibrous structure: A potential scaffold for blood vessel engineering. Biomaterials. 2004;**25**(5):877-886

[41] Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. European Cells and Materials. 2003;**5**(1):1-16

[42] Mouriño V, Cattalini JP, Boccaccini AR. Metallic ions as therapeutic agents in tissue engineering scaffolds: An overview of their biological applications and strategies for new developments. Journal of The Royal Society Interface. 2012;**9**(68):401-419

[43] Verrier S, Blaker JJ, Maquet V, Hench LL, Boccaccini AR. PDLLA/Bioglass® composites for soft-tissue and hard-tissue engineering: An *in vitro* cell biology assessment. Biomaterials. 2004;**25**(15):3013-3021

[44] Tfayli A, Piot O, Draux F, Pitre F, Manfait M. Molecular characterization of reconstructed skin model by Raman microspectroscopy: Comparison with excised human skin. Biopolymers. 2007;**87**:261-274

[45] Allen-Hoffmann BL, Schlosser SJ, Ivarie CAR, Sattler CA, Meisner LF, Connor SLO. Normal growth and differentiation in a spontaneously immortalized near-diploid human keratinocyte cell line, NIKS, Differentiation. 2000;**114**(3):444-455

[46] Marston WA, Hanft J, Norwood P, Pollak R. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: Results of a prospective randomized trial. Diabetes Care. 2003;**26**(6):1701-1705

[47] Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilic J, Pekarik V, Tiscornia G, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nature Biotechnology. 2008;**26**:1276-1284

[48] Zhou Q, Brown J, Kanarek A, Rajagopal J, and Melton DA. *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. Nature. 2008;**455**:627-632

[49] Chen M, Przyborowski M, Berthiaume F. Stem cells for skin tissue engineering and wound healing. Critical Reviews™ in Biomedical Engineering. 2009;**37**(4-5)

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Stem Cells in Clinical Practice and Tissue Engineering is a concise book on applied methods of stem cell differentiation and optimization using tissue engineering methods. These methods offer immediate use in clinical regenerative medicine. The present volume will serve the purpose of applied stem cell differentiation optimization methods in clinical research projects, as well as be useful to relatively experienced stem cell scientists and clinicians who might wish to develop their stem cell clinical centers or research labs further. Chapters are arranged in the order of basic concepts of stem cell differentiation, clinical applications of pluripotent stem cells in skin, cardiac, bone, dental, obesity centers, followed by tissue engineering, new materials used, and overall evaluation with their permitted legal status.

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