IntechOpen

Leukemia

From Biology to Clinic

Edited by Margarita Guenova and Gueorgui Balatzenko

Leukemia - From Biology to Clinic

Edited by Margarita Guenova and Gueorgui Balatzenko

Published in London, United Kingdom

Leukemia - From Biology to Clinic
http://dx.doi.org/10.5772/intechopen.100848
Edited by Margarita Guenova and Gueorgui Balatzenko

Contributors
Edgardo Becerra, Guadalupe García-Alcocer, Valeria J. Soto Ontiveros, Yanitsa Davidkova, Milan Jagurinoski, Gueorgui Balatzenko, Margarita Guenova, Entesar Tebien, Abozer Y. Elderdery, Simon K.H. Szeto, Vivian Wing Ki Hui, Emil Aleksov, Branimir Spassov, Maxim Rykov, Georgij Mentkevich, Igor Dolgopolov, Natalia Subbotina, Gopila Gupta, Vikas Garg, Somanath Padhi, Biswajit Bhuyan, Prabodha Kumar Das, Chinmayee Panigrahi

First published in London, United Kingdom, 2023 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Leukemia - From Biology to Clinic
Edited by Margarita Guenova and Gueorgui Balatzenko
p. cm.
Print ISBN 978-1-80356-905-5
Online ISBN 978-1-80356-906-2
eBook (PDF) ISBN 978-1-80356-907-9

Meet the editors

Professor Margarita Guenova, MD, Ph.D., runs the hematopathology and immunology diagnostics as a head of the laboratory and Professor of Hematology at the National Hematology Hospital, Sofia, Bulgaria. She specializes in the field of blood and lymphoid malignancies. Her scientific interests include the elucidation of critical mechanisms of leukemia pathogenesis and progression, investigation of clinically relevant biomarkers and potential therapeutic targets in leukemias and lymphomas, minimal residual disease and implementation of a multifaceted approach in diagnostics. Margarita Guenova has authored and co-authored many abstracts, articles in peer-reviewed journals and book chapters. She serves on editorial boards and executive boards and committees of medical societies such as the Bulgarian Joint Cancer Network, Bulgarian Society of Hematology, European Hematology Association, etc.

Professor Gueorgui Balatzenko, MD, Ph.D., runs the genetic and molecular diagnostics as a head of the laboratory and Professor of Hematology at the National Haematology Hospital, Sofia, Bulgaria. He has specialized at the Red Cross Blood Bank, Groningen-Drenth, The Netherlands; Institute Paoli-Calmettes-Marseille, and Necker-Enfans Malades-Paris, France. His major scientific interests include molecular aspects of leukaemogenesis, elucidation of incidence and clinical relevance of molecular biomarkers in chronic and acute leukemias and molecular monitoring of treatment response. Gueorgui Balatzenko has authored and co-authored many articles in peer-reviewed journals and book chapters. He is a member of medical societies such as the Bulgarian Society of Hematology, the Bulgarian Society of Genetics, the European Hematology Association, a life member of UICC, and a referee for international journals.

Contents

Preface

Leukemias are still a significant challenge in science and medicine. In recent years, thanks to the introduction of new technologies, we have witnessed significant progress in the understanding of predisposing factors, main pathogenetic mechanisms and the different biological abnormalities, which result in the development of leukemia. This progress is important both for fundamental studies and for routine diagnosis, prognostic stratification of patients, selection of optimal treatment and assessment of the therapeutic response, as well as for the development of new therapeutic approaches and agents.

As a result of this knowledge, new classification approaches are being developed, based on the newly recognized biomarkers, the prognostic stratification of patients has gradually moved from a single factor-based approach to a multifactorial scoring systems, which allows for the better personalization of the therapeutic strategy according to the needs of the individual patient and its optimization during the course of the disease, depending on the therapeutic response achieved.

Therefore, professionals are faced with the need to continuously update their knowledge in a wide range of areas, ranging from fundamental molecular processes and mechanisms to a thorough understanding of clinical features and modern approaches to achieve maximum therapeutic results. This book does not have the ambition to present comprehensively the problems of leukemias, but it is an attempt to acquaint the audience of medical specialists and researchers in the field of hematology with some modern advances and concepts in the three main directions: biology, clinical presentation and treatment.

The biological aspects of the origin, development and therapy resistance of leukemias are within the scope of the first section. An increasing body of evidence indicates that the initiation of malignancies is a complex process involving genetic predisposition as well as diverse environmental factors. In the first chapter, Entesar Tebein and Abozer Y. Elderdery present an interesting intersection within this concept by a comprehensive review of current knowledge on the role of polymorphisms in xenobiotic-metabolizing enzymes (XMEs), which physiologically are one of the first lines of protection against environmental toxins. The majority of genes encoding XMEs are polymorphic, and some of these polymorphic variants result into an altered enzyme activity, thus contributing to the susceptibility to the development of different hematological diseases, including chronic and acute leukemias. In response to different extra-cellular signals and activation of the respective signaling pathways specific transcriptional factors determine the pattern of the gene expression profile of hematopoietic cells. Epigenetic dysregulation or gene mutations may cause aberrant gene expression with a role in further leukemia development and progression. Recent gene expression studies have led to the discovery of a promising group of different leukemic biomarkers that have the potential to influence the leukemia clinical course and to predict the disease severity. An example is the BAALC (brain and acute

leukemia and cytoplasmic) gene overexpression, which is extensively explored in the second chapter by Emil Alexov et al. on the basis of a comprehensive summary of data about the gene functions and interactions, frequency of overexpression and relevance as a predictive marker in myeloid neoplasms. Knowledge and understanding of the molecular basis of primary or acquired leukemic cell resistance to treatment is the other critical issue leading to therapeutic failure, inferior prognosis, and reduced survival. In the third chapter Yanitza Davidkova et al. review important recent studies on various aspects of drug resistance in acute myeloid leukemia, including the role of aberrant expression of cell transporter proteins, abnormalities in drug-metabolizing or other regulating enzymes, structural and/or functional changes in genes and epigenetic modifiers, dysregulation of intracellular signaling pathways, tumor microenvironment, and others.

Specific aspects of clinical presentation are within the scope of the second section. Biswajit Bhuyan et al. demonstrate in the first chapter of this section the successful translation of accumulating knowledge on the underlying mechanisms and clinical features of a disease, namely chronic myeloid leukemia (CML), in refining the diagnosis and achieving one of the most significant successes ever achieved in the treatment of malignant disease. But even with a disease as well-known as CML, as well as with other leukemias, the clinical presentation can include rare, unexpected, not well-known or underestimated complications that have a profound effect on the patient's health and life. In this regard, the second chapter in this section authored by Vivian W.K. Hui and Simon K.H. Szeto is of particular interest as it addresses a real interdisciplinary problem and summarizes the pathogenesis, clinical presentation, specific ophthalmological and imaging findings, as well as the management of leukemic retinopathy. Ocular manifestations, affecting up to 50% of leukemia patients, correlate with unfavorable clinical outcomes and require the competent intervention of both hematologists and ophthalmologists.

Recent advances and current standards in the therapy of acute leukemias are within the scope of the third section. The complex landscape of genetic and molecular abnormalities makes the treatment of acute myeloid leukemias (AML) a difficult prospect. In addition, the frailties and comorbidities frequently present in elderly patients make their management a particularly difficult therapeutic challenge. In the first chapter of this section Gupta Gopila et al. review in an orderly manner how to assess patients with elderly AML and the treatment options available for them in order to overcome the devastating impact of the disease on survival in this age group. Acute lymphoblastic leukemia (ALL) in children is at the opposite pole as modern chemotherapy protocols provide a cure for up to 90% of pediatric patients. However, a portion of children harbor high-risk features, do not respond to upfront therapy, or suffer relapse needing more intensive therapy, which may include hematopoietic stem cell transplantation (HSCT). But given both acute and long-term complications as well as emerging alternatives of novel immunotherapies and better treatment response assessment methods, the transplantation landscape has significantly changed. In the second chapter of this section, Natalia Subbotina et al. provide a comprehensive critical review of the problem as well as their own experience, concluding for careful selection of patients and appropriate regimens. Reasonable hopes for further improvement of therapeutic results are also placed on new sophisticated approaches such as the discovery of new possible targets, new CAR-T cell products, correction of mutations in genes encoding transcription factors, etc. The last chapter

of the book takes us in this direction. Edgardo Becerra et al. present the potential role of novel genome editing technology such as CRISPR-Cas9 to offer better therapeutic results beyond the reach of previous therapies in ALL.

Each chapter of this book is a separate publication that reflects each author's views and concepts. However, in total the book presents an update and introduces novel insights into our current understanding of the biology, clinical presentation, and therapeutic strategies in patients with leukemias.

Margarita Guenova, MD, Ph.D. and Gueorgui Balatzenko, MD, Ph.D.
Professor,
National Specialised Hospital for Active Treatment of Haematological Diseases,
Sofia, Bulgaria

Section 1

Biology

Chapter 1

Genetic Polymorphisms of Xenobiotics-Metabolizing Enzymes Contributing to Leukemia

Entesar Tebein and Abozer Y. Elderdery

Abstract

Polymorphisms in xenobiotic-metabolizing enzymes have been linked to an increased risk of developing leukemia (XMEs). XMEs are found in all higher organisms and are one of the first lines of defense against environmental chemicals. Toxins, including therapeutic agents, are completely metabolized and eliminated from the body by an enzyme system that is encoded by specific genes. The majority of these genes are polymorphic, and some of the polymorphic forms have altered enzyme activity. Phase I XMEs, such as cytochrome P450s (CYPs), and phase II biotransformation enzymes, such as glutathione S-transferases (GST), UDP-glucuronosyltransferases (UGT), and N-acetyltransferases (NAT), are the most important. The majority of genetic variation discovered during clinical testing is due to single-nucleotide polymorphisms (SNPs). The purpose of this chapter is to highlight information about of some genetic polymorphisms of XMEs, contributing to AML, ALL, CML, and ALL. Several keywords were used to search the databases PubMed, Google Scholar, and Web of Science. Currently, numerous manuscripts suggested that genetic polymorphisms of XMEs were associated with ALL, CLL AML, and CML susceptibility.

Keywords: polymorphism, xenobiotic, SNPs, ROS

1. Introduction

Leukemia is a blood cancer that affects the hematopoietic system. Due to a variety of complicated features [1], it has a depraved prognosis. Leukemias are classified primarily by the type of white blood cells affected as myeloid or lymphoid, and as acute or chronic, based on the percentage of blasts or leukemia cells in bone marrow or blood [2]. There are four main types: leukemia, namely acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML) [3]. Despite the fact that leukemogenesis research has been going on for a long time, the mechanisms underlying the development of this hematologic malignancy are still unknown [1]. Leukemia is known to be caused by a number of risk factors, including genetic variables such as constitutional genetic variation in components of DNA damage response pathways, which have become the focus of research [4, 5].

Variants that are more common than mutations in the genome, such as short tandem repeats (STR), variable number tandem repeats (VNTR), and (SNPs), began to be identified more rapidly with the completion of the human genome project and the development of new technologies [6]. This strongly shows that SNPs could be utilized as an useful biomarker for assessing an individual's genetic background for cancer prognosis, and it points to an exciting new area of cancer research [7].

The interaction between environmental contact and genetic factors has been hypothesized as expected cause of various types of cancers [8]. Functional polymorphisms in the genes encoding detoxification enzymes cause inter-individual differences, which contribute to leukemia predisposition [9].

XMEs are found in all eukaryotes, which act as first line against environmental chemicals, toxic materials, and biotransformation of drugs present in the cells. The most important XMEs are classified into phase I such as cytochrome P450s (CYPs), and phase II biotransformation enzymes, glutathione S-transferases (GST), UDP-glucuronosyltransferases (UGT), and *N*-acetyltransferases (NAT) [10].

In recent years, an increasing number of studies have indicated that cancer beginning is a complex process involving genetic predisposition as well as diverse environmental variables [11–13]. As a result, identifying genetic risk factors that contribute to the large burden of disease in the general population is necessary for the development of wide cancer preventive therapy techniques [7]. Therefore, variations in GSTM1, GSTT1, methylenetetrahydrofolate reductase (MTHFR) C677T, and XRCC1 Arg399Gln have been linked to an increased risk of leukemia [14]. The aim of this chapter is to highlight on information of some genetic polymorphisms of XMEs contributing to AML, ALL, CML, and ALL.

2. Xenobiotic

The word xenobiotic is derived from Greek word Xeno meaning strange and biotic meaning life; thus, xenobiotic defined as foreign compound initiating from medicine, food, pesticides, or environment is actively transported and metabolized by the renal proximal tubular cells [15]. *Toxicity in biological systems caused by xenobiotic is variable*, so that the xenobiotics that arrive in living cells are eliminated through the XMEs [16, 17]. Xenobiotic metabolism and biotransformation occur in phases, namely phase 1, 11, and 111 reactions [18]. Hydrophobic molecules are converted into more polar, hydrophilic metabolites during phase I reactions (cytochrome P450), which are more easily excreted by the excretory system via oxidation, reduction, or hydrolysis. Various transferases are involved in phase II biotransformation reactions (also known as the conjugation phase); the most common form of exogenous compound conjugation is the reaction of glucuronide formation catalyzed by UDP-glucuronyl transferases. Conjugates are also more polar and soluble in water than parent substances, so they are excreted from the body more easily. In phase III, these transformations allow for the transport and excretion of xenobiotic metabolites. It is divided into superfamilies: SLC transporters and ATP-binding cassette (ABC) transporters (**Figure 1**) [17, 19, 20].

2.1 Metabolism of xenobiotic

The body eliminates xenobiotics through xenobiotic metabolism. This consists of xenobiotic deactivation and excretion, which occur primarily in the liver. Urine, feces, breath, and sweat are the four excretory routes. Hepatic enzymes are responsible for

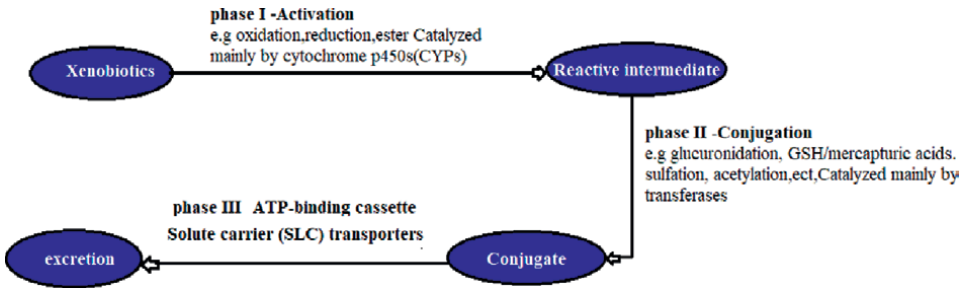

Figure 1.
A schematic representation of the pathways of biotransformation enzymes (phases 1, 11, and 111).

xenobiotic metabolism, which involves first activating, then oxidizing (via cytochrome P450 monoxygenases, flavin-containing monoxygenases, or alcohol/aldehyde dehydrogenases), reduction (*via* cytochrome P450 reductases), hydrolysis and/or hydration (*via* esterases and epoxide hydrolases), and finally conjugating the active secondary metabolite with glucuronic acid, sulfuric acid, or glutathione, and finally by excretion in bile or urine [21].

Biotransformation comes with the generation of reactive oxygen species (ROS) through a multitude of reactions both directly by releasing ROS as part of the respective reaction and indirectly as a consequence of the products generated by transformation of the xenobiotic [17].

The transition from the biotransformation of drugs, xenobiotics, and environmental pollutants causes an increase in free radical production in the body, resulting in lipid peroxidation, oxidative stress, and oxidative damage. Free radicals interact with various macromolecules such as nucleic acids, proteins, and lipids, altering critical intracellular signaling pathways that are responsible for cellular homeostasis maintenance [22].

Free radicals disrupt cellular equilibrium and cause mitogenesis, mutagenesis, genotoxicity, and cytotoxicity, either directly or indirectly through the mediation of oxidative and inflammatory signals. The role of free-radical-mediated damage in the pathophysiology of various diseases such as diabetes, hypertension, atherosclerosis, infertility, and cancer [23].

2.2 Genetic variation in xenobiotic metabolism and leukemia risk

Toxins, including therapeutic agents, are completely metabolized and eliminated from the body by a system of enzymes encoded by specific genes. The majority of these genes are polymorphic, with some polymorphic forms exhibiting altered enzyme activity. Enzymes involved in phase I (bio-activation) and phase II (detoxification) metabolism maintain a critical balance of activation and inactivation of a wide range of chemical exposures that cause ALL, AML, CML, and CLL [12, 24–29].

3. Phase 1 genetic polymorphisms most frequently associated with AML, ALL, CLL, and CML

Cytochrome P450 (CYP450), a phase I biotransformation enzyme, is responsible for the metabolism of both endogenous and exogenous compounds and has the

ability to convert xenobiotics or procarcinogens into DNA reactive metabolites [30]. The human genome contains 57 putatively functional, protein-coding cytochrome P450 (CYP) genes, which have been classified into 18 families and 44 subfamilies based on sequence homology. Over a dozen CYP isozymes from families 1, 2, 3, and 4 are primarily responsible for drug and xenobiotic metabolism [31]. The majority of these polymorphisms, such as T6235C of CYP1A1 (CYP1A1*2A), C-1019T of CYP2E1 (CYP2E1*5B), and A290G of CYP3A4 (CYP3A4*1B), are thought to increase enzymatic activity. They have been linked to the bioactivation of several chemical carcinogens as well as the conversion of polyaromatic hydrocarbons in tobacco smoke into intermediate reactive metabolites, some of which can cause DNA damage [32]. The CYP1 family's expression increases in lymphoblastic and myeloblastic cell lines and plays a role in environmental factor detoxification. As a result, CYP1A1 enzymes may be involved in the development of cancer in hematopoietic cells. Indeed, there is evidence that an increased frequency of the CYP1A1 Val/Val genotype in ALL patients is a risk factor for developing ALL [33].

AML is a group of diseases characterized by clonal expansion of immature myeloid blasts, resulting in hematopoietic failure [34]. Through the inactivation of enzymatic activity, certain SNPs at the CYP genetic loci (CYP2D6, CYP1A1, CYP3A5, and CYP2E1) may be considered risk factors for many types of cancer and hematological malignancies, such as AML, ALL, and myelodysplastic syndromes (MDS) [35].

A number of studies have been undertaken to determine the role of CYP450 polymorphisms in the development of AML leukemia; however, these yielded conflicting findings. Increased susceptibility to AML has been described by Botros *et al*., who state that A CYP2B6 gene mutation increases the risk of developing AML by threefold (odds ratio [OR], 3.0; 95% confidence interval [CI], 1.3–6.9), whereas a CYP3A4 gene mutation increases the risk by approximately fourfold (OR, 3.8; 95% CI, 1.4–10.1) [35]. According to the dominant model, the T3801C polymorphism in CYP1A1 is associated with an increased risk of AML in Asians [14, 36]. According to Gassoum et al., genetic polymorphisms in CYP1A1 heterozygous AG show no significant association with AML, whereas homozygous GG shows a protective effect. CYP2D6 shows no association with AML risk in both heterozygous intermediate metabolizer (IM) and mutant homozygous poor metabolizer (PM) [37].

ALL accounts for about 10% of leukemias in the United States. About 6000 new cases of ALL are diagnosed each year, and ALL accounts for approximately 74% of the leukemia cases among children [38]. Polymorphisms in xenobiotics are thought to influence susceptibility to childhood ALL [39].

Swinney et al. investigated the link between CYP1A1 and ALL susceptibility in three ethnic groups, namely Caucasian, Hispanic, and African-American children. Overall, CYP1A1*2C and *2B homozygous variant alleles have been linked to an increased risk of ALL [40]. Vijayakrishna and Houlston conclude in a meta-analysis that there is a significant association between CYP1A1*2A and childhood ALL [41]. Pakakasma et al., on the other hand, found no significant difference in the distribution of CYP3A4(A290G) polymorphism between case and control [42]. This disparity may be explained by differences in acute leukemia risk factors between children and adults [35].

CML is one of several diseases known collectively as a myeloproliferative disorder of pluripotent hemopoietic stem cells (HSCs). In this case, chromosomal translocation results in an oncogenic BCR-ABL gene fusion that increases tyrosine kinase (TK) activity. Kinases that are abnormally activated disrupt downstream signaling pathways, resulting in abnormal cell proliferation, differentiation, and resistance to cell

death [43]. Joshi *et al*. reported the CYP2C19 gene polymorphisms in susceptibility to CML. Taspiner *et al*. suggested that polymorphic CYP1A1 and GSTT1 genes appear to affect susceptibility to CML [33].

One of the most common types of leukemia is CLL. It most commonly affects elderly patients and has a highly variable clinical course. Specific genomic changes that disrupt the regulation of proliferation and apoptosis in clonal B-cells initiate leukemic transformation [44]. Only one study found that Cytochrome P450 Allele CYP3A7*1C rs45446698 was associated with CLL mortality [45].

4. Phase 11 genetic polymorphisms most frequently associated with AML, ALL, CLL, and CML

Among the XMEs, the NAT and GST enzymes, both of which are phase II enzymes, are of particular interest to hematologists because they metabolize a variety of products, including chemotherapeutic and carcinogenic agents, and they serve as targets for antitumor drug therapies [46].

Phase II enzymes catalyze the conjugation of glutathione or glucuronide with reactive electrophiles and thus detoxify procarcinogens and carcinogens. GST group is widely expressed in mammalian tissues and has broad substrate specificity. GSTs are polymorphic genes and involved in the metabolism of a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and reactive species. The frequencies of GSTs polymorphic alleles especially GSTT1 and GSTM1 have been reported in various cancers [33]. Previous study by Mortazavi *et al.* identified that GSTT1 null genotype can increase the risk of AML, particularly when combined with CYP1A1*2A allele. GSTM1 null genotype can also play a protective role and reduce the risk of AML [25]. A study conducted by Lemas et al. stated that the *GSTM1* analysis failed to reveal any association with the CLL [47].

Regarding the theoretical correlation between NAT2 SNPs and cancer, a variety of reports have been published to date [12, 48–50]. Among these, the data described by Zou *et al*. reported that *NAT2* gene polymorphism rs1799931 was associated with decreased risk of AML and was likely to be a protective factor against AML development [12]. Contradictory to this findings, a study by Abdel Ghafar *et al*. showed that NAT2 gene rs1799931 (G857A) is associated with increased susceptibility to AML in the Egyptian population [51]. A meta-analysis by Jiang *et al*. investigated the relationship between NAT2 polymorphisms and onset risk of acute leukemia and reported there is no significant difference found between the fast-acetylator incidence of NAT2 haplotype and the onset risk of acute lymphoblastic leukemia (ALL, OR = 0.70, 95% CI = 0.45–1.08) or AML, OR = 0.79, 95% CI = 0.46–1.47) [52]. No significant difference was found in NAT2 fast and slow acetylator frequencies between CML patients and controls [47], while Tebien et al. revealed the protective effect of NAT2A 803G and G857A against CML [43].

5. Detection of genetic polymorphisms in leukemia

Clinical genetic testing is being used more frequently in the treatment of cancer patients. By providing information on future illness risk, diagnosis confirmation, and more recently, therapy choice, and prognosis, these tests aid in a range of clinical decisions. SNPs account for the majority of genetic variation found during clinical

testing [53]. DNA extraction is the first step in any SNP detection test [54]. Methods of genotyping commonly used were gel-electrophoresis-based genotyping methods include polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism analysis [55], multiplex PCR [56], as well as allele-specific amplification fluorescent dye-based high-throughput genotyping procedures such as oligonucleotide ligation assay [57], next-generation sequencing (NGS) [58], and TaqMan allelic discrimination [59].

6. Conclusion

XMEs are found in all eukaryotes and serve as a first line of defense against environmental chemicals, toxic materials, and drug biotransformation in cells. The most important XMEs are classified as phase1, phase11, and phase111 biotransformation enzymes. The interaction of environmental and genetic factors has been proposed as a possible cause of various types of cancer. Functional polymorphisms in genes encoding detoxification enzymes cause inter-individual differences that contribute to leukemia susceptibility. Enzymes involved in phase I (CYP enzymes) and phase II (N-acetyltransferases (NAT) and glutathione S-transferases) metabolism regulate the activation and inactivation of a wide range of chemical exposures that cause ALL, AML, CML, and CLL. Several studies have been conducted to determine the role of xenobiotics genetic polymorphisms in the development of AML, ALL, CLL, and CML, with the majority of them yielding significant results.

Author details

Entesar Tebein[1] and Abozer Y. Elderdery[2]*

1 College of Applied Medical Sciences, Shagra University, Saudi Arabia

2 Faculty of Applied Medical Sciences, Clinical Laboratory Sciences, Jouf University, Saudi Arabia

*Address all correspondence to: abozer904@hotmail.com

IntechOpen

References

[1] Tian X, Dai S, Sun J, Jiang S, Jiang Y. Association between TP53 Arg72Pro polymorphism and leukemia risk: A meta-analysis of 14 case-control studies. Scientific Reports. 2016;**6**(1):1-6

[2] Swerdlow SH, Harris NL, Jaffe ES, Pileri SA. WHO Classification of Haematopoietic and Lymphoid Tissues. Lyon: WHO; 2017

[3] Utuama O, Mukhtar F, Pham YT-H, Dabo B, Manani P, Moser J, et al. Racial/ethnic, age and sex disparities in leukemia survival among adults in the United States during 1973-2014 period. PLoS One. 2019;**14**(8):e0220864

[4] Vodicka P, Musak L, Vodickova L, Vodenkova S, Catalano C, Kroupa M, et al. Genetic variation of acquired structural chromosomal aberrations. Mutation Research, Genetic Toxicology and Environmental Mutagenesis. 2018;**836**:13-21

[5] Dokal I, Tummala H, Vulliamy TJ. Inherited bone marrow failure in the pediatric patient. Blood. 2022;**140**(6):556-570

[6] Öztürk R. COVID-19: Pathogenesis, genetic polymorphism, clinical features and laboratory findings. Turkish Journal of Medical Sciences. 2020;**50**(9):638-657

[7] Mandal RK, Dar SA, Jawed A, Wahid M, Lohani M, Panda AK, et al. Impact of LMP7 (rs2071543) gene polymorphism in increasing cancer risk: Evidence from a meta-analysis and trial sequential analysis. Oncotarget. 2017;**9**(5):6572-6585

[8] Minatel BC, Sage AP, Anderson C, Hubaux R, Marshall EA, Lam WL, et al. Environmental arsenic exposure: From genetic susceptibility to pathogenesis. Environment International. 2018;**112**:183-197

[9] Bhatia S. Genetic variation as a modifier of association between therapeutic exposure and subsequent malignant neoplasms in cancer survivors. Cancer. 2015;**121**(5):648

[10] Crocco P, Montesanto A, Dato S, Geracitano S, Iannone F, Passarino G, et al. Inter-individual variability in xenobiotic-metabolizing enzymes: Implications for human aging and longevity. Genes. 2019;**10**(5):403

[11] Pérez-Losada J, Castellanos-Martín A, Mao J-H. Cancer evolution and individual susceptibility. Integrated Biology (Camb). 2011;**3**(4):316-328

[12] Zou Y, Dong S, Xu S, Gong Q, Chen J. Genetic polymorphisms of NAT2 and risk of acute myeloid leukemia: A case-control study. Medicine. 2017;**96**(42):e7499

[13] Zhu K-W, Chen P, Zhang D-Y, Yan H, Liu H, Cen L-N, et al. Association of genetic polymorphisms in genes involved in Ara-C and dNTP metabolism pathway with chemosensitivity and prognosis of adult acute myeloid leukemia (AML). Journal of Translational Medicine. 2018;**16**(1):90

[14] Zhuo W, Zhang L, Wang Y, Zhu B, Chen Z. CYP1A1 MspI polymorphism and acute myeloid leukemia risk: Meta-analyses based on 5018 subjects. Journal of Experimental & Clinical Cancer Research. 2012;**31**(1):1-10

[15] Su R, Xiong S, Zink D, Loo L-H. High-throughput imaging-based nephrotoxicity prediction for

xenobiotics with diverse chemical structures. Archives of Toxicology. 2016;**90**(11):2793-2808

[16] Park Y-C, Lee S, Cho M-H. The simplest flowchart stating the mechanisms for organic xenobiotics-induced toxicity: Can it possibly be accepted as a "Central Dogma" for toxic mechanisms? Toxicology Research. 2014;**30**(3):179-184

[17] Klotz L-O, Steinbrenner H. Cellular adaptation to xenobiotics: Interplay between xenosensors, reactive oxygen species and FOXO transcription factors. Redox Biology. 2017;**13**:646-654

[18] Hoffmann MF, Preissner SC, Nickel J, Dunkel M, Preissner R, Preissner S. The Transformer database: Biotransformation of xenobiotics. Nucleic Acids Research. 2014;**42**(Database issue):D1113-D1117

[19] Rudik AV, Dmitriev AV, Lagunin AA, Ivanov SM, Filimonov DA, Poroikov VV. Computer-aided xenobiotic toxicity prediction taking into account their metabolism in the human body. Biochemistry (Moscow), Supplement Series B: Biomedical Chemistry. 2019;**13**(3):228-236

[20] Phang-Lyn S, Llerena VA. Biochemistry, Biotransformation. StatPearls: StatPearls Publishing; 2021

[21] Das A, Srinivasan M, Ghosh TS, Mande SS. Xenobiotic metabolism and gut microbiomes. PloS One. 2016;**11**(10):e0163099

[22] Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: Harms and benefits for human health. Oxidative Medicine and Cellular Longevity. 2017;**2017**:8416763

[23] Abdel-Daim MM, Moustafa YM, Umezawa M, Ramana KV, Azzini E. Applications of antioxidants in ameliorating drugs and xenobiotics toxicity: Mechanistic approach. Oxidative Medicine and Cellular Longevity. 2017;**2017**

[24] Chokkalingam AP, Metayer C, Scelo GA, Chang JS, Urayama KY, Aldrich MC, et al. Variation in xenobiotic transport and metabolism genes, household chemical exposures, and risk of childhood acute lymphoblastic leukemia. Cancer Causes & Control. 2012;**23**(8):1367-1375

[25] Mortazavi Y, Rahimi R, Azimi F, Rostami S, Moghimi M, Faghihzadeh S, et al. Role of Glutathione S-transferase (GSTM1, GSTT1) and CYP1A1 (cytochrome p450) Gene polymorphisms in susceptibility to acute myeloid leukemia. Middle East Journal of Cancer. 2020;**11**(1):12-20

[26] Kim HS. Association between the Polymorphism of Glutathione S-transferase Genes and Chronic Myeloid Leukemia in Asian Population: A meta-analysis. The Journal of the Korea Contents Association. 2017;**17**(10):289-299

[27] Umay A, Bilgin R, Akgöllü E, Gürkan E, Kis C. Relationship between MTHFR gene polymorphisms (C677T and A1298C) and chronic lymphocytic leukemia in the Turkish population. Meta Gene. 2018;**17**:232-236

[28] Sak K, Kasemaa K, Everaus H. Polymorphisms in COMT and SULT1A1 Genes and Chronic Lymphocytic Leukemia Risk in Estonia. 2019;**3**(4):281-288

[29] Nida S, Javid B, Akbar M, Idrees S, Adil W, Ahmad GB. Gene variants of CYP1A1 and CYP2D6 and the risk of childhood acute lymphoblastic leukaemia; outcome of a case control study from Kashmir, India. Molecular

Biology Research Communications. 2017;**6**(2):77

[30] Chen H-c, Hu W-x, Liu Q-x, Li W-k, Chen F-z, Rao Z-z, et al. Genetic polymorphisms of metabolic enzymes CYP1A1, CYP2D6, GSTM1 and GSTT1 and leukemia susceptibility. European Journal of Cancer Prevention. 2008;**17**(3):251-258

[31] Nebert DW, Wikvall K, Miller WL. Human cytochromes P450 in health and disease. Philosophical Transaction of Royal Society of London. 2013;**368**(1612):431

[32] Bolufer P, Collado M, Barragán E, Cervera J, Calasanz M-J. The potential effect of gender in combination with common genetic polymorphisms of drug-metabolizing enzymes on the risk of developing acute leukemia. Haematologica. 2007;**92**(3):308-314

[33] Taspinar M, Aydos SE, Comez O, Elhan AH, Karabulut HG, Sunguroglu A. CYP1A1, GST gene polymorphisms and risk of chronic myeloid leukaemia. Swiss Medical Weekly. 2008;**138**(1-2):12-17

[34] Shi X, Kitano A, Jiang Y, Luu V, Hoegenauer KA, Nakada D. Clonal expansion and myeloid leukemia progression modeled by multiplex gene editing of murine hematopoietic progenitor cells. Experimental Hematology. 2018;**64**:33-44

[35] Botros SKA, El Gharbawi N, Shahin G, Al Lithy H, El Sherbiny M. Impact of Cytochromes P450 3A4 and 2B6 gene polymorphisms on predisposition and prognosis of acute myeloid leukemia: An Egyptian case-control study. Egyptian Journal of Medical Human Genetics. 2021;**22**(1):1-10

[36] Lu J, Zhao Q, Zhai Y-J, He H-R, Yang L-H, Gao F, et al. Genetic polymorphisms of CYP1A1 and risk of leukemia: A meta-analysis. Oncotargets and Therapy. 2015;**8**:2883-2902

[37] Gassoum SAA-D, Deyab AA, Tebien EM, Hadeil ME. The impact of genetic polymorphisms of cytochrome P450 (CYP1A1&2D6) gene in the incidence of acute myeloid leukaemia. P J M H S. 2020;**14**

[38] Sasaki K, Jabbour E, Short NJ, Jain N, Ravandi F, Pui CH, et al. Acute lymphoblastic leukemia: A population-based study of outcome in the U nited S tates based on the surveillance, epidemiology, and end results (SEER) database, 1980-2017. American Journal of Hematology. 2021;**96**(6):650-658

[39] Bag A, Jyala N, Bag N. Cytochrome P450 1A1 genetic polymorphisms as cancer biomarkers. Indian Journal of Cancer. 2015;**52**(4):479

[40] Swinney RM, Beuten J, Collier AB 3rd, Chen TT, Winick NJ, Pollock BH, et al. Polymorphisms in CYP1A1 and ethnic-specific susceptibility to acute lymphoblastic leukemia in children. Cancer Epidemiology, Biomarkers & Prevention. 2011;**20**(7):1537-1542

[41] Vijayakrishnan J, Houlston RS. Candidate gene association studies and risk of childhood acute lymphoblastic leukemia: A systematic review and meta-analysis. Haematologica. 2010;**95**(8):1405-1414

[42] Pakakasama S, Mukda E, Sasanakul W, Kadegasem P, Udomsubpayakul U, Thithapandha A, et al. Polymorphisms of drug-metabolizing enzymes and risk of childhood acute lymphoblastic leukemia. American Journal of Hematology. 2005;**79**(3):202-205

[43] Tebien EM, Khalil HB, Mills J, Elderdery AY. Evaluation of Genetic

Polymorphisms of N-acetyltransferase 2 and Relation with Chronic Myeloid Leukemia. Asian Pacific Journal of Cancer Prevention. 2020;**21**(12):3711-3717

[44] Hallek M, Al-Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. American Journal of Hematology. 2021;**96**(12):1679-1705

[45] Johnson N, De Ieso P, Migliorini G, Orr N, Broderick P, Catovsky D, et al. Cytochrome P450 allele CYP3A7* 1C associates with adverse outcomes in chronic lymphocytic leukemia, breast, and lung cancer. Cancer Research. 2016;**76**(6):1485-1493

[46] Ouerhani S, Nefzi MA, Menif S, Safra I, Douzi K, Fouzai C, et al. Influence of genetic polymorphisms of xenobiotic metabolizing enzymes on the risk of developing leukemia in a Tunisian population. Bulletin du Cancer. 2011;**98**(12):E95-E106

[47] Lemos M, Cabrita F, Silva H, Vivan M, Placido F, Regateiro F. Genetic polymorphism of CYP2D6, GSTM1 and NAT2 and susceptibility to haematological neoplasias. Carcinogenesis. 1999;**20**(7):1225-1229

[48] Hasanzad M, Ziaei SAM, Montazeri V, Afshari M, Jamaldini SH, Imani M, et al. Association between NAT2 polymorphisms and prostate cancer. Iranian Journal of Cancer Prevention. 2017;**10**(2):1-7

[49] Kabir S, Rehman A. Carcinogenic potential of arylamine N-acetyltransferase in Asian populations. Journal of Cancer Research and Practice. 2018;**5**(4):131-135

[50] Hernández-González O, Ortiz-Zamudio JJ, Rodríguez-Pinal CJ, Alvarado-Morales I, et al. Genetic polymorphisms of arylamine N-acetyltransferases 1 and 2 and the likelihood of developing pediatric acute lymphoblastic leukemia. Leukemia & Lymphoma. 2018;**59**(8):1968-1975

[51] AbdelGhafar MT, Allam AA, Darwish S, Al-Ashmawy GM, Eshra KA, Ibrahim RR. Study of N-acetyl transferase 2 single-gene polymorphism (rs1799931) in patients with acute myeloid leukemia. Egyptian Journal of Haematology. 2019;**44**(3):157-162

[52] Jiang WH, Wang XT, Zheng LD, Yan QQ, Chen LL. Relationship between NAT2 polymorphisms and onset risk of acute leukemia: A systematic review and meta-analysis. European Review for Medical and Pharmacological Sciences. 2019;**23**(21):9259-9266

[53] Judkins T, Hendrickson BC, Deffenbaugh AM, Scholl T. Single nucleotide polymorphisms in clinical genetic testing: The characterization of the clinical significance of genetic variants and their application in clinical research for BRCA1. Mutation Research. 2005;**573**(1-2):168-179

[54] Nayyar A, Ahmed S, Nayyar S. Single nucleotide polymorphism; A molecular marker for genetic variations. International Journal of Pathology. 2021;**2021**:10-13

[55] Saleh R, Musa HH, Hamid GA, Hamid MMA. Genetic polymorphisms of GSTM1 and GSTT1 genes and susceptibility to acute lymphoblastic leukemia in the Yemeni population. Asian Hematology Research. 2022;**6**:1-9

[56] Abdalhabib EK, Jackson DE, Alzahrani B, Elfaki EM, Hamza A, Alanazi F, et al. Combined GSTT1 null, GSTM1 null and XPD Lys/Lys genetic polymorphisms and their association with increased risk of chronic myeloid

leukemia. Pharmacogenomics and Personalized Medicine. 2021;**14**:1661

[57] Halabian R, Arshad V, Ahmadi A, Saeedi P, Azimzadeh Jamalkandi S, Alivand MR. Laboratory methods to decipher epigenetic signatures: A comparative review. Cellular & Molecular Biology Letters. 2021;**26**(1):1-30

[58] Lauschke VM, Ingelman-Sundberg M. Prediction of drug response and adverse drug reactions: From twin studies to next generation sequencing. European Journal of Pharmaceutical Sciences. 2019;**130**:65-77

[59] Chang J-H, Lai T-C, Yang P-J, Shih P-C, Yang Y-C, Lee K-L, et al. Associations of TIMP-3 genetic polymorphisms with EGFR statuses and cancer clinicopathologic development in lung adenocarcinoma patients. International Journal of Molecular Sciences. 2020;**21**(21):8023

Chapter 2

The Role of *BAALC* Gene in the Transformation of Myeloid Progenitor Cells to Acute Myeloid Leukemia

Emil Aleksov, Branimir Spassov, Margarita Guenova and Gueorgui Balatzenko

Abstract

One of the unanswered questions in hematology is the question concerning disorders in the regulation of gene expression in different subtypes of acute myeloid leukemia (AML), leading to changes in the functional activity of certain genes and acting as a component of a series of events in the leukemogenesis. One example of such a gene is *BAALC* gene (brain and acute leukemia and cytoplasmic), localized in chromosome 8, which plays a role in the regulation of myeloid progenitors' differentiation. This role is associated with several other oncogenes, such as *HoxA9*, *ERK*, *and RUNX1*. Gene interactions determine normal proliferation and differentiation of cells, and any disturbances could lead to leukemic development. What is the role of *BAALC* in normal/impaired balance? What are the connections of *BAALC* with the mutations established in AML: *FLT3*, *NPM1*, etc.? What are the correlations of its overexpression with clinical and laboratory findings in AML patients? What are the changes in the expression of *BAALC*, after successful therapy of AML and after therapy failure? Can we use it as a predictive marker in AML patients? This chapter summarizes available data about functions of *BAALC* gene, the frequency of overexpression, and its importance as a predictive marker in the development of AML.

Keywords: *BAALC*, AML, gene overexpression, oncogenes, myeloid proliferation, AML prognosis, drug resistance

1. Introduction

Detailed sequencing of the genome among 200 AML patients, done in the program "the cancer genome atlas (TCGA)," revealed AML as a heterogenic disease with a total of 2585 gene mutations. The authors report 2315 somatic single-nucleotide variants and 270 small insertions and deletions in the genome of AML patients, with an average of 13 mutations per sample [1]. TCGA identifies "at least one potential driver mutation per sample" and confirms the complexity of gene interactions in AML pathogenesis. Some of these mutations could be observed also in myelodysplastic syndromes (MDS)

or in myeloproliferative disorders (MPN), even in healthy individuals, with age-dependent clonal hematopoiesis, increasing the risk for the development of AML [2]. This heterogeneity shows why AML remains one of the serious problems in front of hematology in the twenty-first century, despite the huge advances in the last years. The unclear etiopathogenesis, and the blurred boundaries between the different groups of myeloid diseases, connected with AML, still keep open many questions that continue to challenge researchers. Against the background of large-scale studies on a variety of gene mutations in recent years, some issues are still less clear, such as the abnormalities in the regulation of gene expression in various subtypes of AML, leading to functional impairment of some genes and respectively overexpression or lack of expression, resulting in overproduction/absence of normal proteins of key importance for cell biology. One of these questions was raised with the discovery of *BAALC* (brain and acute leukemia, cytoplasmic) gene in 2001 by Prof. S. Tanner's team at Ohio State University. Increased *BAALC* expression was initially identified in a study of patients with AML and trisomy 8 and was later found in other types of AML as well as in acute lymphoblastic leukemia (ALL) [3, 4].

2. Genetics

BAALC gene is located on the long arm of chromosome 8 at position 22.3 (8q22.3) [5]. It contains 8 exons and extends to 89,613 bases of genomic DNA. The *BAALC* gene has eight different transcriptional forms, resulting in six different protein isoforms [6, 7]. The transcriptional proteins encoded by the corresponding exons consist of 54 to 180 amino acids [5, 8]. The *BAALC* gene is expressed in mammals but is not found in lower-level organisms, such as insects and fungi [2]. The high levels of expression in mammals, as well as the lack of an orthologous gene in lower vertebrates, suggest a specific role of the *BAALC* gene in the mammalian CNS [2]. The *BAALC* gene and the protein encoded by it have high expression in neural tissues, such as the brain and spinal cord, and significantly lower expression in other neuroectodermal tissues, such as the adrenal glands [2, 8]. In neuroectodermal tissue, the highest expression of *BAALC* is found in the frontal cortex of the brain and particularly in the hippocampus and neocortex [3]. Its expression has been found also in bone marrow in CD34-positive white blood cell progenitors [2]. Interestingly, the transcriptional isoforms of *BAALC* in neurons are localized to postsynaptic lipid rafts. The protein encoded by *BAALC* gene is found also in muscle cells (myocardial and skeletal) and localizes attached to the inner side of the cell membrane but polarized only to one end of the cell [8]. The *BAALC*-encoded protein is not expressed in peripheral blood leukocytes, lymph nodes, or non-neural tissue [2, 5]. The expression of *BAALC* by CD34-positive progenitors and neuroectodermal cells suggests that *BAALC* performs certain functions in these tissues [2, 6, 9].

A practical aspect of investigations on *BAALC* overexpression is the question of what to use for the analysis—bone marrow (BM) or peripheral blood (PB)? This question was addressed in different studies and a strong correlation of *BAALC* expression levels in both specimens has been reported and confirmed [10–12].

3. Functions

The *BAALC* gene was originally found in the neuroectodermal tissue [2]. The mechanism and the function of the protein encoded by *BAALC*, are still not clearly

defined, but the current understanding is that the changes in *BAALC* gene expression are connected with a stop in cell differentiation, caused by changes in shape, motility, or adhesion of myeloid precursors [9]. The role of the *BAALC* gene in the development of leukemia has been studied in immature leukemia cells by eliminating gene function by RNA in a human leukemia cell line [13]. The elimination of overexpression of the *BAALC* gene results in a decrease in uncontrolled cell growth and an increase in programmed cell death [9, 14]. Evidence suggests that the *BAALC* protein is an intracellular protein that plays roles in the cytoskeletal network, including regulation of the actin cytoskeleton, associated with a role in the postsynaptic "lipid raft" [2, 3]. The BAALC protein isoforms were found to interact with Ca2+/calmodulin-dependent protein kinase II (CaMKII). CaMKII is a protein kinase whose activation is dependent on Ca2+/calmodulin complex. It is involved in many signaling cascades and is required for Ca2+ homeostasis. CAMKII is thought to be an important mediator in brain tissue in memory and learning [14], as well as in the differentiation and activation of the CD8 T-cell population [15]. BAALC interacts with the CAMKII alpha subunit but not with the CAMKII beta subunit [3]. The interaction with the CAMKII alpha subunit takes place in the regulatory region of the CAMKII alpha protein. The BAALC 1–6-8 isoform targets the postsynaptic "lipid raft", suggesting that it has functions involved in signal transduction, transmembrane traffic, and actin cytoskeleton regulation [3]. *BAALC* may play a role in the regulation of the CAMKII protein by interacting with the alpha subunit [3].

According to Baldus et al. [16], *BAALC* expression is restricted to progenitor cells, and downregulation of *BAALC* occurs with cell differentiation. Authors conclude that *BAALC* is represented in an early progenitor cell common to the myeloid, lymphoid, and erythroid pathways. Heuser et al. also suggested that the function of *BAALC* in the hematopoietic system is to block the differentiation of the myeloid lineage and requires a secondary mutation that favors the proliferation of this clone to induce leukemia [6]. The study by Heuser et al. found that despite the constant activation of the *BAALC* gene, hematopoietic stem cell proliferation did not start, it blocks myeloid differentiation and thus starts leukemogenesis, when combined with a self-renewing oncogene promoter (e.g., HoxA9) [6]. The data of Heuser et al. provide a molecular basis for the role of *BAALC* in regulating the proliferation and differentiation of AML cells and demonstrate the possibility of targeting *BAALC* in AML patients with high *BAALC* expression. Morita et al. found, that *BAALC*-induced leukemic cell cycle progression by sustained activation of extracellular signal-regulated kinase (ERK) interacting with the cellular skeletal protein MEK kinase-1 (MEKK1) inhibiting the interaction between ERK and MAP kinase phosphatase 3 (MKP)/DUSP6). *BAALC* induces chemoresistance in AML cells through enhanced regulation (upregulation) of ATP-binding cassette proteins by ERK—a dependent way that can be therapeutically affected by a target MEK inhibitor. The authors also demonstrated that *BAALC* blocks ERK-mediated monocyte differentiation of AML cells by blocking Krüppel-like factor 4 (KLF4) in the cytoplasm and inhibiting its function in the nucleus. Therefore, MEK inhibition will synergize with the induction of KLF4, and high efficacy is expected against AML cells with high *BAALC* expression [9]. By sequencing the *BAALC* genomic region, Eisfeld et al. identified six informative single nucleotide polymorphisms and they tested them for possible association with *BAALC* overexpression. They showed that *BAALC* overexpression occurs in the presence of a T-allele that creates a binding site for RUNX1 activating transcription factor in the *BAALC* promoter region. The association of high *BAALC* expression with the T-allele and its correlation with RUNX1 expression status have been demonstrated in patients

with cytogenetically normal (CN) AML from different populations. *BAALC* is very likely to act as one of the components of a complex high-risk series of changes in leukemogenesis [17]. There is evidence of a regulatory relationship between *BAALC* expression by FLI1 and c-MYC genes encoding transcription factors leading to cell proliferation [18].

The other fact concerning *BAALC* functions is described by Maki et al. [19], who identified that *BAALC* is connected with drebrin, in a protein complex. Drebrin—an actin-binding cytoplasmic protein located in the cell membrane is encoded from *DBN1* gene, located on chromosome 5. *DBN1* is identified in neural cells [20] and is also expressed in HSCs, which suggests that *DBN1* plays a role in cell skeleton dynamics in hematopoietic cells, as well as in neuronal cells [21]. By remodeling the actin filament, drebrin transforms the cell membrane spine during cell–cell interactions, improving cell adhesion and mobility [22]. *BAALC* physically interacts with *DBN1* and participates in cellular adhesive functions, which may induce drug resistance. The localization of *BAALC-DBN1* complex in the cytoplasm is to the inner cellular membrane, and inhibition of *DBN1* gene activity results in decreased adhesive capacity and better sensitivity to chemotherapy agents of leukemic cells. Taken together, these findings prompted us to hypothesize that *BAALC* plays a role as a molecular transporter between cytoplasm and cellular membrane, recruiting *DBN1* to the cell membrane to activate cellular adhesion to the bone marrow niche. Considering that *BAALC* is exclusively expressed in the stem cell compartment in the hematopoietic system, *BAALC-DBN1* interaction may be important, especially in the immature fraction [19].

4. Frequency of *BAALC* overexpression in myeloid neoplasms

The reported frequency of *BAALC* overexpression in AML patients varies according to different reports and authors, and one of the reasons for this fact is that there are still no defined cutoff ranges on high/low *BAALC* expression.

In a study, Taner et al. tested samples from BM or PB taken from 130 diverse AML. They found that 28% of all AML patients are *BAALC*-positive [4].

Damiani et al. evaluated the effect of *BAALC* overexpression on the outcome of 175 adult AML patients with different cytogenetic risks. *BAALC* was overexpressed in 87/175 (50%) patients, without association with cytogenetic status [23].

Amirpour et al. assessed 47 cases with normal cytogenetic AML and found BAALC gene up-regulation in 47% of the cases and down-regulation in 53% of patients [24].

A similar prevalence of 50% was reported in the investigation of Weber et al. in CN-AML [10].

Significant differences in *BAALC* expression were observed by Zhou et al. among AML subtypes. They investigated samples from 121 de novo patients and found that 67(55.3%) of them have high *BAALC* expression above the cutoff value of 2.35. Among AML FAB subtypes (M0/M1/M2/M3), they found a significantly higher incidence of *BAALC* overexpression in M0/M1 (8/9, 89%) and M2 subtypes (33/48, 68%) than in M3 subtype (6/27, 22%) [25].

The prevalence of *BAALC* overexpression was substantially higher in patients with intermediate (42/70, 60%) and poor karyotypes (9/11, 82%) than it was in patients with a favorable karyotype (13/34, 38%). In contrast to the other patients, those with t(15;17) had the lowest frequency of *BAALC* overexpression, while those with t(8;21) had the highest.

In MDS patients, the observed incidence of *BAALC* overexpression was 50%, with the highest rates in the subtypes: refractory anemia with blast excess (RAEB-2) (30% of all high-expression patients) and refractory anemia (RA) (20% of all patients with high expression) [18].

There are no data on the frequency of overexpression of *BAALC* in MPN—chronic myeloid leukemia, myelofibrosis, polycythemia vera, and essential thrombocythemia.

Concerning overexpression of *BAALC* outside myeloid diseases—in the same study cited before [4], Tanner et al. found 65% *BAALC*-positive ALL patients. In an investigation among pediatric ALL the frequency of *BAALC*-positive ALL patients are reported 60% [26].

Overexpression of *BAALC* has also been found in solid tumors: glioblastoma, melanoma, and gastrointestinal stromal tumors (in children), suggesting its role in oncogenesis [17].

5. Clinical significance and importance of *BAALC* overexpression

5.1 Correlation of *BAALC* overexpression with some clinical, laboratory characteristics, and AML mutational status

In most of the papers in the literature, there are no associations of high *BAALC* gene expression with patients' and clinical characteristics, such as sex, age, white blood cell (WBC) count, PB blasts, BM blasts, or hemoglobin levels, at the time of AML diagnosis [14, 23]. In some of them, the authors found a trend that high *BAALC* expressers are younger age than the low expressers [10]. In an analysis from Shafik et al. a statistical significance between *BAALC* and age, CD34 expression, and close to significant results with some of FAB sub-groups was found [27].

Zhou et al. by applying multivariate analysis, which took into account factors, such as gender, age, WBC, HB, PLT, karyotype classifications, 10 gene mutations (mutant/wild type), and *BAALC*, identified high *BAALC* expression as an independent adverse prognostic factor in AML patients. Multivariate analysis using the same factors, except karyotype classification, among CN-AML patients also supported the conclusion that *BAALC* overexpression is a poor prognostic factor [25].

The results of a meta-analysis conducted in selected studies show a tendency for increased *BAALC* gene expression in poorly differentiated AML (M0/M1/M2) subtypes, while in well-differentiated subtypes the overexpression is rare [14]. There is no evidence of an association between overexpression of the *BAALC* gene and the type of chromosomal abnormalities found. However, an increased incidence of aberrant overexpression has been observed in patients with a normal karyotype.

Association between *BAALC* gene mutations connected with AML has been reported.

FLT3 (ITD/TKD) are some of the most explored mutations in AML. There are sufficient data about the prognostic significance (included in "WHO classification of tumors of hematopoietic and lymphoid tissues"), and in addition, *FLT3* mutations already act as a therapeutic target. Therefore, *BAALC* overexpression and the *FLT3* mutation is an objects of many studies. Some of them [14, 23, 27, 28], failed to find a link between *BAALC* and *FLT3-ITD*, but opposing this conclusion, other authors provide results concluding that there is a strong correlation between high *BAALC* expression and the *FLT3-ITD* mutation [10, 29, 30].

When comparing 307 adult patients with low and high *BAALC* expression, Baldus et al. found that high-expression patients had a higher incidence of primary resistant leukemia. High *BAALC* expression is associated also with a higher cumulative relapse rate and shorter overall survival (OS). According to their conclusion, higher *BAALC* expression is an independent predictive factor for disease resistance. They only confirmed that high *BAALC* expression and the presence of the high *FLT3* mutation/wild-type ratio is a predictive factor for a high incidence of primary resistant leukemia, as well as for reduced OS [29].

FLT3-ITD and *NPM1* mutations were analyzed in several studies and the data were summarized in a meta-analysis [14]. The results obtained do not show a correlation between the high expression of the *BAALC* gene and the *FLT3-ITD* status, but the authors reported an association with the *NPM1* mutation. Patients with high levels of *BAALC* expression at diagnosis were correlated with the mutational status of *NPM1*. This comparison revealed a good correlation between the % *BAALC*/*ABL1* levels with the *NPM1* mutation [10].

As mentioned above, there is evidence that high *BAALC* gene expression blocks myeloid cell differentiation and when combined with a *HOXA9* gene mutation that is associated with cell self-renewal, may induce leukemia [6]. Regarding *IDH1* and *IDH2* gene mutations, *BAALC* overexpression has been reported to be associated with wild-type *IDH1* and *IDH2*, in patients with cytogenetically normal AML [31], but other authors [13], found that there is no direct association between *IDH1* and *IDH2* gene mutations and overexpression of *BAALC*.

Different associations of altered *BAALC* expression with specific molecular aberrations have been shown. For instance, high *BAALC* expression has been demonstrated to correlate with the mutation status of *FLT3-ITD*, *CEBPA*, *and MLL-PTD* as well as with *NPM1wt.* [28–30]. In a cohort of 326 CN-AML patients, the correlation of high *BAALC* expression with *FLT3-ITD*, *CEBPAmut*, *MLL-PTD,* and *NPM1wt* could be confirmed. Authors provided results that show a strong correlation of high *BAALC* expression with *WT1mut*, *biCEBPA*, *IDH2R172mut,* and especially RUNX*1mut* (31 of 33 *RUNX1*-positive AML patients have also high *BAALC* expression), but they did not find correlations to *ASXL1mut*, *IDH1R132mut*, *IDH2R140mut,* or *TET2mut* [10].

Finally, Langer et al. analyzed *BAALC* expression in 172 primary CN-AML patients (<60 years). They found high *BAALC* expression associated with *FLT3-ITD*, *wild-type NPM1*, mutated *CEBPA*, *MLL-PTD*, absent *FLT3-TKD*, and high *ERG* expression. The multidrug resistance gene *ABCB1 (MDR1*) was shown to be one of the most substantially up-regulated genes, which is consistent with the resistant disease [32].

5.2 Predictive value of *BAALC* in AML patients

In contemporary hematology, a crucial part of the therapeutic decision-making process is the proper risk assessment, based on pretreatment genetic markers. The potential effect of *BAALC* in AML could be assessed in two directions: as a prognostic marker at diagnosis and as a minimal residual disease marker after treatment.

There are enough data, confirming that *BAALC* overexpression is associated with adverse prognosis in AML patients. In the last years, several studies have explored the relevance of using *BAALC* overexpression as a part of different score systems aimed at assessing the clinical efficacy of chemotherapy.

We know that approximately 55–60% of cases of AML have specific recurrent chromosomal aberrations, which can be identified by classical cytogenetic techniques.

This information is the basis for classifying patients, at diagnosis, into prognostic categories: favorable, intermediate, and unfavorable risk. According to present recommendations and guidelines, patients with favorable risk AML are treated with chemotherapy only, while patients with adverse risk are referred to allogeneic stem cell transplantation if a suitable donor is available. The optimal therapeutic strategy in patients with intermediate-risk is still debatable. Approximately 40–45% of AML patients, who have no identified cytogenetic abnormality at diagnosis are classified in this group [33]. By finding overexpression of *BAALC* in this group and assessing the outcome, we are able to identify subgroups of patients at adverse risk among those with CN-AML. Xiao et al. reported that patients with AML who overexpress *BAALC* (*BAALC*-positive) have a median EFS (event-free survival) of 5 months, compared to 15 months in *BAALC*-negative patients. The prognosis of patients with AML and *BAALC* overexpression is unfavorable—the significant overexpression of *BAALC* and accumulation of gene products in the cell leads to drug resistance in these patients, which in turn results in a low overall response to therapy and hence to the reduced overall survival of these patients [14].

The importance of identifying prognostic molecular markers in CNAML patients to improve the therapeutic approach was confirmed by a study in which the authors investigated the prognostic effect of altered *BAALC* expression in CN -AML patients [34]. They analyzed bone marrow samples from 30 CN-AML patients for *BAALC* expression levels using RT-PCR. Patients were divided into *BAALC* low and high expression. High expression was present in 70% of patients and the level of expression was not correlated with patients' clinical parameters. In a 2 year follow-up, patients with high *BAALC* expression had a lower incidence of clinical remissions and shorter overall survival (OS). Multivariate analysis confirmed the high expression of *BAALC* as an independent risk factor for OS. *BAALC* overexpression predicts an adverse clinical outcome and may be identified as an important risk factor in CN-OML patients [34].

According to Yahya et al. a similar conclusion about the prognostic relevance of *BAALC* gene expression in adult acute myeloid leukemia was formulated. High expression was found in 22 of 45 patients (48.9%), and there was no link between this expression and the most important treatment-outcome characteristics. High *BAALC* expressers have a higher mortality rate, a significantly shorter DFS, and a shorter overall survival. They also have a lower incidence of CR and a lower mortality rate. The conclusion is that high *BAALC* expression may be a significant prognostic indicator in individuals with a normal karyotype and an independent risk factor for both DFS and OS, according to the authors' findings [35].

In a survey by Weber et al. patients with CN-AML and high expression of *BAALC* had significantly shorter EFS and OS than low expressers. High *BAALC* expression was also linked to a significantly lower survival when OS was analyzed and patients were censored on the day of transplantation, excluding the impact of allogeneic SCT. The authors were able to validate the usefulness of *BAALC* expression as a marker for identifying residual disease patients through their investigation. Parallel investigation of *BAALC* expression showed a substantial association between *BAALC* expression levels at diagnosis and after treatment. Additionally, the authors found that in the cohort of patients with relapsed AML, the mean *BAALC* expression levels at diagnosis and relapse were comparable, confirming *BAALC* expression as a potential MRD marker [10].

What is the role of *BAALC* in patients with AML and abnormal karyotype? In a study of 175 adult patients with AML with different cytogenetic risks, Damiani

et al. assessed the effect of *BAALC* gene overexpression on disease outcome [23]. The patients in the study were distributed as follows: 13 with a favorable karyotype, 117 with an intermediate, and 45 with an unfavorable. The *BAALC* gene was overexpressed in 87/175 (50%) of patients without any association with the cytogenetic status. Complete remission (CR) was achieved in 111 patients (63%) and was maintained till 5th year in 52 ± 7%. *BAALC* overexpression had a negative effect on the achievement of CR but did not affect the likelihood of disease recurrence. The median survival was 22 months with overall survival (OS) of 35%. *BAALC* was identified as one of the factors with a negative impact on the OS. The authors observed a worse outcome in patients with high *BAALC* expression in all cytogenetic risk categories: 5-year OS was 100% versus 71% in patients with a favorable prognosis, 55% versus 40% in patients with an intermediate karyotype, and 34% versus 23% in the group with an unfavorable prognosis. In conclusion, overexpression of the *BAALC* gene identifies patients with an unfavorable prognosis in all cytogenetic groups, and the presence in patients with favorable or unfavorable karyotypes significantly worsens survival [23].

The potential effect of *BAALC* in cytogenetic abnormal AML is explored also by Santamaria et al. [36]. They evaluated *BAALC* expression in AML patients with abnormal karyotypes and found a connection between high *BAALC* overexpression and lower rates of CR and OS.

In meta-analysis involving over 7525 cases of AML from 25 studies, Yuen et al. concluded that at diagnosis, complete remission attainment and survival outcome were negatively impacted by *BAALC* overexpression. Therefore, stratifying AML patients based on *BAALC* gene expression levels at diagnosis could be beneficial for treatment decisions [37].

A survival analysis of 108 patients with follow-up data was conducted to examine the prognostic significance of *BAALC* expression in AML. Both in the entire AML cohort and in CN-AML category, patients with high levels of *BAALC* had significantly lower rates of complete remission (CR) than patients with low levels of *BAALC*. In the entire cohort of AML patients, patients with high *BAALC* expression had significantly shorter overall survival (OS) than those with low *BAALC* expression. Additionally, in the CN-AML subgroup, patients with high *BAALC* expression had also a significantly shorter OS compared to those with low *BAALC* expression [25].

Weber et al. showed data that *BAALC* expression is an independent predictor of shorter EFS, OS, and that it is a useful target for detecting residual disease. Authors showed that a reduction in *BAALC* expression after chemotherapy from the initially detected levels resulted in better EFS [10].

In conclusion, based on the described data, *BAALC* could be regarded as a relevant prognostic marker in AML patients and could be used for treatment response evaluation after treatment in patients with high *BAALC* expression at diagnosis. Future prospective analysis should confirm the predictive value of *BAALC* and if AML patients with high *BAALC* expression at diagnosis may have therapy benefits from *BAALC* detection over the course of their disease.

5.3 Predictive value of *BAALC* in MDS patients

Data on the role of *BAALC* in patients with MDS are scarce, and most of them come from studies investigating multiple genes (including *BAALC*) associated with a poor prognosis in myeloid neoplasms. In a study of 140 MDS patients, Thol et al.

combined the expression data of four genes: *MN1*, *ERG*, *BAALC,* and *EVI1* (MEBE) in an additive score that was validated with an independent cohort of 110 MDS patients. The high MEBE score, defined as high expression of at least two of the four genes, predicted significantly shorter overall survival and time to progression to AML. In the validation cohort of 110 MDS patients, the high MEBE score predicted shorter OS and time to progression to AML. Thus, it can be concluded that a high MEBE score is an unfavorable prognostic marker in MDS and is associated with an increased risk of progression to AML. Almost half of the MDS patients with high *BAALC* expression (48.6%) transformed into AML within the study, compared with only 11.4% of the *BAALC* low expression group [18]. The cited data outline the possible involvement of the *BAALC* gene in the pathogenesis of MDS and clearly indicate the need for further research in this direction.

5.4 Predictive value of *BAALC* in HSCT in AML patients

Concerning the role of *BAALC* in transplantation, we have two main questions: is it possible to use the level of *BAALC* expression as a suitable marker for MRD/response to therapy, and what is the prognostic value of *BAALC* expression on the treatment outcome in transplanted patients.

In an attempt to assess the impact of allogeneic transplantation on treatment outcome, Damm et al. proposed an integrative prognostic risk score (IPRS) for CN-AML patients based on clinical, hematological, and molecular markers, including the expression levels of *BAALC*. Depending on the assessment, authors divided patients with AML into three risk groups: low, intermediate, and high risk. The role of allogeneic stem cell transplantation (alloSCT) for patients in each of the three risk groups was investigated by intent-to-treat analysis, due to the fact that all patients with an available donor were referred to alloSCT. The results showed that the high-risk group of patients have longer overall survival (OS) and relapse free survival (RFS) when alloSCT is performed by a family-related donor than in patients with a nonfamily donor. The intermediate risk group had lower OS and RFS when the alloSCT was performed from a family-related donor compared to patients who were from a nonfamily donor. The effect in the low-risk group is not clear, most likely due to the low frequency of relapses. The data showed that the IPRS may be an additional tool for disease outcome prediction and treatment choice, reflecting the biological heterogeneity in CN-AML patients [38].

Assessing the prognostic value of *BAALC* expression, Weber et al. found that excluding the effect of allogeneic HSCT, patients with high *BAALC* expression have considerably shorter OS [10].

Jentzsch et al. tried to assess how high *BAALC* copy numbers, in peripheral blood, prior to allogeneic transplantation predict early relapse in acute myeloid leukemia patients. They performed absolute quantification of *BAALC* copy numbers in peripheral blood prior (median 7 days) to HSCT in complete remission or CR with incomplete peripheral recovery in 82 acute myeloid leukemia patients. Patients with high *BAALC*/ABL1 copy levels before HSCT were more likely to relapse within 100 days of HSCT [39].

In another study, Zhang et al. investigated 71 de novo AML patients who were treated with allo-HSCT and classified them as low or high expressers based on the median *BAALC* expression levels at diagnosis. They did not find significant differences in event-free survival or overall survival between the two groups [40].

5.5 The role of *BAALC* gene in developing chemoresistance

Interactions between HSCs and the bone marrow niche components, such as osteoblasts, sinusoidal endothelial cells, and mesenchymal stromal cells, are very important because they create a special bone marrow microenvironment, in which processes of self-renewal and differentiation of HSCs are strictly regulated [41]. Cell adhesion to the niche protects HSCs, leukemic cells from toxic exposure, and they can develop resistance to cytotoxic agents [42–44]. Based on the fact that *BAALC* plays an important role in the activation of cellular adhesion to the bone marrow niche some authors rise a hypothesis that high *BAALC* expression in leukemic cells induces drug resistance by adhesion to the niche components [19]. Previously mentioned data suggests that *BAALC* binds to drebrin, and this interaction develops chemoresistance against cytotoxic drugs, such as cytarabine [19].

Several other papers are in alignment with the hypothesis of connections between *BAALC* overexpression and a chemo-resistant disease.

Santamaria et al. analyzed the *BAALC* gene in 127 AML patients categorized as intermediate risk (98 cytogenetically normal and 29 with cytogenetic alterations). They reported that high expressers are more resistant to induction chemotherapy in comparison with low *BAALC* expressers. High expressers also show a lower complete remission rate after reinduction therapy, lower 3-year overall survival, and relapse-free survival. The authors also found similarity in the results when subgroups were analyzed separately according to their cytogenetic status [36].

In a study of gene expression profiles (GEPs) in 312 probe sets, Langer et al. found an association between high *BAALC* expressers with overexpression of one of the genes involved in multidrug resistance (MDR). High *BAALC* expression levels had distinctive features, according to the study. In individuals with high *BAALC* expression levels, the multidrug resistance gene ABCB1 (MDR1) was shown to be one of the most substantially up-regulated genes, which is consistent with the resistant disease that these patients are known to have. The ATP-dependent drug efflux pump MDR1 protein, also known as P-glycoprotein, is in charge of reducing drug accumulation in cells that are resistant to several treatments. It also frequently plays a role in the emergence of anticancer drug resistance [32].

Kuhnl et al. provided another confirmation that *BAALC* gene overexpression induces chemoresistance. They investigated the prognostic significance of *BAALC* in a different from AML area, that is, in B-precursor ALL, and concluded that high *BAALC* expression is associated with an immature chemo-resistant leukemic phenotype and inferior OS [45].

In the paper cited before [10], Weber et al. found that high *BAALC* expression is an independent predictor of shorter EFS and OS and chemotherapy-resistant disease.

In a study of *BAALC*-associated GEPs, Heesch et al. concluded that overexpression of *BAALC* could be associated with adverse outcomes and chemotherapy resistance in adult patients with cytogenetically normal AML [31].

6. Conclusions

Increased *BAALC* expression is identified and connected with the development of AML. Normally, the highest expression of *BAALC* is found in neural tissues, such as frontal cortex of the brain, in the hippocampus, neocortex, and bone marrow in

CD34-positive white blood cell progenitors. *BAALC* gene is expressed in mammals but is not found in lower-level organisms.

The mechanism and the function of the protein encoded by *BAALC* are still not clearly defined, but the current understanding is that the changes in *BAALC* gene expression are connected with a stop in cell differentiation due to changes in shape, motility, or adhesion of myeloid precursors. Evidence suggests that the BAALC protein is an intracellular protein that participates in the cytoskeletal network, including the regulation of the actin cytoskeleton. There is a hypothesis that *BAALC* also plays a role as a molecular transporter between cytoplasm and cellular membrane, acting in the mechanism which activates cellular adhesion to the bone marrow niche.

The reported frequency of *BAALC* overexpression in AML patients varies (28%–55.3%) among different reports and authors because there is still no defined cutoff ranges on high/low *BAALC* expression. Some authors found variety in *BAALC* overexpression in different risk categories or in different AML subtypes according to FAB classification. To our knowledge, only one paper has investigated the frequency of *BAALC* overexpression in MDS–50%, so far. Currently, we have insufficient data about the involvement of the *BAALC* gene in the pathogenesis of MDS and this fact clearly indicates the need for further research in this direction.

In most of the scientific papers, there are no associations of high *BAALC* gene expression with patient and clinical characteristics, such as sex, age, white blood cell (WBC) count, PB blasts, BM blasts, or hemoglobin levels, at the time of diagnosis of AML. Concerning molecular anomalies—there is a discrepancy in the data connecting FLT3 (ITD/TKD) mutations with *BAALC*. Some of the authors failed to find a link between high *BAALC* expression and the FLT3-ITD mutation, but opposing this conclusion, others provide results concluding that there is a strong correlation between high *BAALC* expression and the FLT3-ITD mutation. Obviously, we need additional prospective studies to clarify this question.

Correlations between high *BAALC* expression with CEBPAmut/biCEBPA, MLL-PTD, NPM1wt, WT1mut, IDH2R172mut, high ERG expression, and a strong correlation with RUNX1mut and multidrug resistance gene ABCB1 (MDR1) have been demonstrated.

Based on the fact that *BAALC* plays an important role in the activation of cellular adhesion to the bone marrow niche, some authors rise a hypothesis that high *BAALC* expression in leukemic cells induces drug resistance.

Concerning the predictive value, *BAALC* could be a relevant prognostic marker in AML patients and could also be used for MRD assessment after treatment in patients with high *BAALC* expression at diagnosis. *BAALC* expression is an important risk factor in CN-AML and is associated with an inferior outcome and chemotherapy-resistant disease. In patients with AML with abnormal karyotype, *BAALC* overexpression identifies patients with an unfavorable prognosis across all cytogenetic groups, as survival is significantly worsened both in patients with favorable or unfavorable karyotype. The results about the role of *BAALC* in transplantation are again disputable. The available data cannot confirm it as a suitable marker for MRD or for the response to therapy.

Future prospective analyses to determine the potential role of BAALC expression assessment in the clinical assessment and decision-making process in AML and MDS patients are worthwhile.

Conflict of interest

The authors declare no conflict of interest.

Author details

Emil Aleksov*, Branimir Spassov, Margarita Guenova and Gueorgui Balatzenko
National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

*Address all correspondence to: ealexov@abv.bg

IntechOpen

References

[1] Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. The New England Journal of Medicine. 2013;**368**(22):2059-2074. DOI: 10.1056/NEJMoa1301689 Erratum in: N Engl J Med. 2013 Jul 4;369(1):98. PMID: 23634996; PMCID: PMC3767041

[2] Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, Savage SL, et al. Functional genomic landscape of acute myeloid leukaemia. Nature. 2018;**562**(7728):526-531. DOI: 10.1038/s41586-018-0623-z PMID: 30333627; PMCID: PMC6280667

[3] Baldus CD, Tanner SM, Ruppert AS, Whitman SP, Archer KJ, Marcucci G, et al. BAALC expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: A Cancer and leukemia group B study. Blood. 2003;**102**(5):1613-1618. DOI: 10.1182/blood-2003-02-0359 PMID: 12750167

[4] Tanner SM, Austin JL, Leone G, Rush LJ, Plass C, Heinonen K, et al. BAALC, the human member of a novel mammalian neuroectoderm gene lineage, is implicated in hematopoiesis and acute leukemia. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**(24):13901-13906. DOI: 10.1073/pnas.241525498 PMID: 11707601; PMCID: PMC61139

[5] Database, GeneCards Human Gene. BAALC Gene - GeneCards | BAALC Protein | BAALC Antibody. Available from: https://www.genecards.org/cgi-bin/carddisp.pl?gene=BAALC [Accessed: June 25, 2022]

[6] Heuser M, Berg T, Kuchenbauer F, Lai CK, Park G, Fung S, et al. Functional role of BAALC in leukemogenesis. Leukemia. 2012;**26**(3):532-536. DOI: 10.1038/leu.2011.228 PMID: 21869843

[7] Swiss Model Database. Available from: https://swissmodel.expasy.org/repository/uniprot/Q8WXS3?model=AF-Q8WXS3-F1-model-v2 [Accessed: June 23, 2022]

[8] UniProt Database. Available from: https://www.uniprot.org/uniprot/Q8WXS3#subcellular_location [Accessed: June 24, 2022]

[9] Morita K, Masamoto Y, Kataoka K, Koya J, Kagoya Y, Yashiroda H, et al. BAALC potentiates oncogenic ERK pathway through interactions with MEKK1 and KLF4. Leukemia. 2015;**29**(11):2248-2256. DOI: 10.1038/leu.2015.137 PMID: 26050649

[10] Weber S, Alpermann T, Dicker F, Jeromin S, Nadarajah N, Eder C, et al. BAALC expression: A suitable marker for prognostic risk stratification and detection of residual disease in cytogenetically normal acute myeloid leukemia. Blood Cancer Journal. 2014;**4**(1):e173. DOI: 10.1038/bcj.2013.71 PMID: 24413067; PMCID: PMC3913940

[11] Bienz M, Ludwig M, Leibundgut EO, Mueller BU, Ratschiller D, Solenthaler M, et al. Risk assessment in patients with acute myeloid leukemia and a normal karyotype. Clinical Cancer Research. 2005;**11**(4):1416-1424. DOI: 10.1158/1078-0432.CCR-04-1552 Erratum in: Clin Cancer Res. 2005;11(15):5659. PMID: 15746041

[12] Najima Y, Ohashi K, Kawamura M, Onozuka Y, Yamaguchi T, Akiyama H, et al. Molecular monitoring of BAALC

expression in patients with CD34-positive acute leukemia. International Journal of Hematology. 2010;**91**(4):636-645. DOI: 10.1007/s12185-010-0550-8 PMID: 20376583

[13] Xu B, Chen G, Shi P, Guo X, Xiao P, Wang W, et al. shRNA-mediated BAALC knockdown affects proliferation and apoptosis in human acute myeloid leukemia cells. Hematology. 2012;**17**(1):35-40. DOI: 10.1179/102453312X13221316477499 PMID: 22549446

[14] Xiao SJ, Shen JZ, Huang JL, Fu HY. Prognostic significance of the BAALC gene expression in adult patients with acute myeloid leukemia: A meta-analysis. Molecular and Clinical Oncology. 2015;**3**(4):880-888. DOI: 10.3892/mco.2015.562 PMID: 26171200; PMCID: PMC4486884

[15] Lin MY, Zal T, Ch'en IL, Gascoigne Nicholas RJ, Hedrick SM. A pivotal role for the multifunctional calcium/calmodulin-dependent protein kinase II in T cells: From activation to unresponsiveness. The Journal of Immunology. 2005;**174**(9):5583-5592. DOI: 10.4049/jimmunol.174.9.5583

[16] Baldus CD, Tanner SM, Kusewitt DF, Liyanarachchi S, Choi C, Caligiuri MA, et al. BAALC, a novel marker of human hematopoietic progenitor cells. Experimental Hematology. 2003;**31**(11):1051-1056 PMID: 14585369

[17] Eisfeld AK, Marcucci G, Liyanarachchi S, Döhner K, Schwind S, Maharry K, et al. Heritable polymorphism predisposes to high BAALC expression in acute myeloid leukemia. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(17):6668-6673. DOI: 10.1073/pnas.1203756109 PMID: 22493267; PMCID: PMC3340094

[18] Thol F, Yun H, Sonntag AK, Damm F, Weissinger EM, Krauter J, et al. Prognostic significance of combined MN1, ERG, BAALC, and EVI1 (MEBE) expression in patients with myelodysplastic syndromes. Annals of Hematology. 2012;**91**(8):1221-1233. DOI: 10.1007/s00277-012-1457-7 PMID: 22488406

[19] Maki H, Yoshimi A, Shimada T, Arai S, Morita K, Kamikubo Y, et al. Physical interaction between BAALC and DBN1 induces chemoresistance in leukemia. Experimental Hematology. 2021;**94**:31-36. DOI: 10.1016/j.exphem.2020.12.003 PMID: 33453340

[20] Peitsch WK, Grund C, Kuhn C, Schnölzer M, Spring H, Schmelz M, et al. Drebrin is a widespread actin-associating protein enriched at junctional plaques, defining a specific microfilament anchorage system in polar epithelial cells. European Journal of Cell Biology. 1999;**78**(11):767-778. DOI: 10.1016/S0171-9335(99)80027-2 PMID: 10604653

[21] Steidl U, Bork S, Schaub S, Selbach O, Seres J, Aivado M, et al. Primary human CD34+ hematopoietic stem and progenitor cells express functionally active receptors of neuromediators. Blood. 2004;**104**(1):81-88. DOI: 10.1182/blood-2004-01-0373 PMID: 15016651

[22] Ikeda K, Shirao T, Toda M, Asada H, Toya S, Uyemura K. Effect of a neuron-specific actin-binding protein, drebrin a, on cell-substratum adhesion. Neuroscience Letters. 1995;**194**(3):197-200. DOI: 10.1016/0304-3940(95)11760-t PMID: 7478237

[23] Damiani D, Tiribelli M, Franzoni A, Michelutti A, Fabbro D, Cavallin M, et al. BAALC overexpression retains its negative prognostic role across all cytogenetic risk groups in acute myeloid leukemia patients. American Journal

of Hematology. 2013;**88**(10):848-852. DOI: 10.1002/ajh.23516 PMID: 23760853

[24] Amirpour M, Ayatollahi H, Sheikhi M, Azarkerdar S, Shams SF. Evaluation of BAALC gene expression in normal cytogenetic acute myeloid leukemia patients in north-east of Iran. Medical Journal of the Islamic Republic of Iran. 2016;**30**:418 PMID: 28210583; PMCID: PMC5307615

[25] Jing-dong Z, Lei Y, Ying-ying Z, Jing Y, Xiang-mei W, Hong G, et al. Overexpression of BAALC: Clinical significance in Chinese de novo acute myeloid leukemia. Medical Oncology. 2015;**32**(1):386. DOI: 10.1007/s12032-014-0386-9

[26] Hagag AA, Elshehaby WA, Hablas NM, Abdelmageed MM, Abd El-Lateef AE. Role of *BAALC* Gene in prognosis of acute lymphoblastic leukemia in Egyptian children. Indian Journal of Hematology and Blood Transfusion. 2018;**34**(1):54-61. DOI: 10.1007/s12288-017-0841-9. PMID: 29398800; PMCID: PMC5786634

[27] Shafik NF, El Ashry MS, Badawy RH, Hussein MM, Hassan NM. The prognostic significance of the BMI-1 and *BAALC* genes in adult patients with acute myeloid leukemia. Indian Journal of Hematology and Blood Transfusion. 2020;**36**(4):652-660. DOI: 10.1007/s12288-020-01278-9 PMID: 33100707; PMCID: PMC7573057

[28] Metzeler KH, Dufour A, Benthaus T, Hummel M, Sauerland MC, Heinecke A, et al. ERG expression is an independent prognostic factor and allows refined risk stratification in cytogenetically normal acute myeloid leukemia: A comprehensive analysis of ERG, MN1, and BAALC transcript levels using oligonucleotide microarrays. Journal of Clinical Oncology. 2009;**27**(30):5031-5038. DOI: 10.1200/JCO.2008.20.5328 PMID: 19752345

[29] Baldus CD, Thiede C, Soucek S, Bloomfield CD, Thiel E, Ehninger G. *BAALC* expression and FLT3 internal tandem duplication mutations in acute myeloid leukemia patients with normal cytogenetics: Prognostic implications. Journal of Clinical Oncology. 2006;**24**:790-797

[30] Schwind S, Marcucci G, Maharry K, Radmacher MD, Mrózek K, Holland KB, et al. BAALC and ERG expression levels are associated with outcome and distinct gene and microRNA expression profiles in older patients with de novo cytogenetically normal acute myeloid leukemia: A Cancer and leukemia group B study. Blood. 2010;**116**(25):5660-5669. DOI: 10.1182/blood-2010-06-290536 PMID: 20841507; PMCID: PMC3031412

[31] Heesch S, Schlee C, Neumann M, Stroux A, Kühnl A, Schwartz S, et al. *BAALC*-associated gene expression profiles define IGFBP7 as a novel molecular marker in acute leukemia. Leukemia. 2010;**24**(8):1429-1436. DOI: 10.1038/leu.2010.130 PMID: 20535151

[32] Langer C, Radmacher MD, Ruppert AS, Whitman SP, Paschka P, Mrózek K, et al. High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: A Cancer and leukemia group B (CALGB) study. Blood. 2008;**111**(11):5371-5379. DOI: 10.1182/blood-2007-11-124958 PMID: 18378853; PMCID: PMC2396728

[33] Gregory TK, Wald D, Chen Y, Vermaat JM, Xiong Y, Tse W. Molecular prognostic markers for adult acute myeloid leukemia with normal cytogenetics. Journal of Hematology & Oncology. 2009;**2**:23. DOI: 10.1186/1756-8722-2-23 PMID: 19490647; PMCID: PMC2700131

[34] Eid MA, Attia M, Abdou S, El-Shazly SF, Elahwal L, Farrag W, et al. BAALC and ERG expression in acute myeloid leukemia with normal karyotype: Impact on prognosis. International Journal of Laboratory Hematology. 2010;**32**(2):197-205. DOI: 10.1111/j.1751-553X.2009.01168.x PMID: 19555438

[35] Yahya RS, Sofan MA, Abdelmasseih HM, Saudy N, Sharaf-Eldein MA. Prognostic implication of *BAALC* gene expression in adult acute myeloid leukemia. Clinical Laboratory. 2013;**59**(5-6):621-628. DOI: 10.7754/clin.lab.2012.120604 PMID: 23865362

[36] Santamaría C, Chillón MC, García-Sanz R, Pérez C, Caballero MD, Mateos MV, et al. *BAALC* is an important predictor of refractoriness to chemotherapy and poor survival in intermediate-risk acute myeloid leukemia (AML). Annals of Hematology. 2010;**89**(5):453-458. DOI: 10.1007/s00277-009-0864-x PMID: 19943049

[37] Yuen KY, Lin XY, Zhou YZ, Luo H, Liu Y, Xu LH. Optimal time-points for detecting expression levels of *BAALC*, EVI1, and WT1 genes in patients with acute myeloid leukemia: A meta-analysis. Hematology. 2021;**26**(1):995-1006. DOI: 10.1080/16078454.2021.2006409 PMID: 34871539

[38] Damm F, Heuser M, Morgan M, Wagner K, Görlich K, Grosshennig A, et al. Integrative prognostic risk score in acute myeloid leukemia with normal karyotype. Blood. 2011;**117**(17):4561-4568. DOI: 10.1182/blood-2010-08-303479 PMID: 21372155

[39] Jentzsch M, Bill M, Grimm J, Schulz J, Goldmann K, Beinicke S, et al. High BAALC copy numbers in peripheral blood prior to allogeneic transplantation predict early relapse in acute myeloid leukemia patients. Oncotarget. 2017;**8**(50):87944-87954. DOI: 10.18632/oncotarget.21322 PMID: 29152132; PMCID: PMC5675684

[40] Zhang J, Shi J, Zhang G, Zhang X, Yang X, Yang S, et al. BAALC and ERG expression levels at diagnosis have no prognosis impact on acute myeloid leukemia patients undergoing allogeneic hematopoietic stem cell transplantation. Annals of Hematology. 2018;**97**(8):1391-1397. DOI: 10.1007/s00277-018-3331-8 PMID: 29696374

[41] Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nature Reviews. Immunology. 2006;**6**(2):93-106. DOI: 10.1038/nri1779 PMID: 16491134

[42] Krause DS, Scadden DT, Preffer FI. The hematopoietic stem cell niche--home for friend and foe? Cytometry. Part B, Clinical Cytometry. 2013;**84**(1):7-20. DOI: 10.1002/cyto.b.21066 PMID: 23281119; PMCID: PMC3691061

[43] Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, et al. Identification of the haematopoietic stem cell niche and control of the niche size. Nature. 2003;**425**(6960):836-841. DOI: 10.1038/nature02041 PMID: 14574412

[44] Xie Y, Yin T, Wiegraebe W, He XC, Miller D, Stark D, et al. Detection of functional haematopoietic stem cell niche using real-time imaging. Nature. 2009;**457**(7225):97-101. DOI: 10.1038/nature07639 Erratum in: Nature. 2010 Aug 26;466(7310):1134. PMID: 19052548

[45] Kühnl A, Gökbuget N, Stroux A, Burmeister T, Neumann M, Heesch S, et al. High BAALC expression predicts chemoresistance in adult B-precursor acute lymphoblastic leukemia. Blood. 2010;**115**(18):3737-3744. DOI: 10.1182/blood-2009-09-241943 PMID: 20065290

Chapter 3

Roads of Drug Resistance in Acute Myeloid Leukemia – Is It a Dead End?

Yanitsa Davidkova, Milan Jagurinoski, Gueorgui Balatzenko and Margarita Guenova

Abstract

Acute myeloid leukemia (AML) is a biologically and clinically heterogeneous neoplasm, which is characterized by abnormal proliferation, impaired apoptosis, and differentiation of leukemic immature cells. Nowadays, the first line treatment of AML is the chemotherapy regimen, which combines both cytosine arabinoside and anthracycline. Despite that complete remission (CR) can be achieved in 40–80% of patients depending on age, a considerable number will eventually relapse (acquired resistance) or have refractory disease (primary resistance). Finally, the estimated 5-year overall survival (OS) is less than 30%. Recent investigations reveal various mechanisms, responsible for drug resistance leading to AML persistence and recurrence. In order to improve clinical outcomes and develop successful therapeutic strategies, it is necessary to better explore the major adverse factors for escape from treatment, as well as to explore ways to predict and prevent or target drug resistance.

Keywords: acute myeloid leukemia, chemotherapy, drug resistance

1. Introduction

Acute myeloid leukemia (AML) comprises approximately 80% of all acute leukemias in adults [1]. The median age of the disease is about 69 years with a progressing incidence with advanced age [2]. AML represents a clinically and genetically heterogeneous disease, which is characterized by uncontrolled clonal proliferation, impaired apoptosis, and differentiation of leukemic immature cells. It has poor survival and fatal outcome in those who are untreated. Despite the progress in understanding the pathophysiology of AML and the discovery of novel therapeutic agents, the treatment approach has not changed essentially since the 1970s. According to the European LeukemiaNet (ELN) recommendations for the diagnosis and management of AML in adults (2017), the first-line therapy for the patients, eligible for intensive chemotherapy remains the conventional "3 + 7 regimen" (3 days of anthracycline + 7 days of cytarabine) [3]. Unfortunately, recent studies report that the disease is curable in only 5–15% of patients above 60 years and 35–40% in younger adults with intensive chemotherapy, which is unsatisfactory [4, 5]. Additionally, research data reveals that 10–40% of newly diagnosed AMLs fail to achieve

CR after frontline therapy and are classified as primary refractory AML [6–8]. Lately, ELN determines primary refractory AML as a lack of obtaining CR or complete remission with incomplete hematologic recovery (CRi) after at least 2 courses of intensive induction chemotherapy [3]. One of the major reasons for treatment failure is considered to be drug resistance. Besides, chemoresistance is basically divided into two groups intrinsic (primary) and acquired (secondary). The primary drug-resistant leukemic cells are present already at diagnosis, while the secondary resistance emerges during or after therapy, probably as a result of additionally occurring genetic disorders. In 30–40% of relapsed AML patients, newly developed gene mutations occur, yet in about 25% no molecular alterations emerge [9]. So, these data raise the question that gene alterations are drivers of relapse or refractoriness of AML and whether mutational status is the only cause of disease progression. This review will discuss the potential molecular pathways underlying drug resistance in blast cells and the interactions with the leukemic microenvironment.

2. Leukemogenesis in AML

Two decades ago, Gilliland and Griffin introduced the "two-hit model". In this model, the collaboration of two lesions of two different classes of mutations contributes synergistically to inflict AML [10]. Class I mutations (FLT3, c-KIT, NRAS) comprise mutations that activate signal-transduction pathways and thereby give a proliferation advantage to the hematopoietic progenitor cells. Class II mutations (recurring chromosomal aberrations, which produce fusion transcripts) affect transcription factors and cause impaired differentiation and following apoptosis. However, recent studies report that it is difficult to divide functions between the two classes of mutations [11]. Further, in the last years, genomic sequencing research has encountered new epigenetic genes associated with AML (chromatin-modifying genes: MLL fusions, ASXL1, and EZH2 mutations; methylation-related genes: DNMT3A, TET2, IDH1/2 mutations), which expands the complexity and heterogeneity of AML [12, 13]. Thus, the "two-hit model" of Gilliland and Griffin turns out to be insufficient to explain AML leukemogenesis.

A novel hypothesis for AML development has been formulated recently, based on three types of AML-associated mutations, investigated in mouse models [14]. According to this functional classification the first "type A mutations" (fusion genes) are necessary to initiate or maintain the leukemic phenotype, "type B mutations" (ABL, PDGFR, KIT, FLT3, etc.) support the proliferation and survival of leukemic cells and "type C mutations" (epigenetic modifiers), also called "seed mutations", provide a growth advantage, but still not sufficient to induce leukemia. The study research demonstrates that the combination of mutations of any two types, that is, A + B or A + C or B + C, may result in AML [14]. The model of clonal evolution of AML is considered to be a process of losing specific mutations or gaining a feature, which leads toward resistance. Emerging evidence has revealed that a relapse may present with re-occurring of the initial leukemic clone, assuming that the chemotherapy was unsuccessful or due to further clonal evolution following AML treatment [15, 16].

3. Mechanisms underlying drug resistance

Relapsed and refractory AML (R/R AML) is associated with unfavorable prognosis, due to poor response to conventional antileukemic therapy [17, 18]. Thus, a better understanding of the mechanisms, underlying drug resistance, would improve

Factors	Molecules	Function	Reference
Proteins and enzymes	P-gp, GST, MCL-1, MRP1/ LRP, Topo II, PKC	Affect the drug transport; altered enzyme function	[19–43]
Signal pathways	NF/kB, PI3K/Akt/mTOR	Coordinate complex cellular changes	[44, 45]
Genes and epigenetics	ASLX1, DNMT3, EZH2, FLT3, IDH1/2, TET2, WT1	Cell proliferation and differentiation; regulation of DNA and histones	[46–53]
microRNA	miRNA-155, miRNA-125, miRNA-100, miRNA-223	Control of cell division, self-renewal, DNA damage	[54–56]
Microenvironment	CD44, FGF2/FGFR1, SDF1/ CXCR4, VCAM/VLA4	Cell-to-cell, cell-to-matrix interactions	[57–66]

ASXL1, additional sex combs-like 1; CXCR4, C-X-C motif chemokine receptor 4; DNMT3A, DNA methyltransferase 3A; EZH2, enhancer of zeste homolog 2; IDH1/2, isocitrate dehydrogenase 1/2; FGF2, fibroblast growth factor 2; FGFR1, fibroblast growth factor receptor 1; FLT3, FMS-like tyrosine kinase 3; GST, glutathione S-transferase; LRP, lung resistance protein; MCL-1, myeloid cell leukemia 1; microRNA, microribonucleic acid; MRP1, multidrug resistance-related protein; mTOR, mammalian target of rapamycin; NF/kB, nuclear factor kappa B; P-gp, P-glycoprotein; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; SDF-1, stromal cell-derived factor 1; TET2, ten eleven translocation methylcytosine dioxygenase 2; Topo II, topoisomerase II; VLA-4, very late antigen-4.

Table 1.
Different mechanisms of drug resistance.

the therapeutic approach using novel strategies. Data are available showing that chemoresistance in AML may be due to numerous factors, which include proteins and enzymes with altered function, dysregulation of signaling pathways, mutations in cell cycle control genes, epigenetic modifiers, microRNA as well as impaired interactions with the bone marrow environment, and changes in the immune tolerance (**Table 1**).

3.1 Proteins and enzymes with altered function

Overexpression of the transporter proteins P-glycoprotein (P-gp) and multidrug resistance-related protein (MRP) 1 plays an important role in cross-resistance to drugs. In 1976 Juliano and Ling firstly described the ATP-dependent membrane P-gp, which acts as an efflux pump conferring resistance [19]. P-gp is a 170-kDa protein, encoded by the MDR1 gene (multidrug resistance gene 1) and belongs to the ABC transporter family. It pumps out chemotherapy drugs, maintaining lower drug concentrations intracellularly continuously, so that drug resistance is developed [20]. P-gp is considered to be an independent adverse prognostic factor for response and survival in newly diagnosed or R/R AML [21, 22]. However, according to other research studies, no correlation was identified between the MDR parameters and overall survival of the AML patients [23] and also P-gp activity is not consistently upregulated in relapsed AML [24]. Broxterman et al. found no correlation between the expression of P-gp and the complete response rate, event-free survival, or overall survival after idarubicine-containing induction [25]. The steady-state cellular accumulation of lipophilic idarubicine may circumvent the P-gp-mediated drug resistance in AML patients. The increased expression of P-gp and MRP1 are associated with advanced age, leukocytosis, poor chromosomal abnormalities, shorter overall survival, and also are detected with higher incidence in R/R and secondary AML comparing with de novo cases [26, 27]. Interestingly, an association has been observed between the expression of P-gp and MRP1 and the flow cytometric antigens (CD34 and CD7) and the FAB (French-American-British) classification of AML morphology

(M2, M5a, and M7 types) [26]. Moreover, a recommendation to observe the higher MRP1 expression by flow cytometry as an adverse prognostic marker in AML was proposed by Legrand et al. [28]. In contrast, another study revealed that AML patients relapse despite the lower expression of MRP1, suggesting the involvement of other intracellular mechanisms, possibly leading to cytarabine resistance [29].

LRP (lung resistance protein), a drug efflux transporter, is also assumed to play a role in drug resistance. However, the published data show conflicting evidence of the involvement of LRP in the process of chemoresistance. The overexpression of LRP is found to predict an inferior response in AML, but another study described that higher bone marrow expression of LRP predicts significant favorable therapeutic outcome with increased CR rate and 1-year DFS (disease-free survival) and OS [22, 30, 31].

Glutathione S-transferase (GST) is a drug-metabolizing enzyme, consisting of α, μ, θ, and π-types. It is responsible for controlling cellular oxidative balance, catalyzing the reduced glutathione which leads to diminished cytotoxic drug effects. The decreased enzymatic activity due to GST polymorphisms is associated with cancerogenesis and AML [32]. Indeed, changed detoxification contributes toward drug resistance in AML. Furthermore, patients with higher expression of GSTμ tend to have also MRP1 overexpression which results in increasing the survival of tumor cells and protects them from apoptosis [33, 34]. Recent investigation by Pei et al. demonstrated that primitive leukemic cells acquire aberrant glutathione metabolism and may be selectively eliminated by target therapy against the glutathione pathway [35].

Topoisomerase II (Topo II) is an essential ribozyme that alters the topological properties of DNA. The inhibitors of Topo II may trigger chromosomal translocations that are associated with therapy-related secondary leukemia, often bearing 11q23 translocations involving the *MLL* gene [36]. Decreased or increased expression or mutation in the topoisomerase II genes may lead to chemoresistance to topoisomerase II inhibitors [37, 38].

Somatic mutations in protein kinase genes play a significant role in proliferation, resistance, and apoptosis. The overexpression of PKC (protein kinase C) in AML results in a decline in CR induction and DFS by diminishing intracellular concentration of daunorubicine [39]. The connection between the activation of PKC and the upregulation of P-gp further contributes to chemoresistance in AML [40].

As a BCL-2 family protein member, MCL-1 (myeloid cell leukemia 1) prevents apoptosis. It is upregulated in several hematologic malignancies such as multiple myeloma, AML, and non-Hodgkin lymphoma and is associated with treatment resistance and inferior prognosis [41]. The increased expression or amplification of MCL-1 gene protects tumor cells from programmed cell death and decreases their sensitivity to conventional chemotherapy which appears to be a potential drug resistance mechanism [42]. Besides, the overexpression of MCL-1 correlates with resistance to venetoclax [41]. MCL-1 is described to be regulated by cyclin-dependent kinase (CDK). Recent studies reveal that the treatment with both MCL-1 inhibitors and BCL-2 inhibitors may overcome the acquired resistance to BCL-2 inhibition [43].

3.2 Signaling pathways

Knowledge of aberrantly regulated signal pathways in AML allowed the identification of novel therapy targets. The combination of conventional chemotherapy with targeted agents may potentially overcome resistance. An example is the PI3K/Akt/mTOR signal pathway which is responsible for cell metabolism, proliferation, differentiation, and survival. The upregulation of the PI3K/Akt/mTOR pathway in AML is caused by

mutations in the receptor tyrosine kinases. The FLT3 mutation leads to deregulation of PI3K/Akt/mTOR signaling which results in cytokine-independent survival and proliferation of hematopoietic cells and myeloproliferative neoplasms [44]. In addition, according to lately published data the PI3K/Akt/mTOR pathway plays a significant role in the regulation of therapy-resistant leukemic cells through the pro-inflammatory transcription factor NF-κB (nuclear factor-kappa-light-chain-enhancer of activated B cells). Thus, the mediated chemoresistance is caused by upregulation of anti-apoptotic genes, which leads to tumor cell growth and resistance of apoptosis [45].

3.3 Genes and epigenetic modifiers

FLT3 is normally expressed by hematopoietic stem and progenitor cells, but in AML acts as a protooncogene that stimulates cell proliferation, differentiation, and survival. In approximately 30% de novo AML patients mutations in FLT3 gene emerge. There are two types of mutations, that is, internal tandem duplication (ITD), which is present in about 25% of cases with adverse prognostic impact, and the tyrosine kinase domain (TKD) in about 5%, which prognostic value remains disputable [46]. According to the 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia, the potent significance of the FLT3-ITD mutation depends on the allelic ratio and the presence of NPM1 (nucleophosmin) gene mutations [47]. Published data demonstrated that FLT3-ITD mutation can constitutively activate the receptor and force uncontrollable cell proliferaton, which turns leukemic cells resistant to conventional chemotherapeutic agents [48, 49]. In addition, authors suggested that the mechanisms of drug resistance consist of clonal evolution of resistant leukemic cells, adaptive cellular mechanisms and a protective leukemic microenvironment [50]. FLT3-inhibitors the relapse may occur due to leukemic cells harboring FLT3-TKD mutations or non-FLT3 clones, carrying epigenetic mutations such as IDH1/2, ASXL1, or TET2 [50]. By the advanced methods of whole genome or exome sequencing, several epigenetic modifiers have been determined in AML, regulating DNA methylation (DNMT3A, IDH1/2, TET2) and histone modification (EZH2 and ASXL1) [51]. Research data suggest that epigenetics-modifying gene mutations promote genetic instability and induce FLT3-ITD, leading to drug resistance and relapse [52]. However, the prognostic impact and the precise contribution of these genes to leukemogenesis have not been fully elucidated yet [53].

3.4 microRNA

MicroRNAs are small, 19–24 nucleotide-long, non-coding single-stranded RNAs which play a key role in the control of the expression of several genes involved in the differentiation of hematopoietic stem cells and the development of cancers [54, 55]. The impaired regulation of microRNAs may contribute to the chemoresistance of tumor cells by affecting cell survival and apoptosis-related signaling pathways [56]. Research efforts in the last decade have demonstrated the unquestionable role of microRNAs in reversing drug resistance. However, their implementation into clinical practice is hampered by the inability to ensure sufficient safe and specific entry into tumor cells and further studies are needed [56].

3.5 Tumor microenvironment

The bone marrow microenvironment supports normal hematopoiesis through signaling cascades and affects the evolution, progression, and chemotherapy resistance

of AML [57]. The bone marrow consists of two distinct niches, that is, the osteoblastic (endosteal) and the vascular (endothelial), which act synergistically in order to regulate cell self-renewal, proliferation, and differentiation [58]. Hematopoietic stem cells are maintained by stem cell factor (SCF), CXCL12 (C-X-C Motif Chemokine Receptor 4), Notch ligands, and transforming growth factor-β [59]. Mesenchymal stromal cells secrete SCF and CXCL12, which regulate leukocyte migration [60]. The binding of CXCL12 to its receptor CXCR4 initiates the phosphorylation of CXCR4 and activates prosurvival signaling pathways such as MEK/ERK, JAK/STAT, and PI3K/AKT cascades [58]. Of note, CXCR4 signaling is associated with increased retention to the bone marrow, enhanced chemoresistance of leukemic cells, and poor prognosis in AML [61]. The interaction between the very late antigen-4 (VLA-4) and fibronectin take part in chemokine-mediated homing and mobilization [62]. The adhesion receptor VLA-4 binds to the fibronectin and vascular cell adhesion molecule-1 (VCAM-1), resulting in the retention of the leukemic cells within the bone marrow niche [63]. Wang et al. observed that a higher level of expression of VLA-4 is characterized by poorer survival [58]. The surface marker CD44 is a glycoprotein, that mediates cell adhesion, migration, and homing of leukemic cells [64, 65]. The antigen CD44 is expressed on both hematopoietic and leukemic cells, binding to E-selectin and L-selectin. The inhibition of E-selectin strengthens the influence of the chemotherapeutics daunorubicin and cytarabine [66], and lowers the leukemia burden [57]. As components of the microenvironment have been shown to contribute to drug resistance in AML, novel targeted therapies have been advanced in order to overcome it.

4. Conclusion

The development of drug resistance has emerged as an insurmountable challenge in the treatment of patients with R/R AML. The incompletely understood molecular mechanisms which cause therapeutic failure remain as a major obstacle to the long-term success of leukemic therapy, inferior prognosis, and reduced survival. Further investigations are needed to delineate more precise, genomic-guided, individualized clinical approaches.

Acknowledgements

This work was carried out in collaboration among all authors. All of them read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study and in the writing of the manuscript.

Acronyms and abbreviations

AML	acute myeloid leukemia
ASXL1	additional sex combs-like 1

ATP	adenosine triphosphate
CDK	cyclin-dependent kinase
CR	complete remission
CRi	incomplete hematologic recovery
CXCR4	C-X-C motif chemokine receptor 4
DFS	disease-free survival
DNMT3A	DNA methyltransferase 3A
ELN	European LeukemiaNet
EZH2	enhancer of zeste homolog 2
FAB	French-American-British
FGF2	fibroblast growth factor 2
FGFR1	fibroblast growth factor receptor 1
FLT3	FMS-like tyrosine kinase 3
GST	glutathione S-transferase
IDH1/2	isocitrate dehydrogenase 1/2
ITD	internal tandem duplication
LRP	lung resistance protein
MCL-1	myeloid cell leukemia 1
MDR	multidrug resistance
MRP1	multidrug resistance-related protein 1
mTOR	mammalian target of rapamycin
NF/kB	nuclear factor-kappa-light-chain-enhancer of activated B cells
NPM1	nucleophosmin
P-gp	P-glycoprotein
PI3K	phosphatidylinositol-3-kinase
PKC	protein kinase C
R/R AML	relapsed and refractory acute myeloid leukemia
RNA	ribonucleic acid
SCF	stem cell factor
SDF-1	stromal cell-derived factor 1
TET2	ten eleven translocation methylcytosine dioxygenase 2
TKD	tyrosine kinase domain
Topo II	topoisomerase II
VCAM-1	vascular cell adhesion molecule-1
VLA-4	very late antigen-4
WHO	World Health Organization

Author details

Yanitsa Davidkova[1*], Milan Jagurinoski[1], Gueorgui Balatzenko[2] and Margarita Guenova[1]

1 Laboratory of Hemathopathology and Immunology, National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

2 Laboratory of Cytogenetics and Molecular Biology, National Specialized Hospital for Active Treatment of Hematological Diseases, Sofia, Bulgaria

*Address all correspondence to: yani.tihomirova@gmail.com

IntechOpen

References

[1] Thein MS, Ershler WB, Jemal A, Yates JW, Baer MR. Outcome of older patients with acute myeloid leukemia: An analysis of SEER data over 3 decades. Cancer. 2013;**119**(15):2720-2727

[2] Juliusson G, Antunovic P, Derolf A, Lehmann S, Möllgård L, Stockelberg D, et al. Age and acute myeloid leukemia: Real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. Blood. 2009;**113**(18):4179-4187

[3] Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;**129**(4):424-447

[4] Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. The New England Journal of Medicine. 17 Sep 2015;**373**(12):1136-1152. DOI: 10.1056/NEJMra1406184. PMID: 26376137

[5] Eleni LD, Nicholas ZC, Alexandros S. Challenges in treating older patients with acute myeloid leukemia. Journal of Oncology. 2010;**2010**:943823

[6] Koenig K, Mims A. Relapsed or primary refractory AML: Moving past MEC and FLAG-ida. Current Opinion in Hematology. 2020;**27**(2):108-114

[7] Thol F, Schlenk RF, Heuser M, Ganser A. How I treat refractory and early relapsed acute myeloid leukemia. Blood. 2015;**126**(3):319-327

[8] Ferguson P, Hills RK, Grech A, Betteridge S, Kjeldsen L, Dennis M, et al. An operational definition of primary refractory acute myeloid leukemia allowing early identification of patients who may benefit from allogeneic stem cell transplantation. Haematologica. 2016;**101**(11):1351-1358

[9] Vosberg S, Greif PA. Clonal evolution of acute myeloid leukemia from diagnosis to relapse. Genes, Chromosomes & Cancer. 2019;**58**(12):839-849

[10] Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood. 1 Sep 2002;**100**(5):1532-1542. DOI: 10.1182/blood-2002-02-0492 PMID: 12176867

[11] Naoe T, Kiyoi H. Gene mutations of acute myeloid leukemia in the genome era. International Journal of Hematology. Feb 2013;**97**(2):165-174. DOI: 10.1007/s12185-013-1257-4 Epub 2013 Jan 29. PMID: 23359299

[12] Steensma DP. Clinical consequences of clonal hematopoiesis of indeterminate potential. Blood Advances. 27 Nov 2018;**2**(22):3404-3410. DOI: 10.1182/bloodadvances.2018020222 PMID: 30482770; PMCID: PMC6258914

[13] Gebhard C, Glatz D, Schwarzfischer L, Wimmer J, Stasik S, Nuetzel M, et al. Profiling of aberrant DNA methylation in acute myeloid leukemia reveals subclasses of CG-rich regions with epigenetic or genetic association. Leukemia. Jan 2019;**33**(1):26-36. DOI: 10.1038/s41375-018-0165-2 Epub 2018 Jun 20. PMID: 29925905

[14] Fisher JN, Kalleda N, Stavropoulou V, Schwaller J. The impact of the cellular origin in acute myeloid leukemia: Learning from mouse models. HemaSphere. 2019;**3**(1):e152

[15] Gruszka AM, Valli D, Alcalay M. Understanding the molecular basis

of acute myeloid leukemias: Where are we now? International Journal of Hematologic Oncology. 2017;**6**(2):43-53. DOI: 10.2217/ijh-2017-0002

[16] Grove CS, Vassiliou GS. Acute myeloid leukaemia: A paradigm for the clonal evolution of cancer? Disease Models & Mechanisms. 2014;**7**(8):941-951. DOI: 10.1242/dmm.015974

[17] Thol F, Heuser M. Treatment for relapsed/refractory acute myeloid leukemia. Hema. 1 Jun 2021;**5**(6):e572. DOI: 10.1097/HS9.0000000000000572 PMID: 34095756; PMCID: PMC8171365

[18] Brandwein JM, Saini L, Geddes MN, Yusuf D, Liu F, Schwann K, et al. Outcomes of patients with relapsed or refractory acute myeloid leukemia: A population-based real-world study. American Journal of Blood Research. 25 Aug 2020;**10**(4):124-133 PMID: 32923092; PMCID: PMC7486485

[19] Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochimica et Biophysica Acta. 11 Nov 1976;**455**(1):152-162. DOI: 10.1016/0005-2736(76)90160-7 PMID: 990323

[20] Zeng DF, Zhang J, Zhu LD, Kong PY, Li JP, Zhang X, et al. Analysis of drug resistance-associated proteins expressions of patients with the recurrent of acute leukemia via protein microarray technology. European Review for Medical and Pharmacological Sciences. 2014;**18**(4):537-543 PMID: 24615181

[21] van den Heuvel-Eibrink MM, Wiemer EA, de Boevere MJ, van der Holt B, Vossebeld PJ, Pieters R, et al. MDR1 gene-related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia. Blood. 1 Jun 2001;**97**(11):3605-3611. DOI: 10.1182/blood.v97.11.3605. PMID: 11369657.

[22] Zhang J, Gu Y, Chen B. Mechanisms of drug resistance in acute myeloid leukemia. Oncotargets and Therapy. 11 Mar 2019;**12**:1937-1945. DOI: 10.2147/OTT.S191621 PMID: 30881045; PMCID: PMC6417008

[23] van der Kolk DM, de Vries EG, van Putten WJ, Verdonck LF, Ossenkoppele GJ, Verhoef GE, et al. P-glycoprotein and multidrug resistance protein activities in relation to treatment outcome in acute myeloid leukemia. Clinical Cancer Research. Aug 2000;**6**(8):3205-3214 PMID: 10955805

[24] van der Kolk DM, de Vries EG, Noordhoek L, van den Berg E, van der Pol MA, Müller M, et al. Activity and expression of the multidrug resistance proteins P-glycoprotein, MRP1, MRP2, MRP3 and MRP5 in de novo and relapsed acute myeloid leukemia. Leukemia. Oct 2001;**15**(10):1544-1553. DOI: 10.1038/sj.leu.2402236 PMID: 11587212

[25] Broxterman HJ, Sonneveld P, van Putten WJ, Lankelma J, Eekman CA, Ossenkoppele GJ, et al. P-glycoprotein in primary acute myeloid leukemia and treatment outcome of idarubicin/cytosine arabinoside-based induction therapy. Leukemia. Jun 2000;**14**(6):1018-1024. DOI: 10.1038/sj.leu.2401796 PMID: 10865967

[26] da Silveira Júnior LS, Soares VL, Jardim da Silva AS, Gil EA, Pereira de Araújo MDG, Merces Gonçalves CA, et al. P-glycoprotein and multidrug resistance-associated protein-1 expression in acute myeloid leukemia: Biological and prognosis implications. International Journal of Laboratory Hematology. Oct 2020;**42**(5):594-603. DOI: 10.1111/ijlh.13241 Epub 2020 May 26. PMID: 32452631

[27] Do JH, Oh SH, Song EJ, Chung JS, Kang CD, Lee EY. Treatment outcome of multidrug resistance related mRNA expression and c-jun-N-terminal kinase activity in patients with acute myeloid leukemia. The Korean Journal of Laboratory Medicine. Aug 2007;**27**(4): 229-236. Korean. DOI: 10.3343/kjlm.2007.27.4.229. PMID: 18094581

[28] Legrand O, Simonin G, Beauchamp-Nicoud A, Zittoun R, Marie JP. Simultaneous activity of MRP1 and Pgp is correlated with in vitro resistance to daunorubicin and with in vivo resistance in adult acute myeloid leukemia. Blood. 1 Aug 1999;**94**(3):1046-1056 PMID: 10419897

[29] Funato T, Harigae H, Abe S, Sasaki T. Assessment of drug resistance in acute myeloid leukemia. Expert Review of Molecular Diagnostics. Sep 2004;**4**(5):705-713. DOI: 10.1586/14737159.4.5.705 PMID: 15347263

[30] Kulsoom B, Shamsi TS, Afsar NA. Lung resistance-related protein (LRP) predicts favorable therapeutic outcome in acute myeloid leukemia. Scientific Reports. 23 Jan 2019;**9**(1):378. DOI: 10.1038/s41598-018-36780-8 PMID: 30674943; PMCID: PMC6344578

[31] Scheffer GL, Wijngaard PL, Flens MJ, Izquierdo MA, Slovak ML, Pinedo HM, et al. The drug resistance-related protein LRP is the human major vault protein. Nature Medicine. Jun 1995;**1**(6):578-582. DOI: 10.1038/nm0695-578 PMID: 7585126

[32] Voso MT, D'Alo' F, Putzulu R, Mele L, Scardocci A, Chiusolo P, et al. Negative prognostic value of glutathione S-transferase (GSTM1 and GSTT1) deletions in adult acute myeloid leukemia. Blood. 15 Oct 2002;**100**(8):2703-2707. DOI: 10.1182/blood.V100.8.2703 PMID: 12351375

[33] Sargent JM, Williamson C, Hall AG, Elgie AW, Taylor CG. Evidence for the involvement of the glutathione pathway in drug resistance in AML. Advances in Experimental Medicine and Biology. 1999;**457**:205-209. DOI: 10.1007/978-1-4615-4811-9_22. PMID: 10500795

[34] Karwicka E. Role of glutathione in the multidrug resistance in cancer. Advances in Cell Biology. 2010;**2**(3):105-124. DOI: 10.2478/v10052-010-0006-6

[35] Pei S, Minhajuddin M, Callahan KP, Balys M, Ashton JM, Neering SJ, et al. Targeting aberrant glutathione metabolism to eradicate human acute myelogenous leukemia cells. The Journal of Biological Chemistry. 22 Nov 2013;**288**(47):33542-33558. DOI: 10.1074/jbc.M113.511170 Epub 2013 Oct 2. PMID: 24089526; PMCID: PMC3837103

[36] Cowell IG, Austin CA. Mechanism of generation of therapy related leukemia in response to anti-topoisomerase II agents. International Journal of Environmental Research and Public Health. Jun 2012;**9**(6):2075-2091. DOI: 10.3390/ijerph9062075 Epub 2012 May 31. PMID: 22829791; PMCID: PMC3397365

[37] Beck WT, Morgan SE, Mo YY, Bhat UG. Tumor cell resistance to DNA topoisomerase II inhibitors: New developments. Drug Resistance Updates. Dec 1999;**2**(6): 382-389. DOI: 10.1054/drup.1999.0110. PMID: 11498354

[38] Okada Y, Tosaka A, Nimura Y, Kikuchi A, Yoshida S, Suzuki M. Atypical multidrug resistance may be associated with catalytically active mutants of human DNA topoisomerase II alpha. Gene. 11 Jul 2001;**272**(1-2):141-148. DOI: 10.1016/s0378-1119(01)00554-6 PMID: 11470519

[39] Nicholson R, Menezes AC, Azevedo A, Leckenby A, Davies S,

Seedhouse C, et al. Protein kinase C epsilon overexpression is associated with poor patient outcomes in AML and promotes daunorubicin resistance through p-glycoprotein-mediated drug efflux. Frontiers in Oncology. 30 May 2022;**12**:840046. DOI: 10.3389/fonc.2022.840046 PMID: 35707351; PMCID: PMC9191576

[40] Shtil AA, Ktitorova OV, Kakpakova ES, Holian O. Differential effects of the MDR1 (multidrug resistance) gene-activating agents on protein kinase C: Evidence for redundancy of mechanisms of acquired MDR in leukemia cells. Leukemia & Lymphoma. Dec 2000;**40**(1-2):191-195. DOI: 10.3109/10428190009054896 PMID: 11426620

[41] Carter BZ, Mak PY, Tao W, Warmoes M, Lorenzi PL, Mak D, et al. Targeting MCL-1 dysregulates cell metabolism and leukemia-stroma interactions and resensitizes acute myeloid leukemia to BCL-2 inhibition. Haematologica. 1 Jan 2022;**107**(1):58-76. DOI: 10.3324/haematol.2020.260331 PMID: 33353284; PMCID: PMC8719086

[42] Wang H, Guo M, Wei H, Chen Y. Targeting MCL-1 in cancer: Current status and perspectives. Journal of Hematology & Oncology. 21 Apr 2021;**14**(1):67. DOI: 10.1186/s13045-021-01079-1 PMID: 33883020; PMCID: PMC8061042

[43] Tibes R, Bogenberger JM. Transcriptional silencing of MCL-1 through cyclin-dependent kinase inhibition in acute myeloid leukemia. Frontiers in Oncology. 12 Dec 2019;**9**:1205. DOI: 10.3389/fonc.2019.01205 PMID: 31921615; PMCID: PMC6920180

[44] Nogami A, Oshikawa G, Okada K, Fukutake S, Umezawa Y, Nagao T, et al. FLT3-ITD confers resistance to the PI3K/Akt pathway inhibitors by protecting the mTOR/4EBP1/Mcl-1 pathway through STAT5 activation in acute myeloid leukemia. Oncotarget. 20 Apr 2015;**6**(11):9189-9205. DOI: 10.18632/oncotarget.3279 PMID: 25826077; PMCID: PMC4496211

[45] Darici S, Alkhaldi H, Horne G, Jørgensen HG, Marmiroli S, Huang X. Targeting PI3K/Akt/mTOR in AML: Rationale and clinical evidence. Journal of Clinical Medicine. 11 Sep 2020;**9**(9):2934. DOI: 10.3390/jcm9092934 PMID: 32932888; PMCID: PMC7563273

[46] Daver N, Venugopal S, Ravandi F. FLT3 mutated acute myeloid leukemia: 2021 treatment algorithm. Blood Cancer Journal. 27 May 2021;**11**(5):104. DOI: 10.1038/s41408-021-00495-3 PMID: 34045454; PMCID: PMC8159924

[47] Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 27 May 2016;**127**(20):2391-2405. DOI: 10.1182/blood-2016-03-643544 Epub 2016 Apr 11. PMID: 27069254

[48] El Fakih R, Rasheed W, Hawsawi Y, Alsermani M, Hassanein M. Targeting FLT3 mutations in acute myeloid leukemia. Cell. 8 Jan 2018;7(1):4. DOI: 10.3390/cells7010004 PMID: 29316714; PMCID: PMC5789277

[49] Scholl S, Fleischmann M, Schnetzke U, Heidel FH. Molecular mechanisms of resistance to FLT3 inhibitors in acute myeloid leukemia: Ongoing challenges and future treatments. Cell. 17 Nov 2020;**9**(11):2493. DOI: 10.3390/cells9112493 PMID: 33212779; PMCID: PMC7697863

[50] Lam SSY, Leung AYH. Overcoming resistance to FLT3 inhibitors in the

treatment of *FLT3*-mutated AML. International Journal of Molecular Sciences. 24 Feb 2020;**21**(4):1537. DOI: 10.3390/ijms21041537 PMID: 32102366; PMCID: PMC7073218

[51] Venney D, Mohd-Sarip A, Mills KI. The impact of epigenetic modifications in myeloid malignancies. International Journal of Molecular Sciences. 9 May 2021;**22**(9):5013. DOI: 10.3390/ijms22095013 PMID: 34065087; PMCID: PMC8125972

[52] Wakita S, Yamaguchi H, Omori I, Terada K, Ueda T, Manabe E, et al. Mutations of the epigenetics-modifying gene (DNMT3a, TET2, IDH1/2) at diagnosis may induce FLT3-ITD at relapse in de novo acute myeloid leukemia. Leukemia. Apr 2013;**27**(5):1044-1052. DOI: 10.1038/leu.2012.317 Epub 2012 Nov 8. PMID: 23135354

[53] Conway O'Brien E, Prideaux S, Chevassut T. The epigenetic landscape of acute myeloid leukemia. Advances in Hematology. 2014;**2014**:103175. DOI: 10.1155/2014/103175 Epub 2014 Mar 23. PMID: 24778653; PMCID: PMC3980839

[54] Gabra MM, Salmena L. microRNAs and acute myeloid leukemia chemoresistance: A mechanistic overview. Frontiers in Oncology. 30 Oct 2017;**7**:255. DOI: 10.3389/fonc.2017.00255 PMID: 29164055; PMCID: PMC5674931

[55] Tutar Y. miRNA and cancer; computational and experimental approaches. Current Pharmaceutical Biotechnology. 2014;**15**(5):429. DOI: 10.2174/138920101505140828161335 PMID: 25189575

[56] Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. Clinical Epigenetics. 11 Feb 2019;**11**(1):25. DOI: 10.1186/s13148-018-0587-8 PMID: 30744689; PMCID: PMC6371621

[57] Rashidi A, Uy GL. Targeting the microenvironment in acute myeloid leukemia. Current Hematologic Malignancy Reports. Jun 2015;**10**(2):126-131. DOI: 10.1007/s11899-015-0255-4 PMID: 25921388; PMCID: PMC4447552

[58] Wang A, Zhong H. Roles of the bone marrow niche in hematopoiesis, leukemogenesis, and chemotherapy resistance in acute myeloid leukemia. Hematology. Dec 2018;**23**(10):729-739. DOI: 10.1080/10245332.2018.1486064 Epub 2018 Jun 14. PMID: 29902132

[59] Shin DY. Human acute myeloid leukemia stem cells: Evolution of concept. Blood Research. 30 Apr 2022;**57**(S1):67-74. DOI: 10.5045/br.2022.2021221 PMID: 35483929; PMCID: PMC9057671

[60] Menter T, Tzankov A. Tumor microenvironment in acute myeloid leukemia: Adjusting niches. Frontiers in Immunology. 22 Feb 2022;**13**:811144. DOI: 10.3389/fimmu.2022.811144 PMID: 35273598; PMCID: PMC8901718

[61] Ahn JY, Seo K, Weinberg OK, Arber DA. The prognostic value of CXCR4 in acute myeloid leukemia. Applied Immunohistochemistry & Molecular Morphology. Jan 2013;**21**(1):79-84. DOI: 10.1097/PAI.0b013e3182606f4d PMID: 22914607

[62] Matsunaga T, Takemoto N, Sato T, Takimoto R, Tanaka I, Fujimi A, Akiyama T, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. Nature Medicine. Sep 2003;**9**(9):1158-1165. doi: 10.1038/nm909. Epub 2003 Aug 3. Erratum in:

Nat Med. 2005 May;11(5):578. PMID: 12897778

[63] Becker PS, Kopecky KJ, Wilks AN, Chien S, Harlan JM, Willman CL, et al. Very late antigen-4 function of myeloblasts correlates with improved overall survival for patients with acute myeloid leukemia. Blood. 22 Jan 2009;**113**(4):866-874. DOI: 10.1182/blood-2007-12-124818 Epub 2008 Oct 16. PMID: 18927435; PMCID: PMC2630271

[64] Quéré R, Andradottir S, Brun AC, Zubarev RA, Karlsson G, Olsson K, et al. High levels of the adhesion molecule CD44 on leukemic cells generate acute myeloid leukemia relapse after withdrawal of the initial transforming event. Leukemia. Mar 2011;**25**(3):515-526. DOI: 10.1038/leu.2010.281 Epub 2010 Nov 30. PMID: 21116281; PMCID: PMC3072510

[65] Yu X, Munoz-Sagredo L, Streule K, Muschong P, Bayer E, Walter RJ, et al. CD44 loss of function sensitizes AML cells to the BCL-2 inhibitor venetoclax by decreasing CXCL12-driven survival cues. Blood. 23 Sep 2021;**138**(12):1067-1080. DOI: 10.1182/blood.2020006343 PMID: 34115113

[66] Bolandi SM, Pakjoo M, Beigi P, Kiani M, Allahgholipour A, Goudarzi N, et al. A role for the bone marrow microenvironment in drug resistance of acute myeloid leukemia. Cell. 21 Oct 2021;**10**(11):2833. DOI: 10.3390/cells10112833 PMID: 34831055; PMCID: PMC8616250

Section 2

Clinical Presentation

Chapter 4

Chronic Myeloid Leukemia: Biology, Diagnosis, and Management

Biswajit Bhuyan, Somanath Padhi, Probodha Kumar Das and Chinmayee Panigrahi

Abstract

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm characterized by florid myelo-megakaryocytic proliferation involving peripheral blood, bone marrow, and spleen. These results are due to balanced reciprocal translocation between long arm of chromosome 9 and 22 that produces a truncated chromosome 22 (Philadelphia chromosome) leading to fusion of *BCR-ABL$_1$* genes causing enhanced autonomous activation of tyrosine kinase and downstream cellular proliferation pathway. While targeted therapy with novel tyrosine kinase inhibitors (TKI) has revolutionized the outcome in such patients, occurrence of additional cytogenetic abnormalities, emergence of TKI resistance, and idiosyncratic marrow suppression following higher generation TKI therapy have posed newer management challenges in CML. This chapter is aimed to highlight the recent updates in the disease biology, stepwise diagnostic work-up, and management guidelines in CML with a brief highlight on the prospect of stem cell transplantation in such condition.

Keywords: Ph chromosome, cytogenetics, drug resistance, TKI therapy, bone marrow

1. Introduction

Chronic myeloid leukemia (CML), *BCR-ABL$_1$*-positive, is a clonal myeloproliferative neoplasm (MPN) of hematopoietic stem cell origin characterized by balanced reciprocal translocation between long arm of chromosome (Ch) 9 (q34) and 22 (q11.2) that results in the formation of a truncated Ch 22, so-called Philadelphia (Ph) chromosome. This chromosomal alteration juxtaposes the breakpoint cluster region (*BCR*) gene on chromosome 22 with the *Abelson Murine Leukemia* (*ABL*) proto-oncogene-$_1$derived from Ch 9 producing a characteristic *BCR-ABL$_1$* fusion gene that encodes *BCR-ABL* oncoprotein. The product of such fusion is a 210-kilo Dalton (KD) (p210 *BCR-ABL$_1$*), which causes ligand-independent activation of receptor tyrosine kinase protein producing downstream activation of intracellular pathways with uncontrolled proliferation of maturing myeloid and megakaryocyte lineage, so characteristic of this entity [1].

2. Epidemiology

CML is an uncommon disease with various European registries showing a crude annual incidence of 0.7–1.0 per 100,000 population. The median age at diagnosis is the fifth to sixth decades of life in the Western population; however, in one series of 430 patients, 65% of patients were between 20 and 40 years of age. As compared with Western population, the median age of presentation of CML is one to two decades lower in Indian and Asian population. The incidence of CML in developing countries is low (age-adjusted rate (AAR) =0.71 in males and 0.53 per 100,000 in females) compared with the USA and other developed countries (AAR = 2.0 in males and 1.1 per 100,000 in females). CML is reported to be the most common adult leukemia among Indian subjects with an incidence of 30–60% in comparison to the Western population (15%); the highest prevalence reported from Patna, Bihar (70%), and lowest (17%) from Gujarat [2, 3].

3. Historical perspective (1840: 2021)

A brief summary of the sequence of events in the CML is presented in **Table 1** [4, 5].

Year	Events	Authors
1840	The first leukemia to be described in autopsy studies	Craigie, Bennet, and Virchow
1845	White blood cell leukemia	Virchow
19th Century	"Thick blood" secondary to hyperleukocytosis	Alfred Velpeau
1960	Discovery of Ph chromosome (truncated Ch 22) by cytogenetics testing. First time that a malignancy was linked to a chromosomal abnormality.	Hungerford and Nowell
1970	Identification of the ABL gene in a murine virus and humans	Abelson and Rabstein
1973	t (9, 22) (q34; q11) as the mechanism of Ph chromosome formation	Janet Rowley
1977	CML: a neoplasm of clonal stem cell origin	Fialkow, Jacobson, Papayannopoulou
1984–1990	Breakpoint clustered region (BCR) on Ch 22 and demonstration of BCR-ABL1 fusion in leukemogenesis	Groffen and colleagues
1998	The first clinical therapy using TKI in leukemias	Druker and Lydon
2001	First TKI (Imatinib)	US-FDA
2006–2008	Second-generation TKI (Dasatinib, Nilotinib)	US-FDA
2012	Bosutinib and ponatinib	US-FDA
2021	Asiminib in resistant CML	US-FDA

Table 1.
Historical perspective in chronic myeloid leukemia (1840–2021).

4. Genetics and molecular biology in CML

The breakpoints in CML are located within the so-called Major – Breakpoint Cluster Region [M-bcr], which consists of two intronic regions (intron 13 and intron 14). The commonest *BCR-ABL*$_1$ fusion transcript is from a breakpoint in exon 13 or exon 14 (also known as b2 or b3) in the *BCR* gene on Ch 22, which is fused to the *ABL*$_1$ proto-oncogene at exon a2 (e13a2) (b2a2), (e14a2) (b3a2) on Ch 9. Minority of patients may express atypical transcripts usually due to splicing of alternate *BCR* or *ABL*$_1$ exons. A fusion gene leads to expression of a 210-kilo Dalton (KD) molecular weight **e1a2** transcript, which codes for conventional CML but frequently found in Ph-positive acute lymphoblastic leukemia (ALL) (molecular weight; 190 KD). Less frequently **e19a2** fusion transcript may be found producing a large 230 KD protein found in chronic neutrophilic leukemia (CNL) and rarely in CML. In 2–10% of cases of CML, a variant chromosomal translocation may be found. The 22q11 segment is translocated on chromosome other than nine in simple variant translocations; while in complex variant translocation, there is a third translocation t (9; 22; V), where V is variable partner chromosome. Additional cytogenetic abnormalities (ACA) have been described to occur in 5–10% of CML patients in chronic phase, although same may be present in 30–80% of cases in advanced phase of the disease. Major route abnormalities include trisomy 8, double Ph chromosome, isochromosome 17 (i17), + der (22), while minor route ACA, which are less common include trisomy 21, t (3;12), t (4,6), t (2,16), and t (1,21) [6–8]. In 2–10% patients, a *BCR-ABL*$_1$ rearrangement could be found by Fluorescence in Situ Hybridization (FISH) and/or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in the absence of a Ph-chromosome by means of conventional cytogenetics (so-called cryptic or masked translocations).

The p210 KD protein results in dysregulated tyrosine kinase expression and ligand-independent activation of the downstream intracellular pathways. Major mechanism

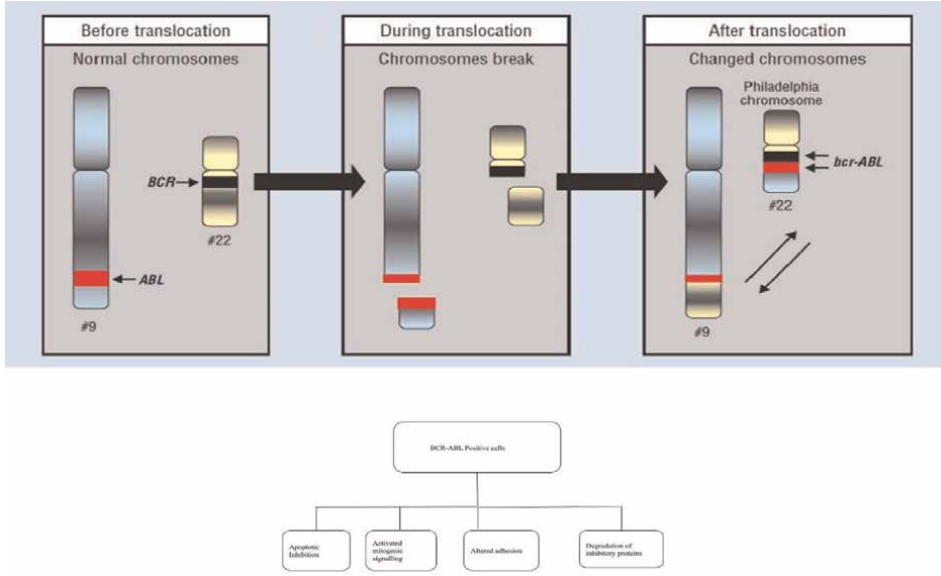

Figure 1.
Molecular event in chronic myeloid leukemia and downstream pathogenetic events.

that has been postulated in pathogenesis of CML includes: a) adhesion to stromal cells and extracellular matrix (ECM), b) constant state of mitogenic activation, c) inhibition of apoptosis, and d) proteasomal degradation of *BCR-ABL*$_1$ inhibitory proteins. (**Figure 1**). Moreover, *BCR-ABL*$_1$ fusion activates the PI3-AKT pathway, which in turn phosphorylates the FOXO transcription factor causing cell cycle arrest and leukemogenesis and along with TGF-β signaling pathway, it has a significant role in survival of leukemic stem cells. Besides these, activation of transcriptional factors such as STAT1 and STAT5 (signal transducer and activation of transcription) contributes to survival advantage and cytokine independency. Other pathways that cause disruption of key cellular process include RAS-mitogen-activated protein kinase (RAS-MAPK pathway) leading to increased proliferation, MYC overexpression, and alteration of Hedgehog signaling pathway [9–12].

5. Clinical presentation

The clinical signs and symptoms depend upon the phase of the disease. During the chronic phase (CP), systemic symptoms such as fatigue, weight loss, diaphoresis, abdominal pain/fullness, and bleeding episodes due to platelet dysfunction are commonly reported to occur in 34%, 20%, 15%, 15%, and 21% of subjects, respectively (25). Rare manifestations such as thrombosis, gouty arthritis (secondary to hyperuricemia), or avascular necrosis of neck of femur and/or priapism (due to hyperleukocytosis) may be present at diagnosis. On the other hand, a significant proportion (up to 50%) of patients may be asymptomatic and detected to have CML during a routine medical examination (26). Compared with males, females are of older age at presentation, have lower hemoglobin, higher platelet counts, and smaller spleen size. Younger adults (<30 years) and elderly patients (>59 years) have higher incidence of splenomegaly than adults (30–59 years old) (27, 28). In accelerated phase (AP) or blast crisis, there may be involvement of extra medullary sites and onset of lymphadenopathy, the latter may point to a lymphoblastic blast crisis [2, 13, 14].

6. Laboratory diagnosis

Recommended diagnostic work-up in CML is summarized in **Table 2** [1, 15–17].

6.1 Complete blood cell count

The most common routine of hematological findings of CML in chronic phase is moderate anemia and an elevated total leukocyte count (TLC) usually above 25×10^9/L (ref. 4 to 11 x 10^9/L) and frequently above 100×10^9/L; and associated normal or even increased platelet count above 500 x 10^9/L (ref. 150 to 450 x 10^9/L); or even thrombocytopenia ($< 100 \times 10^9$/L). The TLC shows granulocytes in all stages of maturation from blast to mature granulocytes with *dual bulge* of myelocyte-metamyelocyte and stab-segment forms forming a "*shoe sox like pattern*" in the complete blood count autoanalyzer WDF scatterplot and up to 2% myeloid blast in a differential count. Basophilia and eosinophilia are common in the absence of significant granulocytic dysplasia and monocytosis (**Figure 2**). Degree of hyperleukocytosis correlates with spleen size in most of the cases.

Physical examination with special attention to spleen and liver size.
Complete blood count with comment on peripheral smear and differential count. Bone marrow aspirate cytology, cytogenetics, biopsy.
Chromosome banding analysis.
Fluorescence in-situ hybridization (FISH) only in case of Ph-negativity.
Reverse transcriptase polymerase chain reaction (PCR) qualitative for the detection of BCR-ABL1 transcripts and identification of the type of transcript.
Electrocardiogram (ECG)
Standard biochemical profile with Hepatitis B-serology.

Table 2.
Recommended diagnostic work-up of CML at baseline.

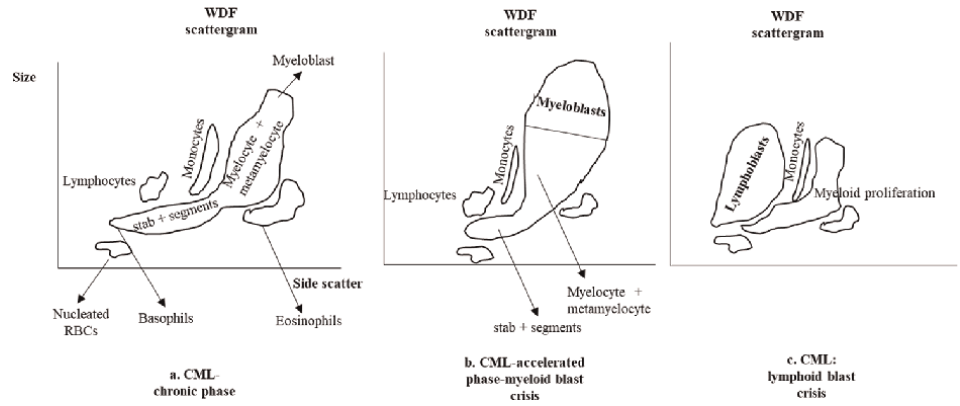

Figure 2.
*Schematic representation of white blood cell differential scattergram in chronic myeloid leukemia. Note the "*shoe sox like pattern*" depicting dual myeloid bulge (stab/segment and myelocyte/metamyelocyte) in chronic phage CML with no excess blast (a), expanded blast window (top) with depletion of maturing myeloid lineage in accelerated phase-myeloid blast crisis (b), and an expanded lymphoid population with increased size representing lymphoid blast crisis in panel c.*

6.2 Bone marrow aspirate

In chronic phase of the disease, bone marrow aspirate (BMA) may be easy or even difficult (hemodiluted, dry tap) as a result of marked hyperleukocytosis as well as associated reticulin fibrosis. Conventionally, BMA tends to be markedly hypercellular (cellularity approaching nearly 100%), with florid granulocytic hyperplasia at all stages of myeloid maturation with up to 09% myeloid blast and 19% basophils, and increased number of characteristic small megakaryocytes with monolobated/hypolobated nuclei (so-called "dwarf megakaryocytes"), which may even show loose *grape-like* clustering (myeloproliferative CML). Florid dwarf megakaryocytic proliferation associated with marked thrombocytosis at presentation (megakaryocyte rich CML) is also not uncommon, and this may be an indicator of disease progression (**Figure 3**). Aspirate may also reveal the presence of sea blue histiocytes (also called pseudo Gaucher cells). BMA is useful not only to confirm the stage of the disease but also characterize the blast phenotype in suspected blast crisis using flowcytometry as well as for submission of sample for cytogenetics and molecular testing [1].

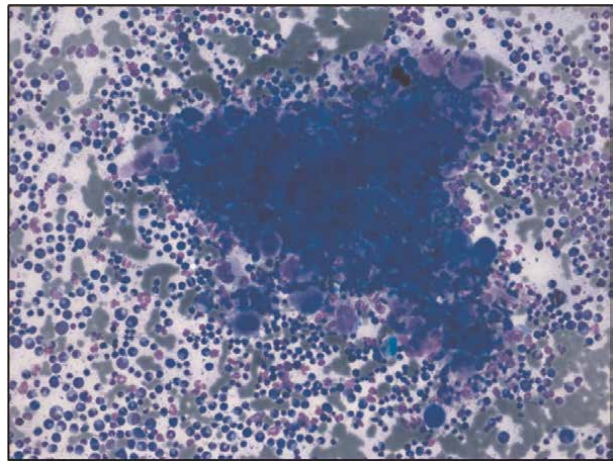

Figure 3.
Bone marrow aspirate smear from a case of chronic phase CML showing myeloid hyperplasia at all stages of maturation, suppressed erythroid islands, no excess blasts (<10% of marrow nucleated differential), and clusters of dwarf megakaryocytes giving a "bunch of grapes" *like appearance (May Grunewald Giemsa stain, x400).*

6.3 Bone marrow trephine biopsy

Although not required for diagnosis, the 2017 WHO classification guidelines suggest adequate trephine biopsy (BMBx) in cases of CML with atypical peripheral smear findings or in those with a difficulty in obtaining cellular marrow aspirates. Additionally, BMBx provides histological and topographic features, proliferation patterns, reticulin fibrosis, confirming the stage of the disease and ruling out disease progression through detection and characterization of *localized* blast collection by applying appropriate panel of antibodies such as CD 34, CD 117, myeloperoxidase, terminal deoxynucleotidyl transferase (TdT), CD 10, CD 19, CD 79a, PAX5, CD 3, CD 7, etc., using immunohistochemical (IHC) technique (**Figure 4**). Demonstration of florid dwarf megakaryocytic proliferation displacing other marrow elements in any of the marrow spaces and associated reticulin fibrosis demonstrated on BMBx is regarded as "presumptive evidence" of disease progression as per 2017 WHO guidelines. Also, the

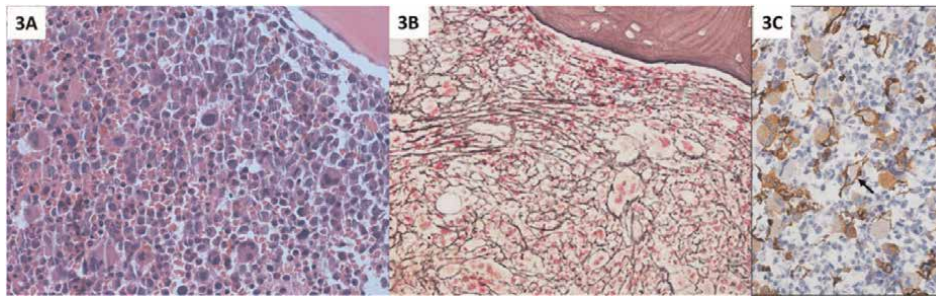

Figure 4.
Bone marrow trephine biopsy in a case of chronic phase CML showing marked hypercellularity (packed marrow) with myeloid hyperplasia, increased number of "dwarf megakaryocytes," and suppressed erythroid islands (a), and associated increased (MF 2) reticulin fibrosis (B). Also note the aberrant CD 34 positivity in dwarf megakaryocytes (C), which should not be interpreted as a feature of disease progression. Reproduced with permission from [18].

WHO criteria (2017) [1]	WHO criteria (2022) [15]
Chronic phase: • Peripheral blood shows leukocytosis (12—1000 x 10^9/L, median: • ~ 80 x 10^9/L); Blast <2% of WBCs • Bone marrow: <5% of marrow cells	Same as 2017
Accelerated phase: Presence of ≥1 of the following hematological/cytogenetic criteria **or** provisional criteria concerning response to tyrosine kinase inhibitor (TKI) therapy: • Persistent or increasing high white blood cell count (> 10 x 10^9/L), unresponsive to therapy • Persistent or increasing splenomegaly, unresponsive to therapy • Persistent thrombocytosis (> 1000 x 10^9/L), unresponsive to therapy • Persistent thrombocytopenia (< 100 x 10^9/L), unrelated to therapy • ≥ 20% basophils in the peripheral blood • 10–19% blasts in the peripheral blood and/or bone marrow • Sheets of dwarf megakaryocytic proliferation replacing proliferating myeloid elements in trephine biopsy (*provisional*) • Additional clonal chromosomal abnormalities in Philadelphia (Ph) chromosome-positive (Ph+) cells at diagnosis, including so-called major route abnormalities (a second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, and abnormalities of 3q26.2 • Any new clonal chromosomal abnormality in Ph + cells that occurs during therapy.	Accelerated phase is *omitted* in favor of an emphasis on high-risk features associated with chronic phase progression and *resistance* to tyrosine kinase inhibitor (TKI)
Blast phase: • ≥ 20% myeloid blasts in the blood or bone marrow **or** • presence of an extramedullary proliferation of blasts. • ≥ 10% lymphoid blasts either in peripheral blood and/or marrow	Blast phase: • ≥ 20% myeloid blasts in the blood or bone marrow. • The presence of an extramedullary proliferation of blasts. • The presence of increased lymphoblasts in peripheral blood or bone marrow.

Table 3.
Diagnostic criteria for CML: A comparison between 2017 [1] and 2022 WHO [15] guidelines.

WHO criteria mandates ≥20% myeloid blast either in PBS or BM to label it as myeloid blast crisis, the optimal cutoff of lymphoblasts and the significance of low level of B-lymphoblasts remain unclear, which warrant additional studies [1, 15]. Moreover, the BMBx is essential in the investigation of unexplained cytopenias during therapy [1, 19, 20].

The previous WHO criteria (2017) for CML have been recently revised in 2022 and are summarized in **Table 3**.

6.4 Conventional G-banded cytogenetics

Conventional metaphase GTG-banded cytogenetics, considered "gold standard in diagnosis," is an essential tool for the diagnosis, prognostication, and follow-up in CML. It is recommended that all patients of CML should have a cytogenetic analysis performed, preferably on BMA sample at diagnosis to look for the presence of

additional cytogenetic abnormalities (ACA) in addition to Philadelphia chromosome. It also helps in assessment of response to TKI therapy.

6.5 Fluorescence in-situ hybridization (FISH)

The drawbacks of conventional cytogenetics are that an invasive procedure is required and is unable to detect sub microscopic or complex chromosomal rearrangements and suboptimal for minimal residual disease (MRD) evaluation. The advantage of FISH is that such analysis can be performed on peripheral blood sample using dual-colored probe in case of dry tap aspirate; and this can be applicable to both interphase and metaphase nuclei. Another advantage is that it can reveal occult BCR-ABL1 genetic fusions that are masked by either an apparently normal or complex cytogenetic findings (**Figure 5**) [16].

6.6 Reverse-transcriptase polymerase chain reaction (RT-PCR)

Polymerase chain reaction (PCR) is a sensitive and specific method to detect Ph-positive cells by the amplification of BCR-ABL1 fusion transcripts. The PCR-based assay has superseded the conventional cytogenetic assessment because of its higher sensitivity and has become the method of choice for monitoring of CML patients on TKI therapy as per 2020 ELN guidelines recommendation. In few cases of CML, the fusion transcript may vary; therefore, a qualitative reverse transcriptase PCR (RT-PCR) on peripheral blood cells is essential at baseline to identify the type of BCR-ABL1 transcripts, which can be then followed up while assessing response to TKI therapy. Laboratories express the BCR-ABL1 transcripts on an international scale, which was developed to standardize levels of BCR-ABL1 RNA across various laboratories. The values at 3 months, 6 months, and 12 months are predictive of subsequent outcomes, which helps to categorize patient and risk of subsequent therapy failure [17].

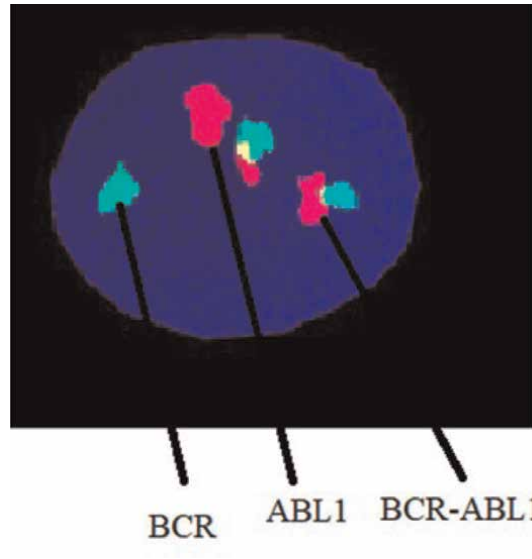

Figure 5.
FISH image depicting the fusion of red (ABL1) – green (BCR) signal representing BCR-ABL fusion gene.

7. Evolution of therapies in CML

Historically, the initial treatment for CML goes back to Fowler's solution, a 1% solution of arsenic trioxide, used in 1865. In the 1950s, chemotherapeutic agents such as busulfan and hydroxyurea became the main therapeutic options for several decades. These drugs could effectively control the TLC, but could not eliminate the leukemic clone nor altered the course of disease progression. The introduction of interferon-α (IFN-α) in the 1980s was a significant advance as it could induce hematologic and cytogenetic *remissions* and improve the overall survival. However, the potential benefits were masked by poor patient tolerance due to frequent and serious side effects. In a randomized trial of 513 CML patients comparing IFN-α vs. busulfan or hydroxyurea, a significant survival advantage was found in IFN-α treated patients over busulfan ($p = .008$) but not over hydroxyurea-treated patients ($p = .44$), thus establishing its superiority over chemotherapy; however, toxicities in IFN-α-treated group were higher than those of other two groups. Hematopoietic stem cell transplantation (HSCT) was an alternative to chemotherapy because of its curative potential; although its applicability was restricted to mainly young and fit patients with a matched donor and also it was associated with considerable morbidity and mortality, with overall 5-year overall survival of nearly 50% and relapse rates of 20% (**Figure 6**) [21, 22].

7.1 First-generation TKIs

The landmark discovery of underlying molecular events in CML paved the way for discovery of targeted therapies in 1996 using a modified 2-phenylaminopyrimidine,

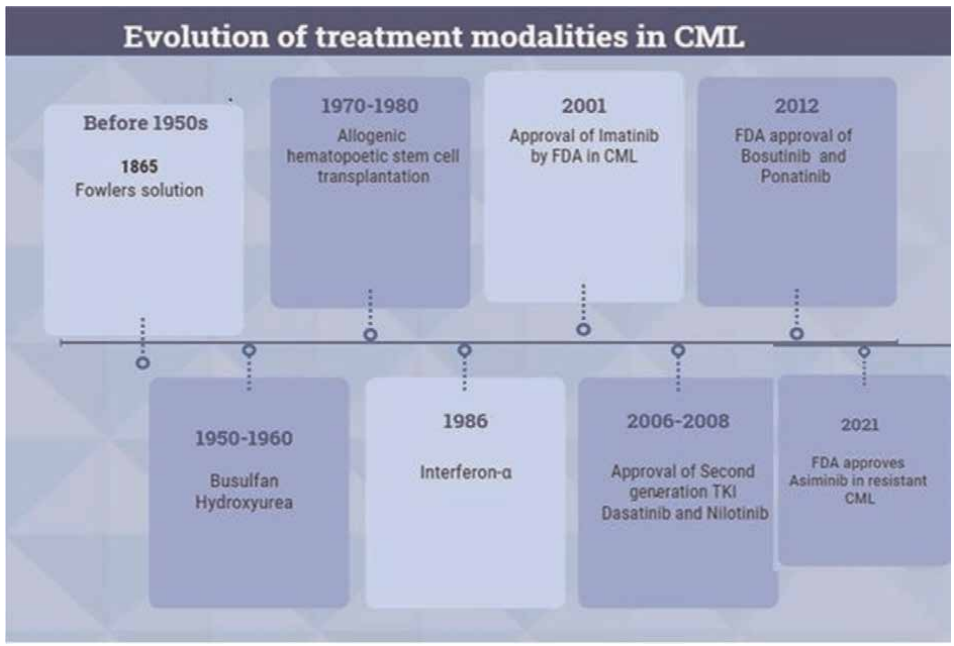

Figure 6.
Evolution of various therapies in CML over time [21].

which led to the successful introduction of Imatinib mesylate (also known as STI571, Gleevec) as an initial oral treatment for newly diagnosed CML patients [23]. The first clinical trial of Imatinib took place in 1998, and the drug received FDA approval in May 2001. Nicholas Lyndon, Brian Druker, and Charles Sawyers bagged the Lasker-DeBakey Clinical Medical Research Award in 2009 for "converting a fatal cancer into a manageable condition" and the Japan Prize in 2012 for their part in "the development of a new therapeutic drug targeting cancer-specific molecules." In 2003, the landmark International Randomized Study of Interferon and STI571 (IRIS) randomized trial on CML published their report comparing Imatinib with IFN-α and low-dose cytarabine, which demonstrated a high response rate to Imatinib as compared with IFN-α group. At a median follow-up of 19 months, rates of major and complete cytogenetics responses were superior in Imatinib group. Moreover, Imatinib was better tolerated than the combination therapy [24]. Long-term data from the IRIS trial show a 10-year overall survival of 83% and the estimated rate of freedom from progression to accelerated or blast crisis of 92.1%; and in approximately half the patients (48.3%) who had been randomly assigned to Imatinib completed study treatment with Imatinib, and 82.8% had a Complete Cytogenetic Response (CCR). Serious adverse events that were considered by the investigators to be related to imatinib were uncommon and most frequently occurred during the first year of treatment.

On the basis of safety and efficacy in phase 3 of IRIS trials, Imatinib at dose of 400 mg OD was the recommended dose in Ph + CML patients in chronic phase, 600 mg OD in accelerated phase, and in blast crisis, 260 mg/m^2 in children. A phase III trial evaluated 160 CML subjects (146 chronic phase, 7 accelerated phase, 7 blast crisis) consisting of children and adolescents (age range 1.3–18.0 years, median 13.2 years) reported PFS of 97% at a median follow up of 18 months and rates of CCR and MMR at 36 months to be 86% and 74% [25]. In the original trial, there was no maximum tolerated dose that was reached. Hence, researchers chose 400 mg OD because it was a relatively safe and biologically active dose. A higher starting dose of Imatinib (600 mg/800 mg) was considered in various trials to achieve deeper remissions. The Tyrosine Kinase Inhibitor Optimization and Selectivity (TOPS) trial evaluated high-dose (800 mg/day; n = 319) vs. standard-dose (400 mg/day, n = 157) Imatinib in frontline settings in newly diagnosed chronic myeloid leukemia in chronic phase. Rates of major molecular response (MMR), event-free survival (EFS), progression-free survival (PFS), and overall survival (OS) were similar between the two arms; however, patients with Imatinib 800 mg achieved faster MMR than with Imatinib 400 mg (8.3 vs. 10.0 month) at the expense of higher-grade 3/4 toxicities and serious adverse events (38.3% in 800 mg arm vs. 26.8% in standard-dose arm [26]. The starting dose of 400 mg once daily Imatinib continues to be the standard of care in newly diagnosed patients with CML in chronic phase based on efficacy and toxicity profile.

7.2 Mechanism of action of Imatinib

Imatinib binds to the BCR-ABL1 protein close to the ATP-binding site blocking ATP from binding and preventing a conformational switch from the inactive to active form. Without binding of ATP substrate, phosphorylation and subsequent signaling are inhibited, which in turn inhibits unregulated cell proliferation and survival (**Figure 7**) [27].

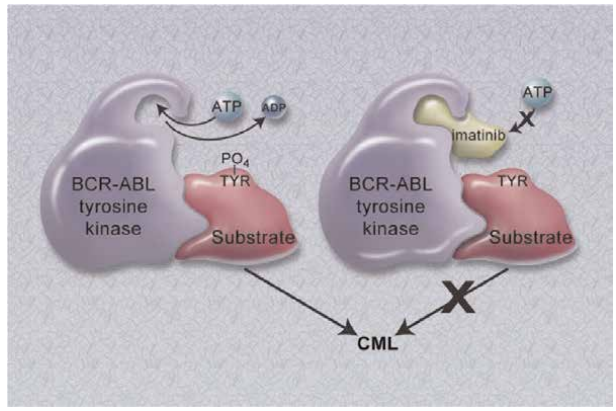

Figure 7.
Mechanism of action of Imatinib mesylate.

7.3 Second-generation TKIs: Guidelines and dosing

Second-generation tyrosine kinase inhibitors (Dasatinib, Nilotinib, and Bosutinib) are generally considered in setting of Imatinib resistance or intolerance to Imatinib. Recently, there has been increasing interest in use of in frontline setting. First-line phase 3 randomized registration studies of Dasatinib, Nilotinib, and Bosutinib vs. imatinib (DASISION17, ENESTnd18, and BFORE19, respectively, have observed the following: (a) more rapid and deep responses have been obtained in case of use of second generation TKI(2G-TKI); (b) Dasatinib and Nilotinib-treated patients develop fewer mutations conferring TKI resistance (40). Dasatinib in lower dose (50 mg OD) in frontline settings studied in 83 CML patients has shown to achieve CCR in 95% of patients and MMR in 81% of patients at a follow-up of 12 months [28]. Nilotinib is an imatinib analog with more specific BCR-ABL1 binding, Bosutinib and Dasatinib are dual SRC/ABL kinase inhibitors. Except T315I mutation Nilotinib, Dasatinib, Bosutinib are active against a large number of BCR-ABL1 kinase mutations [29].

7.4 Third-generation TKIs

Ponatinib is a pan BCR-ABL kinase inhibitor developed specifically to overcome resistance to T315I mutation. In December 2012, Ponatinib gained accelerated approval by the US FDA for T315I-positive CML. In the Ponatinib Ph + ALL and CML Evaluation (PACE) trial, among 267 heavily pretreated CML patients and those harboring T315I mutation, 56% had a major cytogenetic response (51% in patients with resistance or 2G-TKI intolerance and 70% in T315I mutated patients), 46% had a CCR (40% and 66% (T315I) in the two subgroups) and 34% had a MMR (27% and 56% in the two subgroups, respectively). Higher rates of vaso-occlusive events limit its widespread use [30, 31].

7.5 Asciminib

It is a novel drug and, the first-in-class STAMP (**S**pecifically **T**argeting the ***A****BL* **M**yristoyl **P**ocket) inhibiting ABL myristoyl pocket, which targets both native and mutated *BCR-ABL*$_1$, including the gatekeeper T315I mutant. In a trial of 141 heavily

pretreated patients with multiple lines of TKI major molecular response was achieved or maintained by 12 months in 48% of patients who could be evaluated [32].

7.6 Follow-up response assessment

Monitoring of response to TKI therapy is essential for assessing response to TKI and any disease progression. It involves assessment of hematologic, cytogenetic, and molecular responses (**Tables 4** and **5**). Complete blood count with differentials should be assessed every 2 weeks until a complete hematologic response is achieved or can be

Remission status	Definition
Complete hematologic response (CHR)	Platelet count $<450 \times 10^9$/L
	WBC count $<10 \times 10^9$/L
	Differentials: no immature granulocytes, basophils <5%
	Spleen not palpable
Cytogenetic response	
Complete (CCR)	No Ph + fusion positive signals
Partial (PCR)	1–35% Ph + fusion positive signals
Minor	36–65% Ph + fusion positive signals
Minimal	66–95% Ph + fusion positive signals
None	> 95% Ph + fusion positive signals
Molecular response	
Early molecular response (EMR)	Ratio of BCR-ABL1toABL1 $^{\leq 10\% \text{ on}}$ IS at end of 3 months.
Major molecular response (MMR)	Ratio of BCR-ABL1 to ABL1 $^{\leq 0.1\% \text{ on IS.}}$
Molecular response 4 (MR4)	Ratio of BCR-ABL1 to ABL1 $^{\leq 0.01\% \text{ on}}$ IS.
Molecular response 4.5 (MR4.5)	Ratio of BCR-ABL1 to ABL1 $^{\leq 0.0032\% \text{ on IS}}$
Molecular response 5 (MR5)	Ratio of BCR-ABL1 to ABL1 ≤ 0.001% on IS.

Table 4.
Definition of response assessment in CML [17].

Time	Optimal	Warning	Failure
Baseline	NA	High-risk ACA, high-risk ELTS score	NA
3 months	≤10%	>10%	>10% if confirmed within 1–3 months
6 months	≤1%	>1–10%	>10%
12 months	≤0.1%	>0.1–1%	>1%
Any time	≤0.1%	>0.1–1%, loss of ≤0.1% (MMR)	>1%, resistance mutations, high-risk ACA

Table 5.
Milestones for treating CML expressed as BCR-ABL1 on the international scale (IS) [17].

performed more frequently, in case of hematologic toxicity. Cytogenetics may not be sufficiently adequate to monitor responses and a quantitative polymerase chain reaction (RQ PCR) is required for optimal response assessment. The 2020 ELN guideline in CML recommends RQ PCR every 3-month interval even after the achievement of major molecular response (MMR), and response is further stratified as optimal, warning and failure at various time points [17]. Monitoring by FISH may be needed in patients with atypical transcripts.

8. Outcome determinants in CML

8.1 Cytogenetics

Cytogenetic assessment is recommended in all cases of newly diagnosed CML at baseline. Acquisition of additional cytogenetic abnormalities (ACA) during TKI therapy is indicative of disease progression with a worse prognosis; however, the prognostic significance of the presence of ACA at time of diagnosis is not well established [33]. Nevertheless, additional clonal chromosomal abnormalities in Ph + cells at diagnosis, including so-called major route abnormalities (a second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, and abnormalities of 3q26.2 are considered as cytogenetic criteria (WHO, 2017) for accelerated phase [1].

8.2 Degree of BM fibrosis

BM fibrosis was classically thought to be a marker of adverse prognosis in the pre-Imatinib era. However, its relevance in era of TKI is uncertain. In a study of 110 cases of Ph + CML treated with Imatinib reported similar rates of CCR (67 vs. 58%; $p = 0.45$), estimated 4-year survival rates (80 vs. 88%; $p = 0.27$) and failure-free survival rates (69 vs. 77%, $P = 0.34$) between those with severe marrow fibrosis as compared with patients with mild to moderate fibrosis [34].

8.3 Sokal/Hasford/Eutos/ELTS prognostic scores

The Sokal prognostic score was developed in 1984 in era of CML patients receiving conventional chemotherapy and includes age, spleen size, percent blasts, and platelet count. The score allocates patients into three prognostic groups (low risk, intermediate risk, high risk) predicting different overall survival (OS) probabilities for chemotherapy-treated patients with 2-year OS of 90%, 70%, and 65% for low, intermediate, and high-risk groups, respectively [35]. The Hasford score, which adds eosinophilia and basophilia to the calculation, was developed for CML patients receiving treatment with interferon [36, 37]. Both Sokal and Euro Scores were developed in chemotherapy and Interferon era. The EUTOS score was developed, from analysis of 2060 registry patients, using spleen size and basophil percentage, and can discriminate patients into high risk and low risk. The remarkable feature of EUTOS score was done after the advent of imatinib and that it substituted Overall Survival (OS) by Progression-Free Survival (PFS) [37]. The new EUTOS Long-Term Survival (ELTS) score was developed to predict probability of dying from CML taking the age, spleen size, and basophil percentage into consideration [17]. A summary of risk stratification with their corresponding scores in given in **Table 6**.

Sokal score	
Risk Group	**Sokal score**
Low	<0.8
Intermediate	0.8–1.2
High	1.2
EUTOS SCORE	
Low	≤87
High	>87

Table 6.
Risk group definition according to Sokal score and EUTOS score [35–37].

- ***Sokal score calculation:*** Exp 0.0116 × (age in years-43.4) + 0.0345 × (spleen size-7.51) + 0.188 × [(platelet count/700)2–0.563] + 0.0887 × (blast cells-2.10) where Exp is the exponential function.

- ***EUTOS SCORE*** = (7 × basophil) + (4 × spleen) Where

  "Basophil" is basophils as a percentage of peripheral blood leucocytes.

  "Spleen" is spleen palpable below left costal margin in cms.

9. Post TKI issues

9.1 Non-adherence

Non-adherence is a significant issue in any chronic disease. Continuous exposure of Imatinib is critical to remission-free status. Poor adherence can cause loss of cytogenetic and molecular response culminating in relapse.

9.2 Adverse effects

Dose limiting toxicities of various first and second-generation TKI can cause interruptions in TI dosing. Most of the dose limiting toxicities are transient and can be safely ameliorated by temporary interruption of the drug. More serious adverse effects require discontinuation and change to a new class of TI.

9.3 Resistance to TKI

About a quarter of CML patients will switch TKI once in their lifetime due to a either resistance or intolerance. Kinase domain mutation of BCR-ABL is the most common and well-characterized mechanism of TKI resistance in CML. Primary resistance implies failure to achieve time-dependent endpoints of CHR, CCR, and MMR upon initiation of TKI therapy, while secondary (acquired) resistance is defined as the loss of response [38].

9.3.1 Mechanisms of resistance

Point mutation of BCR-ABL1 is the commonest cause of resistance. Point substitutions at just 12 residues (M244, G250, Q252, Y253, E255, V299, F311, T315, F317, M351, F359, and H396) is responsible for most resistance-associated KD mutations [39, 40].

9.4 Mutation analysis

9.4.1 Indications for mutation analysis

The 2020 ELN Guidelines in CML have enlisted the indications of mutation analysis, which should be performed in the following scenarios: (a) patients at diagnosis in cases presenting with accelerated or blast phase, (b) in cases of failure/suboptimal response or loss of MMR on Imatinib therapy, (c) in case of hematologic or cytogenetic failure on second generation TKI.

9.4.2 Methods to detect BCR/ABL mutations

Direct gene sequencing is the commonest method and also the method of choice for detection of BCR/ABL mutation.

9.4.3 Choice of TKI based on mutation analysis

The choice of alternative TKI is based on the mutation detected as follows: a) In V299L, T315A, and F317L/V/I/C mutations, Nilotinib is the TKI of choice. b) In Y253H, E255K/V, and F359V/C/ mutation, Dasatinib is the TKI of choice. Ponatinib is the drug of choice in T315I mutation. d) In other mutation, high dose of Imatinib, Dasatinib, or Nilotinib is drug of choice.

10. TKI-related toxicities (acute and long-term effects of TKI)

With the advent of TKIs, CML has become a chronic disease, and since in CML TI has to be continued for a long time, it's of prime importance to recognize the adverse effects of TKIs.

10.1 Toxicity profile of Imatinib

Generally, Imatinib is considered a safe, well-tolerated drug although mild to moderate toxicities are common. The toxicity of Imatinib depends upon various factors, which include dose of Imatinib used, stage of the disease, and phase of treatment (early vs. later). Imatinib used in advanced phase of CML (accelerated phase or blast crisis) reported increased toxicity as compared with chronic phase that can be attributed to the high dose of Imatinib used in this phase of the disease (600–800 mg per day) and the lack of bone marrow reserves owing to the aggressive biology of the disease. A randomized trial of 476 patients in CML CP started on Imatinib 400 mg (n = 157) OD vs. 800 mg (n = 319) OD reported increased grade 3–4 hematologic toxicities, gastrointestinal toxicity, skin toxicity, edema, in the 800 mg OD arm. Most side effects occur early during treatment and tend to reduce in severity

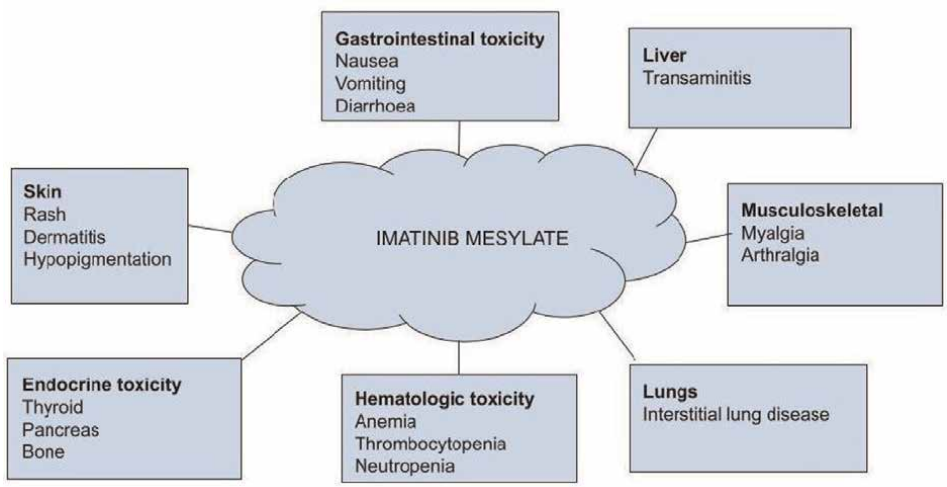

Figure 8.
Organ-specific toxicity profile of Imatinib mesylate [41].

and frequency with time. Toxicity profile of Imatinib can be divided into the following categories: (a) hematological toxicity (b) non-hematological toxicity (**Figure 8**) [41].

10.1.1 Hematological toxicity

It is one of the most common toxicities encountered with Imatinib therapy in CML and can be attributed to the direct toxic effects of Imatinib on stem cells as well delayed restoration of normal hematopoesis following elimination of BCR-ABL-positive leukemic cells. In the landmark IRIS trial comparing Imatinib 400 mg OD vs. INF-α plus cytarabine, grade 3–4 neutropenia was most common hematological toxicity reported (14.3%) followed by thrombocytopenia (8%) and anemia (3%) in Imatinib arm (4). Since mucositis is uncommon with Imatinib therapy, febrile neutropenia is rarely encountered as compared with conventional chemotherapy.

10.1.2 Bone marrow aplasia

There have been case reports of BM aplasia following TKI therapy. Transient BM aplasia is common and resolves spontaneously with discontinuation of drug. However, BM aplasia not improving after drug discontinuation is a potentially serious issue. There is no consensus regarding management of the above condition; best supportive care and allogenic SCT have been tried in some cases with varying results.

10.1.3 Non-hematological toxicity

10.1.3.1 Gastrointestinal toxicity

Nausea, vomiting, and diarrhea are among the most common non-hematologic toxicities encountered with Imatinib therapy.

10.1.3.2 Fluid retention (edema)

Fluid retention leading to superficial edema is the commonest dose limiting toxicity and responsible for dose interruptions in majority of cases on Imatinib therapy. The cause of this phenomenon has been postulated to be the inhibition of platelet-derived growth factor (PDGFR).

10.1.3.3 Other non-hematological toxicity

Other commonly observed non-hematologic toxicities with Imatinib are fatigue and musculoskeletal toxicity manifesting in the form of fatigue, myalgias, cramps, and arthralgia, among which muscle cramps and myalgias were more frequently reported. Severe musculoskeletal toxicity was reported more frequently in cases receiving Imatinib 800 mg daily as compared with cases receiving 400 mg daily.

10.2 Long-term side effects

Since Imatinib has to be continued in a long-term basis in CML in majority of cases, recognition of long-term adverse effects is critical to good patient management. The principal long-term adverse effects of Imatinib are enlisted below:

10.2.1 Cardiotoxicity

There have been instances of cardiotoxicity in the form of congestive heart failure, pericarditis, and hypertension with use of Imatinib. Kerkela et al. demonstrated the cardiotoxicity of Imatinib in cell cultures, as well as in animal model. They postulated that imatinib causes activation of endoplasmic reticulum stress response that subsequently activates Bax and ultimately releases cytochrome c causing apoptosis of myocytes. They also have reported 10 cases (median age = 64) of new-onset congestive cardiac failure while on Imatinib; however, eight of them had preexisting risk factors (type 2 diabetes mellitus or hypertension) or previous cardiac conditions (coronary artery disease) [42].

10.2.2 Pulmonary toxicity

Pulmonary toxicity in presenting in the form of interstitial lung disease (ILD) is rarely reported. A Japanese study reported 27 cases (CML CP (n = 17), CML AP (n = 6), gastrointestinal stromal tumor (GIST) (n = 4), which appeared to develop ILD after initiation of Imatinib therapy; however, majority of the patients had complete resolution of ILD (23 out of 27) on stopping Imatinib and addition of corticosteroids. Due to its rarity, there are no consensus guidelines regarding its optimal management; however, it is recommended to withhold Imatinib therapy till the pulmonary functions recover [43].

10.2.3 Endocrine toxicity

Spectrum of endocrine toxicities ranges from thyroid disorders to pituitary suppression and is illustrated in **Figure 9**.

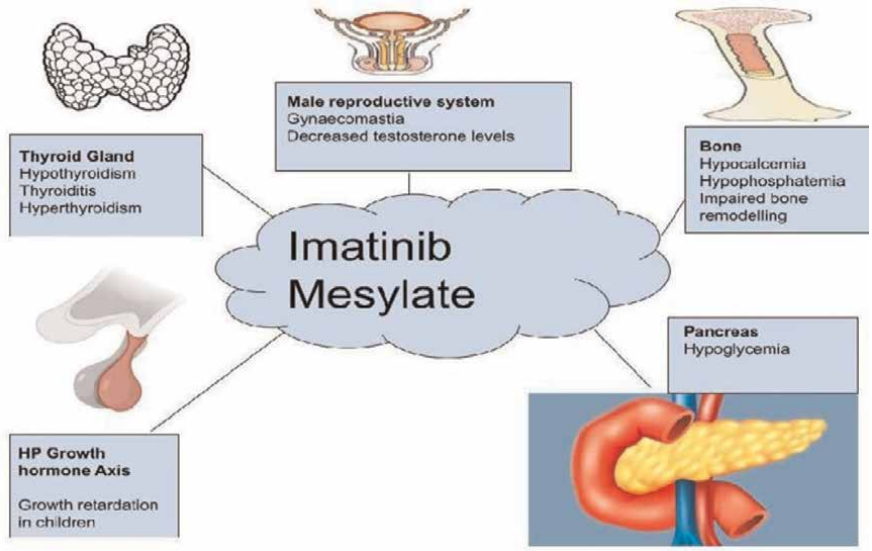

Figure 9.
Endocrine toxicity of Imatinib.

11. Toxicity profile of Nilotinib

Nilotinib is a second-generation TKI, which targets the BCR-ABL, platelet-derived growth factor receptor α (PDGFR-α), PDGFR-β, and c-kit. Nilotinib has been approved for use in frontline as well as in cases refractory to first-generation TKI. As with Imatinib, the most common toxicity includes myelosuppression. Although cardiovascular toxicities are rare, they are important to recognize since they can be life-threatening. The mechanism of A/E of Nilotinib is thought to be the due to its effects on other molecular target apart from *BCR-ABL*. Screening out high-risk groups is recommended before initiating nilotinib therapy. Adverse effects of nilotinib include pancreatitis, cardiac toxicity causing sudden deaths, vaso-occlusive events and hyperglycemia, prolonged QTc. It is important to correct electrolyte abnormalities such as potassium and magnesium before starting Nilotinib. Hepatotoxicity due to nilotinib is a commonly reported side effect as well; however, abnormal liver function test (LFT) results have been reported in asymptomatic cases. When alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are more than five-fold, the upper limit of the normal (ULN) or when the serum total bilirubin level is more than threefold the ULN, dose modification or discontinuation of nilotinib is recommended, resulting in decreased levels of hematological indicators in certain patients with CML. Nilotinib-induced hyperbilirubinemia typically manifests as indirect bilirubinaemia without elevated ALT or AST levels [44].

12. Toxicity profile of Dasatinib

Dasatinib is a second-generation TKI inhibiting BCR-ABL, SRC family kinases. It is 325 times more potent than Imatinib. As with other TKIs, the most important adverse effect is myelosuppression. Among the non-hematologic toxicities, the GI side effects

such as nausea and vomiting are common. About 35% of patients on Dasatinib experience pleural effusion, which is predominantly lymphocytic. Most of the cases of Dasatinib induced pleural effusion are self-limiting and require temporary discontinuation [45]. Diuretics or corticosteroids can be used in symptomatic effusions, not responding to conventional therapies is a rare but life-threatening complication of Dasatinib. Usually, discontinuation of Dasatinib leads to symptomatic improvement; however, more severe cases may require PDE Inhibitors.

13. Role of hematopoietic stem cell transplantation in CML

Twenty years back, CML was a fatal disease with hematopoietic stem cell transplantation being the only curative option. However, with the advent of TKI and their spectacular outcomes, HSCT has taken a back seat and now indicated for only in minority of patients with CML. High rates of transplant-related mortality remain an issue with allo-SCT; however, with the advent of RIC (reduced intensity conditioning) regimens and maintenance TKIs, the mortality and morbidity are steadily decreasing.

13.1 Current indications of SCT in CML

Chronic phase

- Failure of first-line TKI and predicted poor response to second-line TKI [46, 47].
- Failure to respond to first- and second-line TKIs.
- Presence of T315I mutation and/or failure to respond to ponatinib.
- Presence of repeated grade 4 cytopenias in response to treatment with different TKIs despite appropriate dose reduction and cytokine support.

Accelerated phase

- TKI naïve
- TKI naive with suboptimal response to TKI
- TKI resistant

Blast phase

- Acquisition of second CP after TKI or chemotherapy salvage.

13.1.1 Donor selection

The ideal donor is an HLA-matched sibling donor; however, unrelated HLA-matched donor using high-resolution typing can also be considered if MSD is not available. Various studies have demonstrated similar outcomes between MSD and MUD transplant although incidence of CGVHD is more in the unrelated group.

13.1.2 Conditioning regimen

A myeloablative conditioning regimen is preferred in young patients with good performance status. A combination therapy of busulfan (BU) oral/intravenous or cyclophosphamide (CY) regimen is generally used. Fludarabine- or TBI-based non-myeloablative or other reduced-intensity regimens may be considered in patients with comorbidities or elderly.

13.1.3 Post-transplant strategies

Post-transplant strategies are aimed at preventing relapses. These include TKI maintenance, donor lymphocyte infusion (DLI), and interferon. Early molecular relapse should be assessed by RT-PCR/FISH studies. DLI is highly effective therapy for relpase in CML with response rates around 70%.

14. Pediatric CML

Chronic myelogenous leukemia (CML) in children is relatively rare. Since there is of lack of randomized clinical trials, management of CML in children is non-standardized and often follows guidelines developed for adults. Children tend to have a more aggressive clinical presentation than older adults, and prognostic scores for adult CML do not apply to children. Since children have to continue the TKI for a longer period of time, minimizing toxicities and achievement of deep molecular responses are the primary objectives. TKI Imatinib has been shown to cause growth retardation and hormone deficiency in children [48].

15. Recent advances and future perspective

15.1 Discontinuation of TKI

The target of being off drug therapy, also called as treatment-free remission (TFR), is now emerging as the new goal of CML therapy. The advantages of TFR include the following: a) minimizing long-term side effects of TKI and improving quality of life, b) reduced cost to the patients and society. The long-term results of the French stop Imatinib trial (STIM1), which evaluated the outcomes of 100 patients with CML in complete molecular response (undetectable *BCR-ABL* transcripts) for at least 2 years, where Imatinib was discontinued. Sixty-one percent of patients had a molecular relapse occurring within 7 months of discontinuation. However, complete molecular response was again achieved in majority of the patients (55 out of the 61 patients) after Imatinib was restarted [49]. Current guidelines recommend considering stopping TKI therapy for patients in chronic phase of CML who have been on therapy for at least 3 years, sustained deep molecular response (DMR) for at least 2 years with DMR defined as a BCR/ABL_1 level of $<0.01\%$ on the international scale (IS) (equivalent to a 4-log reduction in transcript level from baseline, MR4) [50].

15.2 Novel drugs

Asciminib, a new first-in-class STAMP inhibitor, has shown promising results in heavily pretreated patients. Omacetaxine is approved in the USA for patients with CML resistant or intolerant to ≥2Tis including patients with T315I mutation after TKI failure.

15.3 Leukemic stem cells

Leukemic stem cells have the property of proliferation, self-renewal, and differentiation. Despite achieving molecular responses, there is a persistence of leukemic stem cells, which creates hindrance to eradication of disease by TKI and causes relapse. There exists a potential scope of drugs to potentially target the leukemic stem cells clone.

16. Future perspective

Development of effective therapies for patients of CML not responding to second and third generation remains a critical unmet need in CML. Prognosis still remains unsatisfactory in patients with advanced stage of the disease with SCT being the only option. There remains a need to deepen remssions, achieve a second treatment-free remission, and the management of refractory disease with new and emerging novel therapies.

Author details

Biswajit Bhuyan[1], Somanath Padhi[2*], Probodha Kumar Das[1] and Chinmayee Panigrahi[2]

1 Department of Medical Oncology/Hematology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

2 Department of Pathology and Laboratory Medicine, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

*Address all correspondence to: somanath.padhi@gmail.com

References

[1] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon: International Agency for Research on Cancer; 2017

[2] Singhal MK, Sengar M, Nair R. Summary of the published Indian data on chronic myeloid leukemia. South Asian Journal of Cancer. 2016;**5**(3): 162-165

[3] Hehlmann R, Hochhaus A, Baccarani M. Chronic myeloid leukaemia. The Lancet. 2007;**370**(9584): 342-350

[4] Sampaio MM, Santos MLC, Marques HS, Gonçalves VLS, Araújo GRL, Lopes LW, et al. Chronic myeloid leukemia-from the Philadelphia chromosome to specific target drugs: A literature review. World Journal of Clinical Oncology. 2021;**12**(2):69-94

[5] Wu ZH. Milestone histories and paradigmatic genetic discoveries of chronic myeloid leukemia (CML). In: Wu ZH, editor. Rare Diseases. London, UK.: InTech Open; 2020. DOI: 10.5772/intechopen.83131

[6] Deininger MWN, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood. 2000; **96**:3343-3356

[7] Quintás-Cardama A, Cortes J. Molecular biology of BCR-ABL1–positive chronic myeloid leukemia. Blood. 2009; **113**(8):1619-1630

[8] Marzocchi G, Castagnetti F, Luatti S, Baldazzi C, Stacchini M, Gugliotta G, et al. Variant Philadelphia translocations: Molecular-cytogenetic characterization and prognostic influence on frontline imatinib therapy, a GIMEMA working party on CML analysis. Blood. 2011; **117**(25):6793-6800

[9] Alhuraiji A, Kantarjian H, Boddu P, Ravandi F, Borthakur G, DiNardo C, et al. Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. American Journal of Hematology. 2018; **93**(1):84-90

[10] Hazlehurst LA, Bewry NN, Nair RR, Pinilla-Ibarz J. Signaling networks associated with BCR–ABL–dependent transformation. Cancer Control. 2009; **16**(2):100-107

[11] Ahmed W, Van Etten RA. Signal transduction in the chronic leukemias: Implications for targeted therapies. Current Hematology and Malignancy Report. 2013;**8**(1):71-80

[12] Bar-Natan M, Nelson EA, Xiang M, Frank DA. STAT signaling in the pathogenesis and treatment of myeloid malignancies. Jak-Stat. 2012;**1**(2):55-64

[13] Castagnetti F, Gugliotta G, Baccarani M, Breccia M, Specchia G, Levato L, et al. Differences among young adults, adults and elderly chronic myeloid leukemia patients. Annals of Oncology. 2015;**26**(1):185-192

[14] Savage DG, Szydlo RM, Goldman JM. Clinical features at diagnosis in 430 patients with chronic myeloid leukaemia seen at a referral Centre over a 16-year period. British Journal of Hematology. 1997;**96**(1):111-116

[15] Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al.

The 5th edition of the World Health Organization classification of Haematolymphoid Tumours: Myeloid and histiocytic/dendritic neoplasms. Leukemia. 2022;**36**:1703-1719

[16] Landstrom AP, Tefferi A. Fluorescent in situ hybridization in the diagnosis, prognosis, and treatment monitoring of chronic myeloid leukemia. Leukemia & Lymphoma. 2006;**47**(3):397-402

[17] Hochhaus A, Baccarani M, Silver RT, Schiffer C, Apperley JF, Cervantes F, et al. European LeukemiaNet 2020 recommendations for treating chronic myeloid leukemia. Leukemia. 2020;**34**: 966-984

[18] Bhuyan B, Padhi S. CD34 positive dwarf megakaryocytes in chronic myeloid leukemia are not indicators of disease progression. Indian J Pathol Microbiol [Epub ahead of print] [cited 2022 Nov 1]. 2022. DOI: 10.4103/IJPM.IJPM_601_21

[19] Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2016 update on diagnosis, therapy, and monitoring. American Journal of Hematology. 2016; **91**(2):252-265

[20] Sharma p, Singh T. Bone marrow histology in CML: A continuing relevance. Journal of. Bone Marrow Research. 2013;**1**:1-2

[21] Santos FP, Kantarjian H, Quintás-Cardama A, Cortes J. Evolution of therapies for chronic myelogenous leukemia. Cancer Journal (Sudbury, Mass.). 2011;**17**(6):465

[22] Hehlmann R, Heimpel H, Hasford J, Kolb HJ, Pralle H, Hossfeld DK, et al. Randomized comparison of interferon- α with busulfan and hydroxyurea in chronic myelogenous leukemia. Blood. 1994;**84**(12):4064-4077

[23] Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr–Abl positive cells. Nature Medicine. 1996; **2**(5):561-566

[24] O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. The New England Journal of Medicine. 2003;**348**(11): 994-1004

[25] Suttorp M, Schulze P, Glauche I, Göhring G, von Neuhoff N, Metzler M, et al. Front-line imatinib treatment in children and adolescents with chronic myeloid leukemia: Results from a phase III trial. Leukemia. 2018;**32**(7):1657-1669

[26] Baccarani M, Druker BJ, Branford S, Kim DW, Pane F, Mongay L, et al. Long-term response to imatinib is not affected by the initial dose in patients with Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase: Final update from the tyrosine kinase inhibitor optimization and selectivity (TOPS) study. International Journal of Hematology. 2014;**99**(5): 616-624

[27] Iqbal N, Iqbal N. Imatinib: A breakthrough of targeted therapy in cancer. Chemotherapy Research Practice. 2014;**2014**:357027

[28] Oehler VG. First-generation vs second-generation tyrosine kinase inhibitors: Which is best at diagnosis of chronic phase chronic myeloid leukemia? Hematology. 2020;**2020**(1): 228-236

[29] Naqvi K, Jabbour E, Skinner J, Anderson K, Dellasala S, Yilmaz M, et al. Long- term follow- up of lower dose

dasatinib (50 mg daily) as frontline therapy in newly diagnosed chronic-phase chronic myeloid leukemia. Cancer. 2020;**126**(1):67-75

[30] Gainor JF, Chabner BA. Ponatinib: accelerated disapproval. The Oncologist. 2015;**20**(8):847

[31] Cortes JE, Kim DW, Pinilla-Ibarz JL, Le Coutre P, Paquette R, Chuah C, et al. A phase 2 trial of ponatinib in Philadelphia chromosome–positive leukemias. New England Journal of Medicine. 2013;**369**(19):1783-1796

[32] Hughes TP, Mauro MJ, Cortes JE, Minami H, Rea D, DeAngelo DJ, et al. Asciminib in chronic myeloid leukemia after ABL kinase inhibitor failure. New England Journal of Medicine. 2019; **381**(24):2315-2326

[33] Cortes JE, Talpaz M, Giles F, O'Brien S, Rios MB, Shan J, et al. Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood. 2003;**101**(10): 3794-3800

[34] Kantarjian HM, Bueso-Ramos CE, Talpaz M, O'Brien S, Giles F, Rios MB, et al. The degree of bone marrow fibrosis in chronic myelogenous leukemia is not a prognostic factor with imatinib mesylate therapy. Leukemia & Lymphoma. 2005; **46**(7):993-997

[35] Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, et al. Prognostic discrimination in “good-risk” chronic granulocytic leukemia. Blood. 1984;**63**(4):789-799

[36] Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, et al. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon Alfa writing Committee for the Collaborative CML prognostic factors project group. Journal of the National Cancer Institute. 1998;**90**(11): 850-859

[37] Hasford J, Baccarani M, Hoffmann V, Guilhot J, Saussele S, Rosti G, et al. Predicting complete cytogenetic response and subsequent progression-free survival in 2060 patients with CML on imatinib treatment: The EUTOS score. Blood. 2011;**118**(3):686-692

[38] Patel AB, O’Hare T, Deininger MW. Mechanisms of resistance to ABL kinase inhibition in chronic myeloid leukemia and the development of next generation ABL kinase inhibitors. Hematology/ Oncology Clinics. 2017;**31**(4):589-612

[39] Steegmann JL, Baccarani M, Breccia M, Casado LF, García-Gutiérrez V, Hochhaus A, et al. European LeukemiaNet recommendations for the management and avoidance of adverse events of treatment in chronic myeloid leukaemia. Leukemia. 2016;**30**(8):1648-1671

[40] Hochhaus A. Chronic myelogenous leukemia (CML): Resistance to tyrosine kinase inhibitors. Annals of Oncology. 2006;**17**:274-279

[41] Cortes JE, Baccarani M, Guilhot F, Druker BJ, Branford S, Kim DW, et al. Phase III, randomized, open-label study of daily imatinib mesylate 400 mg versus 800 mg in patients with newly diagnosed, previously untreated chronic myeloid leukemia in chronic phase using molecular end points: Tyrosine kinase inhibitor optimization and selectivity study. Journal of Clinical Oncology. 2010;**28**(3):424

[42] Kerkelä R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, et al.

Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nature Medicine. 2006;**12**(8):908-916

[43] Rix U, Hantschel O, Dürnberger G, Remsing Rix LL, Planyavsky M, Fernbach NV, et al. Chemical proteomic profiles of the BCR-ABL inhibitors imatinib, nilotinib, and dasatinib reveal novel kinase and nonkinase targets. Blood. 2007;**110**(12):4055-4063

[44] Tan YW. Is dose modification or discontinuation of nilotinib necessary in nilotinib-induced hyperbilirubinemia? World Journal of Meta-Analysis. 2021; **9**(6):488-495

[45] Quintás-Cardama A, Kantarjian H, O'brien S, Borthakur G, Bruzzi J, Munden R, et al. Pleural effusion in patients with chronic myelogenous leukemia treated with dasatinib after imatinib failure. Journal of Clinical Oncology. 2007;**25**(25):3908-3914

[46] Craddock CF. We do still transplant CML, don't we? Hematology. 2018; **2018**(1):177-184

[47] Holyoake TL, Vetrie D. The chronic myeloid leukemia stem cell: Stemming the tide of persistence. Blood. 2017; **129**(12):1595-1606

[48] Hijiya N, Schultz KR, Metzler M, Millot F, Suttorp M. Pediatric chronic myeloid leukemia is a unique disease that requires a different approach. Blood. 2016;**127**(4):392-329

[49] Etienne G, Guilhot J, Rea D, Rigal-Huguet F, Nicolini F, Charbonnier A, et al. Long-term follow-up of the French stop Imatinib (STIM1) study in patients with chronic myeloid leukemia. Journal of Clinical Oncology. 2017;**35**(3):298-305

[50] Atallah E, Schiffer CA. Discontinuation of tyrosine kinase inhibitors in chronic myeloid leukemia: When and for whom? Haematologica. 2020;**105**(12):2738

Chapter 5

Clinical and Imaging Features of Leukemic Retinopathy

Vivian Wing Ki Hui and Simon K.H. Szeto

Abstract

Hematological malignancies may be associated with ocular manifestations in up to 50% of cases, and ocular symptoms can be the initial presentation. Retinal leukemic infiltrates may be observed in up to 3% of leukemia patients. Leukemic retinopathy may present more commonly in acute leukemias than chronic leukemias as Roth's spot, multi-level retinal hemorrhages, cotton wool spots, or opportunistic infection secondary to pancytopenia. On the other hand, patients with chronic leukemias, such as chronic myeloid leukemia (CML), may present with leukemic retinal infiltrates and venous stasis secondary to hyperviscosity, which may lead to secondary peripheral microaneurysms and neovascularization. Vascular complication, such as central retinal vein occlusion, may also occur as a result of venous stasis. In addition, leukemic retinopathy is associated with poorer overall survival as pediatric CML patients without ocular manifestation may have twice as high 5-years survival rate compared with those with ocular manifestation. The presence of leukemic retinopathy is associated with more severe systemic disease and is correlated with hematological parameters such as white blood cells count (WBC). In addition, a positive correlation was found between ocular leukemic infiltration and agonal leukocyte count and the severity of systemic disease in an autopsy study. Therefore, the presence of retinal infiltrate may be associated with leukemia with extreme leukocytosis. Optical Coherence Tomography (OCT) is a noninvasive retinal imaging tool that can help diagnose leukemic retinopathy. Inner retina hyper-reflective lesions were observed in areas with intra-retinal hemorrhages or hemorrhagic lesions, while outer retina hyper-reflective lesions were observed in areas with whitish retinal infiltrates. In addition, the loss of the physiological hourglass appearance on cross-sectional OCT scan of retinal vessels may be seen in leukemic retinopathy. It is believed that intraluminal blood flow is responsible for the physiological hourglass appearance, consisting of two paired hyper-reflectivities inside vessel wall on OCT. In leukemic retinopathy, hyperviscosity may disrupt normal intraluminal blood flow, leading to the loss of this physiological appearance. In summary, leukemic retinopathy can be the first presentation of leukemia. Ophthalmologists can play an important role in the diagnosis of leukemia. Noninvasive retinal imaging could help us to monitor and understand the pathophysiology of leukemic retinal infiltrates. Prompt diagnosis and treatment of underlying leukemia may preserve vision and prolong survival rate.

Keywords: leukemia, leukemic retinopathy, leukemic infiltrate, ocular manifestations, retinal hemorrhage

1. Introduction

Leukemia is a neoplastic disorder caused by abnormal proliferation of hematopoietic stem cells that replace the normal bone marrow. Although patients typically present with fatigue, bleeding, and fever, ocular manifestations have been reported to occur in up to 50% of cases at the time of diagnosis [1]. It is vital to assess for ocular symptoms, which may possibly be the first and isolated indicator for disease occurrence, relapse, or progression [2].

Leukemia can either arise from the myeloid or lymphoid cells lines. They can be rapidly progressing (acute) or indolent (chronic). Acute leukemia is fast progressing, generating immature white blood cells (WBC) incapable of normal physiological function, which can lead to imminent death without prompt treatment. Chronic leukemia progresses gradually with higher proportion of mature cells, although they can transform into an aggressive phase. The four major types of leukemia include acute myeloid leukemia (AML), most commonly seen in older adults, chronic myeloid leukemia (CML), with a progressive clinical course, acute lymphoblastic leukemia (ALL), most commonly seen in children with fair survival rate, and chronic lymphocytic leukemia (CLL), a slowly progressing disease with better prognosis. Both acute and chronic leukemia can cause ocular complications, while retina is the most common ocular tissue to be involved.

Patients with leukemic retinopathy may experience a more aggressive course and worse overall disease outcome. Studies have shown a significantly shorter mean survival rate in leukemic patients with leukemic retinopathy compared with those without (21.4% vs. 45.7) [3, 4]. It has also been shown that among patients with leukemic retinopathy, the mean survival rate was significantly shorter in those with cotton wool spots than those without [5]. Hematological parameters such as raised WBC and a low platelet count have been shown to correlate with retinal changes in leukemic patients [6]. Raised WBC has also shown to be positively correlated with the presence of leukemic retinal infiltrates and overall survival rate [7].

2. Pathogenesis

Ocular involvement in leukemia can be divided into: 1) primary (or direct) leukemic infiltration of ocular structures by neoplastic cells, and 2) secondary (or indirect) involvement due to hematological abnormalities (anemia, thrombocytopenia and hyperviscosity), central nervous system (CNS) involvement, opportunistic infection, or treatment-related complication. The term leukemic retinopathy is used to describe the retinal manifestation secondary to hematological abnormalities rather than direct leukemic infiltration [8].

Primary infiltration of neoplastic cells can occur virtually in all ocular tissues including the retina (3%), orbit (1%), choroid (0.3%), and optic nerve (0.3%) [6]. Direct infiltration of the retina occurs across all levels, more commonly in the inner retinal layers [9, 10]. These aggregates of leukemic cells in the retina can cause complete or partial structural destruction [8]. Generally, the internal limiting membrane (ILM) acts as an effective barrier against leukemic cells infiltration, but occasionally invasion into the vitreous can occur [11]. Invasion of leukemic cells into the choroid reduces blood flow in choriocapillaries and alters

the retinal pigment epithelium (RPE), which in turn result in an accumulation of subretinal fluid and serous retinal detachment. Choroidal mass may be evident. Optic nerve involvement may be caused by direct infiltration of optic nerve head, passive swelling due to retrolaminar leukemic infiltration, or passive swelling in papilloedema [6].

Secondary or indirect ocular involvement has been described in up to 39% of leukemic patients [1]. Hematological abnormalities including anemia, thrombocytopenia lead to retinal hemorrhage at all levels, which may be accompanied by white-centered retinal hemorrhage and perivascular sheathing due to accumulation of leukemic cells [12]. Hyperviscosity due to elevated WBC results in vascular stasis and occlusion [12, 13]. Prolonged vascular stagnation especially in chronic leukemia may lead to peripheral retinal ischemia and eventually blinding complications such as proliferative retinopathy or even neovascular glaucoma [14, 15].

3. Clinical presentation

3.1 History

Ocular symptoms: Most patients are asymptomatic. Some may experience unilateral or bilateral blurring of vision, floaters, or visual field defect. Ocular manifestations are more common in acute or relapse phase of leukemia than in chronic leukemia [16].

Systemic symptoms: Acute leukemia presents with symptoms of anemia, hemorrhage, infection, or infiltration of lymphatic organs. On the other hand, symptoms of chronic leukemia are vague, such as fatigue, weight loss, and night sweats. Patients with hyperviscosity syndrome may have headache, hearing impairment, dizziness, and other neurological symptoms.

The review of past history including any past history of leukemia or malignancies, systemic illness, history of infection, any previous treatments received is important in aiding diagnosis of leukemic retinopathy.

4. Physical examination

4.1 Ocular features

Retinal changes can be related to primary leukemic infiltration or secondary retinal manifestations of hematological abnormalities. Secondary changes occur in leukemic retinopathy, including Roth's spot, multi-level retinal hemorrhages, cotton wool spots, and opportunistic infection. These findings present more commonly in acute leukemia [1]. Chronic leukemia develops less rapidly, in which prolonged hyperviscosity results in venous stasis, retinal ischemia, peripheral microaneurysms, and proliferative vitreoretinopathy [1]. Complications such as vascular occlusion, vitreous hemorrhage, neovascular glaucoma, tractional retinal detachment secondary to proliferative vitreoretinopathy may develop in untreated eyes or delayed presentation. These manifestations vary in severity, which can be classified into mild, moderate, and advanced disease depending on the region involved and by visual prognosis (**Table 1**) [17].

	Categorization of ophthalmic manifestations in CML patients
Mild	• Retinal hemorrhages, Roth spots, cotton wool spots, leukemic infiltrates, dilated tortuous vessels, optic disc hyperemia and edema, retinal vessels sheathing
Moderate	• Proliferative retinopathy
	• Retinal vein occlusion
	• Vitreous hemorrhage
Advanced	• Optic disc infiltration
	• Exudative retinal detachment
	• Choroidal infiltration and hemorrhage

Table 1.
Categorization of ophthalmic manifestations in CML patients [17].

a. Venous dilatation and tortuosity

- Vascular congestion is one of the earliest retinal manifestations. It is also the most common manifestation in chronic leukemia [12]

b. Multi-level retinal hemorrhages

- Retinal hemorrhages occur predominantly in the posterior pole
- All retinal layers, especially the inner layers may be involved
    - Preretinal hemorrhages, superficial flame-shaped hemorrhages, intraretinal dot blot hemorrhages, sub-ILM hemorrhages, subhyaloid hemorrhage, and vitreous hemorrhage
- White-centered hemorrhage or Roth's spots result from capillary rupture and extrusion of whole blood, while the white center consists of leukemic cells or platelet-fibrin aggregates [13]
- There is an inconsistent correlation between retinal hemorrhages and hemoglobin or platelet levels [8]

c. Perivascular sheathing

- Perivascular sheathings are gray-white streaks along retinal vessels, which are a collection of leukemic cells [8]

d. Cotton wool spots (CWS)

- CWS is not a unique feature for leukemic retinopathy but is also seen in other pathologies with retinal hypoxia. They are a collection of neuronal debris within nerve fiber layer due to ischemic disruption of nerve axons
- Hypoxic state in leukemic retinopathy results from vascular stagnation and occlusion

- The presence of CWS does not correlate with hematological parameters [18]

e. Microaneurysms (MA)

- MA is a less common manifestation, which tends to locate in the peripheral retina in chronic leukemia. It is considered a manifestation of ischemia and neovascularization secondary to raised WBC [8, 19]

- There is no correlation between the presence of MA and hemoglobin or platelet count [20]

f. Leukemic infiltrates

- Leukemic infiltrates can occur in all ocular tissues, with retina being the most common site of involvement

  - Retinal infiltrates are large gray, white nodules of varying size that can involve all levels of retina, especially common in outer retinal layers. Subretinal infiltrates have been referred as subretinal hypopyon

  - Smaller infiltrates tend to be perivascular, while massive retinal infiltrate can cause total retinal detachment [21]

  - Massive retinal infiltrates may appear as large confluent retinal infiltrates in CML patients with extreme leukocytosis [9, 10].

  - Histological examination demonstrates complete or partial focal tissue destruction with the invasion of retinal infiltrates [8]. However, complete resolution with functional recovery may occur with systemic treatment [22]

  - Other sites of leukemic infiltration including the choroid, vitreous, and the fovea are less frequently seen. Choroidal infiltration often presents as secondary serous retinal detachment, generally shallow at the posterior pole [1]

- Direct leukemic infiltrates correlate positively with increased WBC and severity of systemic disease [7]

g. Optic disc swelling

- Optic disc swelling may occur in direct optic nerve head infiltration, passive swelling due to retrolaminar leukemic infiltration, or passive swelling in papilloedema [6]

- Clinically, papilledema and direct optic nerve head infiltration may be differentiated by the presence of perivascular sheathing in the later, although occasionally the conditions overlap [8]

- Optic nerve dysfunction (drop in visual acuity, color vision deficit, and the presence of relative afferent pupillary defect) may occur when the retrolaminar portion of the optic nerve is involved

- Lumbar puncture and spinal fluid analysis provides information on CNS involvement or raised intracranial pressure but cannot exclude direct optic nerve head infiltration

h. Complications

- Retinal vein occlusion
  - Retinal vein occlusion occurs as a result of hyperviscosity due to leukocytosis [1]
- Serous or exudative retinal detachment
  - This is thought to be a result of choroidal infiltration of leukemic cells or RPE disruption
  - Only a few cases of serous retinal detachment have been reported. Most of the reported cases are of ALL in younger patients. The detachments are generally shallow over the posterior pole
- Proliferative retinopathy
  - Proliferative retinopathy occurs secondary to retinal ischemia in non-perfused retina due to hyperviscosity
  - Peripheral retinal neovascularization is frequent in chronic leukemia, observed in up to 78% of cases in CML [19]. On the other hand, optic disc neovascularization is almost always associated with acute leukemia [23, 24]
  - In untreated eyes, vitreomacular traction, vitreous hemorrhage, and tractional retinal detachment eventually occur [22]
  - Neovascular glaucoma (NVG) and severe loss of vision may occur. The risk of developing NVG may be higher in patients with preexisting diabetic retinopathy [15, 25].

5. Investigation

5.1 Ocular imaging

Optical Coherence Tomography (OCT) is a noninvasive retinal imaging tool that uses low-coherence light waves to capture cross-sectional images of the retina. Lesions across each layer such as retinal infiltration, hemorrhages, and vessels can be visualized. Retinal and choroidal thickness can also be measured. OCT provides a detailed assessment of the structural change in the retina, which is valuable in disease monitoring. For example, the degree and extent of macular edema and serous retinal detachment can be demonstrated if not evident clinically [26]. The resolution of

leukemic infiltrates can also be observed with systemic treatment [9, 27]. The features of leukemic ocular manifestations are described further below:

a. Retinal detachment

- Separation of neurosensory retina from the underlying retinal pigment epithelium (RPE) can be confirmed on OCT, there would be a hypo-reflective space underneath the neurosensory retina

b. Retinal hemorrhages and leukemic infiltrates

- Retinal hemorrhages may present as inner retinal hyper-reflective lesions
- Leukemic infiltrates may appear as outer retinal hyper-reflective lesions, which may invade across retinal layers
- Disruption of photoreceptor layers may be observed after resolution of leukemic infiltrates [9]

c. Macular edema

- Increased central macular thickness (CMT) occurs in macular edema secondary to retinal vein occlusion
- Intraretinal cysts, subretinal fluid, and diffuse thickening may be present

d. Choroidal infiltration

- Increased choroidal thickness due to choroidal infiltration

e. Appearance of retinal vessels cross-section

- Loss of physiological hour-glass appearance over retinal vessels [28]
    - Physiological intraluminal blood flow appears as hourglass-shaped, consisting of two paired hyper-reflectivities inside vessel wall on OCT
    - Venous stasis in leukemic retinopathy disrupts normal intraluminal blood flow, leading to loss of this physiological appearance

Fundus Fluorescein Angiography (FFA) is an imaging technique to demonstrate retinal vasculature with the use of intravenous sodium fluorescein and a specialized camera. Real-time vascular flow with transit time can be recorded, together with the visualization of retinal microvasculature such as microaneurysms, capillary non-perfusion, and neovascularization, all of which may occur in leukemic retinopathy. With the advancement of ultra-widefield FFA in recent years, pathologies in the peripheral retina can be visualized. FFA is usually performed in eyes with delayed presentation, or in cases with high risk of ischemia and proliferative retinopathy due to prolonged vascular stagnation.

FFA features in leukemic retinopathy:

- Microaneurysms (MA)
    - Leukemic retinopathy may share common clinical features with diabetic retinopathy. MAs in leukemic retinopathy are smaller in size, clustered more closely to give a firecracker-appearance on FFA [29]. This picture of miliary MAs can differentiate leukemic retinopathy from a variety of ocular conditions such as Behcets retinal vasculitis, sarcoidosis where MA is not a typical feature.
- Capillary dropout representing areas of non-perfused retina
- Neovascularization at the disc (NVD) and elsewhere (NVE) secondary to retinal ischemia
- Delayed retinal arteriovenous transit time due to vascular stagnation
- Fluorescein leakage and pooling in exudative or serous retinal detachment

Optical Coherence Tomography Angiography (OCT-A) is an emerging noninvasive imaging technique, which generates angiographic images of the retina and the choroid without the need for dye injection. This imaging technique detects laser light reflectance upon moving red blood cells. Apart from structural mapping, the rate of blood flow can also be determined by scanning the same area twice for signal difference. Pathology on OCT-A can be detected even in patients without clinical signs of leukemic retinopathy. Pathological vascular flow loss in the superficial and deep capillary plexus and decrease of vascular density over perifoveal area can be detected with OCT-A in chronic leukemic patients. Currently, there are limited studies on OCT-A features in leukemic retinopathy [30].

6. Systemic investigation

Review of systemic illness should be carried out promptly to facilitate early disease diagnosis, disease staging, and to exclude concurrent infection or other differential diagnosis. The diagnosis of leukemia requires complete blood count with differential, peripheral blood smear, bone marrow examination, histochemical studies, cytogenetic and molecular testing, and immunophenotyping.

- Complete blood count and peripheral blood smear
    - Severe pancytopenia and peripheral blast cells suggest acute leukemia. Leukocytosis in the presence of blast cells excludes infection
    - Elevated white cell count and a normal-to-moderately elevated platelet count may be present in chronic leukemias. Peripheral smear and peripheral blood flow cytometry can further classify types of leukemia
    - Elevated white cell count correlates with the presence of direct leukemic infiltrates and the severity of systemic disease [7]

- Histochemical studies, cytogenetics, and immunophenotyping or molecular studies classify leukemia and provide information on disease prognosis

- Other laboratory abnormalities in leukemia may include hyperuricemia, hyperphosphatemia, hyperkalemia, hypocalcemia, elevated lactate dehydrogenase (LDH), which may indicate a tumor lysis syndrome

- Specific tests are useful in excluding infectious or non-infectious diseases, which mimic leukemic retinopathy

  - C-reactive protein

  - Quantiferon test, purified protein derivative skin test

  - Syphilis serology, HIV test

  - Angiotensin-converting enzyme level, urinary and blood calcium levels

- Imaging

  - Chest X-ray

  - Cranial imaging is done in leukemic patients with CNS symptoms. Cranial and orbital MRI can reveal intracranial lesions, presence of mass effect, presence of orbital involvement, or optic nerve infiltration

7. Management

Close collaboration between hematology-oncology and ophthalmology team is essential for a proper management of leukemic retinopathy. The mainstay of management is to treat underlying leukemia with chemotherapy, targeted therapy, radiotherapy, immunotherapy, or hematopietic stem cell transplant depending on the type and phase of leukemia. Acute general management includes correction of metabolic, infectious, and hyperleukocytosis emergencies. Emergency medical cytoreduction treatment or even therapeutic leukapheresis may be required in cases with extreme leukocytosis.

Regular ophthalmological review with dilated fundal examination is needed to monitor for the development of complication such as proliferative retinopathy and retinal detachment.

The subsequent ophthalmological management depends on disease severity (**Table 1**). In most mild cases, symptoms improved with systemic treatment for underlying leukemia. In moderate and advance cases, ophthalmic treatment together with systemic treatment may be necessary to minimize visual damage.

- In proliferative retinopathy, panretinal laser photocoagulation is applied to reduce ischemic drive from the non-perfused retina

- Non-resolving vitreous hemorrhage, massive subretinal hemorrhage, or tractional retinal detachment may require surgical management with pars plana vitrectomy

- Macular edema secondary to retinal vein occlusion may be treated with intravitreal anti-vascular endothelial growth factor (anti-VEGF)

8. Conclusion

Leukemic retinopathy can be the first presentation of leukemia, which warrants urgent evaluation and early commencement of systemic therapy. Clinical features vary in form and severity, in which milder forms give better visual prognosis. Retinal imaging helps us to understand the pathophysiology of leukemic retinopathy as well as to monitor disease progress. Prompt diagnosis and treatment of underlying leukemia may preserve vision and prolong survival rate.

Author details

Vivian Wing Ki Hui[1,2] and Simon K.H. Szeto[1,2]*

1 Hong Kong Eye Hospital, Hong Kong

2 Department of Ophthalmology and Visual Science, Chinese University of Hong Kong, Hong Kong

*Address all correspondence to: citokaho@gmail.com

IntechOpen

References

[1] Schachat AP et al. Ophthalmic manifestations of leukemia. Archives of Ophthalmology. 1989;**107**:697-700

[2] Ksiaa I et al. Acute lymphoblastic leukemia relapse presenting as retinal vasculitis. Clinical Case Report. 2020;**8**:1467-1471

[3] Ohkoshi K, Tsiaras WG. Prognostic importance of ophthalmic manifestations in childhood leukaemia. The British Journal of Ophthalmology. 1992;**76**:651-655

[4] Curto ML et al. Leukemic infiltration of the eye: Results of therapy in a retrospective multicentric study. Medical and Pediatric Oncology. 1989;**17**:134-139

[5] Abu el-Asrar AM et al. Prognostic importance of retinopathy in acute leukemia. Documenta Ophthalmologica. 1995;**91**:273-281

[6] Reddy SC, Jackson N, Menon BS. Ocular involvement in leukemia--a study of 288 cases. Ophthalmologica. 2003;**217**:441-445

[7] Leonardy NJ et al. Analysis of 135 autopsy eyes for ocular involvement in leukemia. American Journal of Ophthalmology. 1990;**109**:436-444

[8] Kincaid MC, Green WR. Ocular and orbital involvement in leukemia. Survey of Ophthalmology. 1983;**27**:211-232

[9] Szeto SK et al. Massive retinal infiltrates as the presenting sign of chronic myeloid leukemia: Clinical and imaging features of leukemic retinopathy. European Journal of Ophthalmology. 2021;**2021**:1120

[10] Goel N et al. Chronic myeloid leukemia presenting with bilateral central retinal vein occlusion and massive retinal infiltrates. Journal of AAPOS. 2012;**16**:406-408

[11] Kuwabara T. Leukemic miliary nodules in the retina. Archives of Ophthalmology. 1964;**72**:494-497

[12] Ballentine AJ, MIe. Textbook of the Fundus of the Eye. Williams & Wilkins; 1970. pp. 290-292

[13] Allen RA, Straatsma BR. Ocular involvement in leukemia and allied disorders. Archives of Ophthalmology. 1961;**66**:490-508

[14] Rosenthal AR. Ocular manifestations of leukemia. A review. Ophthalmology. 1983;**90**:899-905

[15] Figueiredo LM et al. Chronic myeloid leukemia diagnosed in a patient with uncontrolled proliferative diabetic retinopathy. Retin Cases Brief Report. 2015;**9**:210-213

[16] Yabas Kiziloglu O et al. Recurrence of acute lymphoblastic leukemia manifesting as serous retinal detachments and optic disc swelling. International Ophthalmology. 2018;**38**:1791-1795

[17] Atteya A et al. Evaluation of hepatitis B reactivation among patients with chronic myeloid Leukemia treated with tyrosine kinase inhibitors. Cancer Control. 2020;**27**:107

[18] Guyer DR et al. Leukemic retinopathy. Relationship between fundus lesions and hematologic parameters at diagnosis. Ophthalmology. 1989;**96**:860-864

[19] Duke JR, Wilkinson CP, Sigelman S. Retinal microaneurysms in leukaemia. The British Journal of Ophthalmology. 1968;**52**:368-374

[20] Jampol LM, Goldberg MF, Busse B. Peripheral retinal microaneurysms in chronic leukemia. American Journal of Ophthalmology. 1975;**80**:242-248

[21] Primack JD, Smith ME, Tychsen L. Retinal detachment in a child as the first sign of leukemic relapse: Histopathology, MRI findings, treatment, and tumor-free follow up. Journal of Pediatric Ophthalmology and Strabismus. 1995;**32**:253-256

[22] Yassin MA et al. Ophthalmologic manifestations as the initial presentation of chronic myeloid leukemia: A review. Survey of Ophthalmology. 2022;**67**:530-543

[23] Wiznia RA, Rose A, Levy AL. Occlusive microvascular retinopathy with optic disc and retinal neovascularization in acute lymphocytic leukemia. Retina. 1994;**14**:253-255

[24] Almeida DR, Chin EK, Grant LW. Chronic myelogenous leukemia presenting with bilateral optic disc neovascularization. Canadian Journal of Ophthalmology. 2014;**49**:e68-e70

[25] Roop P et al. Uncontrolled neovascular glaucoma – an alarming manifestation of chronic myeloid leukemia on imatinib therapy - a case report and review of literature. Indian Journal of Ophthalmology. 2019;**67**:285-287

[26] Candoni A et al. Leukaemic infiltration of the retina at onset of Philadelphia-positive acute lymphoblastic leukaemia revealed by stratus optical coherence tomography. British Journal of Haematology. 2006;**133**:455

[27] Le JQ, Braich PS, Brar VS. Optical coherence tomography findings of bilateral foveal leukemic infiltration. International Medical Case Report Journal. 2016;**9**:237-240

[28] Muraoka Y et al. Morphologic and functional changes in retinal vessels associated with branch retinal vein occlusion. Ophthalmology. 2013;**120**:91-99

[29] Kawali A et al. Miliary microaneurysms: An angiographic biomarker of leukemic retinopathy? Indian Journal of Ophthalmology. 2020;**68**:2017-2019

[30] Liu TYA, Mackey K, Linz MO, Scott AW. Retinal vascular changes on optical coherence tomography angiography and ultra-widefield fluorescein angiography in patients with chronic Leukemia. Journal of VitreoRetianl Disease. 2019;**3**:420-427

Section 3

Treatment

Chapter 6

Management of Newly Diagnosed Acute Myeloid Leukemia in Older Adults

Gopila Gupta and Vikas Garg

Abstract

With an increase in the incidence of acute myeloid leukemia with age, there is a worsening in organ function and the patient's ability to tolerate intensive therapies. To deliver the best possible care to this vulnerable group and maintain a good quality of life in patients, physicians need to individualize management to minimize adverse effects while still not compromising the prospects of the cure for fit individuals. In this chapter, we will discuss the tools for assessment in older adults and patients and disease-related parameters to be considered for appropriate classification into the fit, unfit, or frail categories. We will also discuss the treatment as per global fitness status, including novel agents, that have recently become available for older acute myeloid leukemia patients.

Keywords: AML, acute myeloid leukemia, older adults, elderly, management

1. Introduction

Acute myeloid leukemia (AML) is an aggressive hematological malignancy resulting from the uncontrolled proliferation of myeloid precursor cells. Older patients are most at risk for developing AML because its incidence rises with age. The definition of older adults in AML is imprecise, with a cutoff of 60–65 years of age considered in various studies. Outcomes in these patients are poor with 5-year survival rates of less than 15%. Multiple factors are attributed to poor outcomes, including declining performance status, associated comorbidities, higher prevalence of poor risk cytogenetics and molecular aberrations, antecedent hematologic disorders, and drug resistance phenotype. In this chapter, we will focus on the management of newly diagnosed AML in older adults.

2. Epidemiology

AML is the most common hematological malignancy among adults and constitutes around 1% of all malignancies. The incidence of AML increases with advancing age, with a median age at presentation is 68 years and males have slightly higher predilection over females [1–3]. It is more predominant among whites compared to non-white races [4].

Although it is idiopathic in most cases, some patients have known risk factors. Risk factors for the development of AML include exposure to ionizing radiation, chemical exposure (viz. benzene, pesticides), genetic predisposition, and chemotherapeutic drugs (alkylating agents and topoisomerase inhibitors) [5, 6]. Germline mutations in the DDX41 gene have a known association with hematological malignancies in the elderly [7]. AML can also be preceded by myelodysplasia (MDS), myeloproliferative neoplasms (MPN), aplastic anemia (AA), clonal hematopoiesis of indeterminate potential (CHIP), or clonal cytopenia of unknown significance (CCUS) [8, 9].

3. How to define elderly AML

Even though there is no universal consensus, the majority of studies and guidelines classify "elderly AML" or "older adults with AML" as those above the age of 60 years. The National Comprehensive Cancer Network (NCCN), as well as the European Society of Medical Oncology (ESMO) treatment guidelines, have adopted 60 years as a cutoff, while the American Society of Hematology (ASH) has also included 55—60 years in elderly AML [10–12]. Most landmark trials have included patients ≥65 years in their inclusion criteria. While chronological age is an important parameter, other factors, including function status, comorbidities and organ function, must be considered before categorizing as elderly AML [13, 14].

3.1 Prognosis

Although the 5-year survival of patients with AML has significantly improved from 20% in 2000 to around 35% in 2020, survival in patients aged >65 years remains dismal. According to the Surveillance, Epidemiology, and End Results (SEER) database 5-year relative survival in patients aged <50 years, 50–64 years, and ≥ 65 years are 62.2, 36.6, and 9.4%, respectively. Outcomes are even worse in patients aged >75 years with 5-year relative survival of 3.2% [15].

Poor outcomes in elderly patients are attributable to various host, disease, and treatment-related factors (**Table 1**). Elderly patients are more prone to adverse effects due to poor performance status (PS), multiple comorbidities, organ dysfunction, impaired cognition, and poor bone marrow reserves compared to younger ones. As a result, these patients are unfit for intensive chemotherapy, which is considered the standard of care in younger individuals. Additionally, they are more often associated with poor risk features, such as high total leucocyte count (TLC), low platelets, and adverse cytogenetic and molecular features. There is a higher likelihood of antecedent hematological disorders and therapy-related AML in the elderly population. Furthermore, there is inherent chemoresistance due to the increased expression of multidrug-resistance 1 (MDR-1) transporter protein in the cell membranes which facilitates drug efflux [16–19].

3.2 Clinical manifestations

AML can cause constitutional symptoms, symptoms due to bone marrow suppression or organ infiltration. Patients with anemia present with generalized weakness, dyspnea on exertion, and progressive pallor. Bleeding manifestations may result due to thrombocytopenia or coagulopathy. Patients are prone to infections due to a reduction in normal leukocytes. Rarely, hyperleukocytosis may cause leukostasis,

Host factors	Disease factors	Treatment-related factors
Declining performance status.	Higher prevalence of poor risk cytogenetic/molecular features, such as complex cytogenetics and TP53 mutation.	Higher expression of proteins that cause drug efflux.
Comorbidities and poor organ function.	High prevalence of leukocytosis and thrombocytopenia.	Poor tolerance to therapy.
Poor nutritional status.	Higher risk of t-AML.	Polypharmacy and drug interactions.
Impaired cognition	Gene mutations suggestive of antecedent myelodysplastic syndromes and AML-MRC (SRSF2, SF3B1, U2AF1, ZRSR2, BCOR, EZH2, ASXL1, STAG2).	

AML—Acute myeloid leukemia, t-AML—Therapy-related acute myeloid leukemia.

Table 1.
Factors associated with poor outcomes in "elderly AML" [16–20].

leading to blurred vision, respiratory symptoms, or seizures. (20) Extramedullary involvement, more common with monocytic variant can present as gum hypertrophy, leukemia cutis, or central nervous system (CNS) infiltration. Hepatosplenomegaly and lymphadenopathy are observed in minority (<10%). Bone pains can occur due to the expansion of the medullary cavity by growing leukemia cells [21–23].

3.3 Pathogenesis

Elderly patients with AML have a distinct genetic landscape compared with the younger population. The favorable prognostic molecular mutations, such as NPM-1 and CEBPA, are uncommon in elderly AML [24]. Interestingly, FLT3-ITD mutation, which has an adverse prognosis is found in up to one-third of younger patients but only 15–18% in >65 years. Mutations in spliceosomes, epigenetic mutations, and those in DNA repair pathways, such as DNMT3A, SRSF2, IDH1/2, RUNX1, TET2, ASXL1, TP53, and BCOR, are more common in the elderly conferring a poor prognosis and leading to chemo-resistance and inferior survivals [25, 26].

Myeloproliferative neoplasms (MPN), aplastic anemia (AA), and premalignant conditions (**Table 2**), such as clonal hematopoiesis of indeterminate potential (CHIP), clonal cytopenia of unknown significance (CCUS), and myelodysplastic syndrome (MDS), are associated with a higher risk of developing AML. Mutations in DNA methylation genes, such as TET2, ASXL1, DNMT3A, and histone modifiers, such as ASXL-1 and EZH2, keep accumulating in hematopoietic stem cells and increase the risk of developing AML [27, 28]. The risk of progression to AML in patients with CHIP is 1–2% per year. CHIP is also associated with a high risk of atherosclerosis and cardiovascular events in the elderly [9, 29].

With the increasing number of cancer survivors, the incidence of therapy-related myeloid neoplasms (t-MN) is also rising and is estimated to be around 10–15% [30]. Approximately, three-fourth t-MN occurs after alkylating agents and radiation exposure, and has a latency period of 5–6 years after initial exposure. These are often preceded by myelodysplasia and harbor complex karyotype and TP 53 mutation. Whereas, t-MN following exposure to topoisomerase II inhibitors has a shorter latency of 2–3 years, associated with abnormalities in KMT2A (11q23.3) and RUNX1 (21q22.1)

Entity	Definition
Idiopathic cytopenia of undetermined significance (ICUS).	Persistent cytopenia (≥ 6 months), with no somatic mutations, clonal cytogenetic abnormality, or no or mild marrow dysplasia (<10%).
Idiopathic dysplasia of undetermined significance (IDUS).	Marrow dysplasia in ≥10% of any one or more cell lines, with no somatic mutations, clonal cytogenetic abnormality, or cytopenia.
Clonal hematopoiesis of indeterminate potential (CHIP).	One or more myelodysplasia-related somatic mutations with variant allele frequency > 2% or clonal karyotypic abnormality in ≥2 metaphases, no cytopenia, or no or mild marrow dysplasia (<10%).
Clonal cytopenia of unknown significance (CCUS).	One or more myelodysplasia-related somatic mutations with variant allele frequency > 2% or clonal karyotypic abnormality in ≥2 metaphases, with cytopenia (≥ 4 months), but no or mild marrow dysplasia (<10%).

Table 2.
Premalignant conditions associated with myeloid neoplasms.

genes and are usually not preceded by myelodysplasia [31]. According to the European Leukemia Net (ELN), 2022 classification the term therapy-related acute myeloid leukemia (t-AML) is no longer a separate disease entity and is classified according to molecular profiling [32]. Most of these cases present in older adults and are often associated with adverse risk molecular and cytogenetic abnormalities and have inferior survival.

3.4 Diagnosis

Diagnostic evaluation in a patient with suspected hematological malignancy begins with a complete blood count (CBC) and a peripheral smear examination. A bone marrow examination is required for accurate morphological evaluation, immunophenotyping, cytogenetics, and molecular analysis. It aids in confirming the diagnosis, classification, and prognostication. Immunophenotyping provides further information on classification and can be utilized for measurable residual disease (MRD) assessment. Cytogenetic analysis (G banding and FISH analysis) and molecular testing for NPM, CEBPA, and FLT3 (TKD and ITD) with polymerase chain reaction (PCR) are required for prognostication.

In the era of precision medicine, next-generation sequencing (NGS) has become an important part of initial workup as it helps in better risk stratification and therapeutic management. International consensus classification (ICC) has recently published a revision of the World Health Organization (WHO) classification of AML (**Table 3**). It is a more genetically defined classification that includes new genetic entities and excluded therapy-related AML (t-AML) as a separate class [33]. Risk stratification is important for prognostication as well as to decide consolidation post-remission. ELN has risk-stratified AML based on morphological, cytogenetic, fluorescent insitu hybridization (FISH), molecular testing, and NGS (**Table 4**) [34].

Assessment of organ functions and other important tests should be performed before starting therapy including liver and kidney function tests, hepatitis B and C serology, human immunodeficiency virus (HIV), coagulation profile, lactate dehydrogenase (LDH), cardiac functions [electrocardiogram (ECG) and echocardiogram (2D-ECHO) or MUGA scan], and pulmonary function (in patients with

AML and related neoplasms AML with recurrent genetic abnormalities (requiring ≥10% blasts in BM or PB)
t(15;17) (q24.1;q21.2)/PML::RARAb
t(8;21) (q22;q22.1)/RUNX1::RUNX1T1
inv(16)(p13.1q22) or t(16;16) (p13.1;q22)/CBFB::MYH11
t(9;11) (p21.3;q23.3)/MLLT3::KMT2Ac
t(6;9) (p22.3;q34.1)/DEK::NUP214
inv(3) (q21.3q26.2) or t(3;3) (q21.3;q26.2)/GATA2, MECOM (EVI1)
AML with other rare recurring translocations
AML with mutated NPM1
AML with in-frame bZIP mutated CEBPA*
AML with t(9;22) (q34.1;q11.2)/BCR::ABL1#
Categories designated AML (if ≥20% blasts in BM or PB) or MDS/AML (if 10–19% blasts in BM or PB)
AML with mutated TP53~
AML with myelodysplasia-related gene mutations defined by mutations in ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2
AML with myelodysplasia-related cytogenetic abnormalities^
AML not otherwise specified (NOS)
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis associated with Down syndrome
Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukemias of ambiguous lineage
Acute undifferentiated leukemia
MPAL with t (9;22) (q34.1;q11.2)/BCR::ABL1
MPAL with t(v;11q23.3)/KMT2A rearranged
MPAL, B/myeloid, not otherwise specified
MPAL, T/myeloid, not otherwise specified

**AML with in-frame mutation in the bZIP domain of the CEBPA gene, either monoallelic or biallelic.*
#AML with t(9,22)(q34.1;q11.2)/BCR::ABL1 is an exception as it requires bone marrow or peripheral blood blast count of ≥20% due to its overlap with the progression of chronic myeloid leukemia, BCR::ABL1-positive.
~The presence of a pathogenic somatic TP53 mutation (at a variant allele fraction of at least 10%, with or without loss of the wild-type TP53 allele) defines the entity AML with mutated TP53.
^Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities. Unbalanced clonal abnormalities: del(5q)/t(5q)/add(5q); −7/del(7q); +8; del(12p)/t(12p)/(add(12p); i(17q), − 17/add(17p) or del(17p); del(20q); and/or idic(X)(q13).

Table 3.
2022 international consensus classification (ICC) revision of World Health Organization (WHO) classification of acute myeloid leukemia (AML) and related neoplasms [32].

prior respiratory disorders). Serum creatinine, uric acid, potassium, calcium, and phosphate are important to rule out tumor lysis syndrome (TLS). A comprehensive infection workup should be performed in the presence of fever or suspicion of infection. Lumbar puncture and brain magnetic resonance imaging (MRI) are reserved

Favorable	• t(8;21)(q22;q22.1)/RUNX1::RUNX1T1
	• inv.(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11 Mutated NPM1 without FLT3-ITD
	• bZIP in-frame mutated CEBPA
Intermediate	• Mutated NPM1 with FLT3-ITD
	• Wild-type NPM1 with FLT3-ITD
	• t(9;11)(p21.3;q23.3)/MLLT3::KMT2A
	• Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Unfavorable	• t(6;9)(p23;q34.1)/DEK::NUP214
	• t(v;11q23.3)/KMT2A-rearranged
	• t(9;22)(q34.1;q11.2)/BCR::ABL1
	• t(8;16)(p11;p13)/KAT6A::CREBBP
	• inv.(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1)
	• t(3q26.2;v)/MECOM(EVI1)-rearranged
	• −5 or del(5q); −7; −17/abn (17p)
	• Complex karyotype, monosomal karyotype
	• Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2
	• Mutated TP53

Table 4.
European leukemia net (ELN) 2022 risk stratification of acute myeloid leukemia (AML) [34].

for patients with suspected neurological involvement due to AML. Human leukocyte antigen (HLA) typing may be considered in a patient with the potential for hematopoietic stem cell transplant (HSCT).

3.5 Pretreatment assessment

Pretreatment assessment of the patient with a diagnosis of AML includes evaluation of performance status, physical functioning, and comorbidities. Eastern Cooperative Oncology Group performance status (ECOG-PS) helps in assessing general well-being and poor ECOG-PS has been associated with poor outcomes in multiple studies [35–37]. The functional assessment measured by activities of daily living (ADL) and instrumental activities of daily living (IADL) complement ECOG-PS in assessing functioning in elderly patients [38]. In addition, physical assessment using a short physical performance battery (SPPB) has been shown to predict hospitalizations and mortality among the elderly [39, 40].

Comorbidities also play a role in predicting survival and toxicity with treatment. Large population-based studies have shown that approximately half of the elderly patients with AML have at least one preexisting comorbidity, while one-fourth have ≥2 comorbidities. The Charlson comorbidity index (CCI) and the hematopoietic cell transplantation comorbidity index (HCT-CI) are commonly used in hematological malignancies [41]. HCT-CI was initially used for predicting non-relapse mortality in HSCT patients, but recent studies show that it can also predict early death and overall survival (OS) in AML > 60 years after receiving induction therapy [42].

Apart from chronological age, performance status, functional status, and comorbidities, other variables, such as cognition, nutritional status, social support, and

polypharmacy, also play a key role in treatment decisions. Cognitive impairment is more frequent among the elderly and is associated with a higher risk of complications during AML treatment. A modified mini-mental scale (3MS) can be used for brief bedside cognitive assessment and includes assessing various domains of cognition (attention, concentration, orientation, memory, language, constructional praxis, fluency, and abstract thinking) [43].

Many geriatric assessment (GA) scores have been developed considering the above-mentioned domains of patient health. Comprehensive geriatric assessment (CGA) is a multidimensional approach to assess elderly patients and includes functional status, cognition, mental status, comorbidities, social status, and medications. However, it is tedious and may not be feasible for routine clinical use [44]. Lately, screening questionnaire, such as G8 screening tool, for fragility assessment has been devised for use in busy oncology clinics to help identify patients who need geriatric assessment [45]. Multiple tools are also available to categorize elderly patients into those fit for intensive chemotherapy versus those who are not, but the majority of these have been studied in solid tumors [17]. The Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) score can be used in elderly patients with solid tumors as well as hematological malignancies to predict treatment toxicity [46].

Although we have multiple tools, these have not been integrated into clinical practice due to their complexity, paucity of time and manpower required to perform these tests, and lack of uniformity in implementation and interpretation. In general, a score of more than 1 on CCI or HCT-CI, 3-MS score < 77, SPPB <9, and ECOG PS > 2 would predict a higher risk of treatment-related morbidity and mortality with intensive chemotherapy. Patients may be broadly classified into fit, vulnerable, and frail using these variables (**Table 5**). However, the treating physician should base the management on their judgment as these have not been clinically validated [43].

Fitness category	Parameters
Fit	ECOG PS 0–2 CCI ≤ 1, HCT-CI ≤ 1 3-MS score > 77 SPPB >9 No impairment in ADL/IADL
Unfit/vulnerable	ECOG PS 2 CCI < 2, HCT-CI < 2 3MS score < 77 SPPB <9 Mild impairment in ADL/IADL
Frail	ECOG PS ≥ 3 CCI >2, HCT-CI > 2 3MS score < 77 SPPB <9 Impaired ADL/IADL

3-MS—Modified mini-mental scale, ADL—Activities of daily living, AML Acute myeloid leukemia, CCI—Charlson comorbidity index, IADL—Instrumental activities of daily living, ECOG-PS—Eastern Cooperative Oncology Group performance status, HCT-CI—Hematopoietic cell transplantation comorbidity index, and SPPB—Short physical performance battery.

Table 5.
Suggested categorization of "elderly AML" patients.

4. Management

4.1 Management of newly diagnosed AML

Management of AML is based on the patient's age, performance status, comorbidities, and geriatric assessment. It includes either intensive therapies (daunorubicin + cytarabine, 3 + 7), less intensive therapies, such as low-dose cytarabine (LDAC), hypomethylating agents (HMA), or best supportive care. Intensive chemotherapy is considered the standard of care for fit adults with AML, while less intensive therapies are preferred for others [10, 11]. Therefore, it is important to carefully assess patients before initiating any therapy.

Recently, the management of elderly patients (**Table 6**) has transformed with the introduction of targeted therapies (venetoclax and ivosidenib) in combination with HMA markedly improving the responses and survival. Many new targeted therapy approvals in the past 5 years have also modified the landscape of managing older adults with AML. With knowledge of patient physiology and disease biology, we can now better personalize the treatment and tailor it to the individual patient's needs. We will discuss the management according to the fitness of the patients as discussed in the pretreatment assessment.

Therapy	Fit[#]	Unfit	Frail
Induction	• 3 + 7 chemotherapy +/− GO • FLT3 mutated: 3 + 7 + midostaurin • t-AML, AML-MRC: CPX-351 • Complex karyotype/monosomal karyotype/ Tp 53- HMA+ Venetoclax	• HMA+ Venetoclax • LDAC+ Venetoclax • LDAC+ Glasdegib • IDH-1 mutated: ivosidenib+ azacytidine • IDH-2 mutated: enasidenib+ azacytidine	• Clinical trials • Best supportive care (BSC) and transfusion support • LDAC • HMA monotherapy
Consolidation	• HiDAC +/− midostaurin or GO[*] • Allogenic HSCT[**]	• Same as induction • Allogenic HSCT[**] in selected cases	• Same as induction

*AML—acute myeloid leukemia, HSCT—hematopoietic stem cell transplant, GO—Gemtuzumab ozogamicin, t-AML—therapy-related acute myeloid leukemia, AML-MRCacute myeloid leukemia- myelodysplasia related changes, FLT3—Fms like tyrosine kinase, HiDAC—high dose cytarabine, HMA—hypomethylating agents, acute myeloid leukemia, LDAC—low dose cytarabine, and IDH—Isocitrate dehydrogenase.*Favorable risk.*
***Adverse risk.*
[#]Maintenance therapy with oral azacytidine (onureg) or sorafenib (in FLT3 mutated cases).

Table 6.
Summary of management of newly diagnosed AML in older adults.

5. Patients fit for intensive chemotherapy

5.1 Induction therapy

The goal of therapy in these patients is to achieve swift remission with minimizing treatment-related mortality or morbidity. 3+ 7 chemotherapy has been a gold

Response	Response criteria
CR (complete remission)	Bone marrow blasts <5% Absence of circulating blasts Absence of extramedullary disease ANC ≥ 1.0 × 10^9/L Platelet count ≥100 × 10^9/L
CRh (CR with partial hematologic recovery)	All other CR criteria were met except ANC ≥ 0.5 × 10^9/L and platelet count ≥50 × 10^9/L
CRi (CR with incomplete hematological recovery)	All other CR criteria except ANC < 1.0 × 10^9/L or platelet count <100 × 10^9/L
MLFS (morphologic leukemia-free state)	Bone marrow blasts <5%; absence of circulating blasts; absence of extramedullary disease; no hematologic recovery
Relapsed disease	Bone marrow blasts ≥5% Reappearance of blasts in peripheral blood in at least two samples at least 1 week apart, or development of new extramedullary disease.
Refractory disease	Failure to achieve response after two cycles of intensive chemotherapy.

ANC absolute neutrophil count.

Table 7.
Response evaluation in acute myeloid leukemia.

standard with cytarabine (100–200 mg/m2/day daily infusion for days 1 to 7) combined with either daunorubicin (60 mg/m2/day for days 1 to 3) or idarubicin (12 mg/m2/day for day 1 to 3) since almost five decades [47]. Many modifications have been suggested like the addition of 6-thioguanine, dose intensification of daunorubicin to 90 mg/m2, or increasing cytarabine infusion to 10 days but none of them offered any survival benefit [48, 49]. As a result, 3 + 7 chemotherapy remained the standard of care until two seminal studies from the last decade, RATIFY and ALFA-0701, showed improved responses and survivals when 3 + 7 chemotherapy was combined with FLT3 inhibitor midostaurin (in FLT3 positive cases) or anti-CD 33 monoclonal antibody gemtuzumab ozogamicin (GO), respectively [50, 51]. In elderly patients with secondary AML (t-AML or AML MRC) CPX-351 (Vyxeos) is preferred as it has shown improved survival with lesser toxicity compared to the standard 3 + 7 regimen [52]. Response evaluation is based on residual blast percentage on bone marrow examination and recovery of peripheral blood counts (**Table 7**) [34].

5.2 Consolidation therapy

Consolidation therapy post-achieving remission is based on baseline cytogenetic/molecular risk stratification (**Table 4**) [34]. Favorable-risk patients have good long-term outcomes with high-dose cytarabine (HiDAC). However, optimal dose, schedule, and number of cycles are a topic of debate [53–56]. The addition of midostaurin (in FLT3-mutated cases) and GO with consolidation chemotherapy should be considered.

For patients with high risk, allogenic HSCT is preferred due to suboptimal outcomes with chemotherapy alone. It is important to consider the risk of transplant-related mortality (TRM) and expected benefits before proceeding to HSCT. The decision should be made after carefully assessing the risk of TRM based on age,

comorbidities, donor source, and conditioning regimen [57]. Myeloablative conditioning (MAC) improves relapse-free survival (RFS) but is associated with higher TRM. Reduced-intensity conditioning (RIC) regimens may not confer similar benefits as they depend mainly on the graft versus leukemia (GVL) effect but have lower TRM [58]. Recent data suggest that RIC transplant confers a survival benefit compared to chemotherapy in elderly AML patients with intermediate or poor risk cytogenetics (5-year OS 37 vs. 20%, HR-0.67, $p < 0.001$) [59].

Consolidation therapy for patients with intermediate-risk disease has been debated due to the lack of clear survival benefits and risks of TRM with HSCT. Young fit patients without comorbidities may derive greater benefit from HSCT due to lower TRM, while elderly patients with known comorbidities are at risk of increased TRM. However, with advancements in conditioning regimens (NMA and RIC) and greater experience with alternate donor sources (haploidentical, matched unrelated donor and cord blood) more patients are considered eligible for HSCT [60–62]. Maintenance therapy with oral azacytidine (Onureg) has demonstrated a survival benefit in patients who achieved remission but are unable to tolerate an intensive consolidation regimen or are unfit for transplant [63].

5.3 Patients unfit for intensive chemotherapy

The goal of therapy in these patients is to achieve a durable response and maintain it by continuing the therapy until relapse or intolerable side effects. Conventionally, LDAC (20 mg/m2 per day as a subcutaneous injection for 10 consecutive days every 28 days) and HMAs (azacytidine 75 mg/m2 daily for 7 days every 28 days or decitabine 20 mg/m2 daily for 5 days every 28 days) were the mainstay of therapy for elderly AML unfit for intensive chemotherapy. These therapies have low response rates (CR rates ranging from 10 to 30%) and a median OS of fewer than 12 months [14, 64].

With a better understanding of disease biology, a new class of drugs promoting apoptosis in cancer cells has been introduced. The intrinsic pathway of apoptosis is regulated by the B-cell lymphoma 2 (BCL-2) family of proapoptotic and antiapoptotic proteins. Proapoptotic proteins, such as BAX, BIM, and BAK [share BCL-2 homology 3 (BH3) domain], promote apoptosis by triggering mitochondrial outer membrane permeabilization (MOMP). B-cell lymphoma 2 (BCL-2) protein is a key regulator of the apoptotic pathway in mitochondria, which helps in the survival of myeloid blasts by sequestering proapoptotic BAX [65]. Venetoclax is a selective oral BCL-2 inhibitor that acts by binding to the BH-3 binding groove of BCL-2 and displaces BH-3 proteins, such as BAX and BIM, to induce apoptosis. Thus, it is also called the BH-3 mimetic drug. In the VIALE-A study in newly diagnosed AML $\geq$ 75 years or preexisting comorbidities, the combination of venetoclax with azacytidine demonstrated complete response (CR) rates of 36.7% and CR + CR with incomplete hematological recovery (CRi) rate of 66.4%. Median OS was significantly better than azacytidine and placebo, 14.7 months vs. 9.6 months (HR: 0.66, 95% CI 0.52–0.85, p-value <0.001). Venetoclax was administered as a ramp-up schedule in the first cycle, starting at 100 mg on day 1, 200 mg on days 2–3 and 400 mg, thereafter oral once daily for a total 28 days cycle [66]. Similarly, in the VIALE-C study, newly diagnosed AML $\geq$ 75 years or preexisting comorbidities were randomized to venetoclax plus LDAC or LDAC alone. CR + CRi was observed in 48% in combination versus 13% in LDAC alone. At primary analysis, the median OS was better 7.2 months vs. 4.1 months (HR 0.70; 95% CI 0.52–1.07; p-value 0.11) but not statistically significant [67]. Magrolizumab, an anti-CD47 antibody that blocks the "do not eat me signal" on

macrophages, has demonstrated good efficacy in old or unfit treatment naïve as well as relapsed/refractory AML. In a phase 2 study, the combination of magrolizumab, azacytidine, and venetoclax demonstrated CR rates in 86% of newly diagnosed AML patients (82% adverse risk and 47% TP53 mutated) [68].

Somatic mutations in isocitrate dehydrogenase genes IDH-1 and IDH-2 may be detected in up to 10 and 12% of patients with AML. It leads to the generation of D-2-hydroxyglutarate, which disrupts cellular metabolism and epigenetic control resulting in carcinogenesis [69]. Targeted therapy with oral IDH-1 inhibitor drug, ivosidenib either alone or in combination with HMA has been recently approved in elderly AML ≥ 75 years who are ineligible for intensive chemotherapy. In patients with de-novo or secondary AML with IDH-1 mutation ineligible for intensive chemotherapy, ivosidenib 500 mg oral daily achieved CR in 30.3% and CR + CR with partial hematologic recovery (CRh) in 42.4% [70]. A combination of ivosidenib with azacytidine was compared with azacytidine alone in phase 3 AGILE study in newly diagnosed IDH1-mutated AML ineligible for intensive chemotherapy, improved median OS (24 vs. 7.9 months, HR 0.44, 95% CI 0.27–0.73, p-value = 0.001), CR (47 vs. 15%) was observed with the combination [71]. Enasidenib, an oral IDH-2 inhibitor has also been tried in newly diagnosed IDH-2 mutated AML. Overall responses were seen in only 30% with CR in 18%. Similarly, a combination of enasidenib with azacytidine has been tested in newly diagnosed IDH-2 mutated AML in phase Ib/II trial. Responses were observed in 74% in combination compared to 36% with azacytidine alone [72].

Combination of LDAC with oral hedgehog inhibitor glasdegib has also shown improved OS (8.8 months vs. 4.9 months, HR 0.52, 80% CI 0.39–0.67, p-value 0.0004) in AML and high-risk MDS unfit for intensive chemotherapy compared with LDAC alone [73].

Currently, a combination of venetoclax with azacytidine is considered the standard of care for elderly patients with newly diagnosed AML unfit for intensive therapy. In patients, ≥ 75 years with IDH-1 mutation combination of ivosidenib with azacytidine is also approved. However, if these newer molecules cannot be accessed due to cost or availability, HMA remains the treatment of choice for these patients.

6. Frail patients

Management of frail elderly AML patients should aim at symptomatic relief and improving the quality of life. Patients with hyperleukocytosis may be given cytoreductive therapy with hydroxyurea. Supportive care includes blood transfusions and prophylactic anti-microbials. Based on physician discretion LDAC or HMA may be started. Single-agent targeted therapy, such as IDH or FLT-3 inhibitors, may demonstrate some benefit.

Author details

Gopila Gupta[1*] and Vikas Garg[2]

1 Department of Hematology and Bone Marrow Transplant, Fortis Hospital, New Delhi, India

2 Department of Medical Oncology, Dr. B.R.A.IRCH, AIIMS, New Delhi, India

*Address all correspondence to: gopila.gupta805@gmail.com

IntechOpen

References

[1] Surveillance, Epidemiology, and End Results Program (SEER). Available from: https://seer.cancer.gov/ [Accessed: September 6, 2022

[2] Key Statistics for Acute Myeloid Leukemia (AML) [Internet] Available from: https://www.cancer.org/cancer/acute-myeloid-leukemia/about/key-statistics.html [Accessed: September 6, 2022]

[3] Leukemia - Acute Myeloid - AML - Statistics [Internet]. Cancer.Net. 2012. Available from: https://www.cancer.net/cancer-types/leukemia-acute-myeloid-aml/statistics [Accessed: September 6, 2022]

[4] Kirtane K, Lee SJ. Racial and ethnic disparities in hematologic malignancies. Blood. 2017;**130**(15):1699-1705

[5] Godley LA, Larson RA. Therapy-related myeloid leukemia. Seminars in Oncology. 2008;**35**(4):418-429

[6] Song X, Peng Y, Wang X, Chen Y, Jin L, Yang T, et al. Incidence, survival, and risk factors for adults with acute myeloid leukemia not otherwise specified and acute myeloid leukemia with recurrent genetic abnormalities: Analysis of the surveillance, epidemiology, and end results (SEER) database, 2001-2013. Acta Haematologica. 2018;**139**(2):115-127

[7] Yang F, Long N, Anekpuritanang T, Bottomly D, Savage JC, Lee T, et al. Identification and prioritization of myeloid malignancy germline variants in a large cohort of adult patients with AML. Blood. 2022;**139**(8):1208-1221

[8] Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. Blood. 2009;**114**(5):937-951

[9] DeZern AE, Malcovati L, Ebert BL. CHIP, CCUS, and other acronyms: Definition, implications, and impact on practice. American Society of Clinical Oncology Educational Book. 2019;**39**:400-410

[10] Sekeres MA, Guyatt G, Abel G, Alibhai S, Altman JK, Buckstein R, et al. American Society of Hematology 2020 guidelines for treating newly diagnosed acute myeloid leukemia in older adults. Blood Advances. 2020;**4**(15):3528-3549

[11] Pollyea DA, Bixby D, Perl A, Bhatt VR, Altman JK, Appelbaum FR, et al. NCCN guidelines insights: Acute myeloid leukemia, version 2.2021: Featured updates to the NCCN guidelines. Journal of the National Comprehensive Cancer Network. 2021;**19**(1):16-27

[12] Heuser M, Ofran Y, Boissel N, Mauri SB, Craddock C, Janssen J, et al. Acute myeloid leukaemia in adult patients: ESMO clinical practice guidelines for diagnosis, treatment and follow-up†. Annals of Oncology. 2020;**31**(6):697-712

[13] DiNardo CD, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019;**133**(1):7-17

[14] Dombret H, Seymour JF, Butrym A, Wierzbowska A, Selleslag D, Jang JH, et al. International phase 3 study of azacitidine vs conventional care regimens

in older patients with newly diagnosed AML with >30% blasts. Blood. 2015;**126**(3):291-299

[15] Acute Myeloid Leukemia - Cancer Stat Facts [Internet]. SEER. Available from: https://seer.cancer.gov/statfacts/html/amyl.html [Accessed: September 12, 2022]

[16] Pettit K, Odenike O. Defining and treating older adults with acute myeloid leukemia who are ineligible for intensive therapies. Frontiers in Oncology. 2015;**5**:280

[17] Loh KP, Klepin HD. Geriatric assessment in older patients with acute myeloid leukemia. Cancers. 2018;**10**(7):225

[18] Sherman AE, Motyckova G, Fega KR, Deangelo DJ, Abel GA, Steensma D, et al. Geriatric assessment in older patients with acute myeloid leukemia: A retrospective study of associated treatment and outcomes. Leukemia Research. 2013;**37**(9):998-1003

[19] Stone RM, Lindsley C. Older adults with acute myeloid leukemia treated with intensive chemotherapy: "Old" prognostic algorithms may not apply. Haematologica. 2018;**103**(11):1758-1759

[20] Cassileth PA, Sylvester LS, Bennett JM, Begg CB. High peripheral blast count in adult acute myelogenous leukemia is a primary risk factor for CNS leukemia. Journal of Clinical Oncology. 1988;**6**(3):495-498

[21] Karesh JW, Goldman EJ, Reck K, Kelman SE, Lee EJ, Schiffer CA. A prospective ophthalmic evaluation of patients with acute myeloid leukemia: Correlation of ocular and hematologic findings. Journal of Clinical Oncology. 1989;**7**(10):1528-1532

[22] Dores GM, Devesa SS, Curtis RE, Linet MS, Morton LM. Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. Blood. 2012;**119**(1):34-43

[23] Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. Blood. 2011;**118**(14):3785-3793

[24] Silva P, Neumann M, Schroeder MP, Vosberg S, Schlee C, Isaakidis K, et al. Acute myeloid leukemia in the elderly is characterized by a distinct genetic and epigenetic landscape. Leukemia. 2017;**31**(7):1640-1644

[25] Prassek VV, Rothenberg-Thurley M, Sauerland MC, Herold T, Janke H, Ksienzyk B, et al. Genetics of acute myeloid leukemia in the elderly: Mutation spectrum and clinical impact in intensively treated patients aged 75 years or older. Haematologica. 2018;**103**(11):1853-1861

[26] DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable remissions with Ivosidenib in IDH1-mutated relapsed or refractory AML. The New England Journal of Medicine. 2018;**378**(25):2386-2398

[27] Abelson S, Collord G, Ng SWK, Weissbrod O, Cohen NM, Niemeyer E, et al. Prediction of acute myeloid leukaemia risk in healthy individuals. Nature. 2018;**559**(7714):400-404

[28] Shlush LI. Age-related clonal hematopoiesis. Blood. 2018;**131**(5):496-504

[29] Valent P. ICUS, IDUS, CHIP and CCUS: Diagnostic criteria, Separation from MDS and clinical implications. Pathobiology Journal of Immunopathology Molecular and Cellular Biology. 2019;**86**(1):30-38

[30] Morton LM, Dores GM, Schonfeld SJ, Linet MS, Sigel BS, Lam CJK, et al.

Association of Chemotherapy for solid tumors with development of therapy-related myelodysplastic syndrome or acute myeloid leukemia in the modern era. JAMA Oncology. 2019;**5**(3):318-325

[31] Kayser S, Döhner K, Krauter J, Köhne CH, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;**117**(7):2137-2145

[32] Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 ELN recommendations from an international expert panel. Blood. 2022;**140**:1345-1377

[33] Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International consensus classification of myeloid neoplasms and acute Leukemias: Integrating morphologic, clinical, and genomic data. Blood. 2022;**140**(11):1200-1228

[34] Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;**140**(12):1345-1377

[35] Appelbaum FR, Gundacker H, Head DR, Slovak ML, Willman CL, Godwin JE, et al. Age and acute myeloid leukemia. Blood. 2006;**107**(9):3481-3485

[36] Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and response criteria of the eastern cooperative oncology group. American Journal of Clinical Oncology. 1982;**5**(6):649-655

[37] Juliusson G, Antunovic P, Derolf A, Lehmann S, Möllgård L, Stockelberg D, et al. Age and acute myeloid leukemia: Real world data on decision to treat and outcomes from the Swedish acute leukemia registry. Blood. 2009;**113**(18):4179-4187

[38] Min GJ, Cho BS, Park SS, Park S, Jeon YW, Shin SH, et al. Geriatric assessment predicts nonfatal toxicities and survival for intensively treated older adults with AML. Blood. 2022;**139**(11):1646-1658

[39] Bandinelli S, Lauretani F, Boscherini V, Gandi F, Pozzi M, Corsi AM, et al. A randomized, controlled trial of disability prevention in frail older patients screened in primary care: The FRASI study. Design and baseline evaluation. Aging Clinical and Experimental Research. 2006;**18**(5):359-366

[40] Corsonello A, Lattanzio F, Pedone C, Garasto S, Laino I, Bustacchini S, et al. Prognostic significance of the short physical performance battery in older patients discharged from acute care hospitals. Rejuvenation Research. 2012;**15**(1):41-48

[41] Dhakal P, Shostrom V, Al-Kadhimi ZS, Maness LJ, Gundabolu K, Bhatt VR. Usefulness of Charlson comorbidity index to predict early mortality and overall survival in older patients with acute myeloid leukemia. Clinical Lymphoma, Myeloma & Leukemia. 2020;**20**(12):804-812.e8

[42] Giles FJ, Borthakur G, Ravandi F, Faderl S, Verstovsek S, Thomas D, et al. The haematopoietic cell transplantation comorbidity index score is predictive of early death and survival in patients over 60 years of age receiving induction therapy for acute myeloid leukaemia. British Journal of Haematology. 2007;**136**(4):624-627

[43] Teng EL, Chui HC. The modified mini-mental state (3MS) examination.

The Journal of Clinical Psychiatry. 1987;**48**(8):314-318

[44] Klepin HD. Geriatric perspective: How to assess fitness for chemotherapy in acute myeloid leukemia. Hematology. 2014;**2014**(1):8-13

[45] Baitar A, Van Fraeyenhove F, Vandebroek A, De Droogh E, Galdermans D, Mebis J, et al. Evaluation of the Groningen frailty indicator and the G8 questionnaire as screening tools for frailty in older patients with cancer. Journal of Geriatric Oncology. 2013;**4**(1):32-38

[46] Hurria A, Togawa K, Mohile SG, Owusu C, Klepin HD, Gross CP, et al. Predicting chemotherapy toxicity in older adults with cancer: A prospective multicenter study. Journal of Clinical Oncology. 2011;**29**(25):3457-3465

[47] Crowther D, Powles RL, Bateman CJ, Beard ME, Gauci CL, Wrigley PF, et al. Management of adult acute myelogenous leukaemia. British Medical Journal. 1973;**1**(5846):131-137

[48] Burnett AK, Russell NH, Hills RK, Kell J, Cavenagh J, Kjeldsen L, et al. A randomized comparison of daunorubicin 90 mg/m2 vs 60 mg/m2 in AML induction: Results from the UK NCRI AML17 trial in 1206 patients. Blood. 2015;**125**(25):3878-3885

[49] Preisler H, Davis R, Kirshner J, Dupre E, Richards F 3d, Hoagland H, et al. Comparison of three remission induction regimens and two postinduction strategies for the treatment of acute nonlymphocytic leukemia: A cancer and leukemia group B study. Blood. 1987;**69**(5):1441-1449

[50] Castaigne S, Pautas C, Terré C, Raffoux E, Bordessoule D, Bastie JN, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): A randomised, open-label, phase 3 study. The Lancet. 2012;**379**(9825):1508-1516

[51] Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation. The New England Journal of Medicine. 2017;**377**(5):454-464

[52] Lancet JE, Uy GL, Cortes JE, Newell LF, Lin TL, Ritchie EK, et al. CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional Cytarabine plus Daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. Journal of Clinical Oncology. 2018;**36**(26):2684-2692

[53] Miyawaki S, Ohtake S, Fujisawa S, Kiyoi H, Shinagawa K, Usui N, et al. A randomized comparison of 4 courses of standard-dose multiagent chemotherapy versus 3 courses of high-dose cytarabine alone in postremission therapy for acute myeloid leukemia in adults: The JALSG AML201 study. Blood. 2011;**117**(8):2366-2372

[54] Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and leukemia group B. The New England Journal of Medicine. 1994;**331**(14):896-903

[55] Jaramillo S, Benner A, Krauter J, Martin H, Kindler T, Bentz M, et al. Condensed versus standard schedule of high-dose cytarabine consolidation therapy with pegfilgrastim growth factor support in acute myeloid leukemia. Blood Cancer Journal. 2017;**7**(5):e564

[56] Löwenberg B. Sense and nonsense of high-dose cytarabine for acute myeloid leukemia. Blood. 2013;**121**(1):26-28

[57] Loke J, Vyas H, Craddock C. Optimizing transplant approaches and post-transplant strategies for patients with acute myeloid leukemia. Frontiers in Oncology. 2021;**11**:91

[58] Scott BL, Pasquini MC, Logan BR, Wu J, Devine SM, Porter DL, et al. Myeloablative versus reduced-intensity hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. Journal of Clinical Oncology. 2017;**35**(11):1154-1161

[59] Russell NH, Hills RK, Thomas A, Thomas I, Kjeldsen L, Dennis M, et al. Outcomes of older patients aged 60 to 70 years undergoing reduced intensity transplant for acute myeloblastic leukemia: Results of the NCRI acute myeloid leukemia 16 trial. Haematologica. 2022;**107**(7):1518-1527

[60] Cornelissen JJ, van Putten WLJ, Verdonck LF, Theobald M, Jacky E, Daenen SMG, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: Benefits for whom? Blood. 2007;**109**(9):3658-3666

[61] Yanada M, Matsuo K, Emi N, Naoe T. Efficacy of allogeneic hematopoietic stem cell transplantation depends on cytogenetic risk for acute myeloid leukemia in first disease remission: A metaanalysis. Cancer. 2005;**103**(8):1652-1658

[62] Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: Systematic review and meta-analysis of prospective clinical trials. Journal of the American Medical Association. 2009;**301**(22):2349-2361

[63] Wei AH, Döhner H, Pocock C, Montesinos P, Afanasyev B, Dombret H, et al. Oral Azacitidine maintenance therapy for acute myeloid leukemia in first remission. The New England Journal of Medicine. 2020;**383**(26):2526-2537

[64] Kantarjian HM, Thomas XG, Dmoszynska A, Wierzbowska A, Mazur G, Mayer J, et al. Multicenter, randomized, open-label, phase III trial of Decitabine versus patient choice, with physician advice, of either supportive care or low-dose Cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. Journal of Clinical Oncology. 2012;**30**(21):2670-2677

[65] Lessene G, Czabotar PE, Colman PM. BCL-2 family antagonists for cancer therapy. Nature Reviews. Drug Discovery. 2008;**7**(12):989-1000

[66] DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and Venetoclax in previously untreated acute myeloid leukemia. The New England Journal of Medicine. 2020;**383**(7):617-629

[67] Wei AH, Montesinos P, Ivanov V, DiNardo CD, Novak J, Laribi K, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: A phase 3 randomized placebo-controlled trial. Blood. 2020;**135**(24):2137-2145

[68] Daver N, Konopleva M, Maiti A, Kadia TM, DiNardo CD, Loghavi S, et al. Phase I/II study of Azacitidine (AZA) with Venetoclax (VEN) and Magrolimab (Magro) in patients (pts) with newly diagnosed older/unfit or high-risk acute myeloid leukemia (AML) and relapsed/refractory (R/R) AML. Blood. 2021;**138**:371

[69] Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA,

et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a Neomorphic enzyme activity converting α-Ketoglutarate to 2-Hydroxyglutarate. Cancer Cell. 2010;**17**(3):225-234

[70] Roboz GJ, DiNardo CD, Stein EM, de Botton S, Mims AS, Prince GT, et al. Ivosidenib induces deep durable remissions in patients with newly diagnosed IDH1-mutant acute myeloid leukemia. Blood. 2020;**135**(7):463-471

[71] Montesinos P, Recher C, Vives S, Zarzycka E, Wang J, Bertani G, et al. Ivosidenib and Azacitidine in IDH1-mutated acute myeloid leukemia. The New England Journal of Medicine. 2022;**386**(16):1519-1531

[72] DiNardo CD, Schuh AC, Stein EM, Montesinos P, Wei AH, Botton S de, et al. Enasidenib plus azacitidine versus azacitidine alone in patients with newly diagnosed, mutant-IDH2 acute myeloid leukaemia (AG221-AML-005): A single-arm, phase 1b and randomised, phase 2 trial. The Lancet Oncology 2021;**22**(11):1597-1608

[73] Cortes JE, Heidel FH, Hellmann A, Fiedler W, Smith BD, Robak T, et al. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. Leukemia. 2019;**33**(2):379-389

Chapter 7

Allogeneic Transplantation of Hematopoietic Stem Cells (HSCs) for Acute Leukemia in Children – Review of Literature and Experience of Single Center in Russia

Natalia Subbotina, Igor Dolgopolov, Georgij Mentkevich and Maxim Rykov

Abstract

The indications for hematopoietic stem cell transplantation (HSCT) in pediatric leukemias continually change with the risk-stratification-based treatment improvement. Achieving the minimal residual disease (MRD) negativity before transplantation, using RSI when it's appropriate, accurate management of post-transplant complications and GVHD are the factors of improving transplantation outcomes. Graft engineering methods are being worldwide investigated also to make HSCT more effective and less toxic, but still there is no gold standard of graft. Use of haploidentical grafts from relatives is a quick and cost-effective option of anti-leukemic efficacy achievement. Upon our experience in Russian Cancer Center, we believe that differentiated diagnosis-based approach to conditioning regimens in haplo-HSCT setting along with maintaining the manageable level of chronic GVHD could provide positive results in pediatric patients with prognostically the most unfavorable leukemias.

Keywords: pediatric oncology, chemotherapy, allogeneic transplantation of hematopoietic stem cells, acute leukemia

1. Introduction

Modern chemotherapy protocols provide a cure for up to 90% of children with acute lymphoblastic leukemia (ALL). However, there is a risk of disease recurrence after program treatment. Hematopoietic stem cell transplantation (HSCT) is one of the main existing treatments for recurrent ALL. The indications for HSCT in ALL continue to be revised up to the present day, as treatment protocols are improved, and new subtypes of the disease are identified that determine the prognosis. Here in we

review the international studies of allo-HSCT in children with acute leukemia and give the experience of our center in Russia. We highlight some of the unique challenges we face that could affect our outcomes in comparison to other countries.

2. Allogeneic HSCT in leukemia in children. Review of international studies

2.1 HSCT in ALL

Currently, in developed countries, with a positive decision to conduct an HLA-compatible allo-HSCT for ALL in the first remission, it is assumed that standard chemotherapy will provide such a patient with no more than 45% chance of a 5-year event-free survival (EFS). With the current level of accompanying therapy, it is assumed that the 5-year EFS of children who received histocompatible HSCT will be about 60% (in the absence of minimal residual disease (MRD) before HSCT, about 70–75% (1). The final decision on allo-HSCT should be made, based on their own results of standard chemotherapy and transplant program. The ultra-high-risk group with extended indications for allo-HSCT in the first remission includes patients with unfavorable genetic characteristics of the disease (for example, hypodiploidy), as well as with a poor response to induction treatment. For these patients, studies have demonstrated better survival with allo-HSCT [1–3]. The AIEOP-BFM ALL 2009 study protocol clearly articulates the indications for HSCT in first remission, which depend on the depth of response to treatment, as measured by PCR monitoring of MRI, availability of a compatible donor, response to prednisolone, phenotype and genotype leukemia [4].

A special group of patients are children of the first year of life with rearrangements of the 11q23/MLL gene. According to the COG group, in 100 infants with primary ALL and MLL gene rearrangements, allo-HSCT (related or unrelated compatible, or donated cord blood with a compatibility of 4/6 or more) as consolidation did not improve patient survival: 5-year EPS was 48.8% in the group who received HSCT (n = 53) and 48.7% in the group who received only CT (n = 47), p = 0.6 [5]. Interesting data from a large study Interfant 99, which included 277 infants with ALL with rearrangements of the MLL gene, who are in the first remission (93% of the entire study cohort, n = 297). Allogeneic HSCT was statistically significantly associated with better EFS only in patients of the highest-risk group (age under 6 months in combination with hyperleukocytosis or poor response to prednisolone, n = 87). For the rest of the patients, no advantages of HSCT over standard CT were revealed [6].

As for Ph+ ALL, the presence of the "Philadelphia chromosome" itself has ceased to be a genetically determined indication for THCS in 1 remission, and the task of modern research is to try to reduce the intensity of chemotherapy and study the role of tyrosine kinase inhibitors. The indications for HSCT in the first remission of Ph+ ALL are therefore poor response to induction or persistence of MRD after consolidation [7].

Allogeneic HSCT remains a significant therapeutic option for patients with recurrent ALL. According to modern concepts, allogeneic HSCT is indicated for any bone marrow recurrence of T-ALL, relapse of Ph+ ALL, bone marrow relapse of B-ALL after remission lasting less than 36 months, extramedullary relapse of B-ALL after remission lasting less than 18 months [8].

In addition to the above, patients with repeated relapses of ALL are candidates for allo-HSCT. According to the Northern Group of Pediatric Hematology and Oncology NOPHO, about a third of pediatric patients in the third or more remission of ALL can be cured with allo-HSCT [9]. Of course, in order to make a decision to perform allo-HSCT in a patient

with recurrent ALL recurrence, it is necessary to take into account the volume of previous therapy, as well as the fact of achieving repeated stable remission.

A kind of "classic" conditioning regimen for ALL is a myeloablative regimen based on total body irradiation (TOT) in combination with cyclophosphamide (CY). In 2000, the International Bone Marrow Transplant Registry (CIBMTR) presented the results of a long-term multicenter study demonstrating the benefits of a TOT-based conditioning regimen over Busulfan (Bu)-CY regimen in children with related compatible HSCT: 3-year PFS was 50% for the TOT group -CY (n = 451) and 35% for the Bu-CY group (n = 176), p = 0.005 [10].

The high toxicity of TOT for children, especially young children, prompted researchers to develop an effective conditioning regimen that does not include TOT and is characterized by minimal toxicity. For example, the European Community Bone Marrow Transplant (EBMT) Working Group on Childhood Illnesses published data on the use of a treosulfan-based conditioning regimen in children with ALL (n = 71, 72% in second and subsequent remission or out of remission) with allogeneic HSCT from various types of donors. The course dose of treosulfan varied in the study from 39 to 45 g/m^2. The drug was most often combined with cyclophosphamide (120 mg/m^2) or fludarabine (150–180 mg/m^2) and thiophosfamide (8–10 mg/kg). The results of this chemotherapeutic regimen are not inferior to the results of the classical TOT-based regimen; however, the study does not take into account the long-term consequences of both types of pre-transplantation preparation [11].

A significant influence on the result of transplantation is exerted by such factors as the magnitude of the MRD at the time of HSCT, as well as the development of the graft-versus-host reaction in the post-transplant period. The ASCT0431 study, conducted by the COG group and including 143 patients in the first and second remission of ALL, showed that the recurrence rate after HSCT was three times higher, while maintaining an MRD value of ≥ 0.1% before transplantation, according to cytometry [12]. The same authors presented evidence that the development of aGVHD is associated with a lower likelihood of recurrence. According to the results of the work, the authors noted that the presence of grade I-III aGVHD in patients in the post-transplant period was also associated with better EFS (relative risk (RR) = 0.5, p = 0.02) [12]. Data from various studies indicate that the development of both acute and chronic GVHD in the post-transplant period is associated with a statistically significant decrease in the frequency of relapses and an increase in both EFS and OS [8].

2.2 HSCT in AML

The principle of treatment of primary AML is based on chemotherapy with cytarabine and anthracyclines and has not changed significantly over the past 30 years. Improving accompanying therapy has increased the survival rate of children with primary AML up to 65–70%. About a third of children with AML who went into remission relapse later. Allo-HSCT is traditionally considered a consolidating step in the treatment of AML with poor prognosis, relapses of AML, and secondary AML associated with previous chemotherapy or radiation therapy (RT) in children. Currently, methods for determining MRD are being actively introduced to identify the contingent of patients who are indicated for allogeneic HSCT in the first remission. The predictive value of MRD in AML has been shown by large research groups such as COG and St. Jude [13, 14]. The use of modern protocols makes it possible to determine MRD using flow cytometry in 85% of patients.

The use of reduced intensity conditioning regimens (RSIs) is very important for patients with AML [15]. A large meta-analysis of studies on allo-HSCT in leukemia

in adult patients from 1990 to 2013, including data from 13 studies (9754 patients with AML/MDS), showed no difference in 6-year OS between groups of patients who received myeloablative conditioning regimen compared with RSI. Moreover, it should be noted that the average age of patients who received RSI was higher, and also in this group there was a higher proportion of patients in the second or more remission, or not in remission, that is, this group was less favorable prognostically [16]. Similar results were shown by Luger et al. (2012), also conducting a meta-analysis of studies on allo-HSCT in adults (n = 5179, 217 sites) [17]. In children, albeit in smaller patient cohorts, similar results have also been shown comparing survival between myeloablative conditioning regimens and RSI [18, 19].

The relationship between the development of GVHD in the post-transplant period and the antitumor effect of the graft (graft-versus-tumor reaction) has been shown in large cohorts of patients with AML. For example, according to Baron et al. (2012), the development of non-severe chronic GVHD in the post-transplant period was associated with a decrease in the frequency of relapses in patients with AML (RR = 0.72; p = 0.07), which was transformed into a better OS (RR = 1.8; $p < 0.001$). The development of severe cGVHD was also associated with a decrease in the recurrence rate (RR = 0.65; p = 0.02), but at the same time with an increase in transplant-related mortality (RR = 1.8; $p < 0.001$). All patients in this study received a reduced intensity conditioning regimen [20]. Weisdorf et al. also showed that the development of chronic GVHD correlated in patients with AML/MDS (n = 5741) with a decrease in the frequency of relapses of the disease. This effect only affected patients who received RSI. Such observation in patients who received myeloablative conditioning regimens was not noted by the authors [21].

2.3 Sources of HSC for transplantation

Up until now, it has been generally accepted that allogeneic HSCT from an HLA-compatible sibling offers patients in need of this treatment the best chance of recovery [22]. However, only 30% of patients worldwide have such a donor. In Russia, due to the small number of families, the probability of finding a suitable donor among brothers and sisters is even lower. An alternative source of hematopoietic stem cells for transplantation in these cases can be unrelated donors and cord blood from donor registries, as well as partially compatible, usually haploidentical parents of patients. In the first two cases, high-resolution HLA typing is required, which makes it possible to find the most suitable donor material, but it requires time and significant financial costs for the processes of searching, activating the donor, and delivering the material. At the same time, haploidentical HSCT is devoid of these shortcomings. Since the patient inherits a haplotype from each parent, high-resolution HLA typing of the child and parent is usually not necessary. High motivation and proximity of a related donor allow, if necessary, promptly organizing HSC transplantation, about 2 weeks from the moment the indications for it are established; this eliminates the need for the patient to undergo additional courses of CT, which is often done involuntarily during the search for a donor in the registry.

2.4 Development of haploidentical transplantation of hematopoietic stem cells in the world

The course of development of haploidentical transplantation in the world turned out to be interesting for retrospective analysis. The development of severe aGVHD in

patients after haploidentical transplantation in early studies was the impetus for the development of techniques for the depletion of mature lymphocytes from the transplant. However, excessive removal of T cells led to a significant increase (almost 50%) in the frequency of rejection of donor HSCs. Animal studies have shown that this problem can be addressed in several ways: intensification of conditioning regimens, in vivo T-depletion with antibodies, or an increase in the number of transplanted HSCs [23–25]. After the implementation of the CD34+ selection technique in the clinic, Aversa et al. and his followers managed to achieve high rates of engraftment of donor HSCs and a low level of aGVHD [26–28]. However, a survey of transplant centers conducted somewhat later by the European Blood and Marrow Transplant Group (EBMT) showed that when performing CD34+ selection, the mortality of patients from transplant complications approached 50%: recipients died from various infectious complications against the background of delayed immunity recovery after transplantation [29, 30]. Therefore, the next stage in the development of partially compatible transplantation was the development of methods for processing the material, in which the restoration of immunity is not so long. After the presentation on the medical market of a device and a technique for the simultaneous depletion of T and B lymphocytes in 2004, the technique of negative selection (CD3/CD19 depletion) of the graft gained popularity: the immunomagnetic method made it possible to reduce the level of mature T lymphocytes in the material by 3.5–4 orders of magnitude (for comparison: with CD34+ selection—by 4.5–5 orders of magnitude). Most importantly, NK cells, dendritic cells, myelocyte precursors, etc., were preserved in the transplant [31, 32]. In the earliest clinical studies conducted at the St Jude Clinic in the USA using CD3/CD19 depletion of material in haploidentical transplantation in children, the transplant mortality rate was reduced to 16–20% [33, 34]. Building on this work, the

Author	Number of patients, diagnosis	Method cleaning transplant	Transplantation mortality (observation period, years)	Survival
P. Bader et al. [37]	59 ALL 15 AML 14 MDS 2 Solid 18 Benign 10	CD3/CD19	10,7% (3)	3-year event-free survival for leukemias: 68% for those in remission; 0% for those not in remission
G. Dufort et al. [38]	16 ALL 4 AML 5 CML 3 UMML 1 Anemia Fanconi 3	CD3	23,5% * (1,5)	Overall survival 47.6% for leukemias with median observation 31 months after hematopoietic stem cell transplantation
J. Palma et al. [39]	10 ALL 6 AML 4	CD3	10% (1,5)	1-year event-free survival 60%
M. Gonzalez-Vincent et al. [40]	21 ALL 12 AML 9	CD3/CD19	30% (1)	2-year event-free survival 40.7%

Table 1.
Results of partially compatible transplants using T-depletion and reduced intensity conditioning regimens in children.

Tübingen group attempted to further reduce the incidence of transplant complications by reducing the intensity of the conditioning regimen. At first, the results were very encouraging: according to a study published in 2007, out of 38 adult patients, only one patient (2.6%) died from transplant complications [35]. However, when analyzing a later publication by the same authors from 2012, it can be seen that in the group of 61 adult patients, the transplant mortality rate was significantly higher and amounted to 23% by day 100 and 46% by the second year of follow-up [36]. The results of such transplantations in children are presented in **Table 1**. As can be seen from the table, CD3/19 depletion in haploidentical HSC transplantation using reduced-intensity conditioning regimens made it possible to achieve a reduction in transplant mortality and a good survival rate in children with leukemia under the condition in remission at the time of transplantation.

Despite the improvement in the results of partially compatible transplants after moving away from using only CD34+ cells for transplantation, the problem of slow recovery of immunity in patients after T-cell depletion does exist [41–43].

In order to achieve a level of T-depletion comparable to the level during CD34+ selection, and at the same time to achieve a faster immunological recovery of patients after transplantation, a group from Tübingen began to carry out separate depletion—the elimination from the transplant of only mature lymphocytes bearing the αβ-chain of the T-cell receptor (TCR αβ) [43]. The use of this method makes it possible to leave in the transplant T-lymphocytes with TCRγδ, which, according to some data, do not have alloreactivity, but are able to exercise an antitumor effect and control infection [44–46]. Investigators who have performed haploidentical related HSCT with TCRαβ depletion have reported earlier recovery of T-cell immunity in patients compared with CD3/CD19 depletion [47, 48]. In addition to the TCRαβ/CD19 depletion described above, there are other approaches to accelerate the immunological recovery of patients after transplantation of T-depleted HSCs and improve the antitumor control of the graft: adoptive immunotherapy in the post-transplant period with donor T cells after CD8 depletion [49] or selective depletion of allospecific lymphocytes [50, 51]; antigen-specific donor T-cells [52–54]; CD4+CD25+ regulatory cells [55]; alloreactive NK cells [56–58], etc.

Allogeneic HSCT in children with leukemia. Authors' experience

3. Materials and methods

3.1 Patient characteristics and disease status at the time of HSCT

In the Department of Pediatric Bone Marrow Transplantation of the Research Institute of Pediatric Oncology and Hematology, from 2001 to 2018, allogeneic-related HSCT was performed in 64 patients with various oncohematological diseases with an extremely unfavorable prognosis: 29 (45%) with AML, 15 (23%) with ALL, 5 (8%) with CML, 8 (13%) with JMML, 4 (6%) relapses of NHL, 1 (2%) refractory HL, 2 (3%) MDS. The median age of patients at the time of HSCT was 8 (1–19) years. Indications for HSCT for patients with acute leukemia are presented in **Table 2**.

3.2 Choosing a donor for transplantation

The search for donors was carried out in the closely related environment of patients (parents and siblings). Compatibility of related donors was assessed for the A, B, Cw antigens of the first HLA class (serological typing or low-resolution PCR)

Indications for HSCT in acute leukemia	Patients, n (%)
AML	29 (100%)
First remission (slow response to induction)	4 (14%)
Second or more remission	12 (41%)
No remission	6 (21%)
Secondary AML	7 (24%)
ALL	15 (100%)
Second or more remission	6 (40%)
No remission/increase in MRD	9 (60%)

Table 2.
Indications for allogeneic HSCT in acute leukemia.

and for the DRB1 antigens of the second HLA class (low-resolution PCR). The choice was made in favor of the most compatible relative according to the HLA system, taking into account the compatibility of blood according to ABO, age, and weight indicators of the donor and recipient. As mentioned above, due to rather small families in Russia, we were able to find a fully compatible sibling only for two patients. For others, haploidentical relatives became donors, providing the sufficient number of stem cells timely in accordance with patient's treatment protocol. Such timing was critical for our patients with acute leukemias as we didn't wait a long-temp remission after induction of treatment in most of them due to disease aggressiveness. As for those with chronic leukemias, earlier transplantation we believed to be associated with less transfusions and less comorbidity.

3.3 Selecting the conditioning regimen

In patients of the earlier group (until 2012), RSI based on busulfan/treosulfan, fludarabine/cyclophosphamide, and antithymocyte globulin (for haploidentical transplants) was routinely used. Subsequently, the approach to conditioning regimens became more differentiated and depended on the diagnosis, the prevalence of the disease, and the status of the disease at the time of transplantation. We began to use more intensive conditioning regimens for patients with ALL and JMML. In the case of refractoriness of the disease and the absence of the desired antitumor effect by the time of HSCT, drugs were included—modifiers of tumor sensitivity to therapy or RT to potentiate the effect of chemotherapy. The conditioning regimens used in our patients are shown in **Table 3**.

Reduced-intensity standard conditioning regimen: fludarabine 180 mg/m^2 (at 30 mg/m^2 on days – to –1) or cyclophosphamide 60 mg/kg (d –3, –2 for patients with ALL), antithymocyte globulin (Atgam®) 40 mg/kg (10 mg/kg on days –5, –3, –1, +1), and busulfan 8 mg/kg (4 mg/kg on days –3, –2) or treosulfan 30,000–36000 mg/m^2 (at 10000–12000 mg/m^2 on days –4, –3, –2) ± etoposide 30 mg/kg on day –4 (for patients with ALL).

In our work, we individualized the conditioning regimens as follows:

- increasing the dose of an alkylating agent or adding a second alkylating agent (UMML - 3, ALL - 1);

- introduction of a combination of Flu-ARA-C with additional anti-leukemic activity (AML - 1, ALL - 1);
- the use of thiophosfamide as an alkylating agent that penetrates through the BBB (ALL - 1, AML - 1);
- administration of epigenetic agents (5-azacytidine) to increase sensitivity to chemotherapy (AML-3, ALL-2);
- introduction of bortezomib as a modifier of sensitivity to chemotherapy (AML - 1, ALL - 4);
- the use of G-CSF before myeloablation (UMML - 1);
- These methods were used both individually and in combination with each other.

The conditioning regimen was carried out against the background of standard accompanying therapy: crystalloid infusion, antiemetics, intestinal decontamination (fluoroquinolones or gentamicin + fluconazole or nystatin), VOD prevention

Diagnosis	Type of regimen	Diagram of regimen	Number of patients
AML n = 29	standard	Bu-Flu±ATG	13
		Treo-Flu±ATG	13
	individual	5-AZA-Treo-Flu	1
		5-AZA-Vel-Treo-ATG	1
		5-AZA-TT-Flu-Ara-C-ATG	1
ALL n = 15	standard	Bu-Flu±ATG	5
		Treo-Flu±ATG	1
		Treo-VP-CY±ATG	3
	individual	Vel-Treo-Cy-ATG	2
		Vel-Treo-Flu-ATG + КСО 10Гр	1
		5-AZA-Treo-Mel-Flu-ATG	1
		5-AZA-Treo-CY-ATG	1
		Vel-TT-Flu-ARA-C-ATG + КСО 10Гр	1
UMML n = 8	standard	Bu (8)-Flu-ATG	4
		Treo-Flu-ATG	1
	individual	Bu (12)-Flu-ATG	1
		Bu (16)-Flu-ATG	1
		G-CSF + Flu-ARA-C-Mel-Treo-ATG	1
CML n = 5	standard	Bu-Flu±ATG	2
		Treo-Flu±ATG	3
NHL n = 4	standard	Bu-Flu±ATG	3
		Treo-Flu±ATG	1
LH n = 1	standard	Bu-Flu-ATG	1
MDS n = 2	standard	Bu-Flu-ATG	2

Table 3.
Standard and individualized conditioning regimens used in patients before allogeneic HSCT.

(heparin, ursodeoxycholic acids), gastroprotectors, anticonvulsants (when using Bu regimens). The assessment of organ toxicity was carried out according to the same criteria as in patients receiving HDCT [59].

3.4 Obtaining and preparing the graft

On the day of transplantation (day 0), HSCs were collected from the donor. The collection was carried out on a continuous-flow blood cell separator after preliminary stimulation of the G-CSF donor for 4 days. Sessions of HSC collection continued until the required number of CD34+ cells was obtained. The median cellularity of the transfused material during our work was 6.7 ± 0.9 × 106 CD34+ cells/kg (1.3–35.0), 2.4 ± 0.3 × 108 CD3+ cells/kg (0.7–5.1). After the HSC separation process was completed, the material was processed, which consisted of the maximum possible removal of erythrocytes in transplantations incompatible with ABO, as well as the maximum possible reduction in the volume of material transfused to the recipient due to the removal of plasma.

We performed chemical functional depletion of CD3, which was the introduction of vincristine at a rate of 0.0065 mg/ml of graft or 0.0025 mg/108 of nuclear cells in a leukapheresis product into a collection bag with a stem cell separator and methylprednisolone at a rate of 0.35 mg/ml transplant or 0.15 mg/108 nuclear cells in the separate (the method was chosen in which the calculated dose of the drug injected into the bag with the separate, in terms of 1 m^2 of the patient's body surface, was less) at room temperature for 30 minutes. At the end of the incubation, the graft was administered intravenously. In 13 patients, we tried to refuse from chemical depletion. In this group, in 10 (77%) patients, we recorded the early development of the graft engraftment syndrome, which manifested itself in the appearance of fever, skin rash, shortness of breath, and elevated transaminases. The development of symptoms was noted on average at 6 (3–10) days and in all patients was quickly stopped by the appointment of methylprednisolone.

3.5 Evaluation of hematopoietic recovery

From day 0, stimulation of hematopoiesis began in the recipient of G-CSF at an average dose of 5 μg/kg once a day and continued until a steady recovery of neutrophils > 2 × 109/l. The recovery of leukocytes ≥ 1 × 109/l and platelets ≥ 20 × 109/l was considered the first of 3 days of stable establishment of indicators on these figures (without previous transfusions of thromboconcentrate), according to the monitoring of a complete blood count.

After a stable restoration of hematopoiesis, we assessed post-transplant chimerism in the bone marrow and simultaneously recorded the presence and depth of remission (in the case of transplantation in patients with leukemia out of remission or in the presence of a marker for assessing MRD). As a rule, chimerism was assessed once. Reanalysis of chimerism was performed only if a recurrence of leukemia was suspected. In cases where the patient had an informative marker for assessing MRD, MRD was quantified monthly for the first 6 months after HSCT, then once every 2 months until the expiration of 1 year. According to indications, the study was carried out more often and/or continued on a regular basis after a year after HSCT.

3.6 Prevention and treatment of GVHD

GVHD was prevented with calcineurin inhibitors (CNIs: cyclosporin A until 2010, tacrolimus after 2010), a short course of low-dose methotrexate, and administration

of ATH in the post-transplant period (for haploidentical transplants). Cyclosporin A in a single dose of 1.5 mg/kg or tacrolimus in a single dose of 0.0125 mg/kg was administered intravenously for 2–3 hours twice a day, starting from –1 day. The concentration of CNI in the blood was constantly monitored. The recommended therapeutic concentration of cyclosporine A was 200 ± 50 ng/ml, tacrolimus – 5–15 ng/ml. After the restoration of hematopoiesis with adequate control of GVHD and the absence of intestinal toxicity, patients were switched to oral medication. Methotrexate was administered intravenously by bolus once a day at a dose of 10 mg/m^2 on days +1 and +3 and also at a dose of 5 mg/m^2 on day +6. ATH at a dose of 10 mg/kg was administered intravenously for +1 day.

After the onset of GVHD in the post-transplant period, patients were prescribed glucocorticosteroids (GCS) at an average dose of 2 mg/kg/day with their gradual withdrawal after relief of symptoms. Simultaneously with the appointment of GCS, mycophenolate mofetil (MMF) was added to the planned immunosuppressive therapy at an average dose of 30 mg/kg/day. In case of insufficient control of GVHD, alternative methods of treatment were used: extracorporeal photopheresis (ECP), course doses of corticosteroids, administration of ATG, cyclophosphamide, anti-CD20, anti-TNFα. One patient underwent an immunoablative CY-Flu conditioning regimen with transplantation of autologous peripheral blood HSCs mobilized and harvested earlier after two anti-CD20 injections and a CY mobilization course.

Cancellation of immunosuppression was carried out by gradually reducing the dose of immunosuppressants. As a rule, the CNI drug was canceled first. In the case of HLA-compatible transplantations, the withdrawal of immunosuppression began approximately from day +100 in the absence of GVHD. In the case of haploidentical transplantations, the planned decrease in immunosuppression in the absence of GVHD symptoms began no earlier than 6 months after HSCT. In case of reactivation of GVHD against the background of a dose reduction/cancellation of immunosuppression, the patient was prescribed a short course of corticosteroids, and the immunosuppression regimen was returned using the minimum effective doses of drugs. Acute GVHD was assessed according to accepted international modified Keystone criteria [60]. The incidence of acute GVHD was studied among all patients who recovered leukocytes after HSCT. Chronic GVHD was evaluated in patients who survived day +100 after HSCT and had partial or complete donor chimerism. In assessing chronic GVHD, we used the clinicopathological classification used by a group of researchers in Seattle, USA, in a retrospective analysis of our own patients in the late post-transplant period [61]. At the same time, we staged chronic GVHD according to the classification proposed by the GVHD Working Group of the International Bone Marrow Transplant Registry Committee [62].

3.7 Assessment of transplant mortality

Transplant mortality was assessed in the entire group of patients who received allo-HSCT and included mortality in the post-transplant period of patients who were in remission/stabilization for the underlying disease, from toxic, infectious complications associated with HSCT, or GVHD.

4. Results

4.1 Toxicity of conditioning regimens and restoration of hematopoiesis

Grade 4 hematological toxicity was noted in all patients who received conditioning regimens prior to allogeneic HSCT. Grade 3–4 organ toxicity was registered in 9% of patients.

Establishment of stable donor hematopoiesis after allogeneic HSCT occurred in 55 (86%) patients. On average, the recovery time for leukocytes > 1 × 109/l, neutrophils > 0.5 × 109/l was 11 days, platelets > 20 × 109/l –12 days. In three (4%) patients with refractory acute leukemia and grade 3–4 pancytopenia at the start of the conditioning regimen, hematological recovery did not occur. All these children died in the early post-transplant period from infection and progression of leukemia. In six patients with JMML (n = 5)/MDS (n = 1), restoration of their own pathological hematopoiesis was registered due to the displacement of the graft by a tumor clone within 2–7 weeks after transplantation.

4.2 Graft-versus-host disease

Against the background of standard immunosuppressive prophylaxis, the frequency of grade III-IV aGVHD did not exceed 15% (only cases of haplo-HSCT). Chronic advanced GVHD was registered in 24% of patients, and in half of the cases it had a severe course.

4.3 Survival score. Mortality structure

Of the 64 patients at the time of closing the database (March 2019), 22 (34%) people were alive, one patient was lost from observation. The median follow-up time for surviving patients is 56.6 (3.1–182.8) months. Data on the number of surviving patients in various nosological groups and the duration of their follow-up are presented in **Table 4**.

Twenty-three (36%) patients died from recurrent hematological tumors. In one (1.5%) patient after 7 months after allo-HSCT for secondary AML, the primary tumor recurred (Ewing's sarcoma), which caused the death of the patient. Treatment-related

Diagnosis, number of patients	Percentage of survivors	Duration Observations, months
AML, n = 29	14 (48%)	59 (3–183)
ALL, n = 15	3 (20%)	25, 12, and 6
CML, n = 5	2 (40%)	167 and 146
UMML/MDS, n = 10	3 (33%)	157, 97, and 53
NHL, n = 4	0	—
LH, n = 1	0	—

Table 4.
The number of surviving patients in various nosological groups and the period of observation of them.

complications caused the death of 17 (27%) patients: 11 (17.5%) died as a result of cGVHD, 3 (4.5%) as a result of aGVHD and concomitant infectious complications, 3 (4.5%) from a combination of toxicity of therapy and infection.

The death of patients from cGVHD occurred on average after 45.5 (3.1–165.8) months. after HSCT—the period when patients are usually observed at the place of residence. Thus, the problems of adequate follow-up of patients after allo-HSCT away from the transplant center play a certain role in the development of fatal cGVHD.

With the improvement of accompanying therapy and criteria for selecting patients for allo-HSCT, a trend toward a decrease in transplant mortality was noted. Thus, the estimated 5-year transplant mortality in all patients who received HSCT before 2010 was 40%, after 2010, 18.5%. In patients who received partially compatible HSCT, 52.8% and 21.0%, respectively. The decrease in the difference in transplant mortality between the entire group of patients and patients who received haplo-HSCT over time indicates the leveling of differences between fully and partially compatible transplants in terms of the development of severe transplant complications.

4.4 Results of HSCT in patients with AML

The best results of allogeneic HSCT were achieved in patients with AML (n = 29). The curves do not differ very significantly, since only one patient after relapse (myeloid sarcoma 39 months after haplo-HSCT) received combined treatment, including repeated HSCT from the same donor, and alive for 17 months. after repeated HSCT without signs of underlying disease.

The group of patients with high-risk AML is heterogeneous and included a subgroup of 13 patients with an extremely poor prognosis (secondary AML after another malignant disease – 7, AML out of remission at the time of HSCT – 6) (**Table 2**). As can be seen from Figure, the results of overall survival for this subgroup differed significantly from the rest of the patients: in the conditionally "favorable" subgroup, the 5-year OS was 75%, in the unfavorable subgroup, 26%.

In patients with AML, as the largest group of children who received allo-HSCT, we considered it interesting to analyze the correlation of immunological effects of graft-versus-host and graft-versus-tumor. For analysis, patients who received HSCT in the status of clinical and hematological remission of AML were selected, in whom the analysis of cGVHD was possible. The group consisted of 23 patients. In 13 (57%) patients, there were no signs of clinically significant crGVHD, in 10 (43%) patients, signs of crGVHD of varying severity were recorded. Both groups were comparable in terms of the ratio of patients with a "relatively favorable" and "extremely unfavorable" prognosis. In the first group, five relapses were registered, in the second – 3. Thus, a certain correlation of the two immunological effects is certainly present. The results of OS were also better in patients with controlled cGVHD, while the follow-up period for patients was quite long. Thus, the 8-year OS of patients who had signs of cGVHD was 80.8%, and that of those who did not have cGVHD was 46.6%. Thus, in patients with high-risk AML, one should strive to obtain a controlled course of cGVHD in the post-transplant period.

4.5 Results of HSCT in patients with ALL

Of the 15 patients with ALL at the time of closing the database, three (20%) were alive in remission, seven (47%) died from recurrent leukemia, three (20%) died from cGVHD, one (6.5%) died from toxic and infectious complications, and one more (6.5%) was lost from observation.

Thus, the progression of the underlying disease mainly in the first year after allo-HSCT was the main cause of death in the considered patients with ALL of the ultra-high-risk group.

4.6 Results of HSCT in JuMML/MDS Patients

Of 10 patients with JMML/MDS, 6 (60%) relapsed within the first 2 months. after TGSC. Only one of these six patients went into stable remission after retransplantation, but after 63 months. died from hrGVHD. The remaining five relapsed patients died from subsequent relapses. Another patient died from cGVHD and concomitant infection at 40 months. after haplo-HSCT. Thus, by the time the database was closed, three out of 10 patients (33%) were alive, their follow-up periods were 157, 97, and 53 months. (**Table 4**), two (20%) died from crGVHD and associated infectious complications, five (50%) died from progression of JMML/MDS. Relapses after allogeneic HSCT remain the leading cause of death in patients diagnosed with JMML/MDS.

4.7 Results of HSCT in patients with lymphomas and CML

Only in one patient with mediastinal large B-cell lymphoma, it was possible to achieve long-term (24 months) stabilization of the disease against the background of the course of advanced cGVHD; subsequently, this patient died from the progression of lymphoma. The remaining three patients with lymphomas died early after HSCT. Of the five patients with CML at the time of closing the database, two were alive, the follow-up period for them was 147 and 167 months. Three patients died in remission from late complications of HSCT.

4.8 Evaluation of the effectiveness of individualized conditioning regimens in patients with acute leukemia and JMMML/MDS

Individualized conditioning regimens were used in six patients with ALL and three patients with AML from the worst prognostic subgroup. Such regimens in combination with allogeneic HSCT contributed to the achievement/maintenance of clinical and hematological remission for at least 6 months in seven out of nine patients, three patients (two ALL and one AML) remained alive for more than 2 years.

Attempts to escalate the dose of the alkylating agent (busulfan) from 8 mg/kg to 12 and 16 mg/kg to increase the antitumor effect did not lead to a positive result in two younger patients with JMML. Both patients relapsed within the first 2 months after allo-HSCT. A different approach was used in another 3-year-old patient with JMML and monosomy 7. The conditioning regimen for the patient consisted of three components: tumors with a combination of drugs fludarabine and cytarabine; 2 – myeloablation with two alkylating agents; 3 – immunoablation with antithymocyte immunoglobulin. Upon completion of the conditioning regimen, the patient underwent HSCT from a haploidentical mother. At the time of closing the database, the patient remained in remission for 53.5 months after TGSC.

5. Conclusion

The role of HSCT in pediatric oncology is very wide. In cases where the oncological process directly captures the cells responsible for the patient's hematopoiesis,

allogeneic HSC transplantation allows not only to replace the patient's inadequately functioning hematopoietic system, but also to carry out antitumor immunological control. The concept of antitumor control of the donor immune system formed the basis for studying the effect of allogeneic HSCT both in leukemia and in non-hematological oncological diseases.

Due to the specifics of Russian families (a small number of children, often from different fathers), the search for a HLA compatible donor within a family is rarely successful. The use of RSIs, the development of graft rejection and GVHD prevention algorithms, improvement of supportive treatment, along with the lack of budget expenses related to donor searching in registers, all those dramatically actualize using haploidentical transplantations in the country. In the absence of lymphocyte depletion in the graft, patients restore 100% donor hematopoiesis in a short time, after which the time comes for quite painstaking work to correct the activity of the donor's immune system in the recipient's body. Considering that in pediatric oncologists allogeneic HSCT is performed only in patients with extremely unfavorable forms of diseases with a high risk of recurrence, the doctor's goal is to maintain the donor's immune system at a certain level so that antitumor control is ensured and severe forms of GVHD do not develop. Maintaining such a balance, if possible, is the key to successful HSCT. The graft-versus-tumor effect has been described in the literature and shown in our work to a greater extent for myeloid leukemias and to a lesser extent for lymphoid ones. The work also shows a direct correlation between the immunological effects of graft-versus-host and graft-versus-tumor.

According to our data, the use of allogeneic HSCT after completion of the main CT within the framework of modern therapeutic protocols makes it possible to expect the achievement of long-term EFS in approximately 50% of patients with high-risk AML, and the prognosis significantly depends on the achievement of remission by the time of HSCT. For patients with primary AML in remission at the end of CT induction, the 5-year EFS reached 75%. For ALL in our work, the results turned out to be worse, apparently due to the characteristics of the cohort of these patients with the highest likelihood of relapse. The reduced intensity conditioning regimen used in the work is not suitable for such cases. With the development of new approaches in the programmatic treatment of leukemia, the allogeneic HSCT method is given an even smaller role, and the cases of patients receiving this type of treatment are becoming more complex and diverse. Individualization of approaches to conditioning regimens, based on the characteristics of both the patient himself (somatic status) and his tumor (localization, sensitivity to certain drugs and methods of exposure, completeness of response by the time of HSCT, etc.), allows improving the transplantation outcome in each specific case. We have shown this in our work on a cohort of patients with refractory acute leukemia. Only with the help of individualization of approaches to conditioning regimens, we managed to achieve positive results of HSCT in patients with refractory ALL. The same applies to patients with other cancers with extremely high expected recurrence rates (AML, JMML/MDS).

HSC transplantation remains relevant in pediatric oncology and should be used as part of program therapy, based on an analysis of indications and contraindications for each individual patient, as well as the presence/absence of a suitable donor. Setting the right indications, choosing adequate conditioning regimens, along with improving accompanying therapy, are the key to increasing the effectiveness of the HSCT method in children with malignant diseases of an extremely unfavorable prognosis.

DOI: http://dx.doi.org/10.5772/intechopen.107830

Author details

Natalia Subbotina[1*], Igor Dolgopolov[2], Georgij Mentkevich[1] and Maxim Rykov[1,3]

1 Department of Oncology, Tver State Medical University of the Ministry of Health of Russia, Russia

2 Department of Pediatrics, Tver State Medical University of the Ministry of Health of Russia, Russia

3 N.A. Semashko National Research Institute of Public Health, Russia

*Address all correspondence to: docsub@mail.ru

References

[1] Schultz K et al. Philadelphia chromosome-negative very high-risk acute lymphoblastic leukemia in children and adolescents: Results from Children's Oncology Group Study AALL0031. Leukemia. 2014;**28**(4):964

[2] Schrappe M et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. The New England Journal of Medicine. 2012;**366**(15):1371

[3] Pui CH et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. The New England Journal of Medicine. 2009;**360**(26):2730

[4] ALL IC-BFM. et al. 2009. A Randomized Trial of the I-BFM-SG for the Management of Childhood non-B Acute Lymphoblastic Leukemia

[5] Dreyer ZE et al. Analysis of the role of hematopoietic stem-cell transplantation in infants with acute lymphoblastic leukemia in first remission and MLL gene rearrangements: A report from the Children's Oncology Group. Journal of Clinical Oncology. 2011;**29**(2):214

[6] Mann G et al. Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia (MLL)-rearranged acute lymphoblastic leukemia: Results from the Interfant-99 Study. Blood. 2010;**116**(15):2644

[7] Schultz KR 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: Official Journal of the American Society of Clinical Oncology. 2009;**27**(31):5175

[8] Pulsipher MA et al. New frontiers in pediatric Allo-SCT: Novel approaches for children and adolescents with ALL. Bone Marrow Transplantation. 2014;**49**(10):1259

[9] Saarinen-Pihkala UM et al. Pathways through relapses and deaths of children with acute lymphoblastic leukemia: Role of allogeneic stem-cell transplantation in Nordic data. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2006;**24**(36):5750

[10] Davies SM et al. Comparison of preparative regimens in transplants for children with acute lymphoblastic leukemia. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2000;**18**(2):340

[11] Boztug H et al. Treosulfan-based conditioning regimens for allogeneic HSCT in children with acute lymphoblastic leukaemia. Annals of Hematology. 2015;**94**(2):297

[12] Pulsipher MA et al. The addition of sirolimus to tacrolimus/methotrexate GVHD prophylaxis in children with ALL: A phase 3 Children's Oncology Group/Pediatric Blood and Marrow Transplant Consortium trial. Blood. 2014;**123**(13):2017

[13] Carpenter PA et al. Transplantation for AML in children. Journal of American Society of Blood Marrow Transplantation. 2012;**18**(Suppl. 1):33

[14] Alonzo TA et al. Conventional cytogenetics, molecular profiling, and flow cytometric response data allow the creation of a two-tiered risk-group system for risk-based therapy allocation in childhood AML: A report from the

Children's Oncology Group. Blood. 2010;**116**(21):761

[15] Slavin S et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;**91**(3):756

[16] Abdul Wahid SF et al. Comparison of reduced-intensity and myeloablative conditioning regimens for allogeneic hematopoietic stem cell transplantation in patients with acute myeloid leukemia and acute lymphoblastic leukemia: A meta-analysis. Stem Cells and Development. 2014;**23**(21):2535

[17] Luger SM et al. Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. Bone Marrow Transplantation. 2012;**47**(2):203

[18] Ishida H et al. Comparison of a fludarabine and melphalan combination-based reduced toxicity conditioning with myeloablative conditioning by radiation and/or busulfan in acute myeloid leukemia in Japanese children and adolescents. Pediatric Blood & Cancer. 2015;**62**(5):883

[19] Bitan M et al. Transplantation for children with acute myeloid leukemia: A comparison of outcomes with reduced intensity and myeloablative regimens. Blood. 2014:123, 1615

[20] Baron F et al. Impact of graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia: A report from the Acute Leukemia Working Party of the European group for blood and marrow transplantation. Leukemia. 2012;**26**(12):2462

[21] Weisdorf D et al. Graft-versus-host disease induced graft-versus-leukemia effect: Greater impact on relapse and disease-free survival after reduced intensity conditioning. Journal of American Society of Blood Marrow Transplantation. 2012;**18**(11):1727

[22] Shaw PJ et al. Outcomes of pediatric bone marrow transplantation for leukemia and myelodysplasia using matched sibling, mismatched related, or matched unrelated donors. Blood. 2010;**116**(19):4007

[23] Schwartz E et al. Abrogation of bone marrow allograft resistance in mice by increased total body irradiation correlates with eradication of host clonable T cells and alloreactive cytotoxic precursors. Journal of Immunology. 1987;**138**(2):460

[24] Cobbold SP et al. Monoclonal antibodies to promote marrow engraftment and tissue graft tolerance. Nature. 1986;**323**(6084):164

[25] Terenzi A et al. Enhancement of T cell-depleted bone marrow allografts in mice by thiotepa. Transplantation. 1990;**50**(4):717

[26] Aversa F et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: A phase II study in patients with acute leukemia at high risk of relapse. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2005;**23**(15):3447

[27] Aversa F et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood. 1994;**84**(11):3948

[28] Schumm M et al. Isolation of highly purified autologous and allogeneic peripheral CD34+ cells using the CliniMACS device. Journal of Hematotherapy. 1999;**8**(2):209

[29] Klingebiel T et al. Results and factors influencing outcome after fully haploidentical hematopoietic stem cell transplantation in children with very high-risk acute lymphoblastic leukemia: Impact of center size: An analysis on behalf of the Acute Leukemia and Pediatric Disease Working Parties of the European Blood and Marrow Transplant group. Blood. 2010;**115**(17):3437

[30] Ciceri F et al. A survey of fully haploidentical hematopoietic stem cell transplantation in adults with high-risk acute leukemia: A risk factor analysis of outcomes for patients in remission at transplantation. Blood. 2008;**112**(9):3574

[31] Barfield RC et al. A one-step large-scale method for T- and B-cell depletion of mobilized PBSC for allogeneic transplantation. Cytotherapy. 2004;**6**(1):1

[32] Oevermann L et al. New strategies for haploidentical transplantation. Pediatric Research. 2012;**71**:418

[33] Hale GA et al. Haploidentical stem cell transplantation with CD3 depleted mobilized peripheral blood stem cell grafts for children with hematologic malignancies. Blood. 2005;**106**(11):2910

[34] Hale GA et al. Mismatched family member donor transplantation for patients with refractory hematologic malignancies: Long-Term Followup of a Prospective Clinical Trial. Blood. 2006;**108**(11):3137

[35] Handgretinger R et al. Feasibility and outcome of reduced-intensity conditioning in haploidentical transplantation. Annals of the New York Academy of Sciences. 2007;**1106**:279

[36] Federmann B et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: A phase II study. Haematologica. 2012;**97**(10):1523

[37] Bader P et al. Rapid immune recovery and low TRM in haploidentical stem cell transplantation in children and adolescence using CD3/CD19-depleted stem cells. Best Practice & Research. Clinical Haematology. 2011;**24**(3):331

[38] Dufort G et al. Feasibility and outcome of haploidentical SCT in pediatric high-risk hematologic malignancies and Fanconi anemia in Uruguay. Bone Marrow Transplantation. 2012;**47**(5):663

[39] Palma J et al. Haploidentical stem cell transplantation for children with high-risk leukemia. Pediatric Blood & Cancer. 2012;**59**(5):895

[40] Gonzalez-Vicent M et al. Graft manipulation and reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation from mismatched unrelated and mismatched/haploidentical related donors in pediatric leukemia patients. Journal of Pediatric Hematology/ Oncology. 2010;**32**(3):85

[41] Oevermann L et al. Immune reconstitution and strategies for rebuilding the immune system after haploidentical stem cell transplantation. Annals of the New York Academy of Sciences. 2012;**1266**:161

[42] Locatelli F et al. Strategies to optimize the outcome of children given T-cell depleted HLA-haploidentical

hematopoietic stem cell transplantation. Best Practice & Research. Clinical Haematology. 2011;**24**(3):339

[43] Azevedo RI et al. Long-term immune reconstitution of naive and memory t cell pools after haploidentical hematopoietic stem cell transplantation. Journal of American Society of Blood Marrow and Transplantation. 2013;**19**(5):703

[44] Bonneville M et al. Gammadelta T cell effector functions: A blend of innate programming and acquired plasticity. Nature Reviews. Immunology. 2010;**10**(7):467

[45] Godder KT et al. Long term disease-free survival in acute leukemia patients recovering with increased gammadelta T cells after partially mismatched related donor bone marrow transplantation. Bone Marrow Transplantation. 2007;**39**(12):751

[46] Chiplunkar S et al. Gammadelta T cells in cancer immunotherapy: Current status and future prospects. Immunotherapy. 2009;**1**(4):663

[47] Lang P et al. Improved immune recovery after transplantation of TCRαβ/CD19-depleted allografts from haploidentical donors in pediatric patients. Bone Marrow Transplantation. 2015;**50**:6

[48] Kaynar L et al. TcRαβ-depleted haploidentical transplantation results in adult acute leukemia patients. Hematology. 2017;**22**(3):136

[49] Dodero A et al. Haploidentical stem cell transplantation after a reduced-intensity conditioning regimen for the treatment of advanced hematologic malignancies: Posttransplantation CD8-depleted donor lymphocyte infusions contribute to improve T-cell recovery. Blood. 2009;**113**(19):4771

[50] Amrolia PJ et al. Adoptive immunotherapy with allodepleted donor T-cells improves immune reconstitution after haploidentical stem cell transplantation. Blood. 1797;**2006**:108

[51] Mielke S et al. A clinical-scale selective allodepletion approach for the treatment of HLA-mismatched and matched donor-recipient pairs using expanded T lymphocytes as antigen-presenting cells and a TH9402-based photodepletion technique. Blood. 2008;**111**(8):4392

[52] Feuchtinger T et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. Blood. 2010;**116**(20):4360

[53] Feuchtinger T et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. British Journal of Haematology. 2006;**134**(1):64

[54] Lugthart G et al. Simultaneous generation of multivirus-specific and regulatory T cells for adoptive immunotherapy. Journal of Immunotherapy. 1997;**35**(1):42

[55] Di Ianni M. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. Blood. 2011;**117**(14):3921

[56] Passweg JR et al. Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. Leukemia. 2004;**18**(11):1835

[57] Rubnitz JE et al. NKAML: A pilot study to determine the safety and feasibility of haploidentical natural

killer cell transplantation in childhood acute myeloid leukemia. Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology. 2010;**28**(6):955

[58] Rizzieri DA et al. Natural killer cell-enriched donor lymphocyte infusions from A 3-6/6 HLA matched family member following nonmyeloablative allogeneic stem cell transplantation. Journal of American Society of Blood Marrow and Transplantation. 2010;**16**(8):1107

[59] NCI Common Terminology Criteria for Adverse Events. CTCAE 4.03. 2010. Available from: https://evs.nci.nih.gov/ftp1/CTCAE/About.html

[60] Przepiorka D et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplantation. 1995;**15**(6):825

[61] Shulman HM et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. American Journal of Medicine. 1980;**69**(2):204

[62] Lee SJ et al. Severity of chronic graft-versus-host disease: Association with treatment-related mortality and relapse. Blood. 2002;**100**(2):406

Chapter 8

CRISPR-Cas9-based Strategies for Acute Lymphoblastic Leukemia Therapy

Edgardo Becerra, Valeria J. Soto Ontiveros and Guadalupe García-Alcocer

Abstract

Defeating cancer as leukemia has been an up and down challenge. However, leukemia must be treated from the roots. Nowadays, the CRISPR-Cas9 system provided scientists the ability to manipulate the genetic information to correct mutations, rewrite genetic code, or edit immune cells for immunotherapy purposes. Additionally, such system is used for basic and clinical approaches in leukemia therapy. Lymphoid cancers including acute lymphoblastic leukemia (ALL) can be treated by performing gene editing or enhancing immune system through CART cells. Here, we present and detail therapeutic applications of the CRISPR/Cas9 system for immune cell therapy, and knock-out or knock-in of main genes promoting leukemogenesis or ALL progression. We also described current and future challenges, and optimization for the application of CRISPR/Cas9 system to treat lymphoid malignancies.

Keywords: acute lymphoblastic leukemia, gene therapy, CRISPR, Cas9, mutations, homologous direct repair, cancer, cell engineering

1. Introduction

Acute Lymphoblastic Leukemia (ALL) is a type of hematological cancer that affects children and adults around the world. Since the first chemotherapeutic drugs were developed to treat this disease, the survival rate has increased. However, side effects represent one of the main challenges to defeat in ALL. Current therapy for ALL provides selectivity to avoid side effects as much as possible. Still, more specific and effective treatments are necessary. Indeed, gene therapy points to be the most promising future medicine to defeat cancer as ALL. The main objective of gene therapy is to correct mutations that promote leukemogenesis, thereby counteracting or remedying the conditions caused by malfunctioning genes. The most studied and effective technology to alter DNA is the CRISPR-Cas9 system first used to target DNA in 2013. In this chapter, the mechanisms of the CRISPR-Cas9 technology as well as their current and highlight strategies to treat ALL will be explained.

2. Acute lymphoblastic leukemia

2.1 Definition and statistics

Acute lymphoblastic leukemia (ALL) is a hematologic malignancy characterized by impaired differentiation, high proliferation, and accumulation of both T and B lymphoblast in the bone marrow, peripheral blood, and/or extramedullary sites [1, 2]. Children, adolescents, and young adults comprise 70% of ALL cases [3]. The incidence of ALL in the United States (US) is 1.8 per 100,000 for all age groups and 3.5 per 100,000 for ages 0–19. Survival in ALL is strongly influenced by age with five-year relative survival rates of 92.1% in <15 years, 64.1% in 15–39 years, and < 41% in >39 years [4].

2.2 Genetic alterations

2.2.1 B cell ALL

Subtypes of B-ALL are characterized by chromosomal alteration (aneuploidy or chromosomal rearrangements), which promotes deregulation of proteins by chimeric genes formation, and upregulation of genes by juxtaposition with a strong enhancer (**Table 1**). The risk stratification can be established based on such chromosomal abnormalities. Both copy number alterations and sequence mutations on lymphoid transcription factors contribute to leukemogenesis as secondary genomic events [5, 6].

2.2.2 T cell ALL

T-ALL is the result of a multistep process where accumulation of genetic mutations alters the normal control of cell growth, differentiation, proliferation, and survival during thymopoiesis (**Table 2**). The genetics of T-ALL is highly heterogeneous, with abnormalities in almost all patients, and it accounts for approximately 15% and 25% of pediatric and adult ALLs, respectively [5, 6].

2.3 Treatments

2.3.1 Chemotherapy

Front-line treatment for ALL consists of a multiagent chemotherapy regimen, typically divided into 3 phases: induction (combination of steroids, anthracyclines, and vincristine), consolidation, and long-term maintenance (over a 2 to 3- year period), and recommend central nervous system (CNS) prophylaxis [5]. Despite high rates of complete remission (CR) (80–90%), about 20% of pediatric and 40% of adult patients will relapse [1, 7]. This treatment can also affect some normal cells in the body, leading to side effects such as hair loss, diarrhea, constipation, and loss of appetite, among others. Besides, affects normal cells in bone marrow leading to leukocytopenia (increased risk of infections), thrombocytopenia (easy bruising or bleeding), and/or anemia (fatigue and shortness of breath) [5, 8].

2.3.2 Immunotherapy

Immunotherapy is the use of medicines to help patient's immune system to recognize and destroy cancer cells in a more effective way [9]. A broad range of

Category	Frequency	Description	Prognosis
High hyperdiploid (gain ≥5 chromosomes)	Children 25% AYAs and adults 3%	Mutations in RTK-RAS signaling pathway and histone modifiers	Favorable
Near-haploid (24–31 chromosomes)	Children 2% AYAs and adults <1%	Ras-activating mutations Inactivation of IKZF3	Poor
Low-hypodiploid (32–39 chromosomes)	Children <1% AYAs 5% Adults >10%	TP53 mutations Inactivation of IKZF2 Deletions of CDKN2A/B and RB1	Very poor
MLL (KMT2A) rearrangements	Infants >80% Children <1% AYAs 4% Adults 15%	Low number of additional somatic mutations, commonly in kinase PI3K-RAS signaling pathway	Very poor
t(12;21)(q13;q22) translocation encoding ETV6-RUNX1	Children 30% AYAs and adults <5%		Favorable
t(1;19)(q23;p13) translocation encoding TCF3-PBX1	Children, AYAs, and adults ≈5%	Association with CNS relapse	Favorable
t(1;19)(q23;p13) variant of the TCF3-HLF	<1% ALL		Poor
Philadelphia chromosome, t(9;22)(q34;q11) translocation encoding BCR-ABL1	Children 2–5% AYAs 6% Adults >25%	Common deletions of IKZF1, CDKN2A/B, and PAX5	Poor (improved with tyrosine kinase inhibitors)
Philadelphia chromosome -like ALL	Children 10% AYAs 25–30% Adults 20%	Rearrangement of CRLF2 (≈50%) Rearrangement of ABL-class tyrosine kinase genes (≈12%) Rearrangement of JAK2 (≈10%) EPO receptor (≈ 3–10%) Mutations activating JAK–STAT (≈ 10%) and Ras (≈2–8%) signaling pathways	Poor
DUX4- and ERG deregulated ALL	5–10% ALL	Distinct gene expression profile; most cases present focal ERG deletions	Favorable, including if coexistence of IKZF1 mutation (≈ 40% patients)
ZNF384-rearranged ALL	Children 5% AYAs and adults 10%	ZNF384 rearranged with a transcriptional regulator or chromatin modifier (EP300, CREBBP, TAF15, SYNRG, EWSR1, TCF3, ARID1B, BMP2K, and SMARCA2)	Intermediate

Table 1.
Main genetic subtypes of B-cell acute lymphoblastic leukemia.

immunotherapy strategies has been developed to overcome the failure of front-line treatment as well for patients with relapsed/refractory (R/R) disease [10], which includes the following:

Category	Frequency	Description	Prognosis
TAL1 deregulation	50% of T-ALL Children<adults	Overexpressed oncogenic transcription factor t(1;7)(p32;q35) and t(1;14)(p32;q11) translocations and interstitial 1p32 deletion	Generally favorable
LMO2 deregulation	50% of T-ALL children	Overexpressed oncogenic transcription factor t(11;14)(p15;q11) translocation and 5′ LMO2 deletion	Generally favorable
TLX1 (HOX11) deregulation	50% of T-ALL Children<adults	Overexpressed oncogenic transcription factor t(10;14)(q24;q11) and t(7;10)(q35;q24) translocations	Good
TLX3 (HOX11L2) deregulation	50% of T-ALL Children and adults	Overexpressed oncogenic transcription factor t(5;14)(q35;q32) translocation; commonly fused to BCL11B, also a target of deletion and/or mutation	Poor
MLL rearrangements	Children	Multiple partners; disruption of HOX gene expression and of self-renewing	Poor
9q34 amplification encoding NUO214-ABL1	8% of T-ALL Children	Amenable to tyrosine kinase inhibitors, also identified in high risk B-ALL; other kinase fusions identified in T-ALL include EML1-ABL1, ETV6-JAK2, and ETV6-ABL1	
t(7;9)(q34;q34.3)	Children	Rearrangement of NOTCH1	
NOTCH1 mutations	80% of T-ALL; children and adults	Constitutive activation of NOTCH signaling Impairment of differentiation and proliferation	Overall favorable
FBXW7 mutations	Present in 80% of patients; children and adults	Loss of function mutations Impairment of differentiation and proliferation, usually evaluated in combination with NOTCH1	
Early T-cell precursor ALL	Children and adults	Immature immunophenotype; expression of myeloid and/or stem-cell markers; poor outcome; genetically heterogeneous with mutations in hematopoietic regulators, cytokine and Ras signaling, and epigenetic modifiers	

Table 2.
Main genetic subtypes of T-cell acute lymphoblastic leukemia.

Monoclonal antibodies include unconjugated antibodies and antibody-drug conjugate (ADC). Antibody therapy can lead to direct apoptosis, complement-dependent cytotoxicity (CDC), and antibody-dependent cell-mediated cytotoxicity (ADCC). The targets for ALL include CD19, CD20, CD22, and CD52 [11, 12].

Bispecific antibodies are single chain variable fragments (Scfv) consisting of at least two different specific antibodies one for tumor-associated surface antigens and the other for surface antigens on effector cells (CD3 ε on T cells) redirecting T cells to lyse malignant cells: CD3/CD19 and CD3/CD20 bispecific T-Cell Engagers (BiTE) [13, 14].

Chimeric antigen receptor -T (CART) are genetically engineered cell membrane binding receptors, which can activate T cells by linking from the extracellular antigen

binding region to the intracellular signal domain via the spacer, whose effect on target antigen is independent of major histocompatibility complex MHC [15].

Even though immunotherapy is the most promising tumor treatment, the advances in molecular biology tools have made it possible to manipulate human DNA by different strategies to treat diseases at the roots. One of the main tools for precise gene editing is CRISPR-Cas9, which allows simple, cost-efficient human cells and another eukaryotic genome editing.

2.4 Overview of CRISPR-Cas9

Clustered regularly interspersed short palindromic repeats (CRISPR), first found in 1987, function as part of an adaptive prokaryotic immune system (CRISPR-associated system, Cas) against phage infection and plasmid transfer in nature [16, 17]. CRISPR-Cas systems are grouped into two classes and subdivided into six types. Class 1 consists of types I, III, and IV, while class two consists of types II, V, and VI. Class 2 systems act by single-subunit effector proteins such as Cas9 and Cas12a and are more precise and efficient for genome engineering than class 1 systems. CRISPR-Cas9 recognizes target DNA through CRISPR RNA (crRNA) and trans activating RNA (tracrRNA), which form a single guide RNA (sgRNA) that activates and guides Cas9 to bind target DNA, which is subsequently cleaved to form a double-strand break (DSB) (**Figure 1**) [18].

Since DSB is repaired in a host cell, it may introduce DNA mutations leading to genetic changes when non-homologs end joining (NHEJ) predominates as repair mechanism. NHEJ is an error-prone repair mechanism that often leads to insertions or deletions (indels). These indels can cause frameshift mutations, large deletions or inversions using two adjacent DSBs, genomic rearrangements, NHEJ-mediated homology-independent knock-in, premature stop codons, or/and nonsense mediated decay to the target gene, resulting in a loss-of-function. In contrast, homology-directed repair (HDR), considered a high-fidelity repair, uses assisted recombination of DNA donor templates to reconstruct DSBs. This mechanism can be exploited to introduce well-defined mutations by transferring altered donor templates into targeted cells, which provides the basis to perform precise gene modification, such as gene knock-in/knock-out, insertion, deletion, correction, or replacement [16, 18].

In addition, a nuclease-deficient Cas9 modification (dCas9) can be fused to a variety of effector domains to mediate specific local DNA manipulations as [19]:

- Transcriptional regulation:
    - CRISPR interference (CRISPRi) inhibits transcription by sterically blocking the RNA polymerase [20].
    - CRISPR activation (CRISPRa) binds to the upstream promoter regions and recruits the RNA polymerase, and further activates transcription [20].
- Epigenetic modification:
    - Increasing/repressing DNA methylation of CpG motifs in targeted genomic regions [21–24].

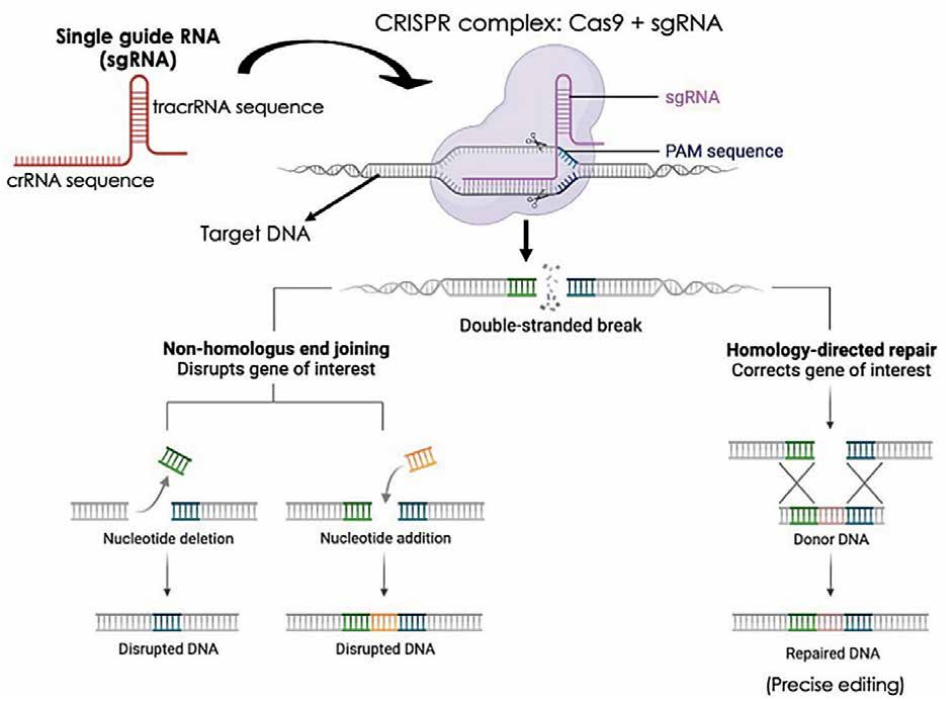

Figure 1.
CRISPR-Cas9 mechanism to disrupt or edit genes precisely.

- Modifying acetylation of histones to increase the accessibility of genomic regions [25].

- Nucleotide editing: Cas9-nickases (nCas9) were developed to change single DNA nucleotides without introducing DSBs or requiring any homology-directed repair. The system consists of dCas9 coupled to cytidine deaminase domains to induce targeted transitions from C to T or G to A [26–28].

2.4.1 CRISPR-Cas9-based therapies for acute lymphoblastic leukemia

The next-generation sequencing (NGS) has allowed researchers to analyze genes in a better way and thus, obtain a better correlation between genotype and phenotype. Integrating gene editing to gene sequencing enables researchers to manipulate any described gene or noncoding sequences in a wide variety of organisms including humans. Here, we present and discuss relevant subgroups of genes that were edited as well as therapy strategies based on CRISPR-Cas9.

2.4.1.1 Improving immunotherapy via CRISPR-Cas9

Since immunotherapy provides the most promising clinical outcomes in leukemia, it is attractive to perform the generation of CART cells via CRISPR-Cas9. First, autologous T cells are collected from patients and further genetically engineered to recognize and attack cancer antigens in *ex vivo* experiments. Cells expressing CART are then transferred into the patient. The use of CRISPR-Cas9 to produce CART cells will allow

to treat efficiently different types of cancers and provide higher safety than conventional therapy [29]. Besides, CRISPR-Cas9 can be used to disrupt genes that code signaling molecules or T cell inhibitory receptors to enhance the CART cell's function.

Immunotherapy can now be improved by using CRISPR-Cas9 to increase the efficacy of CART cells. Such improvement can be performed through knock-out and knock-in mutations in the T-cell receptor α constant (TRAC) locus and CAR against CD19. Previous studies reported that CAR inserted in the AAVS1 locus, which is under control of endogenous regulatory elements, reduces tonic signaling, and blocks differentiation and T cell depletion leading to an increased therapeutic function of CART cells [30]. The disadvantage of CART therapy is due many patients are unable to receive engineered autologous T-cells but can be faced by generating universal infusion products obtained from healthy donors. Nevertheless, the use of "off-the-shelf" CART cell products can promote the induction of graft-versus-host disease (GVHD) because the allogenic T-cells were *ex vivo* activated causing rejection by the host [31]. To solve this issue, endogenous αβ T-cell receptors (TCRs) are knocked out by CRISPR-Cas9 on transferred donor lymphocytes that interact with/recognize alloantigens, which avoid GVHD. Besides, the deletion or elimination of the subunit of human leukocyte antigen class I (HLA-I) beta-2-microglobulin (β2M) would decrease rapid depletion of allogenic cells expressing foreign HLA molecules [32]. According to preclinical data regarding CRISPR-Cas9-related clinical trials, stimulating NHEJ by cleaving endogenous TCR and β2M through CRISPR-Cas9 will generate universal CD19-directed CART cells to avoid rejection.

The function of CART cells may be negatively regulated by overexpression of negative checkpoint regulators plus up-regulation of cognate inhibitory ligands in the tumor microenvironment. The negative checkpoint regulator programmed cell death 1 (PD-1) as well as other inhibitory receptors such as T-cell Ig and ITIM domain (TIGIT), T cell membrane protein-3 (TIM-3), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), and lymphocyte-activation protein-3 (LAG-3) act in a synergistic way to reduce immune cell availability [33, 34]. Currently, the administration of checkpoint inhibitors has been the most effective strategy to reinvigorate such lymphocytes. Unfortunately, severe immune-related side effects affect some patients. In this line, knocking-out multiple genes by CRISPR-Cas9 allows the production of universal, allogenic T-cells lacking multiple negative regulators ready to be transferred into patients [31].

The modification of CART cells against CD19 (CAR19) has been used to effectively treat refractory relapsed B-ALL in infants. The transcription activator-like effectors nucleases (TALENs) technology was used to produce CAR19 and set the basis for engineering T-cells using CRISPR-Cas9 to improve the outcomes [35]. It seems that some type of resistance to CART is observed in 10–20% of treated patients who suffer relapses. These relapses are due to the partial loss of the CD19 epitope [36]. The NGS analysis indicated that frameshift mutations and total loss of exon 2 resulted in alternative splicing of CD19. In this regard, CRISPR-Cas9 was applied to ALL cell lines to eliminate CD19 and to further be reconstituted with different isoforms. It was observed that the depleted isoform of exon 2 was deposited in the cytosol and not in the cell membrane, thus, escaping from CAR19 [37]. The approach of CRISPR-Cas9 was also useful to better understand the mechanisms of immunotherapy resistance.

2.4.1.2 Regulation of transcription factors by CRISPR-Cas9

Transcription factors (TFs) control several cellular processes, and any deregulation could lead to pathogenic mechanisms promoting leukemogenesis. In leukemia, TFs

can be amplified, rearranged, deleted, or affected with punctual mutations resulting in gain or loss of function [38]. In consequence, correcting such mutations by CRISPR-Cas9 represents a promising therapeutic strategy in ALL. The TFs PAX5 and IKZF1 are metabolic repressors that limit the amount of ATP available, which decreases cell proliferation rate, but such TFs are altered in about 80% of patients with B-ALL. At this point, CRISPR-Cas9 was used to screen all the possible PAX5 and IKZF1 targets, where NR3C1, TXNIP, and CB2 genes were identified as the effectors of B-cells restriction of glucose and energy supply [38] and proposed as new targets for B-ALL therapy. Also, point mutations on PAX5 and IKZF1 could be corrected by using CRISPR-Cas9 and promoting the HDR mechanism.

On the other hand, TAL1 oncogene is deregulated in T-ALL cells, whose function lies in the regulation of the cell cycle progression. Frequently, deregulation of TAL1 is produced by translocations or deletions, but many patients do not present mutations, which indicates epigenetic-related deregulation [39]. To study the effect of insertion or deletions on the expression of TAL1, CRISPR-Cas9 was used in T-ALL cells. It was observed that methylation acetylation pattern in H3K27 changed in mutant cells [29]. This indicates that mutagenesis and epigenetic modulation cooperate to regulate TAL1 expression. This is an adequate scenario where dCas9 can be used fused with DNA methyl transferases or demethylases, or DNA acethyl or deacetylases to mediate specific local epigenetic modulation. Another potent oncogene deregulated in T-ALL is LMO2, which is a central effector for the formation of large transcriptional complex. It was observed that its overexpression is related to leukemogenesis. Unfortunately, only few LMO2 mutations have been described and its overexpression mechanisms are poorly understood. However, several mutations were introduced into LMO2 by CRISPR-Cas9 identifying those mutations in noncoding regions that promote deregulation in LMO2 expression [40]. In the same manner, such identified mutations can be repaired by CRISPR-Cas9 or epigenetically modulated by dCAS9 leading to normal LMO2 expression.

2.4.1.3 Discovering drug targets by CRISPR-Cas9

Most times, drug failure is related to mutations in genes that code the drug target, but mutations also help to understand the mechanism of action of drugs. Sometimes it is necessary to induce several mutations to study the drug-protein interactions, thus, elucidating their mechanism of action. A notable example is ibrutinib, an inhibitor kinase used to treat ALL where BCR signaling is damaged. The elucidation of its mechanism of action was determined through the generation of different knock-out in ALL cells as follows: Bruton tyrosine kinase (BTK), B lymphocyte kinase (BLK), and BTK/BLK via CRISPR-Cas9. Since overexpression of both BTK and BLK were reported in ALL, the strategy was to eliminate one or both kinases. The BTK/BLK knock-out reduced cell proliferation similar to ibrutinib. However, when ibrutinib was administrated in those ALL cells, the decrease in proliferation was greater suggesting that such drug targets other proteins [41].

In addition, drug specificity and binding site in a protein have been demonstrated by CRISPR-Cas9. Exportin 1 (XPO1) mediates the transport through the nucleus of cell cycle regulatory proteins and tumor suppressor proteins. Such transports are related to development of ALL and poor prognosis. XPO1 inhibitors bind to Cys528 blocking the transport of charged proteins to the cytoplasm. To determine the precise binding site of XPO1 inhibitors as well as its selectivity, a non-synonymous mutation was inserted at Cys528. Such mutation resulted in notorious drug resistance, therefore, demonstrating the high specificity of the inhibitors to XPO1 [38].

Drug efflux-related chemoresistance is the main concern in ALL, where ABC transporter proteins are involved. The proteasome inhibitor carfilzomib (CFZ) significantly improves favorable clinical outcomes for refractory childhood ALL, except in those ALL cells with P-glycoprotein positive t (17;19). By using CRISPR-Cas9, the gene ABCB1 that codes for P-glycoprotein (P-Gp) was knocked-out resulting in sensitization of P-Gp-positive t (17;19) ALL cells to CFZ [42], which is a P-Gp substrate. This finding highlighted the application of CRISPR-Cas9 to combat chemoresistance.

2.4.2 Safety: off-targets

The main side effect of the application of CRISPR-Cas9 is the risk of off-targets, which can knock-out, knock-down, or knock-in other genes permanently. To significantly reduce off-targets, it is recommended to use appropriate sgRNA designing tools, which allow gRNA selection to enhance the high specific DNA manipulation. It has been proposed the prediction of all possible off- target effects for any designed gRNA, at least in clinical trials. Nevertheless, unexpected off-targets have been observed even in off-target free sgRNA [43]. Even though off-target predicting tools (*in silico*) are not completely precise, such tools must be combined with *in vitro* assays and NGS techniques to evaluate and identify off-targets and DSBs consequences prior to clinical or preclinical stages. Regarding clinical stages, cell-based genome-wide (CBGW) assays are well recommended to identify cleavage sites under experimental conditions. This is a notorious tool because provides higher safety to patients. To date, CBGW assays such as genome-wide unbiased identification of DSBs enabled by sequencing (GUIDE-seq) as well as linear amplification-mediated high-throughput genome-wide translocation sequencing (LAM-HTGTS) are reported to have substantial potential at clinical stages [44].

Since there is not a perfect strategy to detect and avoid off-targets for clinical purposes, current reports indicate that other options available are sgRNA-Cas9 modification. This means the use of Cas9 variants with higher precision or using another type of Cas protein such as Cas12 (Cpf1) in immunotherapy at preclinical stages [45–47]. Another consideration is the expression of tumor suppressor gene p53 due to its expression in normal cells decreases gene editing by CRISPR-Cas9 system when the HDR mechanism is used. On the other hand, leukemia cells lack p53 function, which makes them easier to edit by CRISPR-Cas9. This is a relevant highlight because, theoretically, most of edited cells would be leukemic cells instead of normal cells [48, 49]. Such statement set the mandatory screening of p53 function in immune cells prior to using CRISPR-Cas9 to produce CART cells.

2.5 Delivery of CRISPR-Cas9 components into target cells

CRISPR-Cas9 components can be introduced as plasmid encoding Cas9 and sgRNA or as Cas9 protein and sgRNA in complex called ribonucleoprotein (RNP). In *ex vivo* gene editing, microinjection and electroporation have been used successfully to introduce plasmid or RNP. Recently, the transiently mechanical membrane deformation with microfluidic devices provides highly efficient delivery and low cell mortality for *ex vivo* assays, particularly used to transfect breast cancer and leukemia cells [50]. For preclinical and clinical stages, the transmembrane internalization assisted by membrane filtration (TRIAMF), the induced transduction by osmocytosis, and propane betaine (iTOP) methods are widely and recently used to introduce RNP [51, 52].

Delivering CRISPR-Cas9 components for in vivo applications is more complicated because we must consider the consequences related to off-target mutations in a complete organism as well as immunological responses. The most used method is the use of viral systems to transduce cells. However, such systems have disadvantages such as immune activation by adenoviral vectors and long expression of Cas9 protein by use of lentiviral vectors, which lead to a high frequency of off-targets. The viral systems are being replaced by nonviral or synthetic methods based on the envelop of plasmids or RNP into polymeric, lipid, or inorganic nanoparticles, which are widely used in preclinical and clinical stages [46, 53, 54]. The use of gold nanoparticles has successfully been used to deliver RNP to treat Duchene muscular dystrophy in a mouse model. Also, previous reports indicate that gold nanoparticles are nontoxic, which makes them one of the best options to deliver CRISPR-Cas9 components in RNP format [50].

3. Conclusions

ALL is one of the most common cancers diagnosed in children from 0 to 14 years old. Despite continual improvement in drug development and immunotherapy, it is necessary to focus on the root causes of the disease. For years, scientists dreamt about the possibility to manipulate DNA to correct mutations that promote cancer initiation and progression. Now with the use of CRISPR-Cas9-based gene editing, it is possible to develop effective and specific treatments against ALL. There has been a substantial advance in gene editing technology but still is not possible to ensure 100% specificity to avoid off-target leading side effects. Nevertheless, current research has made it possible to identify the main target genes in ALL and how to edit them by CRISPR-Cas9, mostly through *in vitro* and *ex vivo* assays. According to previous reports, CRISPR-Cas9 points to be the most effective tool to develop gene therapy for ALL, but still needs to be optimized to enhance precise gene editing and thus, avoid off-targets.

Conflict of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.106702

Author details

Edgardo Becerra[1,2], Valeria J. Soto Ontiveros[1,2] and Guadalupe García-Alcocer[1,2]*

1 Posgrado en Ciencias Químico Biológicas, Facultad de Química, Universidad Autónoma de Querétaro, Querétaro, México

2 Centro Universitario, Unidad de Investigación Genética, Facultad de Química, Universidad Autónoma de Querétaro, Querétaro, México

*Address all correspondence to: leguga@email.com

IntechOpen

References

[1] Paul S, Kantarjian H, Jabbour EJ. Adult acute lymphoblastic leukemia. Mayo Clinic Proceedings. 2016;**91**(11):1645-1666. DOI: 10.1016/j.mayocp.2016.09.010

[2] Al UO, Gupta N, Bakhribah H, Griffiths E, Wang E, Wetzler M. Clinical updates in adult acute lymphoblastic leukemia. Critical Reviews in Oncology/Hematology. 2016;**99**:189-199

[3] Shiraz P, Jehangir W, Agrawal V. T-Cell acute lymphoblastic leukemia—current concepts in molecular biology and management. Biomedicine. 2021;**9**(11):1621

[4] Cancer Stat Facts: Leukemia- Acute Lymphocytic Leukemia. 2022. Available from: https://seer.cancer.gov/statfacts/html/alyl.html

[5] Malard F, Mohty M. Acute lymphoblastic leukaemia. Lancet. 2020;**395**(10230):1146-1162

[6] Iacobucci I, Mullighan CG. Genetic basis of acute lymphoblastic leukemia. Journal of Clinical Oncology. 2017;**35**(9):975-983

[7] Fattizzo B, Rosa J, Giannotta JA, Baldini L, Fracchiolla NS. The physiopathology of T- cell acute lymphoblastic leukemia: Focus on molecular aspects. Frontiers in Oncology. 2020:10

[8] Team TACS Medical and Editorial Content. Chemotherapy for Acute Lymphocytic Leukemia (ALL). 2018. Available from: https://www.cancer.org/cancer/acute-lymphocytic-leukemia/treating/chemotherapy.html

[9] American Cancer Society. Treatment of Children With Acute Lymphocytic Leukemia (ALL). 2017

[10] Shang Y, Zhou F. Current advances in immunotherapy for acute leukemia: An overview of antibody, chimeric antigen receptor, immune checkpoint, and natural killer. Frontiers in Oncology. 2019;**9**:917. DOI: 10.33897FONC.2019.00917. e Collection 2019

[11] Kantarjian H, Thomas D, Wayne AS, O'Brien S. Monoclonal antibody-based therapies: A new dawn in the treatment of acute lymphoblastic leukemia. Journal of Clinical Oncology. 2012;**30**(31):3876-3883

[12] Ai J, Advani A. Current status of antibody therapy in ALL. British Journal of Haematology. 2015;**168**(4):471-480

[13] Löffler A, Gruen M, Wuchter C, Schriever F, Kufer P, Dreier T, et al. Efficient elimination of chronic lymphocytic leukaemia B cells by autologous T cells with a bispecific anti-CD19/anti-CD3 single-chain antibody construct. Leukemia. 2003;**17**(5):900-909

[14] Sun LL, Ellerman D, Mathieu M, Hristopoulos M, Chen X, Li Y, et al. Anti-CD20/CD3 T cell–dependent bispecific antibody for the treatment of B cell malignancies. Science Translational Medicine. 2015;**13,** 7(287): 287ra70. DOI: 10.1126/scitranslmed.aaa4802

[15] Barrett DM, Singh N, Porter DL, Grupp SA, June CH. Chimeric antigen receptor therapy for cancer. Annual Review of Medicine. 2014;**65**(1):333-347

[16] Jiang F, Doudna JA. CRISPR–Cas9 structures and mechanisms. Annual Review of Biophysics. 2017;**46**(1):505-529

[17] Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: A review

of the challenges and approaches. Drug Delivery. 2018;**25**(1):1234-1257

[18] Zhan T, Rindtorff N, Betge J, Ebert MP, Boutros M. CRISPR/Cas9 for cancer research and therapy. Seminars in Cancer Biology. 2019;**55**:106-119

[19] Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;**152**(5):1173-1183

[20] Kampmann M. CRISPRi and CRISPRa screens in mammalian cells for precision biology and medicine. ACS Chemical Biology. 2018;**13**(2):406-416

[21] McDonald JI, Celik H, Rois LE, Fishberger G, Fowler T, Rees R, et al. Reprogrammable CRISPR/Cas9-based system for inducing site-specific DNA methylation. Biology Open. 2016;**5**(6):866-874

[22] Stepper P, Kungulovski G, Jurkowska RZ, Chandra T, Krueger F, Reinhardt R, et al. Efficient targeted DNA methylation with chimeric dCas9–Dnmt3a–Dnmt3L methyltransferase. Nucleic Acids Research. 2017;**45**(4):1703-1713

[23] Vojta A, Dobrinić P, Tadić V, Bočkor L, Korać P, Julg B, et al. Repurposing the CRISPR-Cas9 system for targeted DNA methylation. Nucleic Acids Research. 2016;**44**(12):5615-5628

[24] Ku B, Liang C, Jung JU, Oh B-H. Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX. Cell Research. 2011;**21**(4):627-641. Available from: http://www.nature.com/articles/cr2010149

[25] Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, et al. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nature Biotechnology. 2015;**33**(5):510-517

[26] Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, et al. Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. Nature. 2017;**551**(7681):464-471

[27] Nishida K, Arazoe T, Yachie N, Banno S, Kakimoto M, Tabata M, et al. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. Science (80-). 2016;**353**(6305)

[28] Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;**533**(7603):420-424

[29] Cheng X, Fan S, Wen C, Du X. CRISPR/Cas9 for cancer treatment: technology, clinical applications and challenges. Briefings in Functional Genomics. 2020;**19**(3):209-214. Available from: https://academic.oup.com/bfg/article/19/3/209/5734960

[30] Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJC, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;**543**(7643):113-117. Available from: http://www.nature.com/articles/nature21405

[31] Salas-Mckee J, Kong W, Gladney WL, Jadlowsky JK, Plesa G, Davis MM, et al. CRISPR/Cas9-based genome editing in the era of CAR T cell immunotherapy.

Human Vaccines & Immunotherapeutics. 2019;**15**(5):1126-1132. DOI: 10.1080/21645515.2019.1571893

[32] Ren J, Zhang X, Liu X, Fang C, Jiang S, June CH, et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncotarget. 2017;**8**(10):17002-17011. DOI: 10.18632/oncotarget.15218

[33] Odorizzi PM, Wherry EJ. Inhibitory receptors on lymphocytes: Insights from infections. Journal of Immunology. 2012;**188**(7):2957-2965. DOI: 10.4049/jimmunol.1100038

[34] Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. Journal of Experimental Medicine. 2010;**207**(10):2187-2194 Available from: https://rupress.org/jem/article/207/10/2187/40732/Targeting-Tim3-and-PD1-pathways-to-reverse-T-cell

[35] Xu X, Sun Q, Liang X, Chen Z, Zhang X, Zhou X, et al. Mechanisms of relapse after CD19 CAR T-cell therapy for acute lymphoblastic leukemia and its prevention and treatment strategies. Frontiers in Immunology. 2019;**10**. DOI: 10.3389/fimmu.2019.02664/full

[36] Topp MS, Gökbuget N, Zugmaier G, Klappers P, Stelljes M, Neumann S, et al. Phase II trial of the anti-CD19 bispecific T cell–engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. Journal of Clinical Oncology. 2014;**32**(36):4134-4140. DOI: 10.1200/JCO.2014.56.3247

[37] Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discovery. 2015;**5**(12):1282-1295. Available from: https://aacrjournals.org/cancerdiscovery/article/5/12/1282/5329/Convergence-of-Acquired-Mutations-and-Alternative

[38] Montaño A, Forero-Castro M, Hernández-Rivas J-M, García-Tuñón I, Benito R. Targeted genome editing in acute lymphoblastic leukemia: A review. BMC Biotechnology. 2018;**18**(1):45. DOI: 10.1186/s12896-018-0455-9

[39] Navarro J-M, Touzart A, Pradel LC, Loosveld M, Koubi M, Fenouil R, et al. Site- and allele-specific polycomb dysregulation in T-cell leukaemia. Nature Communications. 2015;**6**(1):6094. Available from: http://www.nature.com/articles/ncomms7094

[40] Rahman S, Magnussen M, León TE, Farah N, Li Z, Abraham BJ, et al. Activation of the LMO2 oncogene through a somatically acquired neomorphic promoter in T-cell acute lymphoblastic leukemia. Blood. 2017;**129**(24):3221-3226 Available from: https://ashpublications.org/blood/article/129/24/3221/36322/Activation-of-the-LMO2-oncogene-through-a

[41] Kim E, Hurtz C, Koehrer S, Wang Z, Balasubramanian S, Chang BY, et al. Ibrutinib inhibits pre-BCR+ B-cell acute lymphoblastic leukemia progression by targeting BTK and BLK. Blood. 2017;**129**(9):1155-1165 Available from: https://ashpublications.org/blood/article/129/9/1155/36592/Ibrutinib-inhibits-preBCR-Bcell-acute

[42] Takahashi K, Inukai T, Imamura T, Yano M, Tomoyasu C, Lucas DM, et al. Anti-leukemic activity of bortezomib and carfilzomib on B-cell precursor ALL cell lines. PLoS One. 2017;**12**(12):e0188680. DOI: 10.1371/journal.pone.0188680

[43] Scott DA, Zhang F. Implications of human genetic variation in CRISPR-based therapeutic genome editing. Nature Medicine. 2017;**23**(9):1095-1101. Available from: http://www.nature.com/articles/nm.4377

[44] Tsai SQ, Nguyen NT, Malagon-Lopez J, Topkar VV, Aryee MJ, Joung JK. CIRCLE-seq: A highly sensitive in vitro screen for genome-wide CRISPR–Cas9 nuclease off-targets. Nature Methods. 2017;**14**(6):607-614. Available from: http://www.nature.com/articles/nmeth.4278

[45] Dai X, Park JJ, Du Y, Kim HR, Wang G, Errami Y, et al. One-step generation of modular CAR-T cells with AAV–Cpf1. Nature Methods. 2019;**16**(3):247-254. Available from: http://www.nature.com/articles/s41592-019-0329-7

[46] Zhang Y, Long C, Li H, McAnally JR, Baskin KK, Shelton JM, et al. CRISPR-Cpf1 correction of muscular dystrophy mutations in human cardiomyocytes and mice. Science Advances. 7 Apr 2017;**3**(4):e1602814. DOI: 10.1126/sciadv.1602814

[47] Romito M, Rai R, Thrasher AJ, Cavazza A. Genome editing for blood disorders: State of the art and recent advances. Emerging Topics in Life Sciences. 2019;**3**(3):289-299 Available from: https://portlandpress.com/emergtoplifesci/article/3/3/289/219698/Genome-editing-for-blood-disorders-state-of-the

[48] Ihry RJ, Worringer KA, Salick MR, Frias E, Ho D, Theriault K, et al. p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. Nature Methods. 2018;**24**(7):939-946. Available from: http://www.nature.com/articles/s41591-018-0050-6

[49] Haapaniemi E, Botla S, Persson J, Schmierer B, Taipale J. CRISPR–Cas9 genome editing induces a p53-mediated DNA damage response. Nature Medicine. 2018;**24**(7):927-930 Available from: http://www.nature.com/articles/s41591-018-0049-z

[50] Mehravar M, Roshandel E, Salimi M, Chegeni R, Gholizadeh M, Mohammadi MH, et al. Utilization of CRISPR/Cas9 gene editing in cellular therapies for lymphoid malignancies. Immunology Letters. 2020;**226**:71-82. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165247820303515

[51] D'Astolfo DS, Pagliero RJ, Pras A, Karthaus WR, Clevers H, Prasad V, et al. Efficient intracellular delivery of native proteins. Cell. 2015;**161**(3):674-690. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867415003153

[52] Yen J, Fiorino M, Liu Y, Paula S, Clarkson S, Quinn L, et al. TRIAMF: A new method for delivery of Cas9 ribonucleoprotein complex to human hematopoietic stem cells. Scientific Reports. 2018;**8**(1):16304. Available from: http://www.nature.com/articles/s41598-018-34601-6

[53] Finn JD, Smith AR, Patel MC, Shaw L, Youniss MR, van Heteren J, et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell Reports. 2018;**22**(9):2227-2235. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2211124718301827

[54] Yin H, Song C-Q, Dorkin JR, Zhu LJ, Li Y, Wu Q, et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nature Biotechnology. 2016;**34**(3):328-333. Available from: http://www.nature.com/articles/nbt.3471

Edited by Margarita Guenova and Gueorgui Balatzenko

Medical professionals and researchers in the field of leukemias are faced with the need to continuously update their knowledge in a wide range of areas, ranging from fundamental molecular processes and mechanisms to a thorough understanding of clinical features and modern approaches to achieve maximum therapeutic results. This book does not have the ambition to present comprehensively the problems of leukemias, but it is an attempt to acquaint the audience of specialists involved in clinical management and experimental research with some modern advances and concepts in the three main directions: biology, clinical presentation and treatment. The biological aspects of the origin, development and therapy resistance of leukemias are within the scope of the first section. Comprehensive and concise information on the clinical assessment and treatment of leukemia patients is provided, as well as on some specific interdisciplinary clinical scenarios such as leukemic retinopathy, in the second section. Finally, challenges, current standards and recent advances in the therapy of acute leukemias comprise the third section of the book.

Published in London, UK

IntechOpen

ISBN 978-1-80356-907-9