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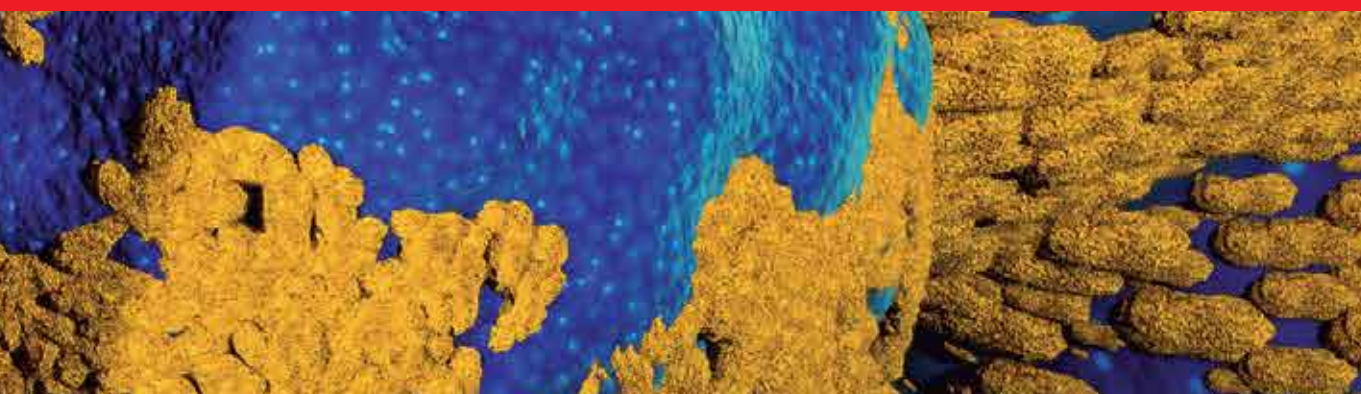
Ovarian Cancer

From Pathogenesis to Treatment

Edited by Omer Devaja and Andreas Papadopoulos



OVARIAN CANCER - FROM PATHOGENESIS TO TREATMENT

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and **Andreas Papadopoulos**

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Meet the editors

Dr. Omer Devaja graduated from the Medical School of Novi Sad, Serbia. He was awarded a PhD for his research in the field of molecular biology of ovarian and endometrial carcinoma at Guy's and St Thomas' Hospital, London, and ICRF, UK. In 2000 he completed his subspeciality training in gynaecologic oncology at Guy's and St Thomas' Hospital. In the 2003 he was awarded a scholarship to attend advanced gynaecologic oncology laparoscopic training in the University of Québec, Canada, and the National Cancer Institute, Cairo, Egypt. He is currently working as a gynaecologic oncology surgeon at the Kent Oncology Centre, Maidstone, UK. He has written on a wide range of topics in the field of gynaecologic oncology and edited the atlas on laparoscopic surgery in gynaecology. He is director of subspeciality training in gynaecological oncology and visiting professor at the Medical School, University of Novi Sad, Serbia. His research interests include sentinel node detection and minimal access surgery in gynaecologic oncology.

Dr. Andreas Papadopoulos trained at King's College School of Medicine, University of London. He completed his general training in gynaecology and gynaecologic oncology at Guy's and St Thomas' Hospital, London. His research MD was in the field of immunobiology of ovarian cancer. He was awarded an RCOG scholarship to attend both the MD Anderson Cancer Center, Houston, Texas, USA, and the Tata Memorial Hospital, Mumbai, India. He has been a lead cancer clinician as well as a gynaecologic oncology cancer lead at the Kent Oncology Centre, UK. He is currently a gynaecologic oncology surgeon and MDT lead at the Kent Oncology Centre. He has written a wide range of articles in gynaecologic oncology, including ovarian cancer. His research interests include immunobiology of ovarian cancer, sentinel node detection, and minimal access surgery in gynaecologic oncology.

Contents

Preface

Our understanding of ovarian malignancy is constantly changing and over the last few years research into cancer genetics and molecular biology has revealed a huge amount of information. This knowledge has been used to develop management strategies as well as new and novel treatment agents. The role of clinical genetics to guide treatment protocols and preventative surgical options has also entered the clinical domain. The place of surgery, be it as a primary manoeuvre, or as an adjunct to primary chemotherapy as well as in the recurrent setting, is still being defined. This book brings together a variety of up-to-date concepts in the field of ovarian cancer management and addresses some of these issues. Specialists from around the world have contributed a variety of topics, including pathogenesis, molecular biology, diagnosis, screening, and both surgical and chemotherapeutic management. In addition, newer novel treatment strategies have also been outlined. This book outlines the breadth of scientific knowledge that forms the basis of new concepts in this disease and addresses the traditional treatment strategies. We hope the reader will be encouraged to explore this subject further and also contribute to the research of this devastating disease.

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Section 1

Pathogenesis and Molecular Biology of Ovarian Cancer

Chapter 1

Ovarian Cancer Genetics: Subtypes and Risk Factors

Jeff Hirst, Jennifer Crow and Andrew Godwin

Additional information is available at the end of the chapter

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Abstract

The genetics of ovarian cancer are a complex, ever evolving concept that presents hurdles in classification, diagnosis, and treatment in the clinic. Instead of common driver mutations, genomic instability is one of the hallmarks of ovarian cancer. While ovarian cancer is stratified into different clinical subtypes, there still exists extensive genetic and progressive diversity within each subtype. In high-grade serous ovarian cancer, the most common subtype, *TP53* is mutated in over 90% of all patients while the next most common mutation is less than 20%. However, next-generation sequencing and biological statistics have shown that mutations within DNA repair pathways, including *BRCA1* and *BRCA2*, are common in about 50% of all high-grade serous patients leading to the development of a breakthrough therapy of poly ADP ribose polymerase (PARP) inhibitors. This is just one example of how a better understanding of the complex genetic background of ovarian cancer can improve clinical treatment. A thorough review of ovarian cancer genetics and the effect it has on disease development, diagnosis, progression, and treatment will enhance the understanding of how to better research and treat ovarian cancer.

Keywords: genetics, subtypes, pathogenesis, *BRCA1*, *BRCA2*, *TP53*, risk factors

1. Introduction

Ovarian cancer is a generic term used to classify cancers involving the ovaries though they can arise from many different cell types within the Müllerian compartment. Ovarian cancer presents as a distinct subset of cancers with a wide variety of genomic variation (*e.g.*, somatic *TP53* mutations, germline *BRCA1/2* mutations, copy number gains in *BRAF*, *CCNE1*, *TERC*, *TERT*, and copy number loss of *RB1* and/or *PTEN*) as demonstrated through a Pan-Cancer analysis using The Cancer Genome Atlas (TGCA) database (**Figure 1**). The pathogenesis and the debate of cellular origins of ovarian cancer will be discussed in Section 4.

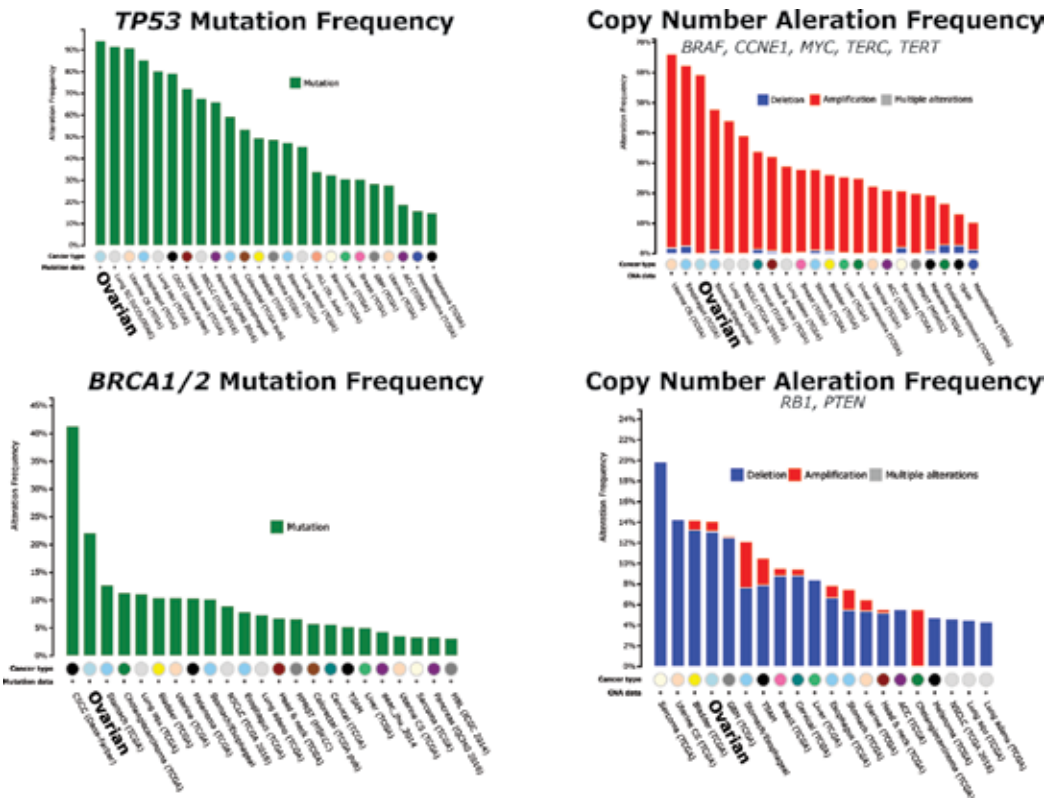

Figure 1. Common genetic alterations in ovarian cancer represented across pan-Cancer analysis from the TCGA. Bar graphs depict % of cases with mutations (green), amplification (red), and/or deletion of commonly dysregulated genes across a panel of cancers in the TCGA.

Ovarian cancer is a pathological and genetically diverse disease that presents many hurdles towards clinical detection and treatment. These clinical barriers have prevented significant improvement in patient survival for the past three decades. The heterogeneity of ovarian cancer is one of the driving factors limiting clinical progress. In this chapter, we will discuss the diversity of ovarian cancers and how these genetic factors effect clinical detection, progression, and treatment. A better understanding of the genetic differences in ovarian cancer will open up new areas for research and treatment.

Ovarian cancer can be classified into subclasses based on pathological and genetic observations. Each subclass has distinct genetic alterations, disease pathogenesis, tumor progression, and survival outcomes in response to therapy. Not only does each subclass behave differently, heterogeneity within specific subclasses presents challenges in regards to treatment options, drug resistance, and overall clinical response. Genetic diversity has greatly limited the development of targeted therapies, which have been successful in other cancers, such as *HER2* amplified breast cancers (trastuzumab, Herceptin®), *BCR-ABL* fusion in chronic myelogenous leukemia (CML) or *KIT* mutant gastrointestinal stromal tumor (imatinib mesylate, Gleevec®), and *BRAF* V600E mutant melanoma, vemurafenib (Zelboraf®). However, understanding genetic vulnerabilities such as deficiencies in homologous DNA repair prompted the development of poly

ADP ribose polymerase (PARP) inhibitors, a breakthrough in the treatment of specific ovarian cancer patients.

Finally, we will discuss genetic and lifestyle factors that can contribute to the development or progression of ovarian cancer. Since ovarian cancer is difficult to detect at early stages, knowing genetic and lifestyle risk factors for the development of the disease is critical. In fact, studying familial breast and ovarian cancer led to the discovery of inherited mutation in either *BRCA1* or *BRCA2* and improved detection of patients at risk for both cancers. While germline *BRCA1/2* mutations are two of the highest risk factors for developing ovarian, other genetic and lifestyle factors have been shown to influence the risk of disease development. A more thorough understanding of the risks of ovarian cancer is needed to stratify the chances of developing ovarian cancer for each patient.

2. Classification of ovarian cancer

Ovarian cancers of epithelial cell origin account for more than 85% of all ovarian tumors when compared to tumors that arise from germ, epidermoid, stromal, and border cells [1]. Since EOCs are the most common and deadly form of ovarian cancer, we will refer to EOC as ovarian cancer for the remainder of this chapter and primarily discuss ovarian cancers of epithelial origin [2, 3]. Typically, EOC is classified into five different histological subtypes: high-grade serous (HGS), low-grade serous (LGS), endometrioid, clear cell and mucinous [3, 4] (**Table 1**). Low-grade and high-grade disease can typically be distinguished based on the extent of nuclear atypia and mitosis [5]. Low-grade tumors are slower growing, more genetically stable and do not respond to chemotherapy as well as the faster growing, gnomically instable high-grade tumors [6–8]. High-grade serous carcinomas are the most common ovarian cancer subtype (more than 70%) followed by endometrioid, clear cell and low-grade serous [9]. Mixed ovarian cancers that represent more than one subtype are more rare, accounting for less than 1% of all ovarian cancers [10, 11]. Globally, each subtype follows a similar distribution of incidence outside of Asia, where clear cell and endometrioid tumors are more frequent compared to other locations [12]. Each subtype behaves as a discrete disease with differences in presentation, progression, mutation profile, association with hereditary cancer syndromes, and response to chemotherapy (**Table 1**) [13]. The 10-year survival for each subtype can be influenced by each of these factors and ranges from mucinous (87%), endometrioid (59.7%), clear cell (58.7%), to serous (24.4%) [14, 15].

Each subtype has distinct histological protein expression patterns, mutations and even epigenetic signatures. Further classification based on molecular profiles may provide insights into improving therapy selection [16, 17]. Recent studies have helped to further stratify the genomic differences between each subtype where 12 different loci contribute to the susceptibility of serous (3q28, 4q32.3, 8q21.11, 10q24.33, 18q11.2, 22q12.1, 2q13, 8q24.1 and 12q24.31), mucinous (3q22.3 and 9q31.1) and endometrioid (5q12.3) subtypes of ovarian cancer [18]. Molecular classification has been shown to stratify low-grade diseases into separate clusters, whereas high-grade diseases have less genetic separation [19–21], indicating early pathogenesis of the disease might be the best time to molecularly phenotype or develop targeted therapies.

Sub Type	Mutations	Clinical Prognosis	Frequency
High-grade serous	*TP53*, *BRCA1*, *BRCA2*, *CDK12*	Often diagnosed at late stage and chromosomally unstable.	~65%
Low-grade serous	*BRAF*, *KRAS*, *NRAS*, *ERBB2*	Often diagnosed in younger patients, less aggressive, gnomically stable.	~5%
Endometrioid	*PTEN*, *CTNNB1*, *PPP2R1α*, MMR deficient	Favorable prognosis and response to chemotherapy.	~20%
Clear cell carcinoma	*PIK3CA*, *KRAS*, *PTEN*, *ARID1A*	Low response to chemotherapy and intermediate prognosis.	~5%
Mucinous	*KRAS*, *HER-2* amplification	Low response to chemotherapy.	~5%

Table 1. Subtypes of ovarian cancer.

Within each subclass ovarian cancers are diagnosed and staged after primary cytoreductive surgery which attempts to remove any visible mass within the peritoneal cavity. The International Federation of Gynecology and Obstetrics (FIGO) have established guidelines for the staging of ovarian cancer. These guidelines are established based on disease localization from ovaries only (Stage I), pelvic extension (Stage II), peritoneum spread (Stage III), to distant metastases (Stage IV). While the 5-year relative survival for localized disease is over 90%, the majority of patients are diagnosed with regional (15%) or distant (60%) disease where the 5-year survival is 73% and 28.9% respectively [22]. While molecular characterization of each stage is still progressing, some data suggest there is a stepwise progressing in gene expression that could be exploited for enhanced staging [23].

In the next sections of this chapter we will discuss each subtype of ovarian cancer. We will focus primarily of specific genomic alterations, clinical pathogenesis, and responses to therapy.

2.1. High-grade serous tumors

High-grade serous tumors account for both the majority of ovarian cancer diagnoses and deaths [5, 9]. HGS tumors show a broad range of histological phenotypes with papillary, micropapillary, glandular, cribriform and trabecular structures involving columnar cells with pink cytoplasm [24, 25]. HGS is a separate disease from its LGS counterpart (and not different grades of the same neoplasm) and is identified by high mitotic index and high-grade nuclear features [5, 26] (**Figure 2**). HGS disease can be identified from other malignancies such as uterine cancer and endometrioid cancer through positive staining in WT-1, p53, and p16 [27–31]. The majority of HGS tumors are diagnosed at late stages when a complete resection of the tumor is difficult. In fact, less than 5% of HGS cancers are diagnosed at a Stage 1 (when the tumor is confined to the ovaries). Finally, while extremely rare, there is some evidence to support the progression of LGS or borderline tumors into high-grade disease. These cases have been identified through concurrent mutations in *KRAS* and *TP53* in both a borderline lesion and HGS carcinoma [32]. This progression could be due to a secondary mutation of *TP53* in borderline or low-grade tumors [33].

HGS tumors are associated with genomic instability [2, 34] since almost all (>95%) high-grade serous cancers have somatic *TP53* mutations and over half have homologous DNA repair

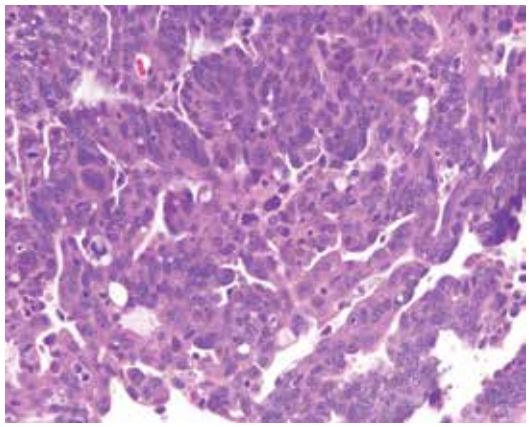

Figure 2. Representative H&E staining of high-grade serous ovarian carcinoma.

pathway deficiencies mainly represented by defects in BRCA1, BRCA2, or related proteins [35–38]. Many of these genomic alterations are similar to basal-like breast cancer, opening the opportunity for comparative studies [39]. In fact, when compared to other cancers HGS ovarian cancer had the most genomic instability when comparing copy number alterations to mutation rates [40]. Other genetic alterations that have been identified in HGS disease include cyclin E1 (*CCNE1*) amplifications. *CCNE1* amplification in HGS disease is associated with poor prognosis and platinum resistance [41]. Likewise, HGS genomic instability leads to inactivation of tumor suppressor genes through gene breakage [42]. Loss of expression of *PTEN* in tumor specific cells is predictive of poor patient survival in ovarian cancer [43].

To provide an example of this, we utilized data available through TCGA to demonstrate genetic aberrations within 34 common cell cycle control genes from 316 HGS ovarian cases with complete mutation, copy number alteration, and mRNA data [44] (**Figure 3**). While some alterations were fairly consistent across patient samples (such as up-regulation or amplification of *MYC* in ~30% of cases, down-regulation of *RBL2* in ~25% of cases, and up-regulation or amplification of *CCNE1* in ~20% of cases) the remaining 31 queried genes had between 3 and 29% alteration rates of which there was little discernable pattern. As a comparison, *TP53* is shown to be altered in most of the cases.

Examples such as this demonstrate just how difficult high-grade EOC is to treat with single molecularly-targeted therapies [45, 46]. However, one of the major breakthroughs for the treatment of ovarian cancer has been the development and FDA approval of PARP inhibitors, olaparib (Lynparza), rucaparib (Rubraca), and niraparib (Zejula). Specifically, in BRCA deficient or other homologous repair deficient cells, PARP inhibitors induce the error prone DNA repair pathway non-homologous end joining [47]. Therefore, PARP inhibitors were investigated for efficacy in ovarian cancer due to the high number of patients with BRCA and/or homologous recombination (HR) deficient tumors [48]. Rucaparib, an oral PARP-1, −2 and −3 inhibitor, has been approved for treatment in patients with *BRCA* mutations (somatic or germline) who have received at least two prior chemotherapy treatments [49, 50]. Another PARP inhibitor, niraparib, was approved in early 2017 for the maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer, regardless of the *BRCA* mutation status. However, in the Phase III trial of niraparib, the progression

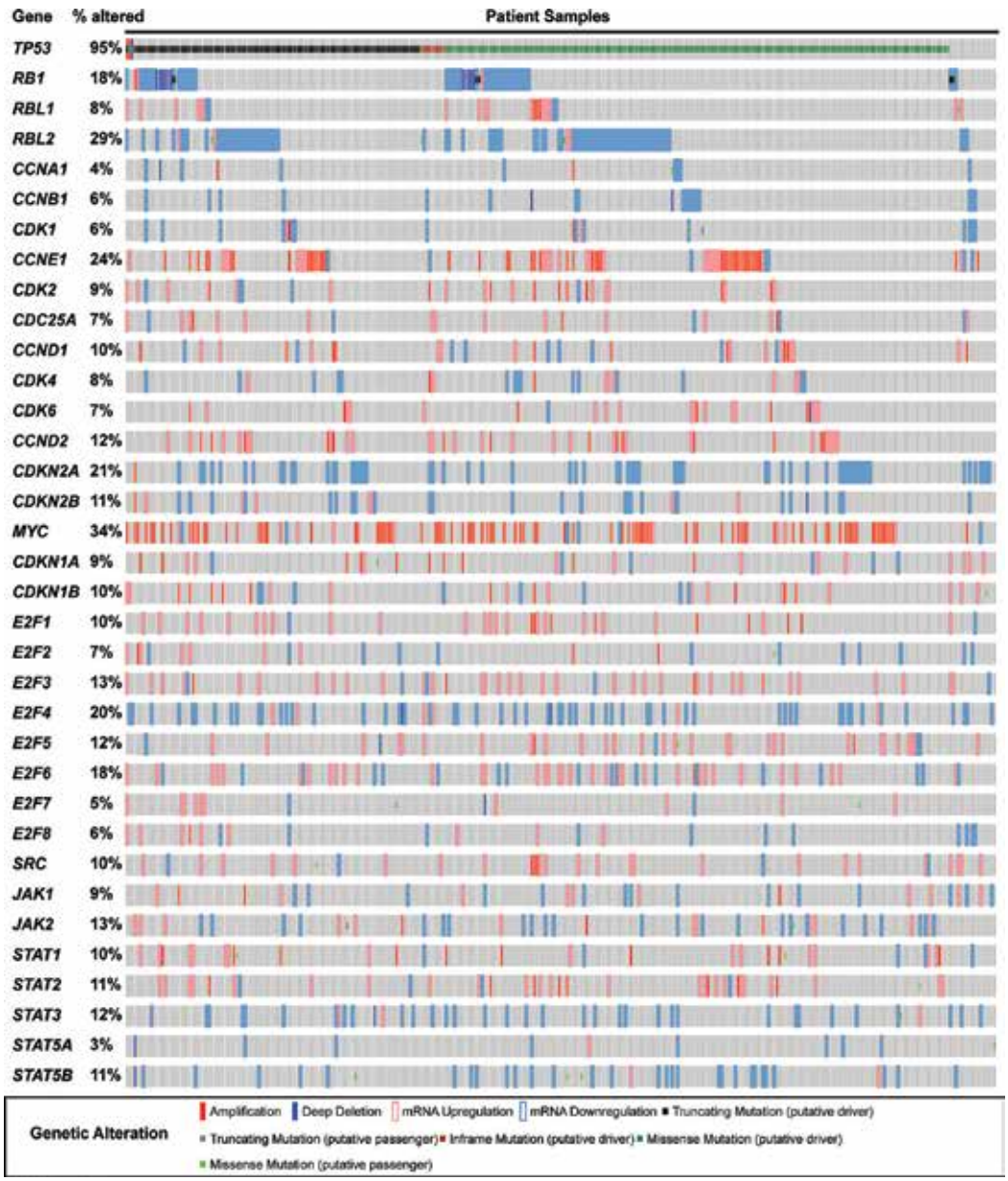

Figure 3. Genetic Dysregulation in high grade serous ovarian cancer. Data from the TCGA showing mutation, copy number alteration, and mRNA dysregulation of 34 cell cycle control genes and *TP53* alteration status (as a comparison) within 316 cases of high grade serous ovarian cancer demonstrates the overall heterogeneity of the disease.

free survival (PFS) was superior only for germline *BRCA* mutant patients when compared to standard of care (22 months vs. 9 months) versus *BRCA* competent patients compared to standard of care (9.3 months vs. 3.9 months) [51], indicating better activity in the BRCA deficient tumors. To address this limitation, our laboratory has shown that alisertib (MLN8237) can inhibit DNA double strand break repair as well as BRCA expression which sensitizes resistant

cells to PARP inhibitors [52]. Using therapies to mimic different genetic phenotypes such as BRCAness has promising clinical application for ovarian cancer in trying to identify target therapies in a genetically diverse disease. Both of these therapies show that and understanding of the dynamic genes expressed in ovarian cancer can be used to mimic more sensitive disease (synthetic lethality) and improve therapy efficacy in the laboratory.

To add to this hurdle, while HGS tumors are initially responsive to platinum-chemotherapy, most patients' tumors recur which are resistant to standard chemotherapy, thus limiting treatment options for these women. The deficiencies in DNA repair pathways associate with widespread copy number alterations and make HGS cancer initially sensitive to platinum-based chemotherapy (and PARP inhibitors) but develop therapy resistance. Specifically the genomic instability can drive changes that reverse the initial sensitivity to PARP inhibitors through reversion of BRCA1/2 mutants to wild-type function [42, 53]. Similar to PARP inhibitors, patients with *BRCA* mutations are initially more sensitive to chemotherapy; however, reversion of the *BRCA1*/2 mutations promotes cisplatin resistance [53, 54]. Further, specific expression of many different genes such as *ABC1* [55–59], *ABC2* [60, 61], and *GSH1* [62, 63] correlate to disease progression and drug resistance. The expression of mesenchymal genes such as *SNAIL*, *SLUG*, and *TWIST* through the epithelial to mesenchymal transitions (EMT) promotes chemotherapy resistance [64, 65]. EMT is a dynamic cellular process that can be transferred from on cell to the next through many cellular pathways including extracellular vesicles [66, 67]. Since EMT is a dynamic process, therapies that reverse the process and promote the expression of epithelial genes are an intriguing area for drug development to reverse cell growth into more sensitive phenotypes [68]. The relative success of PARP inhibitors and lack of clinical efficacy of more specific targeted therapies shows the value of identifying and exploiting the underlining molecular vulnerabilities of ovarian cancer.

2.2. Low-grade serous and borderline tumors

Low-grade serous (LGS) account for approximately 10% serous tumors. LGS tumors are more common in younger patients with an average age at diagnosis of 55.5 years compared to 62.6 years for their high-grade counterpart. LGS ovarian cancer is more commonly diagnosed at early stages, with bilateral involvement, and without invasive potential [69]. Patients with non-invasive tumors have a significantly higher 7-year survival (95.3%) compared to those with invasive tumors (66%) [70, 71]. LGS tumors appear with extensive papillary features and psammoma bodies, uniform round to oval nuclei, evenly distrusted chromosomes, and ~10 mitoses/HPF (**Figure 4**).

When compared to high-grade disease, LGS tumors are typically slower growing and have more frequent mutations in *KRAS*, *BRAF,* and *ERBB2*, and tend to lack *TP53* mutations [72–74]. Mutations in *KRAS, BRAF,* and *ERBB2* in LGS tumors are mutually exclusive. However, each gene mutation are signatures of activated mitogen-activated protein kinase (MAPK) pathways. MAPK activation is higher in LGS compared to HSG and correlates with paclitaxel sensitivity and an improved 5-year survival [75]. Along with having functional p53, LGS tumors have a more stable genome with less rearrangements, mutations, and tumor heterogeneity [76]. However, due to more competent DNA repair pathways, LGS tumors do not respond

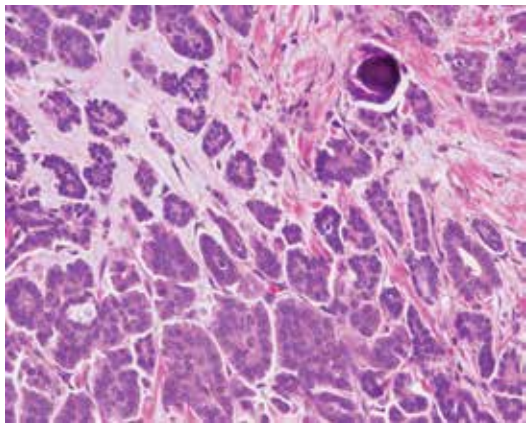

Figure 4. Representative H&E staining of low-grade serous ovarian carcinoma.

to front-line chemotherapy as well as HGS tumors [77]. Consequently, a patient with optimal debulking surgery with minimal residual tumor is the best predictor of survival [78]. The involvement of MAPK regulation of cell cycle is thought to be strongly associated with LGS chemoresistance [75], but in turn provides a potential subpopulation for targeted therapeutic development [79]. Selumetinib, a MEK1/2 inhibitor, showed some activity in recurrent LSG, leading to further investigation of MAPK pathway inhibitors for the treatment of LSG [80].

LGS tumors are thought to be borderline tumors formed step-wise from the ovarian surface [73]. Borderline tumors are epithelial tumors that appear to represent and intermediates step between benign cystadenomas and adenocarcinomas with histological features such as cellular atypia without stromal invasion. Progression of LGS tumors from borderline tumors is also thought be from recurrence of undetected borderline tumors [81–83]. While borderline tumors can be diagnosed as either serous or endometrioid the majority of such cases are diagnosed as serous tumors [26]. Borderline tumors account for ~15% of all ovarian cancer diagnoses with a large percent of cases diagnoses at early stage (~75%) and a high rate of overall survival [84]. Diagnosis at an early age (mean age of ~45 years) and minimal invasive disease are primary factors for the favorable survival [26]. While rare, invasive borderline tumors (Stages II-IV) account for the majority of deaths in borderline tumor patients [85]. Borderline tumors have a similar activation of MAPK compared to LGS tumors [75], but a higher frequency in *BRAF* mutations [86]. *BRAF* mutations are more common in early stage tumors as well as in late stage tumors that do not recur in the patient [87]. However, it is possible many LGS progress independent of borderline tumors and the pathogenesis of LGS requires further elucidation [88].

2.3. Endometrioid tumors

Endometrioid tumors account for about 10–20% of all ovarian cancers. Their morphology is described as having a smooth outer surface with solid, cystic areas inside while the pathological phenotype involves high amounts of proliferative cells that resemble squamous or endometrioid differentiations with secretory cell features. Tumors contain cystic spaces lined by gastrointestinal-type mucinous epithelium with stratification and may form filiform papillae with at least minimal stromal support. Histologic review find that endometrioid tumors possess

nuclei that are slightly larger than cystadenomas; mitotic activity is present; goblet cells and sometimes Paneth cells (most commonly found in the small intestine) are present, but stromal invasion is absent [89, 90] (**Figure 5**). Endometrioid ovarian tumors are histologically similar to endometrial neoplasms. In fact, approximately one third of all endometrioid cases experience synchronous endometrial carcinoma or endometrial hyperplasia. This is not surprising given that endometrioid tumors are believed to arise from endometrial precursor cells and/or transformed endometrioses, possibly from back flow during menstruation that implants onto the ovarian surface epithelium [91–95].

The 5-year survival rate for endometrioid tumors is between 40 and 80%, and the 10-year survival is promising at~60%. This is mostly due to early stage presentation of the disease; however, there is no survival difference when matched with serous patients of the same age and stage of diagnosis [96, 97]. Likewise, with serous tumors, endometrioid tumors can be both high- and low-grade with similar growth patterns distinguishing the two [98]. High-grade endometrioid tumors are very similar to HGS tumors in terms of genome instability and response to chemotherapy [99]. The primary treatment regimen consists of surgical debulking followed by platinum-based chemotherapy. Mutation profiles of endometrioid tumors reveal frequent activating mutations in *CTNNB1* and *PIK3CA* [100, 101], as well as *ARID1A* (which helped link their origin to endometriosis) [102]. *PTEN* is altered in ~20% of endometrioid tumors, and to a lesser extent *KRAS* and *BRAF* [103, 104]. Given this mutational profile, it has been hypothesized that a subset of endometrioid tumors may be responsive to mTOR inhibitors; however, results of Phase I and II trials have shown minimal increases in overall response rate [105]. Ongoing studies emphasize a need for better molecular screening to identify individuals who could potentially benefit from a limited number of targeted therapies.

2.4. Mucinous ovarian cancer

Mucinous ovarian cancer (MOC) are primarily unilateral, can be very large (mean size of 10 cm and can range up to 48 cm) [106–108], and are diagnosed at early stages (most are stage I or II). Invasive disease accounts for less than 10% of all MOC cases [108, 109]. Mucinous ovarian tumors are rare when compared to other subtypes with reports of the overall incidence ranging from

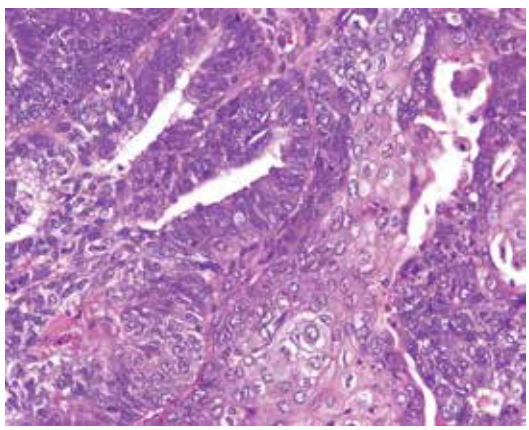

Figure 5. Representative H&E staining of endometrioid ovarian cancer.

~12% [110] to as low as 3% [3, 111]. Patients with invasive disease (FIGO Stage III or IV) have higher risk of death and shorter survival than patients with early disease (FIGO Stage I or II) [112]. The pathological definition of MOC dictates intracytoplasmic mucin is mandatory, although many mucinous tumors lack obvious apical mucin in large parts of tumor, thereby imparting an endometrioid appearance. Mucinous tumors are often heterogeneous contain endocervical-like or intestinal-like cells with gastric superficial/foveolar and pyloric cells, enterochromaffin cells, argyrophil cells, and Paneth cells (**Figure 6**). While cytokeratin 7 and 20 staining is used to define MOC pathologically, it is limited in distinguishing primary ovarian tumors from secondary metastases of gastrointestinal tumors [113, 114]. Secondary pathological markers such as SATB2, CDX2, and PAX8 have potential to help diagnose MOCs [115–117].

While the overall survival for mucinous ovarian disease is high due to the majority of cases being diagnosed at early stage, invasive disease has a worse clinical outcome [118] and low response rates to chemotherapy due to the high expression of genes involved in drug resistance, including the ABC transporters [119]. Mucinous disease is mostly thought to originate from the gastrointestinal tract [120], though the molecular mechanisms of the disease are still not fully elucidated. *KRAS* mutations, which are found in other ovarian cancer subtypes, are the most common genetic alterations found in MOC [29, 121, 122], followed by *HER2* amplifications [123]. Other mutations such as *BRAF*, *TP53*, and *CDKN2A* have been reported in MOC [124].

Extensive clinical studies of MOC are difficult to perform due to low number of cases and complex diagnosis and lead to early trial terminations such as GOG241 [125]. Small trials have shown that *HER2* amplifications in recurrent MOC are a potential therapeutic target with trastuzumab [126]. While most ovarian cancer trials of HER2 inhibitors have shown limited efficacy, the prevalence of *HER2* amplifications in MOC disease to other subtypes makes it a prospect for preselection if enough patients can be recruited [127].

2.5. Ovarian clear cell carcinoma

Ovarian clear cell carcinoma (CCC) accounts for approximately 5% of all ovarian cancer patients in the United States; however, it is more common in Asian women (~11%) than in African American

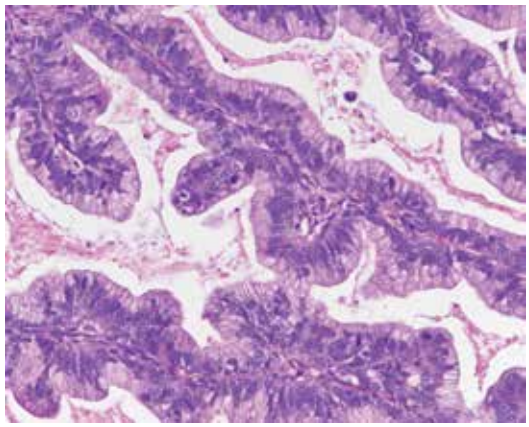

Figure 6. Representative H&E staining of mucinous ovarian cancer.

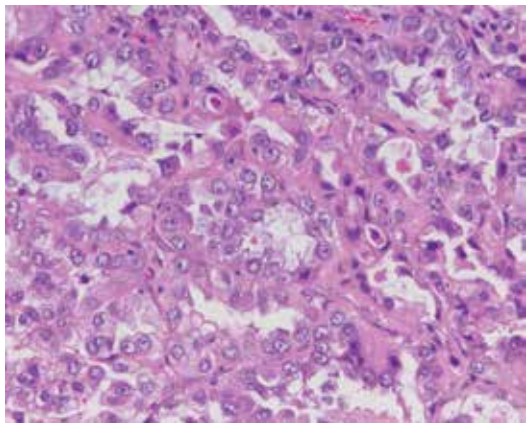

Figure 7. Representative H&E staining of ovarian clear cell carcinoma.

(~3%) or Caucasian (~5%) women [3, 128, 129]. CCCs are generally large (can grow over 15 cm), unilateral tumors that display only papillary, tubulocystic, and solid architectures with hobnail cells containing clear cytoplasm (**Figure 7**). While the pathogenesis of CCC is unknown, gene expression studies indicate clear cell ovarian cancer does not cluster with other ovarian cancers and more closely resembles lung cancers, endometriosis, and renal cell carcinoma [99, 130–132]. In terms of molecular mechanisms, CCCs are complex at the genomic level and can have mutations in *ARID1A*, *PIK3CA*, *KRAS* and *PTEN* [133, 134]: *ARID1A* is mutated in ~50% and *PIK3CA* mutated in ~33% of patient tumor samples [102, 135]. In contrast, CCCs are usually wild-type for *TP53* and have a lower frequency of *BRCA1* and *BRCA2* mutations [136, 137].

Clinically, CCCs are typically diagnosed at an early stage; however, they are less responsive to front-line platinum-based chemotherapy, especially at later FIGO stages. When compared to matched serous disease, early stage CCC (I-II) had a better overall survival than serous, but late stage CCC (III-IV) had a worse prognosis than both serous [138] and endometrioid adenocarcinoma [137]. Interestingly, some evidence suggests that drug response can be correlated to *CD44*-10v isoform expression [139]. Like endometrioid, clinical trials aimed at treating CCC include mTOR inhibitors, including a Phase II trial investigating the addition of temsirolimus to standard first-line chemotherapy (NCT01196429). Additionally, CCC is characterized by overexpression of the pro-inflammatory cytokine IL-6, which could prove to be an alternative therapeutic target [140].

3. Ovarian cancer pathogenesis

EOCs were, for years, believed to arise primarily from the ovarian surface epithelium. However, two novel hypotheses for the pathogenesis of HGS ovarian cancer have been proposed. In the first mechanism, genetic alterations occurring within the normal ovarian surface epithelium or inclusion cysts which either proceed via a high-grade pathway with no perceivable intermediate histology or a low-grade pathway encompassing several, benign and non-invasive steps (**Figure 8**). This first hypothesis was established in the 1970s and proposed that

ovarian surface epithelial cells underwent repeated stress through multiple rounds of ovulation, leading to inflammation, DNA damage, and the initiation of tumorigenesis [141]. This hypothesis was in part supported by evidence on the decreased risk of ovarian cancer with the use of oral contraceptives, which inhibit complete ovulation [142, 143]. Other evidence supported the correlation between the number of lifetime ovulation cycles and the increase in ovarian cancer incidence [144]. Likewise, ovarian cancers are rare in other primates which have fewer ovulations cycles than humans [145]. However, ovarian tumors are more common in hens which have been induced to frequently ovulate [146, 147]. To further study the incessant ovulation theory, additional animal models will clearly be needed. In fact, Godwin and colleagues were some of the first investigators to establish ovarian surface epithelial cultures from rat and human ovaries and use model incessant ovulation *in vitro* as a mechanism for transformation and tumorigenesis [148–161]. Inactivation of p53 and Rb1 in mouse ovarian surface cells also led to tumorigenic transformation [162].

The second theory, which has gain much traction over the past decade, describes a progression model in which ovarian cancer precursors develop in the fimbria from occult serous tubal intraepithelial carcinoma (STIC), prior to metastasis to the ovary [163, 164]. Due to the aggressive nature of HGS tumors and the presence of early genomic instability, it is hypothesized that HGS ovarian tumors are instead metastatic lesions from the fallopian tube epithelial cells (**Figure 8**). To reduce the risk of HGS ovarian cancer in women *BRCA* mutation carriers it is beneficial to undergo a bilateral salpingo-oophorectomy (removal of both the ovaries along with the fallopian tubes) instead of just an oophorectomy (removal of only the ovaries) [165, 166]. The primary risk reduction for ovarian cancer following salpingo-oophorectomy was found to be serous disease [167]. Not only did these studies suggest a fallopian origin for serous disease, the use of salpingo-oophorectomy for preventative treatment for high-risk patients gave researchers and pathologist tissue to study and search for early ovarian cancer or precursor lesions. Microdissection of the fallopian tube epithelium following salpingo-oophorectomy from patients with a disposition to ovarian cancer showed lesions with *BRCA* and *TP53* alterations that resemble HGS tumors [168–171]. To follow-up, extensive evaluation of both the fallopian tube and ovarian surface from *BRCA* mutant patients also showed common precursor lesions in the fimbria and not the ovarian surface [164, 172–174]. In genetic mouse models, conditional inactivation of commonly mutated ovarian cancer genes (*BRCA1*, *TP53* and *RB1*) in ovarian surface epithelium cells leads to the formation of leiomyosarcomas and not HGSC following implantation into the mouse bursal sack [175]. Along with genetic alterations, fallopian lesions from *BRCA* patients showed gene expression profiles that mimicked HGS cancers [176]. Immortalization of human fallopian tube secretory epithelial cells (using hTERT and SV40 large T antigen) were transformed *in vivo* and *in vitro* by oncogenic *RAS* or *MYC* [177]. In contrast to ovarian surface epithelial cells, the inactivation of *Brca*, *Tp53* or *Pten* in *Pax8* over expressing mouse fallopian tubal secretory cells led to the development of HGSC [178]. Other genomic alterations common in HGS disease such as *CCNE1* amplification and other copy number alterations are also found in STIC lesions and might be an early step in the progression of HGS ovarian cancer [179, 180]. For example, *CCNE1* amplifications are common in both tubal lesions and HGS tumors, while centrosome amplification is more pronounced in HGS disease, indicating *CCNE1* copy number gain is an early step in tumorigenesis that later promotes centrosome amplification [181]. However, some evidence exists to show an independent clonal evolution between tubular lesions and

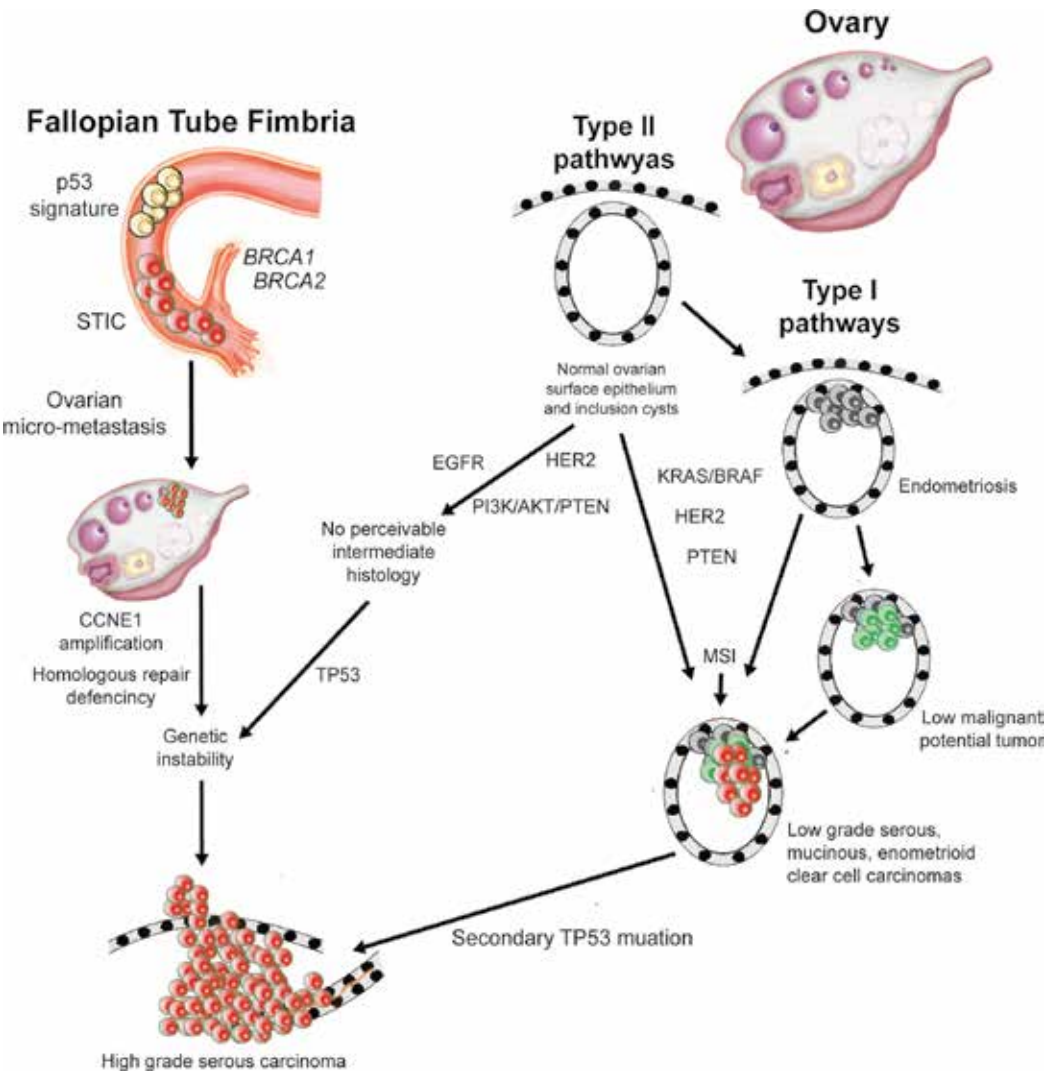

Figure 8. Pathogenesis pathways of ovarian cancer. Schematic representation of the prevailing theories behind ovarian cancer development.

the patient's synchronous carcinoma, indicating small number of fallopian tube lesions may be micrometastases from uterine endometrioid carcinomas [182].

Other studies suggest a different route of the pathogenesis of cancers, where somatic stem cells undergo oncogenic mutation and create cancer stem cells that populate tumors [183–187]. While this mechanism has been contested with evidence that cancer cell plasticity can induce a stem cell phenotype in cancer cells from differentiated tissue [188], understanding any stem cell niche in ovaries and fallopian tubes may provide insight into the pathogenesis of ovarian cancer. Both the ovarian surface epithelium and fallopian tube epithelium have stem cell niches with cells with regenerative properties that could serves as progenitor cells for ovarian cancer [189–191]. Some evidence supports there could be a stem cell niche within the junction between

the ovarian surface the fallopian tube that helps repair the damage to the ovarian surface following follicle release [192]. Notch and Wnt, canonical stem cell pathways, have been shown to regulate differentiation in fallopian tube organoids and could contribute to fallopian tube repair [193]. Fallopian stem-like cells ($CD44^+$ and $PAX8^+$) can be isolated from distal end of the tube and are capable of clonal growth and self-renewal [194, 195]. Since these stem cell niches are located near the areas of ovarian and fallopian surface repair and precursor lesions they could be hotspots for the development of tumors from mutations in somatic stem cells. One recent study has shown that *SOX2* is overexpressed in the fallopian tubes of patients with HGS disease and in *BRCA1*/*BRCA2* mutation carries [196], indicating a possible stem cell precursor lesion. The role of stem cells in cancer and cancer progression will remain an influential area of research and can provide potential insight into ovarian cancer pathogenesis in the future.

Taken together, these data support that the pathogenesis of ovarian cancer is complex and thus contributes to the clinical difficulties in detecting the disease early. As our understanding of the genomic complexities of ovarian cancer continues to evolve and the cell type of origin is further defined, we should be able to use this information to improve detection at a time when disease can be cured and develop more precise therapies based on tumor profiling and precision medicine.

4. Ovarian cancer risk factors

4.1. Hereditary and genetic risk factors

Ovarian cancer risk is causally linked to both lifestyle and genetics. Firstly, hereditary ovarian cancer accounts for approximately 5–15% of all cases [197] and are often diagnosed at an earlier age than sporadic disease. Furthermore, hereditary ovarian cancer tends to be of the high-grade serous subtype [198]. Therefore, patients with a first or second-degree relative with ovarian cancer have an increased risk of developing the disease (**Figure 9**). Specifically, there is a 2.5% risk of ovarian cancer in woman who report a sister EOC and a 9% risk if their mother has been previously diagnosed [197]. Familial ovarian cancer was first observed in Lynch syndrome (a disease associated with familial cancer due to inherited mutations in DNA repair machinery) in the 1970s [199, 200]. Multiple group and genomic mapping studies of breast and/or ovarian cancer-prone families ultimately led to the identification of inherited mutations in *BRCA1* [201] and later *BRCA2* [202, 203]. The prevalence of *BRCA1* or *BRCA2* mutations in the populations has been estimated from 0.1–0.3%, and 0.1–0.7%, respectively, in Caucasians with European origins [204–206]. *BRCA1* and *BRCA2* are mutated in the germline of approximately 9–13% patients with hereditary ovarian cancer [207–209]. For mutations in *BRCA1,* the estimated average risk of ovarian cancers ranges from 20 to 50% [210–214]. For *BRCA2,* average risk estimates range from 5 to 23% [210–214]. Mutation-specific cancer risks have been reported that suggest ovarian cancer cluster region (OCCR) exist in both *BRCA1* and *BRCA2* [211, 215]. The prevalence and spectrum of mutations in *BRCA1* and *BRCA2* have been reported in single populations with the majority of reports focused on Caucasians in Europe and North America. The Consortium of Investigators of Modifiers of *BRCA1* or *BRCA2 (*CIMBA) has assembled data on more than 26,000 *BRCA1* and nearly 17,000 *BRCA2* female mutation carriers from 69 centers in 49 countries on six continents [216–222]. Ongoing studies by Tim Rebbeck and the

CIMBA consortium have comprehensively evaluated the characteristics of the over 1600 unique *BRCA1* and more than 1700 unique *BRCA2* deleterious (disease-associated) mutations found in the carriers [215]. The most common mutation types in these genes are frameshift mutations, followed by nonsense mutations. Therefore, understanding the type of mutations in *BRCA1* or *BRCA2* is important for risk assessment and determining medical management for patients. Most subtypes of ovarian cancer have been linked to *BRCA1* or *BRCA2* germline mutations but the development of HGS disease is the most common in these women carriers [223]. *BRCA1* and *BRCA2* mutations are more common in Ashkenazi Jewish women [206, 224, 225] due to the three common Jewish founder mutations *BRCA1* c.5266dup (5382insC) and *BRCA1* c.68_69del (185delAG) and *BRCA2* c.5946del (6174delT) which have long been used as a primary genetic screening test for women of Jewish descent. Other mutations that are relatively common in specific populations, referred to as founder mutations, can be used to in limited screening tests. For example, in Iceland, only two mutations have been reported: the common founder mutation *BRCA2* c.771_775del and the rarer *BRCA1* c.5074G > A [226]. Despite having a higher risk for developing ovarian cancer, *BRCA1/2* carriers have a better clinical outcome in terms of survival, with *BRCA2* carriers having a more favorable outcome than *BRCA1* carriers [54]. This

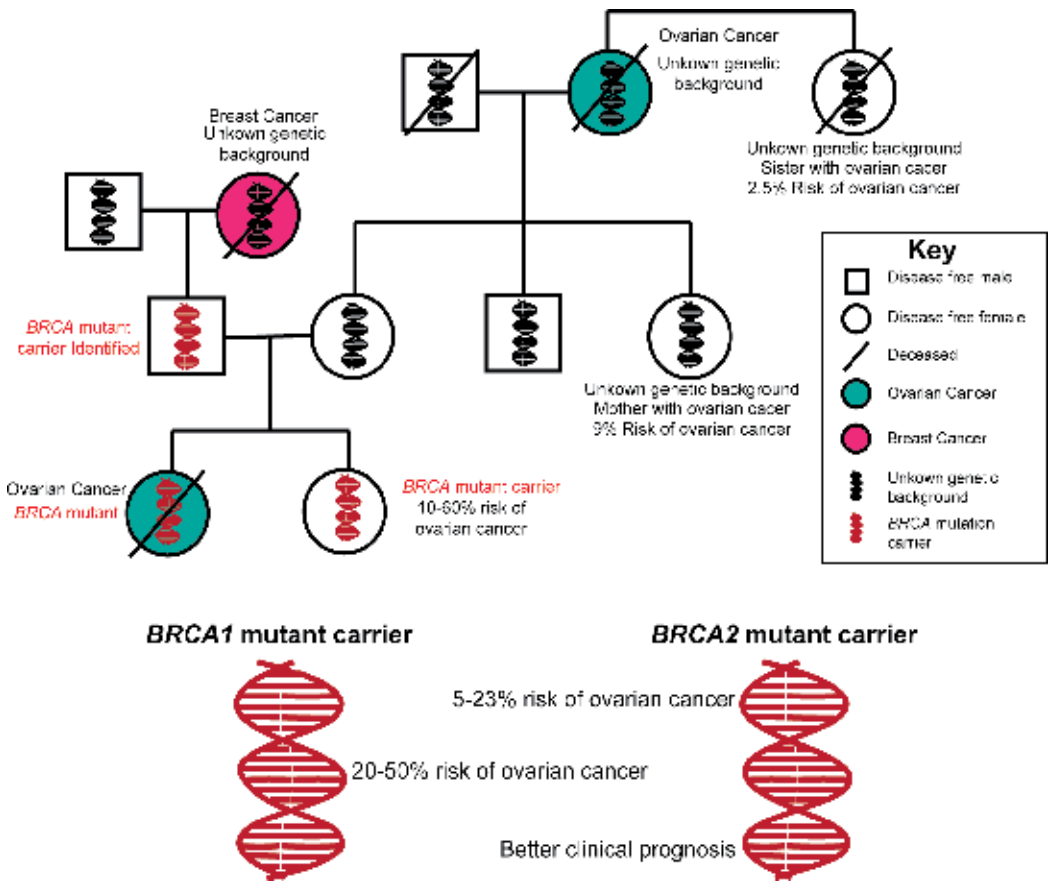

Figure 9. Hereditary ovarian cancer and BRCA mutations. Pedigree descripting "BRCAness" and risk of ovarian cancer (top). The relative risk and prognosis for women with germline *BRCA1/2* mutations.

phenomenon is thought to be due to *BRCA2* carriers responding better to platinum-based chemotherapy [227]. However, the survival benefit decreases when examined over 10 years in HGS instead of 5 years [228]. Over time, this could be possible due to secondary intragenic mutations in *BRCA1* and *BRCA2* that restore the wild-type reading frame (conversion back to a functional BRCA) and losing favorable responses to chemotherapy [229].

As indicated, the location of the alteration within *BRCA1* or *BRCA2* may vary the risk of breast and ovarian cancer [215], but other studies including genome-wide association study (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with risk of ovarian cancer for women in the general population [230]. Four of these SNP, *i.e.,* rs10088218, rs2665390, rs717852, rs9303542, were associated with ovarian cancer risk in *BRCA2* carriers, while two loci (rs10088218 and rs2665390) were associated with ovarian cancer risk in *BRCA1* carriers [217]. Inherited variants in other loci along with *BRCA1* or *BRCA2* mutations can better predict the risk of either breast or ovarian cancer [220], indicating the need to better understand concurrent sequence variants in women with deleterious *BRCA1* or *BRCA2* mutations. Concurrent mutations in 1p36 (*WNT4*), 4q26 (*SYNPO2*), 9q34.2 (*ABO*), and 17q11.2 (*ATAD5*) increased risk of all EOC subtypes while 1q34.3 (*RSPO1*) and 6p22.1 (*GPX6*) mutations increased the risk of serous ovarian cancer in *BRCA* carriers [231]. *BRCA1* mutation carries can have reduced risk with concurrent sequence variants in *CASP8, i.e.,* the D302H polymorphism [232]. Other genetic markers of risk, such as a variant allele of *KRAS* at *rs61764370*, referred to as the *KRAS*-variant, which disrupts a *let-7* miRNA binding site in this oncogene, is associated with sporadic and familial ovarian cancer without *BRCA1/2* mutations [233]. *PALB2*, encoding for a BRCA2 interacting protein, has increased promoter hypermethylation which results in decreased *BRCA2* function and increased risk of ovarian cancer [234]. Recent data have shown that copy number variation in *BRCA1* or *BRCA2* mutation carriers can either increase the risk (*OR2A*) or decrease the risk (*CYP2A7*) of ovarian cancer [235]. A better understanding of secondary genetic alteration in *BRCA1/2* mutant carriers can help determine the best clinical approach for managing the risk of disease.

Genetic risk factors outside of *BRCA1* or *BRCA2* mutations are not as well defined but often take place in genes involved in genomic integrity, most commonly DNA mismatch repair (MMR). SNPs in the *TERT* locus (rs2242652 and rs10069690) were associated with decreased telomere length and increased breast and ovarian cancer risk in *BRCA* mutation carriers [236]. A study that sequenced 12 genes for germline mutations in patients with ovarian cancer found *BARD1*, *BRIP1*, *CHECK2*, *MREA11*, *MSH6*, *NMN*, *PALB2*, *RAD51C*, or *TP53* were mutated in 24% of the 360 patients enrolled [237]. Genes within the Fanconi anemia pathway are also associated with developing ovarian cancer, including *RAD51C*, *RAD51D*, and *BRIP1* [238, 239]. Other MMR genes have been associated with Lynch syndrome and ovarian cancer risk *MLH1*, *PMS2*, *MSH2*, and *MSH6* [240–242].

4.2. Lifestyle risk factors

Environment and lifestyle also play a risk for developing both hereditary and sporadic ovarian cancer by either increasing or decreasing the lifetime risk of developing ovarian cancer. Like many cancers, age is a risk factor for ovarian cancer with most cases being diagnosed after the

age of 60 and the disease being extremely rare in patients under 40 years of age [243]. As previously discussed, surgical procedures such as tubal ligation, salpingectomy and unilateral or bilateral oophorectomy have varying degrees of success for the development of ovarian cancer by removal of the organs from which the cancer develops [244, 245]. In women with a *BRCA1* or *BRCA2* mutation, risk-reducing salpingo-oophorectomy (RRSO) decreased the lifetime risk of developing ovarian and breast cancer [165]. In a multicenter study, RRSO was associated with an 85% reduction in *BRCA1*-associated gynecologic cancer risk (hazard ratio [HR] = 0.15; 95% CI, 0.04 to 0.56), while protection against *BRCA2*-associated gynecologic cancer (HR = 0.00; 95% CI, not estimable) was suggested, its effect did not reached statistical significance [246]. The effects of RRSO can influence risk for each subtype given the nature of development from different tissues, hence why bilateral oophorectomy has a stronger influence on the development of HGS disease, since it is believed to develop from the fallopian tubes. Lifestyle factors which influence complete cycling during menstruation have some of the strongest effects on the risk of developing ovarian cancer. This hypothesis is attributed to incessant ovulation, in which the release of eggs from the ovary, the fusion on the fallopian tube and the rebuilding of the uterine wall all contribute to pathogenesis of ovarian cancer [141, 148]. One of the most common factors which can alter complete cycling is the use of oral contraceptives [243]. The increase in use of oral contraceptives could be attributed to the decrease in ovarian cancer in the last decade. The longer use of oral contraceptives has been shown to correlate to lower risk of developing ovarian cancer [247, 248]. The risk is reduced in both *BRCA* wild-type and mutant carriers [249] [250]. The risk of developing each subtype is decreased following oral contraceptive use, with the exception of clear cell carcinoma [251]. However, the associated side effects make it a poor treatment for prevention alone [252]. Another factor that can influence menstrual cycles and the risk of ovarian cancer is child birth [253], in specific the age at first birth and the number of births. In fact, it was discovered the risk of ovarian cancer decreases by approximately 10% for each 5-year increment in age at first birth [254]. Also, the number of births for a given women has additive decrease in the risk of ovarian cancer, decreasing by about 8% for each birth [255], while the age of each woman at the onset of menopause had a weak association [129, 256].

Other lifestyle factors can influence the risk of ovarian cancer, such as hormone replacement therapy, breast feeding, obesity and inflammation. Hormone replacement therapy increases the risk of developing ovarian cancer, depending on the therapy. For instance, the use of estrogen increases the risk of developing ovarian cancer by 22%, while the combination of estrogen and progesterone only has about a 10% chance of developing ovarian cancer [257–259]. A meta-analysis showed a similar risk for developing both HGS and endometrioid ovarian cancer in menopausal women [260]. Conversely, hormone replacement given for menopause symptoms may improve survival of ovarian cancer patients [261]. Another reproductive factor is breastfeeding, in *BRCA1* mutant carriers breastfeeding lead to a reduced the risk of developing ovarian cancer [129, 243]. Meta-analysis also suggests the duration of lifetime breastfeeding is additive in reducing the risk of developing ovarian cancer [262]. Like many other cancers, cigarette smoking and alcohol consumption have at least some association with increasing the risk of developing ovarian cancer. Specifically, smoking is associated with an increased risk of developing clear cell and endometrial ovarian cancer but not serous [263]. Smoking increased the risk of mucinous ovarian cancer, but cessation returns can reduce the

risk over time [264] while heavy smoking (>10 packs per day) more than doubles the risk of developing ovarian cancer [265]. Alcohol consumption increased the risk of ovarian cancer, but seems to have an effect only in heavy drinkers. Consumption of more than 20 drinks per week is associated with increased risk [266] while with moderate use the risk is less pronounced or significant [267, 268]. Obesity is associated with less common subtypes of ovarian cancer and not HGS [269] and the lifetime risk decreases with recreation physical activity [270]. Finally, inflammation increases the risk of developing ovarian cancer [271] while the use of aspirin was shown to reduce risk of developing ovarian cancer from between 20 and 34% [272]. The use of other non-steroidal anti-inflammatory drugs (NSAIDs) showed a reduction in risk but was not significant.

5. Conclusion

Genetically, ovarian cancer is a heterogeneous and dynamic disease that presents several clinical and research challenges. While epithelial ovarian cancer is categorized pathologically into five basic subtypes, within each subtype exist genetic diversity that limits the development of target therapies. To add to this complexity, one of the hallmarks of serous ovarian cancer is genomic instability, which is driven by frequent *TP53* mutations and deficiencies in DNA repair pathways. While this genomic alterations have led to the development of breakthrough therapies (PARP inhibitors), they also contributes to the dynamic cell growth and frequent genomic alterations and gene expression changes which contribute to the adaptation to therapy. Likewise, the pathogenesis of ovarian cancer remains a debated field with the recent insights of progression of a subset of serous ovarian cancer from fallopian tube epithelial lesions. Progression from the fallopian tube means tumors detected on the ovarian surface are already metastatic disease, leading to quick progression and limited response to therapy. Overall, while many genetic and genomic abnormalities have been identified in ovarian cancer, additional discovers are needed to (1) improve early detection of the disease (at a time when current treatment might be curative), (2) further define molecular classifiers of response to therapy, and (3) develop therapies that will be more effective across or specific to the different molecular subtypes. Other than the very common *TP53* mutation in high-grade serous ovarian cancer (96% of cases), which to date is undruggable, and the previously mentioned *BRCA* mutations (approximately 10–12% of ovarian cancers), only a small overall percentage of tumors from patients with this malignancy will be found to possess a specific causative mutation that can be effectively targeted therapeutically. Therefore, implementation of genomic-based medicine remains a challenge for the management of women with ovarian cancer.

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Chapter 2

Signaling Pathways Related to Nerve Growth Factor and miRNAs in Epithelial Ovarian Cancer

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Additional information is available at the end of the chapter

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Abstract

Epithelial ovarian cancer (EOC) is a disease that causes 140,000 deaths every year. Nerve growth factor (NGF) and its high affinity receptor TRKA play important roles in follicular maturation, follicle-stimulating hormone (FSH) receptor acquisition and ovulation in normal ovary. Also, NGF has many roles in EOC cells: increasing survival, proliferation, cyclooxigenase-2 (COX-2), vascular endothelial growth factor (VEGF) and metalloproteinase ADAM17 expression. Besides, NGF inhibits calreticulin translocation from the endoplasmic reticulum to cell surface, possibly diminishing the efficacy of immunogenic therapies in EOC. Additionally, NGF acts as an angiogenic factor by a direct stimulation of migration, differentiation and proliferation of endothelial cells. Among the numerous factors actually described to be important in many types of cancer, including EOC, are the microRNAs (miRs). Indeed, it has been found that miR-143 is downregulate in EOC, which correlates with an increase of COX-2; concomitantly, NGF increases COX-2 as mentioned. Furthermore, NGF increases miR-222 and its target is the metalloproteinase inhibitor TIMP3, increasing the ADAM17 function. Also, NGF increases cMYC transcription factor in EOC, which decreases miR-23 levels regulating proteins involved in cell cycle and tumor growth. Therefore, NGF/TRKA signaling pathways alter the expression of many proteins and deregulate miRs in EOC, leading to the progression of this cancer.

Keywords: epithelial ovarian cancer, nerve growth factor, vascular endothelial growth factor, ciclooxigenasa-2, prostaglandin-E2, calreticulin, c-MYC, DAM17, microRNAs

1. Introduction

Ovarian cancer is a deadly disease that causes around 225,000 new cases and 140,000 deaths every year, remaining a major health problem worldwide [1]. Moreover, epithelial ovarian cancer (EOC) is more common in elderly women who are no longer experiencing reproductive cycles [1]. This cancer is characterized by the non-specificity of its symptoms and the lack of efficacy for therapies at advanced stages. Therefore, EOC is diagnosed at late stages and has a low overall 5-year survival below 45% [2].

A key process for EOC growth and metastasis is angiogenesis, the formation of new blood vessels from pre-existing vasculature. It is a complex process regulated by the balance between pro- and anti-angiogenic factors [3]. In the normal reproductive ovary, angiogenesis is a physiological process that occurs during every cycle in a controlled manner [4]. In cancer, pro-angiogenic factors are overexpressed and angiogenic regulation is lost. Among these factors, neurotrophins have an important role in controlling angiogenesis in the normal and neoplastic ovary, being also implicated in the regulation of other physiological and pathological processes [5]. The roles of neurotrophins in the normal ovary and in EOC are discussed in the next sections.

2. Roles of nerve growth factor in the normal ovary and in epithelial ovarian cancer

Neurotrophins are small polypeptides that were first discovered as a growth factor on the nervous system, subsequently named nerve growth factor (NGF) [6]. Besides NGF, there are four other neurotrophins: brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), neurotrophin 4/5 (NT-4/5) and neurotrophin 6 (NT-6). Besides the nervous system, most of these peptides are also found in several other systems and organs, including the ovary [7].

To induce a biological effect, neurotrophins need to interact with cell-surface receptors. All neurotrophins interact with two different types of receptors: the p75 neurotrophin receptor ($p75^{NTR}$) and a member of the tyrosine receptor kinase (TRK) family. All neurotrophins can bind to $p75^{NTR}$ with low affinity, but every different TRK receptor can bind to a specific neurotrophin with high affinity [8]. The TRK family is constituted by three members: TRKA, TRKB and TRKC. NGF binds to TRKA; BDNF and NT4/5 bind to TRKB; and NT-3 binds to TRKC. Moreover, alternative splicing can generate different TRK isoforms and some of them can initiate signal transduction pathways [9]. On the other hand, $p75^{NTR}$ and also TRK receptors can dimerize, forming either homodimers or interacting with each other (heterodimers) [10].

Nerve growth factor can induce cell survival on several systems, including the nervous, cardiovascular, immune, endocrine and reproductive systems [7]. Upon binding to TRKA, the receptor homodimerizes and autophosphorylates its tyrosine residues, inducing signaling pathways that induce trophic and anti-apoptotic effects [11]; NGF deficiency, conversely, activates apoptosis (**Figure 1**) [12]. The NGF/$p75^{NTR}$ pathway can lead to proliferation, survival or

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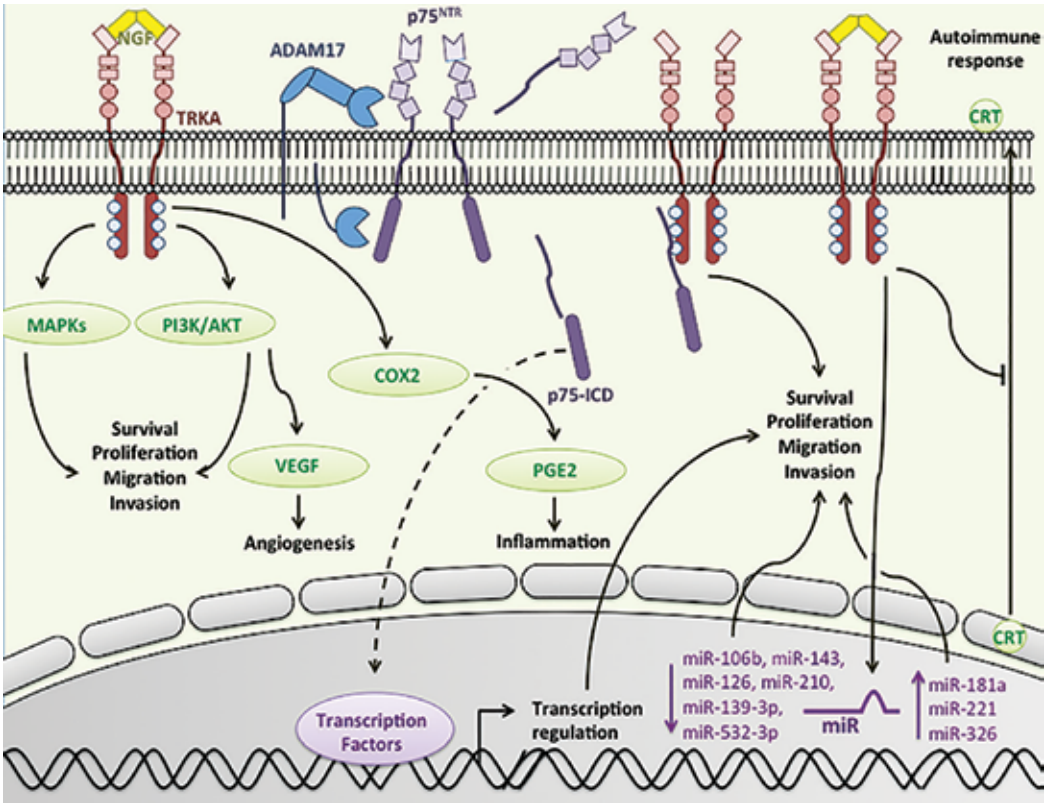

Figure 1. Several NGF-related signaling pathways are involved in epithelial ovarian cancer. NGF, a protein whose levels are elevated in EOC, activates several signaling pathways leading to carcinogenesis. Upon binding to its high affinity receptor, TRKA, NGF activates the MAPKs and PI3K/Akt signaling pathways, inducing cell survival, proliferation, migration and invasion. Through TRKA activation, NGF also increases VEGF, COX-2 and PGE 2 levels, which promotes angiogenesis and inflammation, respectively. Besides, NGF inhibits CRT translocation from the endoplasmic reticulum to the cell surface, potentially inhibiting anticancer immune responses. NGF's low affinity receptor, $p75^{NTR}$, is also present in ovarian cancer cells. This receptor can be cleaved by ADAM17, a cell surface metalloproteinase, producing an intracellular domain (p75-ICD) that could be responsible for the regulation of different processes through transcription control. Furthermore, p75-ICD can interact with TRKA, increasing its activity. Several microRNAs (miRNAs) are regulated by NGF, and these miRNAs could be responsible for NGF-mediated effects.

cell death, depending on the cell context, availability of adaptors and expression of co-receptors. While NGF can trigger apoptosis through the activation of the Jun N-terminal Kinase (JNK)/c-Jun death pathway, it can also activate the canonical NFκB signaling cascade, which promotes cell survival by increasing anti-apoptotic molecules levels [13]. The receptor $p75^{NTR}$ can also enhance TRKA phosphorylation by increasing the TRKA ability to bind to NGF [14].

Neurotrophins are involved in normal ovarian development and functioning, regulating follicular assembly, folliculogenesis and ovulation. Concerning ovarian development, $p75^{NTR}$ is expressed in the stromal cells surrounding the oocytes of human fetuses previously and during follicular assembly [15]. NGF and TRKA also seem to be necessary for follicular assembly, because mutations on these genes reduce the number of primordial follicles in mice [16].

Besides, NGF increases follicle-stimulating hormone receptor (FSHR) protein levels and the ovary response to FSH, collaborating in the growth of pre-antral follicles of 2-day-old rat ovaries [17]. Neurotrophins also participate in folliculogenesis, since they are involved in the differentiation of primordial follicles into primary follicles and in the development of secondary follicles from primary follicles [18].

In humans, NGF is present in the oocyte and granulosa cells from follicles at primordial and secondary stages, suggesting that NGF is necessary for follicle maturation after the primordial stage [16]. $p75^{NTR}$, on the other hand, is not detected on human stromal cells after birth, but theca cells from growing follicles do express this protein [15]. Concerning TRKA, this receptor is found in granulosa cells and oocytes of neonatal mice ovaries; its expression is higher on primary follicles and diminishes with folliculogenesis [15].

In human antral follicles, both granulosa and theca cells express NGF and TRKA. Furthermore, NGF has a role in ovulation, since in human ovarian granulosa cells, NGF increases FSHR and estradiol secretion [19]. Nerve growth factor contributes to ovulation by decreasing gap junctions, stimulating the proliferation of theca cells and inducing the release of prostaglandin E2 (PGE2), which acts on granulosa cells and is necessary for successful ovulation [20, 21]. Indeed, PGE2 is a paracrine mediator of luteinizing hormone (LH), and LH induces an increase of intrafollicular levels of PGE2, controlling key molecular events of ovulation, including the facilitation of follicle rupture and the release of the oocyte [22].

Angiogenesis is a key process in the normal ovarian functioning, necessary for the growth of ovarian follicles and the development and maintenance of the corpus luteum [22]. The expression and secretion of the vascular endothelial growth factor (VEGF), an important proangiogenic molecule, is key for normal adult reproductive function, and its expression is induced by the activation of FSHR and the LH receptor (LHR) [23]. VEGF production is also stimulated by NGF in cultures of human granulosa cells through the MAPK and PI3K/AKT signaling pathways [23]. Besides, NGF can directly regulate angiogenesis by acting on endothelial cells [24]. Thus, NGF participates in normal ovarian angiogenesis through its high affinity receptor TRKA.

While NGF plays a physiological role in the ovary, regulating its development and ovulation, it can also participate in cancer-related processes, particularly through its TRKA receptor [25], as seen in **Figure 1**. In cancer cells, these pathways are linked to proliferation, survival, migration and invasiveness. Interestingly, whilst in normal epithelial ovarian cells NGF and TRKA expression is only found on a small percentage of cells, both of these proteins are present in EOC tissues [26]. The active or phosphorylated form of TRKA is highly elevated in EOC compared to normal tissues, making it a possible marker for poor prognosis [27].

The NGF/TRKA signaling pathway has also been linked to several transduction cascades that stimulate cancer progression, including VEGF production and secretion [26], the COX2/PGE2 inflammatory response [28], ADAM17 activity [29] and alterations on calreticulin (CRT) subcellular localization [30]. All the molecules mentioned above have a role in the development or progression of ovarian cancer by altering processes such as inflammation, angiogenesis, immune evasion, survival and metastasis.

Angiogenesis is a vital process necessary for solid tumors to grow, develop and metastasize [31]. Several molecules are known to promote angiogenesis, in several cancer tissues including EOC; however, VEGF is considered the main angiogenic factor [32]. Its expression is controlled by the hypoxia-inducing factor (HIF-1α), a transcription factor that is produced in cells with low oxygen levels, a condition typically found on cancer cells from solid tumors [33]. VEGF induces angiogenesis by binding to its tyrosine kinase receptors located on the surface of endothelial cells, promoting their proliferation, migration and increasing their permeability [34]. In EOC explants, NGF induces an increase of VEGF levels through TRKA activation, increasing VEGF secretion [26]. Also, the NGF-conditioned medium secreted by EOC explants and by A2780 cells (an immortalized EOC cell line) induces proliferation, migration and differentiation of human endothelial Eahy926 cells [27]. Importantly, NGF, total TRKA and p-TRKA molecules are present in endothelial cells from cancer tissues. Therefore, NGF acts on EOC cells by inducing VEGF expression, besides its direct angiogenic effect by acting on the TRKA receptor found on endothelial cells [26, 35].

Moreover, given the role of NGF in the promotion of ovulation through the increase of PGE2, this neurotrophin has been linked to pro-inflammatory responses in the ovary. Interestingly, cancer has been linked to chronic inflammation, since different inflammatory pathways are activated in tumor tissues, including pathways involving cyclooxygenase (COX) proteins [36]. PGE2 is synthesized by members of the COX family: COX-1 and COX-2 [37]. COX-2 expression is inducible by external stimuli, and several molecules found in cancer, including cytokines, growth factors, oncogenes and chemicals, can induce its expression [37]. As for PGE2, this prostaglandin induces cell growth, angiogenesis, invasiveness, inhibition of apoptosis and inflammation [38]. Importantly, non-steroidal anti-inflammatory drugs (NSAIDs), which act by selectively binding to COX-1 or COX-2 and inhibiting the arachidonic acid pathway, have preventive and inhibitory effects on carcinogenesis, highlighting the importance of COX-2 in cancer [39]. Moreover, COX-2 levels have been found to be elevated in several types of cancer, including colon, gastric, breast, pancreatic, bladder and prostate cancer [40]. Therefore, COX-2 has become a focus for cancer research as a potential therapeutic target [41].

In EOC, COX-2 levels have been found to be elevated in human ovarian cancer samples compared to normal ovaries [28]. In theca cells from bovine ovaries, NFG increases COX-2 and PGE2 levels [42] and on prostate cancer cell lines, PGE2 promotes VEGF secretion [43]. Therefore, our research group explored a possible connection between NGF, COX-2, PGE2 and VEGF. In vitro experiments on A2780 epithelial ovarian cancer cells showed that NGF induces COX-2 expression and increases PGE2 levels, suggesting that NGF could stimulate inflammatory processes [28].

Other proteins that are involved in inflammatory responses are metalloproteinases, including a disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) [44]. ADAM17 is expressed in granulosa cells, being important in ovary signaling during oocyte development and follicular fate determination [45].

ADAM17 is ubiquitously expressed; it is primarily active during inflammation and in cancer tissues; therefore, ADAM17 has become another focus for cancer research [46]. In lung cancer, for instance, ADAM17 protein levels are increased, and ADAM17 inhibitors aid cancer

treatment when the tumor has developed resistance mechanisms [47]. In breast cancer, ADAM17 protein levels are also overexpressed, which has been linked to tumor progression and metastasis [48]. Additionally, ADAM17 levels and activity have also been found to be elevated in colorectal, pancreatic, kidney, prostate and ovarian cancer [46].

An important ADAM17 target is TRKA, where its dimerization with $p75^{NTR}$ favors ADAM17 activation, which in turn induces $p75^{NTR}$ cleavage [49] through γ-secretase, resulting in a cytoplasmic fragment (p75-ICD) that can bind to the intracellular domain of TRKA, increasing TRKA signaling activity [50]. In human ovarian cancer samples, $p75^{NTR}$ levels are lower compared to normal ovarian tissues. In A2780 cells, ADAM17 cleaves $p75^{NTR}$, possibly decreasing p75 anticancer effects. The p75-ICD, on the other hand, increases TRKA activation, potentially inducing pro-carcinogenic processes. Besides, NGF stimulation activates TRKA, ADAM17 and γ-secretase, reducing $p75^{NTR}$ levels and increasing p75-CTF and p75-ICD levels, favoring cell survival [29]. Also, there is evidence that suggests that p75-ICD could act as a transcription regulator, enhancing TRKA cancer activity [51].

2.1. NGF effect on calreticulin subcellular localization: potential consequences for immunotherapy

Cancer cells are exposed to higher levels of endoplasmic reticulum (ER) stress, since they are exposed to stressful conditions such as hypoxia, nutrient deprivation and pH changes, among others [52]. In order to adjust to these changes, cancer cells activate the unfolded protein response (UPR), composed of three branches initiated by three proteins: IRE1α, PERK and ATF6 and sensors of ER stress [53]. In this context, calreticulin (CRT), a chaperone resident of the endoplasmic reticulum, plays a role in the adaptation of cancer cells to changes in the microenvironment [54]. CRT, a multifunctional, buffering and ubiquitous protein, is mainly involved in protein folding and the maintenance of calcium homeostasis; as a chaperone, CRT participates in protein folding quality control [54]. Under conditions of ER stress, calreticulin levels increase to restore the cell to homeostasis [55]. CRT protein levels are elevated in different cancer tissues, including EOC [37, 65], and while this increase could be associated with an adaptation to ER stress, CRT expression has also been linked to proliferation, metastasis, invasion and angiogenesis [56]. Moreover, in EOC cells, NGF induces an increase of CRT levels, which could be associated with the acquirement of carcinogenic properties [30, 57].

Importantly, despite the pro-carcinogenic effects of CRT, when this protein is found in the cell surface it can induce an anti-immune response against cancer cells [58]. In human ovarian cancer cells, our research group found that mitoxantrone, a direct ER stress inducer, can trigger CRT translocation from the ER to the cell surface [30]. Previous studies have shown that ER stress is a necessary step for CRT transport to the cell surface, and concordantly, in EOC cells, CRT translocation was accompanied by activation of the UPR protein PERK and its substrate eIF2α [59].

Interestingly, several reports show that NGF can inhibit the effects of ER stress, which could hinder cells' ability to translocate CRT from the ER to the cell surface [60–62]. Indeed, when A2780 cells were incubated with both NGF and mitoxantrone, CRT levels on the cell surface were diminished compared to cells stimulated with mitoxantrone alone [30]. Therefore, an

anticancer immune therapy based on drugs that induce CRT translocation from the ER to the cell surface could have limited efficiency in ovarian cancer patients, since NGF levels inhibit CRT translocation.

As described above in EOC, NGF is involved in many processes such as cellular survival, proliferation, angiogenesis and response to therapy. NGF could be regulating these processes through microRNA modulation; therefore, it is important to describe the role of microRNAs in EOC and its relation with NGF.

3. Role of microRNAs (miRs) in the progression of ovarian cancer and their relation with nerve growth factor

New targets of NGF and its receptor TRKA include various microRNAs (miRs). Since the 1990s, deregulation of miRs has become important in several pathological processes, including several types of cancer [63]. Currently, miRs could be used as new biomarkers and/or for therapy in various diseases [64]. Particularly in ovarian cancer some miRs are downregulated or upregulated [65], and NGF and its receptor TRKA could be implicated in the deregulation of some miRs.

MicroRNAs are the biggest family of non-coding RNAs; they are ~22-nucleotides (nts) long and regulate mRNAs post-transcriptionally [66]. The first step on miR biogenesis is the synthesis of a long primary miR (pri-miR) by an RNA polymerase II. Then, the pri-miR is cleaved, producing a pre-miR [67] that is transported to the cytoplasm to be enzymatically cleaved in its loop structure, releasing a double-strand miR called duplex [68]. This duplex has two strands, one called "mature" or "guide" miR and the other named "passenger", which is released and degraded [69]. Mature miR has ~22 nts and binds to the three-prime untranslated region (3′-UTR) of a target mRNA in order to regulate protein expression. This regulation depends on miR-mRNA complementary: total complementarity of miR with its mRNA target is a signal to cleave or degrade the mRNA. On the other hand, partial complementarily induces deadenylation of the mRNA target (facilitating its degradation) or inhibition of its translation [70]. In normal cells, microRNAs have an important role maintaining their normal functioning; however, a deregulation in their expression can lead to cellular alterations. Most studies concerning miR roles in pathologies evaluate whether there are changes on miR expression; therefore, miR targets are still being described. Regarding these targets, one miR has several targets, meaning that one miR can be involved in the development of different pathologies.

Cancer development involves miR deregulation. Cancer-related miRs are divided in two groups: oncogenic (oncomiR) and tumor suppressor (oncosuppressor) miRs; oncomirs regulate the mRNA of tumor suppressor genes, while oncosuppressors control the mRNA of oncogenes. Both of these types of miRs are normally in equilibrium; however, during carcinogenesis, they exhibit a deregulation on their expression [71]. One miR can regulate the same mRNA targets in different types of cancer, which makes them an attractive target for the development of new therapies.

Besides their potential as therapeutic targets, currently, miRs' profiles are being described in order to obtain more accurate and reliable biomarkers for cancer development and/or progression [64]; in EOC, several miRs have been found to be upregulated [72].

Interestingly, it has been found that eight miRs could be regulating 89% of the miR-associated genes [73]. Thus, to produce a more accurate clinical diagnosis, it would be beneficial to have miR profiles as biological markers.

EOC development and progression is regulated by several miRs. OncomiRs and tumor suppressor miRs modulate different processes of the hallmarks of cancer, such as proliferation, angiogenesis, migration, invasion, survival and apoptosis, among others (**Table 1** summarizes the most important miRs involved in different cancers, including EOC).

As discussed above, NGF is overexpressed in EOC and it has a significant role in the progression of this disease [35]. Interestingly, studies show that NGF could regulate the expression of some miRs. Most of these studies have been done in PC12 cells: in these cells, NGF stimulation increases the expression of several miRs [74].

Importantly, in EOC, miR-143 is downregulated [75], which is correlated with an increase of COX-2 levels [76]. As stated in the previous section, NGF increases COX-2 levels [28]. It also decreases the expression of miR-143 in PC12 cells [74]. Therefore, in EOC, the NGF-mediated COX-2 increase could be regulated through miR-143. Another miR regulated by NGF is miR-222 [77], which targets a metalloproteinase inhibitor (TIMP3) [78]. TIMP3 inhibits ADAM17 function [79]; then, NGF could increase miR-222 in order to decrease TIMP3 levels, allowing the ADAM17 activity. Consequently, NGF regulation of miR-143 and miR-222 could be important for EOC development, through the regulation of COX-2 levels and ADAM17 activity, respectively (summarized in **Table 2**).

miR	Regulation	Cancer	Targets	References
Let-7 family	↓	Lung, hepatocellular, breast and ovarian	RAS, HMGA2, cyclin D2, c-myc	[83–86]
miR-17-92	↑	Myeloma, breast, gastric and colon cancer	BIM, E2F1 PTEN	[85, 87–89]
miR-21	↑	Oral, colon, breast, glioma, ovarian and cervical cancer	PTEN, DKK2, PDCD4, TGFbR2	[85, 90–93]
miR-23a/b	↓	Colon, pancreatic and ovarian cancer	MAP3K1, Cyclin G1, RRAS2, TGFβR2	[72, 82, 94, 95]
miR-122	↓	Hepatocellular cancer	Wnt1, TCF4, Cyclin G1, B-catenin	[84, 96]
miR-143	↓	Gastric cancer	COX2	[97]
miR-125 family	↑	Renal cell carcinoma, endometrial and breast cancer	ERBB2, P53INP1, HDAC5	[85, 98–100]
	↓	Ovarian cancer	SET	[101]

One miR can be deregulated in different types of cancer; simultaneously, several miRs can be deregulated in one type of cancer. Some examples are described in the table, including oncomiRs and tumor suppressor miRs. miRs can have a dual role. A few of their mRNA targets are also depicted.

Table 1. List of miRs and some of their targets de-regulated in cancer.

NGF-related miR	Regulation	Cancer	References
miR-92a	↑	Neuroblastoma	[102]
miR-21	↑	Pheocromocitoma	[103]
miR-221/222	↑	Pheocromocitoma	[77]
miR-23b	↓	Ovarian cancer	[80]
miR-143	↓	Pheocromocitoma	[75, 76]

NGF stimulation regulates miRs in these cancers through the upregulation of several miRs, including miR-92a, miR-21 and miR-221/222, while it downregulates other miRs, such as miR-23b and miR-143.

Table 2. List of miRs regulated by NGF.

Besides, in EOC, an increase of NGF levels induces the expression of c-MYC transcription factor [80], and c-MYC downregulates the miR-23b expression [81]. This miR levels decrease in EOC, and we described that after NGF stimulation, EOC cells diminish miR-23b levels [80]. Therefore, in this cancer, NGF could reduce miR-23b levels through c-Myc. miR-23b targets cell cycle and tumor growth proteins, regulating cyclin-G1 [82] and SP-1 transcription factor [81], respectively.

4. Conclusion

Solid scientific evidences indicate that NGF has important roles in the progression of EOC by promoting the expression or activation of several proteins involved in the different carcinogenic processes, including cell proliferation, angiogenesis and in therapy resistance. For instance, NGF interaction with its TRKA receptor can activate AKT and ERK signaling, promoting cell proliferation and survival. TRKA activation by NGF also increases COX-2 and PGE2 levels, contributing to inflammatory processes, which are important to cancer progression. Besides, NGF can act on the ADAM17 metalloproteinase, which cuts the $p75^{NTR}$ receptor in EOC cells, leaving an intracellular fragment that can activate transcription and that can interact with TRKA, increasing its carcinogenic effects. Furthermore, NGF could modulate the immune response, since it can reduce CRT translocation from the endoplasmic reticulum to the cell membrane, reducing cancer cells' recognition by immune cells.

Additionally, it is relevant to point out that recent reports describe how NGF regulates the expression of different miRs, which in turn could affect the translation of protein participants of the abovementioned processes. Some examples include miR-143, whose levels are downregulated EOC and correlate with an increase of COX-2 levels. Another miR regulated by NGF is miR-222, which targets the metalloproteinase inhibitor TIMP3, an ADAM17 inhibitor. Furthermore, NGF stimulation reduces miR-23b levels through c-Myc, targeting the cell cycle and tumor growth proteins. Therefore, there is evidence to suggest that NGF-dependent miR regulation could lead to tumor development. Nevertheless, further studies are needed to confirm NGF's role in EOC; therefore, it is important to evaluate new miRs associated with EOC. These findings could result in new biomarkers used for diagnosis or target molecules that could allow the development of new therapies.

Abbreviations

ADAM17	a disintegrin and metalloproteinase domain-containing protein 17
COX	cyclooxygenase
CRT	calreticulin
EOC	epithelial ovarian cancer
ER	endoplasmic reticulum
FSH	follicle-stimulating hormone
FSHR	follicle-stimulating hormone receptor
LH	luteinizing hormone
LHR	luteinizing hormone receptor
miR	micro-RNA
NGF	nerve growth factor
Nts	nucleotides
p75NTR	p75 neurotrophin receptor
PGE2	prostaglandin E2
TRK	tyrosine receptor kinase
VEGF	vascular endothelial growth factor

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Chapter 3

Ovarian Cancer Overview: Molecular Biology and Its Potential Clinical Application

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Additional information is available at the end of the chapter

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Abstract

Over the previous two decades, there has been a shift in the ovarian cancer paradigm to consider it as a multiplicity of disease types rather than a single disease, requiring specialized medical management from molecular diagnosis through to treatment. Despite the achieved improvements in diagnosis, surgery, and systemic treatment, ovarian cancer remains the leading cause of death from gynecological tumors in western countries. The study of ovarian cancer at a molecular level could reveal potential biomarkers of disease diagnosis and progression, as well as possible therapeutic targets in areas such as angiogenesis and homologous recombination deficiencies. Although this area of research is proving invaluable concerning newer therapeutic approaches, platinum-based chemotherapy continues to be the core of the first-line treatment. Genomic screening focusing on the identification of prognostic and predictive markers is considered one of the leading areas for future ovarian cancer research.

Keywords: ovarian cancer, epithelial ovarian cancer, clinics, molecular biology, diagnosis, treatment, prognostic biomarkers, predictive biomarkers

1. Introduction

Ovarian cancer (OC) represents almost 4% of all cancer diagnoses among women worldwide. It is the eighth most common cause of death by cancer, resulting in 152,000 deaths (4.3% of all cancer deaths) [1, 2]. Besides its low incidence, OC is associated with a high mortality rate attributable, in part, to the frequent diagnosis at an advanced stage. The late diagnosis of OC is due to several factors including symptomatology absence and/or nonspecificity to the

inexistence of an effective screening method and to the aggressive biology of this tumor with the ability to disseminate.

The geographic variability in OC incidence is considerable, being frankly higher in developed countries with rates surpassing 7.5/100,000 women. The highest continental rate is registered in Europe, where 65,584 new OC cases were observed. In opposite, the lowest continental values were registered in African regions, with incidence rates below 5/100,000 women [1]. Concerning mortality, for women with less than 75 years, the average risk of dying from OC is twice as high in more than less-developed regions. Inclusively, for developed countries, OC stands as the fifth most lethal cancer among women [1]. In Europe, in 2012, 42,749 deaths were observed, which corresponds to more than 25% of all worldwide OC deaths [3, 4]. Among the gynecological tumors, OC is the leading cause of death even being only the third most common, preceded by cervical and endometrial cancers [2–4].

Nevertheless, the numerous attempts to characterize the ovarian carcinogenesis and etiology, age is considered as a major determinant for OC development: there is an increased disease risk after the menopause, being 63 the median age at diagnosis [5].

Beyond age, an important risk factor for OC is the familiar history. Although the germline mutations in genes that predispose to OC are relatively rare in the general population, they are responsible for approximately 10–20% of all cases [6, 7]. The critical genes involved in hereditary OC are *BRCA1* and *BRCA2*, associated with hereditary breast/ovarian cancer syndrome. The risk of spontaneous OC development, throughout life, is around 1.7%, while the heritage of germline mutations that alter *BRCA1* gene function confers a cumulative risk from 40 to 60%, mainly to serous carcinoma. The presence of pathogenic mutations in *BRCA2* gene lowers the risk for about a half (10–30%). OC hereditary women tend to develop the disease nearly 10 years earlier than women with sporadic OC [8–10].

Moreover, reproductive and endocrine factors seem to be important, whereby the nulliparity, early menarche (<12 years), late menopause (>52 years), endometriosis, polycystic ovary syndrome, and the recent exposure to hormone replacement treatment might be associated with a higher risk to develop OC [11–14]. Therefore, some behaviors and lifestyles were associated with a decrease in OC incidence, namely breastfeeding, multiparity, and the oral contraceptives use [11, 13]. Surgical procedures such as tubal ligation, hysterectomy with salpingectomy, and oophorectomy correlate with a lower incidence of this tumor but are mainly reserved for women with higher disease risk, after the completion of familiar planning.

Standard treatment of epithelial ovarian cancer (EOC) is based on cytoreductive surgery, followed by platinum-based first-line chemotherapy. This neoplasia is considered chemosensitive, yielding 40–60% of complete responses rates for advanced disease stages. Despite the apparent efficacy of treatment, up to 75% of patients will relapse and become candidates for second-line chemotherapy. As a result, the high percentage of late-stage diagnosis and the occurrence of tumor recurrence limit the treatment efficacy, and the overall 5-year survival rate remains only around 45%.

In the clinical practice, several pathological factors are considered prognostic for EOC patients, and many efforts are made to identify those that will improve patient's stratification and be

useful tools for therapeutic decisions. Current research is focusing on the identification of both prognostic and predictive biomarkers that would help to optimize EOC treatment strategies and to improve the cost-effective incorporation of emerging biological agents.

2. Clinics and diagnosis

Upon the detection of an adnexal mass suspected of malignancy, the diagnostic approach should be based on a careful clinical history that should include the overall physical examination, as well as gynecological, rectal, and abdominal evaluation. After the clinical evaluation, additional diagnostic and biochemical tests should be requested, judiciously and objectively, to aid in the differential diagnosis of a pelvic mass. Among the complementary diagnostic tests, transvaginal ultrasonography (TVU) and CA125 tumor marker determination are mandatory [8, 12, 15]. Other markers are also used in the diagnostic investigation for suspected EOC cases, such as CEA and CA19.9.

In the suspicion of ovarian neoplasia, abdominal-pelvic computed tomography (CT) should be requested to confirm and characterize the presence of lesions, to evaluate the tumor extension, to identify unresectable disease, and to exclude nonovarian metastatic disease. Nevertheless, the EOC diagnosis is surgical as only the anatomopathological exam confirms the definitive diagnosis. Diagnostic radiologically guided aspiration/biopsy or laparoscopy should be requested, whenever neoadjuvant chemotherapy is being considered [8, 16].

Late disease diagnosis explains, in part, the high mortality rate of these patients [12, 17]. Over the past 25 years, there has been little improvement in the survival rate, being around 37% in the early 1970 and 44% in 2000, despite the advances in the medical treatment [18]. However, the currently available tests lack adequate sensitivity and specificity, promoting a noneffective screening strategy. Prospective studies have shown that the combined use of serum CA125 and TVU improved the specificity of the tests and allowed the detection of a number of OC cases in the preclinical phase (this is discussed in detail in another chapter).

3. Histopathology

Ovarian tumors are classified according to the World Health Organization (WHO) proposal for gynecological tumors. Ovarian cancer has high cellular heterogeneity, and most of the primary ovarian tumors can be integrated into three major groups, namely epithelial, sex cord and ovarian stroma, and germ cell tumors [2].

Although the ovarian epithelial surface represents only a small fraction of all ovarian cell types, EOC is the most common, corresponding to almost 60% of all ovarian tumors [19, 20]. According to the criteria proposed by the WHO in 2014, EOC can be divided into seven histological subcategories, namely serous, mucinous, endometrioid, clear cell, Brenner, seromucinous, and undifferentiated [2]. All the mentioned histological subtypes, except for the undifferentiated type, are further subdivided into benign, borderline, and malignant neoplasia, depending on the optical microscopy characteristics.

Sex cord and stroma tumors arise from the ovarian connective tissue, often responsible for hormone secretion. These tumors encompass a vast group of tumors, for which the subgroup of "pure" ovarian stromal tumor is the most frequent (9% of all OC), usually with benign behavior. Also in this group of tumors, granulosa cell tumors are associated with aggressive behavior and represent 1% of all OC. Regarding the germ subgroup, a mature cystic teratoma is very common (32% of all OC), although the remaining germ cell tumors, both benign and malignant, are rare, representing 3–5% of all OC cases [2, 21].

4. Staging

Ovarian cancer staging is surgical, being performed according to the International Federation of Gynecology and Obstetrics (FIGO) criteria [22]. CT or magnetic resonance imaging scans, although of limited impact for OC early diagnosis, allow to establish a surgery plan and to determine tumor irresectability criteria for 70–90% of all patients. The ability to detect peritoneal implants in both exams depends upon their location, size, and the presence of ascites. However, CT is the imaging modality of choice for OC staging, since it is indispensable for the preoperative evaluation to optimize maximal cytoreduction surgery or to help in the decision of neoadjuvant chemotherapy.

Ovarian cancer dissemination can occur through all known propagation routes, i.e., lymphatic, hematogenic, transcavitary, and contiguous. The transcavitary course is undoubtedly the most clinically relevant and, in the vast majority of cases, has an impact on the patient prognosis [23, 24]. The dissemination to the peritoneal cavity is an early phenomenon in the natural history of the disease, since the malignant cells follow the peritoneal fluid, flow concerning intra-abdominal pressure variations. Ovarian cells are characterized as anchorage-dependent cells, meaning that they could only survive when adherent to the extracellular matrix or in contact with neighbor cells. However, when OC cells exfoliate into the peritoneal cavity, they can avoid anoikis (apoptosis process triggered by the loss of binding to the extracellular matrix) and survive even when isolated. Cancer cells in this state can survive and disseminate into the peritoneum, depositing accordingly to the passive flow distribution of peritoneal fluid, predominantly into the paracolic gutters, diaphragmatic surfaces, liver capsule, intestine surface, and omentum. The adhesion of malignant cells to the peritoneum precedes the local invasion and the secondary metastasis, namely to the pleural cavity by the transdiaphragmatic pores (Stage IV) [25]. The transcavitary route seems to be related to the OC cells predilection for the abdominal cavity (homing) rather than the deposition in other organs such as liver, lungs, brain, or bone (rarely in these latter two locations). The dissemination by contiguity is also important and of particular interest for organs like fallopian tubes, uterus, contralateral appendix and bladder, rectum, and pouch of Douglas. The iatrogenic route by contiguity, for example, to the abdominal wall is less frequent. Lymphatic dissemination is frequently observed when the disease is confined to the ovary, being found in almost 15% of FIGO I–II cases [26]. In fact, for a proper FIGO staging, lymphadenectomy is required, and the removal of bulky lymph nodes should be performed to achieve complete macroscopic resection. Although the systematic lymphadenectomy in advanced OC surgical management

is still discussed, it has an impact in early disease stages not only to define FIGO staging but also to establish the need for adjuvant treatment, with a significant impact in survival [27, 28]. Blood dissemination is less frequent and usually occurs in advanced disease stages [23, 24].

5. Prognostic factors

A considerable number of clinical-pathological factors have been implicated in OC prognosis. Disease stage, tumor size, histological subtype, differentiation degree, and residual tumor after surgery are considered as the classic prognostic factors. More specifically, the extent of residual disease after surgery is regarded as a major prognostic factor, shown to influence the chemotherapy response and survival [29–33]. Inclusively, a recent meta-analysis has shown that residual tumor is a more powerful prognostic determinant than FIGO stage [31]. The correct histological classification of EOC is also crucial, since it is an independent prognostic factor and provides a guideline for therapeutic management [8, 27]. Performance status (PS) and age are also important factors having an impact on the prognosis and, ultimately, in the decision of medical treatment [27].

Numerous studies have been conducted to assess the clinical significance of molecular alterations in OC. However, so far, the obtained results do not allow a prognostic biomarker to be universally accepted, although the determination of *BRCA* germline mutations has been recently approved as a predictive biomarker for OC. Recently, the development and the application of new genomic technologies have allowed the description of molecular signatures integrated into prognostic and predictive models. In particular, the Cancer Genome Atlas Project (TCGA) has been critical in adding to our knowledge, as it has been used to confirm the importance of *BRCA* genes to serous OC patients survival, as well as being able to help to describe a transcriptional signature with prognostic relevance [34] (this will be investigated further in a separate chapter).

6. Treatment

6.1. First-line treatment

The therapeutic strategy for EOC is based on cytoreductive surgery and staging, followed by adjuvant chemotherapy with the duplet platinum/taxane [8, 20]. As mentioned above, the extent of surgery is a determinant for survival and response to chemotherapy, since these parameters vary significantly depending on the success (optimal or suboptimal) of the surgical procedure [33]. Systemic therapy with cytotoxic agents plays a fundamental role in the treatment of this neoplasia. Chemotherapy is generally recommended in the EOC, including early stages with histopathological criteria of poor prognosis (FIGOIA/IB G3, FIGO IC, FIGO II, or clear cell histology at any stage). However, stage IA or IB G1 or G2 tumor patients, if adequately staged (i.e., with peritoneal washings, assessment of the contralateral ovary and fallopian tube, pelvic and para-aortic node assessment and omentectomy), have a better prognosis and can be treated with surgery alone without the need for adjuvant chemotherapy [35–37] (**Figure 1**).

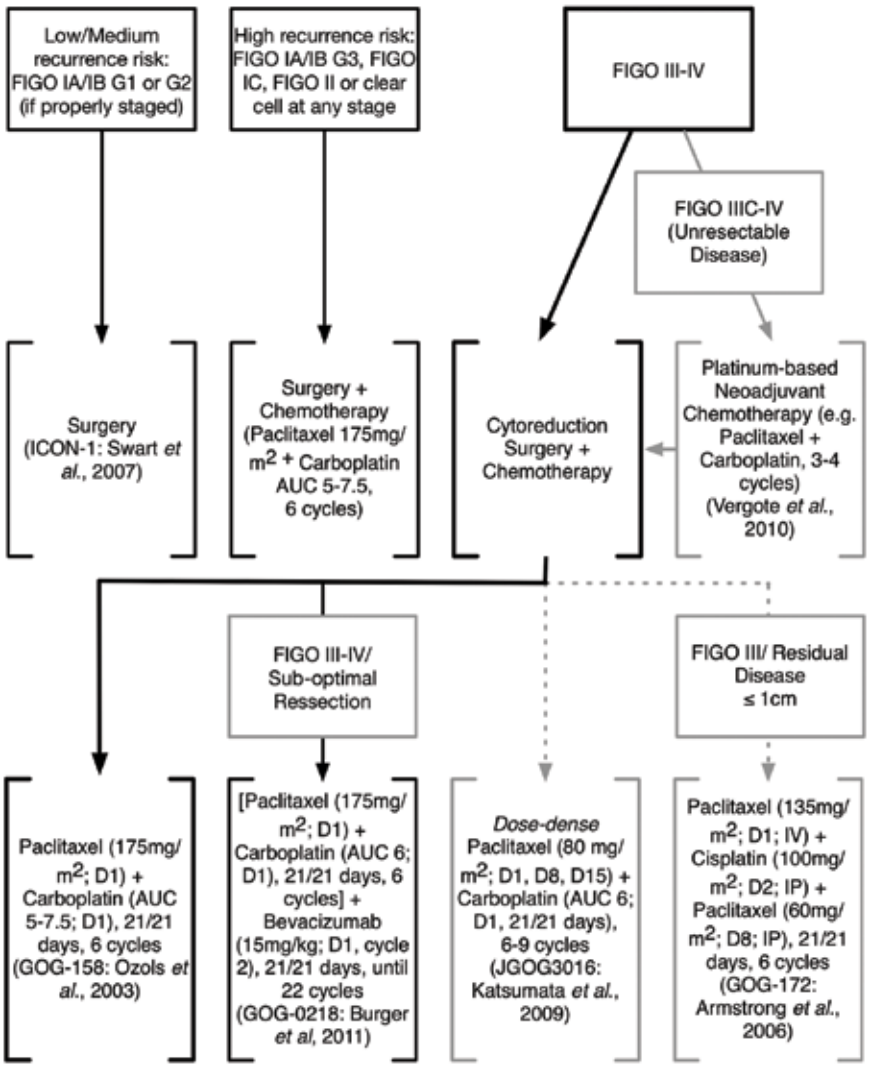

Figure 1. EOC first-line treatment algorithm according to the clinical trial that determines their approval.

The last decades have brought significant advances in the medical treatment of EOC. The association of paclitaxel with platinum has been shown to prolong both progression-free survival (PFS) and overall survival (OAS) of advanced stage patients when compared to the previous nontaxane treatment regimens. Globally, the inclusion of paclitaxel in the adjuvant chemotherapy scheme resulted in a 30% reduction in the risk of death [38–40]. Thus, the intravenous combination of paclitaxel (175 mg/m^2) and carboplatin (AUC 5–7.5), every 3 weeks, for six cycles, was established as the standard primary adjuvant chemotherapy for advanced stage disease, after cytoreductive surgery (**Figure 1**) [8, 38, 39, 41–43].

This treatment regimen has been the standard for more than 15 years, and the clinical trials conducted in the last decades for the introduction of a third agent, as in the ICON-5/GOG182

clinical trial, have not shown any improvement in the survival [44]. For patients that develop allergy or toxicity to paclitaxel, namely hypersensitivity or neurotoxicity, the combination of docetaxel/carboplatin or pegylated liposomal doxorubicin (PLD)/carboplatin can be considered as an alternative [42, 45]. The cisplatin/paclitaxel duplet is equally valid but associated with increased toxicity and less convenience in the administration, being currently reserved for patients who have developed hypersensitivity to carboplatin [8, 41].

The inclusion of bevacizumab, an anti-VEGF (Vascular Endothelial Growth Factor) monoclonal antibody, is recommended for advanced OC patients with poor prognostic characteristics (Stage IV or suboptimal resection). This targeted therapy should be administrated concomitantly with paclitaxel/carboplatin (after the first cycle) and be maintained after the six cycles of chemotherapy. Regarding the dose and duration of maintenance, the results are not clear, although a similar benefit is obtained with the administration of 7.5 mg/kg and 15 mg/kg for 12 and 15 months, respectively [46, 47]. Although not licensed in the United States of America and not consistently used in Europe, bevacizumab was approved by the European Medicines Agency (EMA) at a dose of 15 mg/kg for 22 cycles (15 months) [8, 46].

To improve the efficacy of the primary treatment, several clinical trials have evaluated the addition of a third cytotoxic agent (such as epirubicin, topotecan, gemcitabine, or PLD) to the first-line regimen, but none have demonstrated a benefit for triplets [8]. In addition, the Japanese JGOG-3016 trial evaluated the impact of a dose-dense therapeutic regimen (paclitaxel, weekly, 80 mg/m^2) on the chemotherapy effectiveness for OC patients. The results were promising for the benefits in PFS and OAS although associated with higher toxicity, especially myelotoxicity. Although it was a trial with potential impact on the clinical practice, because of the pharmacogenetic differences between the Japanese and Caucasian populations, further study was required to confirm these results. The European MITO-7 study did not confirm these findings in Caucasian patients, showing no benefit in the PFS and OAS with the weekly carboplatin (AUC 2) and paclitaxel (60 mg/m^2) regimen [48]. In the absence of new data, paclitaxel dose-dense administration can only be considered as an option [8].

Clinical data demonstrate that, despite the high response rate to the first-line treatment, a significant proportion of OC patients will develop disease recurrence, which in most cases is confined to the abdominal cavity. Based on this particular feature, intraperitoneal chemotherapy administration was associated with an improvement in PFS and OAS in phase III randomized studies (GOG 104, 114 and 172), in combination with intravenous chemotherapy [49, 50]. However, this strategy is not widely used in clinical practice due to its high toxicity [8]. Chemotherapy administered directly in the abdominal cavity might also be performed in the surgical setting using hyperthermal intraperitoneal chemotherapy (HIPEC). The justification for the use of the last therapeutic approach is based on studies that demonstrated that high temperatures help to overcome the resistance to cisplatin, as a result of increased penetration and cellular accumulation of this drug when administrated intraperitoneally in association with hyperthermia [51]. Although it represents a promising strategy, the use of HIPEC remains controversial.

Numerous studies have shown that neoadjuvant chemotherapy is feasible in advanced disease (Stage IIIC–IV), for which the disease is considered unresectable or when optimal

primary cytoreduction is not possible due to the disease extension and/or comorbidities that increase the surgical risk [30, 52]. Neoadjuvant chemotherapy is associated with some advantages, including tumor size and disease extension reduction, improvement of optimal cytoreduction rate, less extensive surgery with lower morbidity/mortality, improvement of patients' PS before surgery, and evaluation of tumor chemosensitivity. The chemotherapy scheme to be applied should be based on platinum (often a paclitaxel/carboplatin combination), and it is not recommended to perform more than 3–4 cycles to avoid the emergence of resistant clones [8, 30]. Consequently, the use of primary chemotherapy with interval surgery has become widely accepted, whereas the role of secondary interval debulking surgery after primary surgery (suboptimal cytoreduction and three cycles of chemotherapy) is less clear, as improved survival was reported by the European Organization for Research and Treatment of Cancer (EORTC) trial [32] but not confirmed by the Gynecological Oncology Group (GOG) [53]. Also, the "second look" diagnostic laparoscopy or laparotomy to evaluate the intraperitoneal condition is obsolete and should not be considered an option [8].

6.2. Recurrent disease treatment

The maximal surgery resection strategy combined with adjuvant chemotherapy achieves complete clinical remission in about 75% of EOC patients. However, after 12–18 months, approximately 75% of these patients will develop recurrent disease and be subjected to further treatment. The OC recurrence is defined according to the progression-free interval (PFI) after the end of the initial treatment (**Figure 2**) [8, 21, 41, 54–56].

The prognosis and the likelihood of response to second-line therapy (and subsequent lines) are dependent on the PFI after the last cycle of the previous chemotherapy line. This categorization defines "platinum-refractory," when disease progresses during therapy or within 4 weeks after the last cycle; "platinum-resistant," whose progression occurs within 6 months after platinum therapy completion; "partially platinum-sensitive," for disease which progression occurs between the 6 and 12 months; and "platinum-sensitive," whose progression occurs in a period superior to 12 months [57]. The biological behavior of tumors in these groups is quite variable, with distinct response rates and variable symptoms with different treatment needs. If relapse occurs 6 months after the completion of first-line chemotherapy, a platinum-based regimen should be performed, since the disease is considered platinum sensitive. For patients with platinum-sensitive relapses, there are several therapeutic strategies available which, since this phenomenon can occur repeatedly, allows the selection of different therapeutic combinations [8]. However, the time to subsequent relapse will be progressively shorter until the tumor becomes virtually resistant to these agents [21].

The platinum re-administration is associated with a response rate around 30%, similar to the improvement seen in the PFI. Available treatment options for OC platinum-sensitive relapse are ideally based on the association of platinum with paclitaxel, gemcitabine (with or without bevacizumab), or with PLD [58–62] (**Figure 2**). The therapeutic scheme selection should consider the toxicity profile of each regimen, the residual toxicities of the previous regimens, and the patients' preferences.

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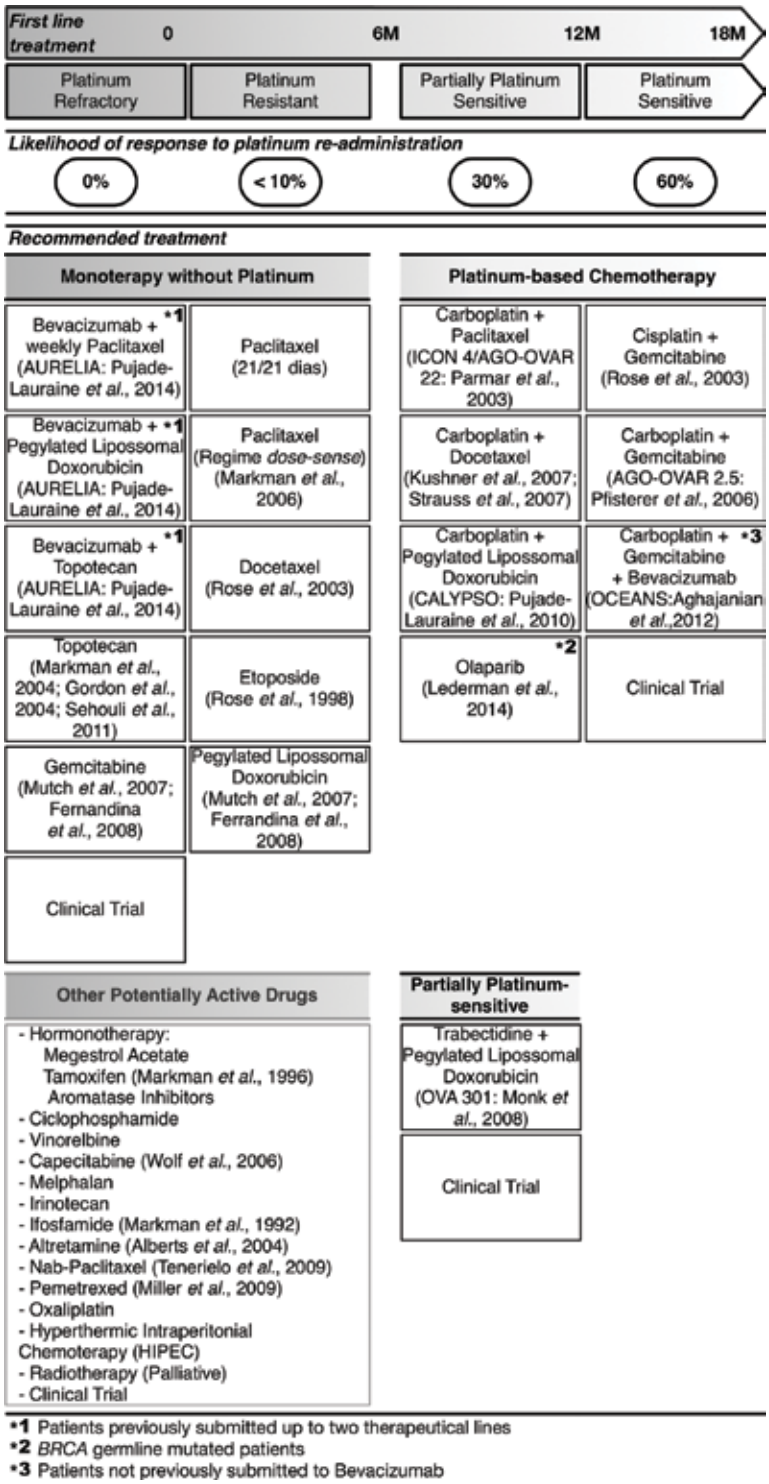

Figure 2. EOC recurrence treatment algorithm based on platinum-free interval, according to the clinical trial that determines its approval.

The administration of bevacizumab in combination with carboplatin/gemcitabine, followed by maintenance until progression or toxicity, was approved by EMA as the first-line treatment for platinum-sensitive relapse (for bevacizumab-naïve patients), being associated with an improvement in PFS despite no impact on OAS [8, 63]. In December 2016, US Food and Drug Administration (FDA) also approved bevacizumab administration to platinum-sensitive recurrent patients, either in combination with carboplatin/paclitaxel or carboplatin/gemcitabine, followed by bevacizumab alone.

Patients with PFI between 6 and 12 months, considered as partially sensitive, also benefit from platinum-based second-line therapy, although with a lower therapeutic effect (**Figure 2**). For these patients, the administration of trabectedin associated with PLD might also be an option, according to the results of the OVA301 trial, probably by restoring platinum sensitivity due to the artificial prolongation of the platinum-free interval [8, 64].

For relapses linked to a disease-free interval less than 6 months, the tumor is defined as platinum resistant and another treatment strategy should be instituted, with monotherapy regimens being recommended [8, 40, 65]. The treatment of platinum-resistant/refractory patients is mostly directed toward improvement in the quality of life and symptom control, as these patients are usually associated with a reduced prognosis with a reduced OAS (generally less than 12 months) [8]. Surgery as a therapeutic alternative in these cases might be considered only in need of symptom palliation. Monotherapy regimens with paclitaxel (preferably weekly), PLD, gemcitabine, and topotecan, among others, have shown similar response rates (not exceeding 15%) and PFS between 3 and 4 months [8, 66–73]. Thus, the choice for one of these agents should be based on previously performed therapies, toxicity profiles, administration convenience, cost, and patient opinion. Combination therapy regimens did not significantly improve response rates or survival for platinum-resistant disease, when compared to monotherapy regimens, even when considering toxicity [8, 40].

Recently, promising results have been achieved with biological maintenance treatments, in particular with anti-angiogenic agents (bevacizumab, pazopanib, and trebananib) and PARP (poly(ADP-ribose) polymerase) inhibitors (olaparib, niraparib, and rucaparib) [74]. Bevacizumab was the first antiangiogenic agent to demonstrate clinical benefit in platinum sensitive and resistant relapse, concomitantly with chemotherapy and as maintenance therapy. As previously mentioned, according to the results published in the OCEANS trial, EMA approved the combination of bevacizumab with carboplatin/gemcitabine for patients with platinum-sensitive OC relapse, if there was no previous exposure to this antiangiogenic drug [63]. According to the AURELIA results, the addition of bevacizumab to the chemotherapy (weekly paclitaxel, PLD or topotecan) in patients with platinum-resistant OC (previously treated with up to two therapeutic lines) has been shown to be associated with an improvement in PFS, response rates, and quality of life, although without impact on OAS [75]. Therefore, this regimen could be an alternative in this subgroup until the development of toxicity or progression (**Figure 2**).

In addition to bevacizumab, olaparib is also considered as a target therapy option in OC recurrence. This drug was the first PARP inhibitor to be authorized by EMA as maintenance treatment of *BRCA*-mutated patients, with partial or complete responses to platinum-based chemotherapy. The results have shown almost a 7-month extension in PFS for patients with

BRCA mutations exposed to Olaparib (11.2 versus 4.3 months; HR, 0.18), although the impact on OAS was not observed [76]. Response rates to this drug correlate with the platinum-free interval, being 69.2, 45.8 and 23.1% for the sensitive, resistant, and platinum-refractory disease, respectively [77]. Furthermore, olaparib administration allows for a time extension for a subsequent therapy which suggests that its administration did not adversely affect the treatment recurrence.

With the existence of several treatment alternatives that allow for sequential approaches and the emergence of new targeted therapies, most of which are well tolerated, it is possible to administrate extended therapeutic regimens concomitantly with significant symptomatic control and a positive impact in the quality of life.

6.3. Emergent therapeutic approaches

The duplet platinum/taxane is considered the standard first-line therapy for advanced OC treatment. Nevertheless, chemotherapy response rates remain disappointing, and the introduction of newer treatment strategies at recurrence is essential to increase the long-term survival. The recent adoption of molecular therapies targeting the inhibition of angiogenesis and DNA repair is a step forward in the OC medical treatment, aiming to delay disease progression and the re-treatment with chemotherapy [33]. The encouraging study results with bevacizumab, in first-line treatment and both platinum sensitive and resistant recurrence, illustrate the importance of angiogenesis inhibition in the success of OC treatment [46, 63, 75].

PARP is an enzyme involved in the response to DNA single-strand breaks, and so it was initially suggested that its inhibition could be used to enhance the effects of chemotherapy [78]. However, the finding that the survival of tumor cells carrying *BRCA* homozygous deletions is significantly lower with the administration of PARP inhibitors prompted the development of a new therapeutic strategy for OC [79, 80]. The molecular rationale for this association is based on the fact that cells with BRCA defective proteins are not able to repair DNA double-strand breaks by homologous recombination (HR), depending on other pathways to repair the damage, namely the base excision repair (BER) pathway, in which PARP is involved. In the BER pathway, PARP is responsible to detect single-strand breaks and to activate effector proteins to repair the damage. Thus, homologous recombination deficiencies (HRD), as in the presence of *BRCA* mutations, in concomitance with PARP inhibition lead to cell death due to the excessive accumulation of unrepaired damage. This phenomenon is designated as synthetic lethality and occurs when two nonlethal defects are combined to culminate in cell death. This strategy is also of benefit in that toxicity is reduced for normal tissues, as nontumor cells can repair DNA by the HR pathway [80, 81].

As molecular and genetic knowledge of OC is increasing, studies with PARP inhibitors are indicating that more patients with OC may benefit. According to data published by the TCGA project, the presence of *BRCA* mutations is identified in about 20% of high-grade serous ovarian cancer (HGSOC), and about 50% of these tumors have a positive HRD phenotype, even in the absence of a familial history of breast/ovarian cancer [34, 78]. In addition to the excellent results obtained with olaparib for the subgroup of patients with *BRCA* mutations, the study published by Ledermann et al. also demonstrated that PARP inhibition is also useful for *BRCA* wild-type patients, although to a less extent [76].

The promising results achieved with olaparib encouraged the development of new PARP inhibitors, including niraparib and rucaparib [82, 83]. Maintenance clinical trials ongoing with both agents include *BRCA* wild-type patients to test the effect of PARP inhibitors in this major group, incorporating additional molecular tests for HDR. Namely, for patients with platinum-sensitive recurrence, the PFS mean duration is significantly higher for patients receiving niraparib when compared to placebo, regardless of the presence/absence of *BRCA* germline mutations or HRD status [83]. Thus, clinical trials are being developed to evaluate not only the impact of PARP inhibitors on limiting recurrence, as in the SOLO2 trial [84], but also as a maintenance strategy for first-line treatment, as in SOLO1 [85]. In addition, the GOG3005 trial evaluates the addition of the PARP inhibitor veliparib to first-line therapy (carboplatin/paclitaxel), as well as its role in subsequent maintenance [78].

A possible synergy between PARP inhibitors and other pathways inhibitors, such as anti-angiogenic, has also been hypothesized. In fact, preclinical studies have demonstrated an additive effect on the association of inhibitors of these two pathways, since hypoxia leads to a decreased expression of DNA repair proteins, thereby increasing the sensitivity for PARP inhibitors [86, 87]. Thus, a recent phase I clinical trial, which combined a tyrosine kinase inhibitor of VEGF receptor, cediranib, with olaparib, achieved an objective response rate of 44% in recurrent disease [88]. The results of this study prompted the development of a randomized phase II trial, demonstrating an improvement in PFS and in the objective response rate for the cediranib/olaparib combination when compared to olaparib alone (17.7 versus 9.0 months; HR, 0.42; 95% CI, 0.23–0.76; $P = 0.005$ and 79.6% versus 47.8%; OR, 4.24; 95% CI, 1.53–12.22; $P = 0.002$) in patients with platinum-sensitive recurrence [89]. Although the results must be interpreted carefully, due to the low number of recruited patients, they are of high interest as it suggests a synergistic action for the combined use of angiogenesis/DNA repair inhibitors. Thus, numerous clinical trials exploring these pathways are under development, either isolated or in combination, for first-line therapy or maintenance, with the prospect of increasing the treatment opportunities for OC patients.

6.4. Monitoring treatment response

The treatment response evaluation in OC is based on CT, following RECIST criteria, complemented by the CA125 serum measurement following Gynecologic Cancer InterGroup (GCIG) criteria [90]. In fact, despite the limitations as a diagnostic biomarker, CA125 is a good predictor of relapse as it proved to be a useful biomarker for monitoring treatment response in more than 80% of OC patients [91]. Normalization of CA125 serum levels following first-line therapy does have clinical implications, especially when considering maintenance treatment in OC. However, even the systemic therapy in early recurrence stages had the potential to improve survival, studies have demonstrated that the premature treatment in asymptomatic patients with single elevation of CA125 levels (without clinical or radiological evidence) had no positive impact [92].

In Medical Oncology clinical practice, high heterogeneity in the response and toxicity to cytotoxic agents are observed. There are subgroups of patients who, despite being at an early disease stage, have a higher risk of tumor progression. In these cases, surgery and classic prognostic factors do not allow to predict the biological behavior of these tumors correctly. In

the current era of individualized therapy, and according to the OC heterogeneity, biomarkers need to be developed to identify patients at an early disease stage but with the potential to progress and disseminate [93].

6.5. Predictive factors

Several biomarkers are considered to have prognostic relevance, independent of the therapeutic approach. In OC, as previously mentioned, FIGO staging, histological subtype, or the extent of residual disease are considered as key prognostic factors. The identification and characterization of predictive biomarkers for OC have proven to be a challenge, and none of the molecular determinants that underlie platinum-sensitivity/resistant phenotypes have reached the clinical setting [91].

Additionally, the inability to select those patients that will benefit from bevacizumab to maximize survival and minimize toxicity and costs complicates treatment planning. Several studies have been performed to unravel the role of the VEGF signaling pathway and the key drivers of response to antiangiogenic agents in OC. VEGF serum levels are thought to be representative of the VEGF-mediated OC angiogenesis, but the results were not systematically concordant [94]. Also, VEGFR-2 plasma levels were not predictive for patients treated with bevacizumab in the GOG-218 trial [95]. Translational research conducted within the ICON7 trial identified three candidate biomarkers (mesothelin, VEGFR-3, and alpha-1-acid glycoprotein) for patients treated concomitantly with this antiangiogenic agent and first-line chemotherapy. Each of these biomarkers was considered as an independent factor and, in combination with CA125 measurement, was included in a predictive nomogram for bevacizumab [96]. However, though several promising candidate angiogenesis biomarkers for OC were identified, it was neither possible to achieve meaningful results for their use in routine clinical practice nor possible to select patients for this targeted therapy [97, 98].

Failure to improve the therapeutic strategies in OC has resulted in studies focusing on genomic features, such as the TCGA project. This project aims to determine the impact of OC genomic and epigenomic changes and, thus, to identify molecular markers influencing clinical outcome and possible therapeutic targets for OC. One of the most interesting findings obtained from this study is the presence of HRD in about 50% of HGSOC, which could represent a patient subgroup which could benefit from PARP inhibitor treatment [34]. In fact, the presence of *BRCA* mutations and an HRD positive phenotype is both positive predictive factors for PARP inhibition, thus indicating personalized OC therapy defined by a genetic biomarker [76, 83]. The impact of *BRCA* mutations as predictive biomarkers has been published for other agents such as PLD and trabectedin [99, 100]. The implications of these advances are still being investigated, and as a result, genetic testing for *BRCA* mutations should be offered for all patients with nonmucinous tumors, regardless of age or familial history. The test should be performed at diagnosis, as it provides information on the likelihood of response to chemotherapy and can then be systematically incorporated into clinical practice to promote an individualized therapeutic strategy [33]. The TCGA project also provided the opportunity to identify four OC subtypes based on the expression of marker genes (differentiated, immunoreactive, mesenchymal, and proliferative), and several retrospective subanalyses have already demonstrated that is possible to correlate distinct outcomes between the subgroups [101, 102].

Based on the TCGA data, other studies have also proposed molecular signatures, namely the prognostic model "Classification of Ovarian Cancer" (CLOVAR), for which 23 genes involved in the platinum-induced DNA damage repair are predictive of treatment response among HGSOC patients [103]. Recently, the ARIEL2 clinical trial showed that the combination of *BRCA* mutational status with the degree of genome-wide loss of heterozygosity (LOH) in the tumor could predict the rucaparib treatment response. *BRCA*-mutated patients (germline or somatic) or *BRCA* wild-type with high LOH had longer PFS and clinical response to rucaparib, when compared with *BRCA* wild-type and low LOH patients [104].

The concept of *BRCA*ness must be promptly clarified, as the associated phenotypes define a clinical subpopulation of EOC patients with common characteristics. These include high response rates to both first-line platinum-based treatment and to relapse therapies (including platinum based), long treatment-free intervals (even in recurrent disease), and improved OAS and include mainly serous tumors. The HRD phenotype (somatic or germline) might be complemented with other molecular defects, beyond *BRCA* deficiencies, which lead to an analogous clinical profile and be targeted for PARP inhibition [34, 105]. Commercial tests are already available, and multiple clinical trials (as ARIEL3 and NOVA) are ongoing to investigate PARP inhibition in *BRCA* wild-type patients and to identify a putative predictive signature.

7. Pharmacogenomics for future predictive marker definition

Although *BRCA*ness signature definition can provide valuable information regarding the magnitude of the benefit of targeted therapy, these biomarkers may not be unique for the determination of the likelihood of treatment sensitivity/resistance. To date, besides *BRCA* mutations and HRD status, platinum sensitivity remains the best biomarker of PARP inhibitor response. Platinum sensitivity correlates with HRD, and platinum-sensitive tumors are more responsive to PARP inhibitors than platinum-resistant tumors, whatever the genetic background [33, 78]. Therefore, perhaps the PARP inhibitors administration should be offered to all OC patients that respond to platinum-based treatment.

Platinum-based compounds are among the most active and used cytotoxic agents in the clinical practice. They exert their biological effect by acting as alkylating agents by the ability to covalently bind to DNA, leading to the formation of intrastrand and interstrand DNA adducts that promote cell-cycle arrest and tumor cell apoptosis. The mechanisms underlying the development of chemoresistant phenotypes in OC are not fully recognized. Interindividual variation in platinum-drug response might be a major determinant for OC. This is suggested from the wide variability in the PFI and its direct association with a platinum response, as well as the finding that intrinsic resistance to these compounds, occur in up to a fifth of OC patients [106–109]. Mechanisms involved in platinum resistance are likely to be multifactorial although seems to be greatly determined by the platinum detoxification pathway and DNA damage repair ability [54, 108, 110–113].

While platinum therapy is prescribed to achieve a target exposure based on renal function, the dose of taxanes is based on body surface area. Taxanes are microtubule-stabilizing drugs,

inducing cell cycle arrest and activating proapoptotic signaling. The cellular toxicity to taxanes is controlled by the action of multiple mediators, namely those involved in transport (i.e., ABCB1, ABCC1, and ABCC2), metabolism, and metabolism-associated proteins (cytochrome P450s and nuclear receptors), as well as pharmacodynamics (i.e., TP53 and CDKN1A), which appear to play a role in taxane efficacy [54, 108, 114–116]. However, to date, no reliable biomarker or signature exists to predict the sensitivity or resistance to paclitaxel. Although the duplet platinum/taxane is associated with better outcome, rather than platinum alone, the results of the GOG132 trial showed that only 42% of patients are likely to benefit from paclitaxel administration [117], and thus, further study into the mechanisms of resistance is needed.

8. Conclusion

Achieving an individualized therapeutic strategy will only be possible through the identification of feasible, validated, and reproducible biomarkers in the clinical practice that will allow the prediction of the likelihood of response to a given treatment. Biomarker validation is crucial, both in respect of predictive ability and sensitivity/specificity, and should be stated previously in the definition of treatment subgroups [106, 107, 118, 119].

Research in OC treatment evolution and improvement needs to focus on the identification of interindividual determinants, which is often associated with genetic polymorphisms to identify potential biomarkers and/or treatment targets. Circulating tumor cells or tumor nanovesicles (as exosomes) may help to identify the molecular targets. Consequently, the incorporation of molecular and genetic information into integrated clinical models may be a potential approach in order to define predictive nomograms. Pharmacogenomics will be important in clinical practice to improve efficacy, reduce toxicity, and predict nonresponders to several therapies, thus allowing for individualized treatment strategies.

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Chapter 4

New Insights into the Pathogenesis of Ovarian Cancer: Oxidative Stress

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Additional information is available at the end of the chapter

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Abstract

Ovarian cancer is the leading cause of death from gynecologic malignancies yet the underlying pathophysiology is not clearly established. Epithelial ovarian cancer (EOC) has long been considered a heterogeneous disease with respect to histopathology, molecular biology, and clinical outcome. Treatment of ovarian cancer includes a combination of cytoreductive surgery and combination chemotherapy, with platinums and taxanes. Despite initial success, over 75% of patients with advanced disease will relapse around 18 months and the overall 5-year survival is approximately 50%. Cancer cells are known to be under intrinsic oxidative stress, which alters their metabolic activity and reduces apoptosis. Epithelial ovarian cancer has been shown to manifest a persistent pro-oxidant state as evident by the upregulation of several key oxidant enzymes in EOC tissues and cells. In the light of our scientific research and the most recent experimental and clinical observations, this chapter provides the reader with up to date most relevant findings on the role of oxidative stress in the pathogenesis and prognosis of ovarian cancer, as well as a novel mechanism of apoptosis/survival in EOC cells.

Keywords: ovarian cancer, oxidative stress, chemoresistance, apoptosis, nitrosylation, caspase-3

1. Introduction

Ovarian cancer is the fifth leading cause of cancer death; the leading cause of death from gynecologic malignancies, and the second most commonly diagnosed gynecologic malignancy; yet the underlying pathophysiology continues to be delineated [1, 2]. Epithelial ovarian cancer

has long been considered a heterogeneous disease with respect to histopathology, molecular biology, and clinical outcome. It comprises at least five distinct histological subtypes, the most common and well-studied being high-grade serous ovarian cancer (HGSOC) [3]. The majority of advanced-stage tumors are of epithelial cell origin and can arise from serous, mucinous, or endometrioid cells on the surface epithelium of the ovary or the fallopian tube [2]. The most obvious clinical implication of tumor heterogeneity is that molecular-targeted therapy, while being effective at one tumor site, may not be as effective at all of them [3].

Because early-stage ovarian cancer presents with nonspecific symptoms, most often diagnosis is not made until after the malignancy has spread beyond the ovaries [4]. Mortality rates for this type of malignancy are high because of a lack of a sensitive and specific early-stage screening method [4]. Surgical cytoreduction followed by platinum/taxane chemotherapy results in complete clinical response in 50–80% of patients with stage III and IV disease, but most will relapse within 18 months and ultimately develop chemoresistant disease [2]. Resistance to chemotherapy can either be intrinsic, occurring at the onset of treatment, or acquired, when the disease recurs despite an initially successful response [5–7]. Attempts to overcome drug resistance are central to both clinical and basic molecular research in cancer chemotherapy [5, 8]. Cancer cells are known to be under intrinsic oxidative stress, resulting in increased DNA mutations or damage, genome instability, and cellular proliferation [9–13]. The persistent generation of cellular reactive oxygen species (ROS) is a consequence of many factors including exposure to carcinogens, infection, inflammation, environmental toxicants, nutrients, and mitochondrial respiration [14–17].

The origin and causes of ovarian tumors remains under debate. Injury to surface epithelial ovarian cells due to repeated ovulation is thought to induce tumorigenesis in these cells and is known as the "incessant ovulation hypothesis." Additionally, hormonal stimulation of the surface epithelium of the ovary has been described to initiate tumorigenesis in surface epithelial cells and is known as the "gonadotropin hypothesis." Moreover, the fallopian tube, and not the ovary, has been suggested to be the origin for most epithelial ovarian cancer [2, 18, 19]. Nevertheless, many cases of ovarian cancer continue to be described as *de novo*.

Histopathologic, clinical and molecular genetic profiles were successfully utilized to clearly discriminate between type I and type II ovarian tumors [19]. Accordingly, type I ovarian tumors develop from benign precursor lesions that implant on the ovary and include clear cell, endometrioid, low-grade serous carcinomas, mucinous carcinomas and malignant Brenner tumors [19]. Type II ovarian tumors develop from intraepithelial carcinomas of the fallopian tube and can then spread to involve both the ovary as well as other sites, such as high-grade serous carcinomas which comprise morphologic and molecular subtypes [19]. Additionally, high-grade endometrioid, poorly differentiated ovarian cancers, and carcinosarcomas are also classified as type II tumors.

Attempts to identify specific genes in ovarian tumors to help in early detection of the disease and serve as targets for improved therapy had failed to identify reproducible prognostic indicators [2, 20–22]. Several oncogenic mutations and pathways have been identified in ovarian cancer. Specific inherited mutations in the *BRCA1* and *BRCA2* genes that produce tumor suppressor proteins, are known to be associated with a 15% increased risk of ovarian cancer overall [2]. Ovarian cancers associated with *BRCA1* and *BRCA2* mutations are much more common in

younger age patients as compared with their nonhereditary counterparts. Additionally, somatic gene mutations in RAD51C and D, HNPCC, NF1, RB1, CDK12, P53, BRAF, KRAS, PIK3CA, and PTEN have been identified in epithelial ovarian cancer. Somatic mutations in BRAF and KRAS genes are relatively common in type I tumors, while p53 mutations, RAS signaling and PIK3CA are common in type II. Additional genetic variations have been hypothesized to act as low to moderate alleles, which contribute to ovarian cancer risk, as well as other diseases [23].

Ovarian tumors are distinct from many other type of cancers as they rarely metastasize outside of the peritoneal cavity [24]. Ovarian tumors are spread into the peritoneal cavity when cells from the primary tumor detach and travel into the peritoneum where they implant into the mesothelial lining [25]. Metastases beyond the peritoneum are usually restricted to recurrent or advanced disease; however, pleural metastases were reported to be present at initial diagnosis. Moreover, the recent discovery of ovarian cancer stem cells, which manifest properties of typical cancer stem cells, in ascites is a new additional contributing factor to not only to metastasis but also to chemoresistance [25, 26].

2. Oxidative stress

Homeostasis, the balance between the production and elimination of oxidants, is maintained by mechanisms involving oxidants and antioxidant enzymes and molecules. If this balance is altered, it leads to an enhanced state of oxidative stress that alters key biomolecules and cells of living organism [13]. Oxidant molecules are divided into two main groups; oxygen-derived or nitrogen-containing molecules. Oxygen-derived molecules, also known as reactive oxygen species (ROS), includes free radicals such as hydroxyl ($HO^{\bullet}$), superoxide ($O_2^{\bullet-}$), peroxyl ($RO_2^{\bullet}$), and alkoxyl ($RO^{\bullet}$), as well as oxidizing agents such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), and singlet oxygen ($^1O_2$) that can be converted to radicals [13, 27]. Nitrogen containing oxidants, also known as reactive nitrogen species (RNS), are derived from nitric oxide (NO) that is produced in the mitochondria in response to hypoxia [13]. Exposure to inflammation, infection, carcinogens, and toxicants are major sources of ROS and RNS, *in vivo* [13, 16, 27, 28]. Additionally, RNS and ROS can be produced by various enzymes including cytochrome P450, lipoxygenase, cyclooxygenase, nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase complex, xanthine oxidase (XO), and peroxisomes (**Figure 1**) [13, 28, 29].

To maintain the redox balance, ROS and RNS are neutralized by various important enzyme systems including superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione (GSH), thioredoxin coupled with thioredoxin reductase, glutaredoxin, glutathione peroxidase (GPX), and glutathione reductase (GSR) (**Figure 1**) [27]. Superoxide dismutase is known to convert $O_2^{\bullet-}$ to H_2O_2, which is then converted to water by CAT. Glutathione S-transferase is involved in detoxification of carcinogens and xenobiotics by catalyzing their conjugation to GSH that will aid in expulsion from the cell (**Figure 1**) [27]. Indeed, the GSH-to-oxidized-GSH (GSH/GSSG) ratio is a good indicator of cellular redox buffering capacity [30, 31]. Under enhanced oxidative stress, the GSH/GSSG complex is known to stimulate the activity of the GS-X-MRP1 efflux pump, which removes toxins from cells. This mechanism has been investigated in the development of resistance to chemotherapeutic drugs [30, 31].

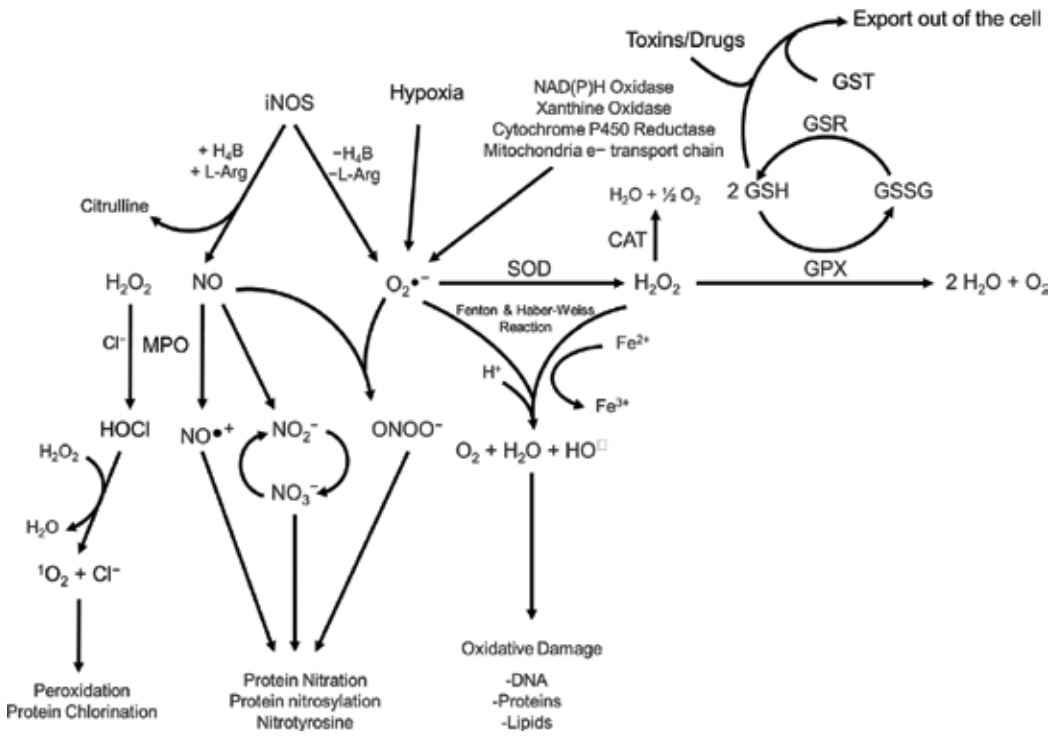

Figure 1. Summary of key oxidant and antioxidants in cancer [1]. Abbreviations are CAT, catalase; Cl^-, chloride ion; Fe_2^+, iron (II); Fe_3^+, iron (III); GPX, glutathione peroxidase; GSH, glutathione; GSR, glutathione reductase; GSSG, reduced glutathione; GST, glutathione S-transferase; H_2O_2, hydrogen peroxide; H_4B, tetrohydrobiopterin; $HO^•$, hydroxyl radical; HOCl, hypochlorous acid; iNOS, inducible nitric oxide synthase; L-Arg, L-arginine; MPO, myeloperoxidase; NAD(P)H, nicotinamide adenine dinucleotide phosphate; $NO^{•+}$, nitrosonium cation; NO_2^-, nitrite; NO_3^-, nitrate; $O_2^{•-}$, superoxide; $ONOO^-$, peroxynitrite; SOD, superoxide dismutase.

3. Oxidative stress and cancer

Oxidative stress has been implicated in the etiology of several diseases, including cancer. Alteration of the cellular redox balance modulates the initiation, promotion, and progression of tumor cells [13, 27]. The continuous generation of oxidants and free radicals affects key cellular mechanisms that control the balance of cell proliferation and apoptosis, which play a major role in the initiation and development of several cancers. Depending on the concentration of ROS and RNS in the cellular environment, oxidants can initiate and promote the oncogenic phenotype or induce apoptosis, and thus act as antitumor agents [32]. Several transcription factors that modulate the expression of genes critical to the development and metastasis of cancer cells are known to be controlled by oxidative stress. This includes hypoxia inducible factor (HIF)-1α, nuclear factor (NF)-κB, peroxisome proliferator-activated receptor (PPAR)-γ, activator protein (AP)-1, β-catenin/Wnt, and Nuclear factor erythroid 2-related factor 2 (Nrf2) [13]. The transcription factor regulator Nrf2 is known to control the expression of some key antioxidant enzymes that are needed to scavenge oxidants and free radicals [13, 33]. The activation of Nrf2 involves the suppressor protein, Kelch-like ECH-associated protein 1

(Keap1), that binds Nrf2 in the cytoplasm and prevents its translocation into the nucleus, where it binds to promoters of antioxidant enzymes [13, 33]. Additionally, oxidative stress is known to activate certain signaling pathways, specifically, the MAPK/AP-1 and NF-κB pathways, which are critical for the initiation and maintenance of the oncogenic phenotype [34].

More importantly, ROS and RNS are known to induce genetic mutations that alter gene expression as well as induce DNA damage, and thus have been implicated in the etiology of several diseases, including cancer [2, 13, 35]. Damage to DNA by ROS and RNS is now accepted as a major cause of cancer, and has been demonstrated in the initiation and progression of several cancers including breast, hepatocellular carcinoma, and prostate cancer [34]. Oxidative stress is known to modify all the four DNA bases by base pair substitutions rather than base deletions and insertions. Modification of GC base pairs usually results in mutations, whereas, modification of AT base pairs does not [36]. Modification of guanine in cellular DNA, causing G to T transversions, is commonly induced by ROS and RNS [34]. If not repaired, the transversion of G to T in the DNA of oncogenes or tumor suppressor genes can lead to initiation and progression of cancer. Oxidation of DNA bases, such as thymidine glycol, 5-hydroxymethyl-2′-deoxyuridine, and 8-OHdG are now accepted markers of cellular DNA damage by free radicals [35].

Oxidants and free radicals are known to enhance cell migration contributing to the enhancement of tumor invasion and metastasis, main causes of death in cancer patients [2, 13]. Reactive oxygen species, through the activation of NF-κB, regulate the expression of intercellular adhesion protein-1 (ICAM-1), a cell surface protein in various cell types [13]. In response to oxidative stress, the interleukin 8 (IL-8)-induced enhanced expression of ICAM-1 on neutrophils enhances the migration of neutrophils across the endothelium, which is key in tumor metastasis [13]. Another important player that controls cell migration and consequently, tumor invasion, is the upregulation of specific matrix metalloproteinases (MMPs), essential enzymes in the degradation of most components of the basement membrane and extracellular matrix, such as type IV collagen [13, 37]. The expression of MMPs, such as MMP-2, MMP-3, MMP-9, MMP-10, and MMP-13 is enhanced by free radicals, specifically H_2O_2 and NO, through the activation of Ras, ERK1/2, p38, and JNK, or the inactivation of phosphatases [13, 37]. Indeed, the major source of cellular ROS, the NAD(P)H oxidase family of enzymes, has been linked to the promotion of survival and growth of tumor cells in pancreatic and lung cancers [2, 13].

Oxidants and free radicals are also known to enhance angiogenesis, a key process for the survival of solid tumors [13]. Angiogenesis involves the upregulation of vascular endothelial growth factor (VEGF) or the downregulation of thrombospondin-1 (TSP-1), an angiogenesis suppressor in response to oxidative stress [13]. This process is controlled by several oncogenes and tumor-suppressor genes such as Ras, c-Myc, c-Jun, mutated p53, human epidermal growth factor receptor-2, and steroid receptor coactivators [38, 39]. Additionally, oxidants and free radicals are known to stabilize HIF-1α protein and induce the production of angiogenic factors by tumor cells.

4. Cancer cells are under intrinsic oxidative stress

Cancer cells are continuously exposed to high levels of intrinsic oxidative stress due to increased aerobic glycolysis (Warburg effect), a known process in cancer cell metabolism [10, 40].

Thus, cancer cells trigger several critical adaptations that are essential for their survival such as suppression of apoptosis, alteration of glucose metabolism, and stimulation of angiogenesis [10, 29]. Oxygen depletion, due to a hypoxic microenvironment, significantly stimulates mitochondria to produce high levels of ROS and RNS which is known to activate HIF-1α and consequently promote cell survival in such an environment [29]. The half-life of HIF-1α is extremely short as it is rapidly inactivated through hydroxylation reactions mediated by dioxygen, oxaloglutarate, and iron-dependent prolyl 4-hydroxylases, located in the nucleus and cytoplasm [40, 41]. Nitric oxide and other ROS, as well as H_2O_2 efflux into the cytosol due to dismutation of $O_2^{\bullet-}$, can inhibit prolyl 4-hydroxylases activity, leading to the stabilization of HIF-1α [29, 42]. More importantly, stabilization of HIF-1α, under hypoxic conditions, can be blocked when inhibiting ROS production in mitochondria that lack cytochrome c [29, 43].

Pro-oxidant enzymes such as myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS) and NAD(P)H oxidase have been associated with initiation, progression, survival, and increased risk in cancers such as breast, ovarian, lung, prostate, bladder, colorectal and malignant melanoma [21, 44]. Moreover, the expression of those key pro-oxidant enzymes was found to change based on the histological type and grade of the tumor [21, 45, 46]. Likewise, antioxidants have also been associated with initiation, progression, survival, and increased risk in cancers such as lung, head and neck, and prostate cancer [47–50]. The expression of GSR and GPX, key antioxidant enzymes, has also been reported to be altered in various types of cancer [21]. The activity and expression of SOD, a powerful antioxidant enzyme, has been reported to be decreased in colorectal carcinomas, pancreatic, lung, gastric, ovarian, and breast cancers [21, 45, 46]. Likewise, the expression and activity of CAT, a key antioxidant enzyme, was reported to be decreased in breast, bladder, and lung cancers but increased in brain cancer [21, 45, 46]. Antioxidant enzymes play a critical role in maintaining the redox balance in the presence of microenvironment stress, and thus, alteration of this balance may provide a unique and complex microenvironment for cancer cell survival.

5. Ovarian cancer cells manifest a persistent pro-oxidant state

Recent evidence suggests that oxidative stress is a critical factor in the initiation and development of several cancers, including ovarian cancer [40, 51]. Consistently, it has been reported that ovarian cancer patients manifested significantly decreased levels of antioxidants and higher levels of oxidants [10, 22, 40, 51–53]. An enhanced redox state, resulting from increased expression of key pro-oxidant enzymes and decreased expression of antioxidant enzymes, has been extensively described in epithelial ovarian cancer (EOC) [52–54]. We have previously reported that MPO, a hemoprotein present solely in myloid cells that acts as a powerful oxidant, and iNOS, a key pro-oxidant enzyme, are highly expressed and co-localized to the same cell in EOC cells [53]. These two enzymes, MPO and iNOS, work together to inhibit apoptosis, a hallmark of ovarian cancer cells. Nitric oxide, produced by iNOS, is used by MPO as a one-electron substrate to generate nitrosonium cation (NO^+), a labile nitrosating species, resulting in a significant increase in S-nitrosylation of caspase-3, which inhibits apoptosis [53, 55, 56]. Indeed, attenuating oxidative stress by inhibiting MPO or iNOS significantly induced

apoptosis in EOC cells [54]. Moreover, the remarkably higher levels of iNOS/NO, produced by EOC cells, resulted in the generation of high levels of nitrate and nitrite, powerful protein nitration agents that are known to stimulate the initiation and progression of tumor cells [53]. Under oxidative stress, where both NO and $O_2^{\bullet-}$ are elevated, MPO was reported to serve as a source of free iron which reacts with H_2O_2 and generated highly reactive hydroxyl radical ($HO^{\bullet}$), further increasing oxidative stress [22, 53]. Additionally, EOC cells are also characterized by enhanced expression of NAD(P)H oxidase, a potent oxidant enzyme that is known to be the major source of $O_2^{\bullet-}$ in the cell. Such high levels of $O_2^{\bullet-}$ combined with significantly high levels of NO generates peroxynitrite, another powerful nitrosylation and nitration agent, which modifies proteins and DNA structure and function in cells [57].

Recently we have gathered compelling evidence demonstrating that talc, through alteration of the redox balance, can generate a similar pro-oxidant state in both normal ovarian epithelial and ovarian cancer cells. Talc and asbestos are both silicate minerals, and the carcinogenic effects of asbestos have been extensively studied and documented in the medical literature [58]. Asbestos fibers in the lung initiate an inflammatory and scarring process, and it has been proposed that ground talc, as a foreign body, might initiate a similar inflammatory response [58]. Although there is strong epidemiological evidence to suggest an association between talc use and ovarian cancer, the direct link and precise mechanisms have yet to be elucidated. We investigated the effect of talc on both oxidants and antioxidants in normal ovarian epithelial and ovarian cancer cell lines. There was a marked increase in mRNA levels of the pro-oxidant enzymes, iNOS and MPO in talc treated ovarian cancer cell lines and normal ovarian epithelial cells, all as compared to their control, as early as 24 hours. Additionally, there was a marked decrease in the mRNA levels of the antioxidant enzymes CAT, GPX, SOD3, but with a marked increase in GSR, and no change in GST, in talc treated ovarian cancer cell line and in normal ovarian epithelial cells, all compared to their control, as early as 24 hours (*data not published*). Thus, there is a direct effect of talc on the molecular levels of oxidant and antioxidants, elucidating a potential mechanism for the development of ovarian cancer in response to talc.

6. Biomarkers for the early detection of ovarian cancer

The discovery of MPO expression in ovarian EOC cells and tissues was surprising, as it is only expressed by cells of myeloid origin. Intriguingly, the combination of serum MPO and free iron was reported to potentially serve as biomarkers for early detection of ovarian cancer [22]. A robust detection method based on molecular profiles for ovarian cancer has not yet been developed because the disease exhibits a wide range of morphological, clinical and genetic variations during its progression. The search for non-invasive, cost-effective ovarian cancer biomarker tests has been ongoing for many years. Immunizations of mice with ovarian cancer cells has led to hybridoma validation by ELISA, while flow cytometry analysis permitted the discovery of cancer antigen (CA)-125 and mesothelin [59]. Furthermore, the screening of an array of 21,500 unknown ovarian cDNAs hybridized with labeled first-strand cDNA from ten ovarian tumors and six normal tissues led to the discovery of human epididymis protein 4 (HE4) [60]. Most interestingly, HE4 is overexpressed in 93% of serous and 100% of endometrioid

EOCs, and in 50% of clear cell carcinomas, but not in mucinous ovarian carcinomas [61]. Thus, HE4 was identified as one of the most useful biomarkers for ovarian cancer, although it lacked tissue-specificity [60, 62–64]. Secreted HE4 high levels were also detected in the serum of ovarian cancer patients [65]. Additionally, combining CA-125 and HE4 is a more accurate predictor of malignancy than either alone [66–68].

Multi-marker panels have the potential for high positive predictive values (PPVs), but careful validation with appropriate sample cohorts is mandatory and complex algorithms may be difficult to implement for routine clinical use [59]. Panels of biomarkers have been extensively investigated to improve sensitivity and specificity and have included some of the most promising reported markers such as CA72–4, M-CSF, OVX1, LPA, prostacin, osteopontin, inhibin and kallikrein [69–71]. However, most of these tests frequently require certain equipments and complex computational algorithms that may not be available in a standard immunoassay laboratory, [32]. Among postmenopausal women in the U.S., only 1 in 2500 women are reported with ovarian cancer. Due to this low prevalence of the disease, a screening method that yield a 75% sensitivity and 99.6% specificity to achieve a PPV value of 10% to be effective for the detection of all stages of ovarian cancer [72]. To date, there is no single biomarker available that met these requirements.

The established role of MPO in oxidative stress and inflammation has been a leading factor in the study of MPO as a possible marker of plaque instability and a useful clinical tool in the evaluation of patients with coronary heart disease [73]. Recent genetic studies implicated MPO in the development of lung cancer by demonstrating a striking correlation between the relative risk for development of the disease and the incidence of functionally distinct MPO polymorphisms [74]. Myeloperoxidase levels reported for various inflammatory disorders are coincidentally lower than those levels found in all stages of ovarian cancer. A previous study reported normal serum MPO and iron levels as 62 ± 11 ng/ml and 96 ± 9 μg/dl, respectively [75]. However, there was a significant increase in serum MPO and iron levels to 95 ± 20 ng/ml and 159 ± 20 μg/dl, respectively, in asthmatic individuals [75]. Although there was an increase in this reported serum iron, these levels still fell within the normal range (50 to 170 μg/dl) [22, 75]. Other studies have showed that an elevated MPO levels, reaching up to 350 ng/ml, in serum plasma, was indicative of a higher risk for cardiovascular events in patients hospitalized for chest pain [76, 77]. A recent study showed a significant correlation between MPO levels and the stage of ovarian cancer, as is the linear trend for MPO with increasing stage [22]. Similarly, there was a significant difference in the level of free iron in serum and tissues obtained from stage I as compared to combined stages II, III, and IV ovarian cancer. There was an overlap between stage I ovarian cancer and inflammation (endometriosis) serum MPO levels, however serum free iron levels were significantly higher in stage I ovarian cancer as compared to inflammation. There was no significant change in free iron levels between the healthy control group, benign gynecologic conditions group, and inflammation group [22].

Due to the overlap of MPO levels in early-stage ovarian cancer and inflammatory conditions, there is a potential for a false positive with MPO alone in patients with cardiovascular, inflammation, and/or asthmatic disorders. It has been reported that MPO heme destruction and iron release is mediated by high levels of both HOCl (a product of MPO) and oxidative stress (i.e. cancer) [22]. The free iron generated by hemoprotein destruction not only contributes to elevation of

serum iron levels, but may also induce oxidative stress, which can promote lipid peroxidation, DNA strand breaks, and modification or degradation of biomolecules [78–80]. Iron reacts with H_2O_2 and catalyzes the generation of highly reactive hydroxyl radicals, which in turn further increases free iron concentrations by the Fenton and Haber–Weiss reaction [81]. Several studies from our laboratories have provided a mechanistic link between oxidative stress, MPO, higher levels of HOCl and higher free iron that could explain the observed accumulation of free iron in epithelial ovarian cancers tissues [53, 82–85]. Utilizing serum iron levels alone as a biomarker is also not sufficient for early detection of ovarian cancer due to many uncontrolled variables, i.e. dietary intake, supplements, effects of other iron-generating enzymes or factors, and more importantly they are not as specific as MPO levels. Specifically, in iron deficiency anemic patients, their free iron levels may become a confounding factor in its utilization for early detection of ovarian cancer. Thus, anemia should be ruled out to eliminate any overlap that would lead to misdiagnosis. The incorporation of iron deficiency anemic patients in a logistic regression model will help determine its overlap with early-stage ovarian cancer. Additionally, currently available clinical studies focused on either biochemical or more recently, genetic markers of iron overload have reported conflicting results regarding the use of iron levels alone for diagnosis [86–89].

Thus, the combination of serum MPO and iron levels should yield a higher power of specificity and sensitivity that should distinguish women with early-stage ovarian cancer from other disorders, specifically inflammation [22]. Additionally, combining serum MPO and iron levels with the best currently existing biomarkers through the creation of a logistic regression model may increase the overall predictive values. Collectively, there is a role for serum MPO and free iron in the pathophysiology of ovarian cancer, which thereby qualifies them to serve as biomarkers for early detection and prognosis of ovarian cancer.

7. Modulation of oxidative stress

Several studies have reported the beneficial effects of modulating the redox status of cancer cells, however few studies have been reported for ovarian cancer [90–92]. Inhibition of pro-oxidant enzymes, such as NAD(P)H oxidase, has been shown to significantly induce apoptosis of cancer cells [93, 94]. We investigated whether NAD(P)H oxidase-mediated generation of intracellular reactive ROS lead to anti-apoptotic activity and thus a growth advantage to EOC cells. Diphenyleneiodonium (DPI) has been used to inhibit ROS production mediated by NAD(P)H oxidase in various cell types [95–97]. Our results showed that NAD(P)H oxidase is over-expressed in EOC tissues and cells as compared to normal ovarian tissues and cells [52]. Indeed, high levels of NAD(P)H oxidase are known to promote tumorigenesis of NIH3T3 mouse fibroblasts and the DU-145 prostate epithelial cells [98].

Inhibition of NAD(P)H oxidase has also been reported to decrease the generation of $O_2^{\bullet-}$, H_2O_2, as well as other oxidants [93, 94]. Cancer cells are known to manifest enhanced intrinsic oxidative stress and metabolic activity that lead to mitochondrial failure [99, 100]. Indeed, it was previously reported that ovarian tumors are characterized by increased ROS levels as evident from increased $O_2^{\bullet-}$ generated from NAD(P)H oxidase as well as mitochondrial malfunction [101]. The NAD(P)H oxidase redox signaling is controlled by mitochondria, and thus loss of

this control is thought to contribute to tumorigenesis [101]. Others have also shown that inhibition of NAD(P)H oxidase induced apoptosis in cancer cells [102]. Continuous ROS production by the cell and the environment further induces the inhibition of phosphorylation of AKT and subsequent suppression of AKT-mediated phosphorylation of ASK1 on Ser-83, resulting in significant decrease in apoptosis [102–104]. Furthermore, paclitaxel, a chemotherapeutic agent used in the treatment of ovarian cancer and other cancers, induced apoptosis of ovarian cancer cells by negative regulation of AKT–ASK1 phosphorylation signaling [102–104]. On the other hand, activation of AKT by ROS provided protection against apoptosis [102–104].

Data from our laboratory clearly demonstrated that treatment of EOC cells with DPI, which inhibits ROS production mediated by NAD(P)H oxidase, significantly reduced SOD3 and HIF-1α mRNA and protein levels as early as 30 minutes after treatment with a concomitant increase in apoptosis [52]. The association between increased HIF-1α expression and decreased cellular apoptosis has also been demonstrated in lung and hepatoma cancer cells [94, 105]. Overexpression of HIF-1α is thought to decrease apoptosis by the upregulation of anti-apoptotic proteins, Bcl-2 and Bcl-xL and down regulation of pro-apoptotic proteins, BAX and BAK [106]. Inhibition of HIF-1α by rapamycin increased apoptosis by decreasing the expression of apoptosis inhibitor Bcl-2 in ovarian cancer xenografts [107]. Additionally, inhibition of HIF-1α by rapamycin enhanced apoptosis through the inhibition of cell survival signals in several other cell lines [107].

Most of the NAD(P)H oxidase-generated $O_2^{\bullet-}$ is utilized to produce H_2O_2 by nonenzymatic or SOD-catalyzed reactions [108–110]. Hydrogen peroxide serves as the precursor of more toxic hydroxyl radicals and thus is extremely destructive to cells and tissues [109–111]. The expression of SOD3 was reported to increase in response to intrinsic oxidative stress in ovarian cancer cells [112]. It has been demonstrated that overexpression of the SOD3 gene significantly suppressed lung cancer metastasis as well as inhibited the growth of B16-F1 melanoma tumors in mice [113, 114]. However, in a somewhat controversial study, it has been shown that inhibition of SOD selectively induced apoptosis of leukemia and ovarian cancer cells [10].

Under hypoxic conditions, SOD3 is overexpressed and has been reported to significantly induce the expression of HIF-1α in tumors through unknown mechanisms however, steady state levels of $O_2^{\bullet-}$ and the stabilization of HIF-1α have been proposed to play a role in this mechanism [107, 115]. Therefore, inhibition of NAD(P)H oxidase and the consequent reduction of $O_2^{\bullet-}$ levels may destabilize HIF-1α, and subsequently increase apoptosis by lowering SOD3 levels. Thus, we conclude that lowering oxidative stress, possibly through the inhibition of NAD(P)H oxidase-generated $O_2^{\bullet-}$, induces apoptosis in ovarian cancer cells and may serve as a potential target for cancer therapy. This effect was attributed to the modulation of key enzymes that are central to controlling the cellular redox balance.

8. Modulation of metabolism

Cancer cells are known to favor anaerobic metabolism, even when oxygen is present and is known as the “Warburg effect” [116, 117]. Aerobic glycolysis is known to decrease ATP yield as well as increase lactate production by cancer cells [116–118]. To compensate for this decrease in

ATP, cancer cells significantly increase glucose uptake through upregulation of glucose receptors [40, 41, 118]. Increased lactate in cancer cells enhances lactic acidosis, which is significantly toxic to the surrounding tissues and can facilitate tumor growth through the stimulation of ECM degradation, angiogenesis, and metastasis [118]. Additionally, aerobic glycolysis in cancer cells activates HIF, an oxygen-sensitive transcription factor that plays an important role in initiation and maintenance of the oncogenic phenotype [118]. In this regard, HIF induces the expression of several glucose transporters and glycolysis enzymes as well as induces the expression of pyruvate dehydrogenate kinase (PDK), an enzyme that stimulates pyruvate entry into the mitochondria for oxidation [41, 118, 119]. Thus, shifting glucose metabolism in cancer cells from glycolysis to glucose oxidation may have therapeutic value [120]. Indeed, inhibiting PDK by dichloroacetate (DCA) has been reported to induce apoptosis in tumor cells and significantly decreased HIF-1α expression [40]. More importantly DCA is currently in the clinical use for the treatment of hereditary mitochondrial diseases as well as lactic acidosis [41, 121]. The use of DCA at a dose of 35 to 50 mg/kg decreased lactate levels by more than 60% [41, 122]. Dichloroacetate treatment has been shown to significantly induce apoptosis, through the stimulation of caspase-3 activity, in a dose-dependent manner in EOC cells as well as other cancers, such as glioblastoma, endometrial, prostate, and non-small cell lung cancers [40, 123]. Aerobic glycolysis is associated with resistance to apoptosis in cancer cells as many of the enzymes in the glycolysis process are known to modulate gene transcription of apoptotic proteins [40, 41, 69, 124]. Stimulation of pyruvate entry into the mitochondria by DCA, through activation of PDH and inhibition of PDK, is an ideal method to shift aerobic glycolysis to glucose oxidation as inhibiting aerobic glycolysis results in ATP depletion and necrosis, not apoptosis [41, 125].

An additional approach to induce apoptosis in cancer cells is through scavenging high levels of oxidants produced by cancer cells utilizing antioxidants [126]. Deficiency in SOD or inhibition of SOD enzyme activity causes accumulation of $O_2^{\bullet-}$ which is the precursor for several toxic free radicals that are critical to the oncogenic process [127]. Elevated levels of oxidants and free radicals are also known to induce cellular senescence and necrosis, and thus can kill tumor cells [40, 128]. The precise effect of high levels of oxidants and free radicals in cancer cells will depend on the type of cells and tissues, the site of production, and the type and concentration of oxidants [13].

9. Chemotherapy and the acquisition of chemoresistance in EOC cells

Resistance to taxanes and platinums, chemotherapy drugs in current use for ovarian cancer treatment, remains a major obstacle to a successful treatment of ovarian cancer patients [6]. Resistance to chemotherapy not only limits the use of the initial drug but also limits the use of other agents, even those with different mechanisms of action [129]. Chemotherapy drugs exert their actions by the initiation of cell death either directly through the generation of oxidative stress or as an indirect effect of exposure, as observed with several chemotherapeutic agents [130]. The development of chemoresistance to drugs is dependent on several factors that include: influx/efflux of drugs that decrease platinum accumulation in tumor cells, enhanced GSH and GST levels, upregulation of anti-apoptotic proteins such as Bcl-2, loss of tumor necrosis factor receptor ligand which induces apoptosis, increased DNA repair through up-regulation of repair

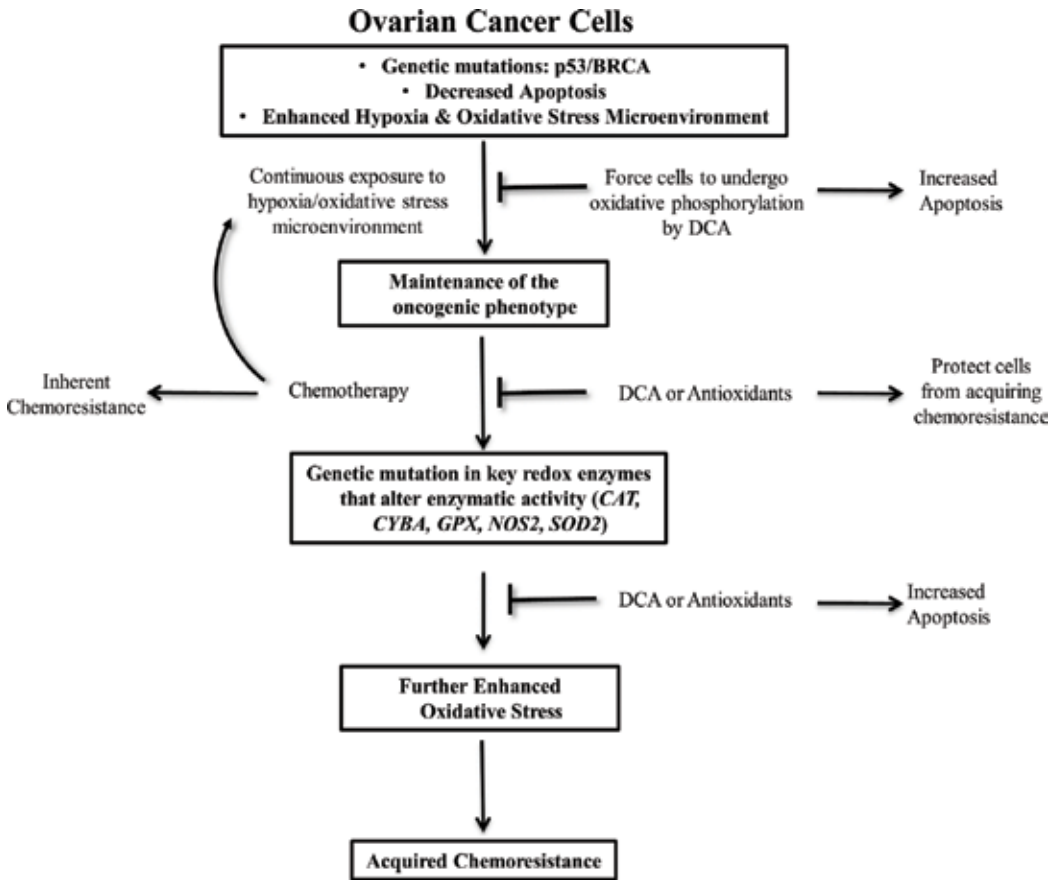

Figure 2. Summary of the role of oxidative stress in the development of sensitive and chemoresistant ovarian cancer [1].

genes, and loss of functional p53 that augments NF-κB activation [13, 131]. We have previously shown that chemoresistant EOC cells manifested increased iNOS and nitrate/nitrite levels as well as a decrease in GSR expression as compared to sensitive EOC cells, suggesting a further enhancement of the redox state in chemoresistant cells [1, 45]. Additionally, CAT, GPX, and iNOS were shown to be significantly increased while, GSR, SOD, and the NAD(P)H oxidase subunit ($p22^{phox}$) were decreased in chemoresistant EOC cells as compared to their sensitive counterparts [21]. These finding supports a key role for oxidative stress, not only in the development of the oncogenic phenotype, but also in the development of chemoresistance (**Figure 2**).

10. Common polymorphisms in redox enzymes are associated with ovarian cancer

A single nucleotide polymorphism (SNP) occurs as a result of gene point mutations with an estimated frequency of at least one in every 1000 base pairs that are selectively maintained and distributed in populations throughout the human genome [132]. An association

between common SNPs in oxidative DNA repair genes and redox genes with human cancer susceptibility has been established [28]. Common SNPs in the redox enzymes are known to be strongly associated with an altered enzymatic activity in these enzymes, and may explain the enhanced redox state that has been linked to several malignancies, including ovarian cancer [40, 52]. Additionally, it may further explain the observation of significantly decreased apoptosis and increased survival of EOC cells [53]. It is therefore critical to determine the exact effect of common SNPs in various redox enzymes on all process involved in the development of the oncogenic phenotype [21, 46, 133, 134]. Such studies can be linked to other studies focusing on determining the effects of genes involved in carcinogen metabolism (detoxification and/or activation), redox enzymes, and DNA repair pathways [133]. Numerous SNPs associated with change of function have been identified in antioxidant enzymes including *CAT*, *GPX1*, *GSR*, and *SOD2* [21, 134]. Additionally, the association between genetic polymorphisms in genes with anti-tumor activity and those involved in the cell cycle has been reported in ovarian cancer [135, 136]. Recently, several genetic variations have been identified in genome-wide association studies (GWAS), and were found to act as low to moderate penetrant alleles, which contribute to ovarian cancer risk, as well as other diseases [23, 137].

There is now an association of specific SNPs in key oxidant and anti-oxidant enzymes with increased risk and overall survival of ovarian cancer [21, 46]. A common SNP that reduced CAT activity (rs1001179) was utilized as a significant predictor of death when present in ovarian cancer patients and was also associated with increased risk for breast cancer [21, 46, 134, 138]. This SNP is also linked to increased risk, survival, and response to adjuvant treatment of cancer patients, including ovarian [46, 139]. Another common SNP that reduced CYBA activity (rs4673) was also reported to be associated with an increased risk for ovarian cancer [21, 46]. The mutant genotype of the *CYBA* gene has been shown to both decrease and increase activity of the protein, thereby altering the generation of $O_2^{\bullet-}$ [21, 46]. Moreover, functionally distinct *MPO* polymorphisms, such as (rs2333227) have been linked to relative increased risk for development of ovarian cancer as well as other cancers [21, 44, 46]. Additional SNPs that influenced the risk of EOC have been successfully identified from the GWAS studies including rs3814113 (located at 9p22, near BNC2), rs2072590 (located at 2q31, which contains a family of HOX genes), rs2665390 (located at 3q25, intronic to TIPARP), rs10088218 (located at 8q24, 700 kb downstream of MYC), rs8170 (located at 19p13, near MERIT40), and rs9303542 (located at 17q21, intronic to SKAP1) [21, 46]. Thus, the genetic component of increased ovarian cancer risk may be attributed to SNPs that result in point mutations in the redox genes and potentially other genes [140].

11. Chemoresistance is associated with point mutations in key redox enzymes in EOC cells

To date, the acquisition of chemoresistance in ovarian cancer is not fully understood. The enhanced oxidant state reported in chemoresistant EOC cells may be linked to point mutations in key redox enzymes [21]. Chemoresistant EOC cells manifested increased levels of CAT, GPX, and iNOS and decreased levels of GSR, SOD, and NAD(P)H oxidase as compared to their sensitive counterparts [21]. Interestingly, chemoresistant EOC cells, and not their sensitive counterparts,

manifested specific point mutations that corresponded to known functional SNPs, in key redox enzymes including *SOD2* (rs4880), *NOS2* (rs2297518), and *CYBA* (rs4673) [1]. However, altered enzymatic activity for CAT and GSR observed in chemoresistant EOC cells did not correspond to the specific SNP of interest in those enzymes, indicating involvement of other possible functional SNPs for those enzymes [21]. Coincidently, chemotherapy treatment induced point mutations that happen to correspond to known functional SNPs in key oxidant enzymes subsequently led to the acquisition of chemoresistance by EOC cells. Indeed, the induction of specific point mutations in *SOD2* or *GPX1* in sensitive EOC cells resulted in a decrease in the sensitivity to chemotherapy of these cells [21]. In fact, the addition of SOD to sensitive EOC cells during chemotherapy treatment synergistically increased the efficacy to chemotherapy [21].

Alternatively, the observed nucleotide switch in response to chemotherapy in EOC cells may be the result of nucleotide substitution, a process that includes transitions, replacement of one purine by the other or that of one pyrimidine by the other, or transversions, replacement of a purine by a pyrimidine or vice versa [21]. Indeed, hydroxyl radicals are known to react with DNA causing the formation of many pyrimidine and purine-derived lesions [21]. The oxidative damage to 8-Oxo-2′-deoxyguanosine, a major product of DNA oxidation, induces genetic alterations in oncogenes and tumor suppressor genes has been involved in tumor initiation and progression [21]. A GC to TA transversion has been reported in the *ras* oncogene and the *p53* tumor suppressor gene in several cancers. However, the GC to TA transversion is not unique to hydroxy-2′-deoxyguanosine, as CC to TT substitutions have been identified as signature mutations for oxidants and free radicals [21].

Moreover, the observed nucleotide switch in response to chemotherapy in EOC cells can be due to the fact that acquisition of chemoresistance generates an entirely different population of cells with a distinct genotype. Hence, chemotherapy kills the bulk of the tumor cells leaving a subtype of cancer cells with ability for repair and renewal, known as cancer stem cells (CSCs) [21]. Indeed, cancer stem cells have been isolated from various types of cancer including leukemia, breast, brain, pancreatic, prostate, ovarian and colon [21]. Interestingly, CSC populations were present in cultures of SKOV-3 EOC cells and have been shown to be chemoresistance in nature [21].

12. Further increasing pro-oxidant enzymes: potential survival mechanism

Apoptosis is a tightly regulated molecular process that removes excess or unwanted cells from organisms. Resistance to apoptosis is a key feature of cancer cells and is involved in the pathogenesis of cancer. We have previously reported that EOC cells have significantly increased levels of NO, which correlated with increased expression in iNOS [54]. We have also reported that EOC cells manifested lower apoptosis, which was markedly induced by inhibiting iNOS by L-NAME, indicating a strong link between apoptosis and NO/iNOS pathways in these cells [54]. Caspase-3 is known to play a critical role in controlling apoptosis, by participating in a cascade that is triggered in response to proapoptotic signals and culminates in cleavage of a set of

proteins, resulting in disassembly of the cell [141–144]. Caspase-3 was found to be S-nitrosylated on the catalytic-site cysteine in unstimulated human lymphocyte cell lines and denitrosylated upon activation of the Fas apoptotic pathway [145]. Decreased caspase-3 S-nitrosylation was associated with an increase in intracellular caspase activity. Caspase-3 S-nitrosylation/denitrosylation is known to serve as an on/off switch regulating caspase activity during apoptosis in endothelial cells, lymphocytes and trophoblasts [146–149]. The mechanisms underlying S-nitrosothiol (SNO) formation *in vivo* are not well understood.

Myeloperoxidase typically uses H_2O_2, in combination with chloride to generate hypochlorous acid [55, 150–153]. We, and others, have demonstrated that MPO utilizes NO, produced by iNOS, as a one-electron substrate generating NO^+, a labile nitrosating species that is rapidly hydrolyzed forming nitrite as end-product [55, 56, 154, 155]. The ability of MPO to generate NO^+, from NO, led us to believe that not only does MPO play a role in S-nitrosylation of caspase-3 in EOC cells, but also highlights a possible cross-talk between iNOS and MPO. Indeed, we observed that MPO is responsible for the S-nitrosylation of caspase-3, which led to the inhibition of caspase-3 in EOC cells. Silencing MPO gene expression induced apoptosis in EOC cells through a mechanism that involved S-nitrosylation of caspase-3 by MPO.

Molecular alterations that lead to apoptosis can be inhibited by S-nitrosylation of apoptotic proteins such as caspases. Thus, S-nitrosylation conveys a key influence of NO on apoptosis signaling and may act as a key regulator for apoptosis in cancer cells. It has been known that the effects of NO on apoptosis are not only stimulatory but may also be inhibitory. These paradoxical effects of NO on apoptosis seem to be influenced by several factors. It has been suggested that biological conditions, such as the redox state, concentration, exposure time and the combination with O_2, $O_2^{\bullet-}$ and other molecules, determines the net effect of NO on apoptosis [156]. Also, NO is implicated in both apoptotic and necrotic cell death depending on the NO chemistry and the cellular biological redox state [57, 156]. As described earlier, we have previously demonstrated that the EOC cell lines, SKOV-3 and MDAH-2774, manifested lower apoptosis and had significantly higher levels of NO due to the presence of elevated levels of iNOS [54, 157]. We have also reported significant levels of MPO expression, which was found to be co-localized with iNOS, in both EOC cell lines SKOV-3 and MDAH-2774 [53]. We have demonstrated that 65% of the invasive epithelial ovarian carcinoma specimens tested expressed MPO in the neoplastic cells. The co-localization of MPO and iNOS has been demonstrated by immunohistochemical studies in cytokine-treated human neutrophils and primary granules of activated leukocytes [158]. Both plasma levels and tissue expression of MPO in gynecologic malignancies were previously evaluated and it was found that gynecologic cancer patients had higher plasma MPO compared to control subjects [159]. Using immunostaining, it was also demonstrated that MPO expression was higher in cancer tissues compared to control [159].

We have now characterized chemoresistant EOC cells to manifest an even further increase in pro-oxidant enzymes including MPO, and NO, a surrogate for iNOS activity in conjunction with a further increase in the S-nitrosylation of caspase-3 (*data not published*) and a concurrent decrease in the level of apoptosis [21]. Thus, we hypothesized that the decrease in apoptosis observed in chemoresistant EOC cells is a consequence of a further increase in the degree of S-nitrosylation of caspase-3. Since resistance to apoptosis is a hallmark of tumor

growth, identifying mechanisms of this resistance such as S-nitrosylation may be a key in cancer progression and the development of chemoresistance. S-nitrosylation is reversible and seemingly a specific post-translational modification that regulates the activity of several signaling proteins. S-nitrosylation of the catalytic site cysteine in caspases serves as an on/off switch regulating caspase activity during apoptosis in endothelial cells, lymphocytes, and trophoblasts [147–149]. Targeting MPO may be a potential therapeutic intervention to reverse the resistance to apoptosis in sensitive and chemoresistant EOC cells.

13. Ovarian cancer immunotherapy and oxidative stress

It is well established that tumorigenic cells generate high levels of ROS to activate proximal signaling pathways that promote proliferation, survival and metabolic adaptation while also maintaining a high level of antioxidant activity to prevent buildup of ROS to levels that could induce cell death [160]. Moreover, there is evidence that ROS can act as secondary messengers in immune cells, which can lead to hyperactivation of inflammatory responses resulting in tissue damage and pathology [160]. Ovarian cancer is considered an ideal tumorogenic cancer because ovarian cancer cells have no negative impact on immune cells [161].

Effective immunotherapy for ovarian cancer is currently the focus of several investigations and clinical trials. Current immunotherapies for cancer treatment include therapeutic vaccines, cytokines, immune modulators, immune checkpoint inhibitors, and adoptive T cell transfer [162]. The discovery of a monoclonal antibodies (such as bevacizumab) directed against VEGF have been shown to improve progression free survival compared to cytotoxic chemotherapy alone was a major outcome of these clinical trials [163]. Other monoclonal antibodies currently approved for other cancers such as trastuzumab for breast cancer or cetuximab for colon cancer exhibited limited activity in ovarian cancer [163]. Several clinical trials are ongoing for the utilization of immune checkpoint blockade in ovarian cancer immune therapy [164]. Most recently tested were the programmed death (PD)-1 inhibitors, pembrolizumab and nivolumab, which showed a consistent response rate of 10–20% in phase 2 studies and then failed to improve outcomes in confirmatory trials [164]. Ultimately, larger phase 3 studies are needed to validate these findings for checkpoint inhibitors, particularly with regard to the duration of response seen with these agents. Additionally, the direct intraperitoneal delivery of interleukin (IL)-12, a potent immunostimulatory agent, exhibited some potential therapeutic efficacy in ovarian cancer [165]. Recently, targeting folate receptor alpha, which is found to be expressed in ovarian cancer, has shown promising therapeutic value. The targeting of the folate receptor was achieved by either a blocking monoclonal antibody (farletuzumab) or antibody conjugates of folate analogs, such as vintafolide [166].

14. Summary and conclusion

Oxidative stress has been implicated in the pathogenesis of several malignancies including ovarian cancer. Epithelial ovarian cancer is characterized to manifest a persistent pro-oxidant

state through alteration of the redox balance, which is further enhanced in their chemoresistant counterparts, as summarized in **Figure 2**. Forcing ovarian cancer cells to undergo oxidative phosphorylation rather than glycolysis has been shown to be beneficial for eliminating cells via apoptosis (**Figure 2**). Collectively, there is convincing evidence that indicated a causal relationship between the acquisition of chemoresistance and chemotherapy-induced genetic mutations in key redox enzymes, leading to a further enhanced oxidative stress in chemoresistant EOC cells. This concept was further confirmed by the observation that induction of point mutations in sensitive EOC cells increased their resistance to chemotherapy. Also, a combination of antioxidants with chemotherapy significantly sensitized cells to chemotherapy. Identification of targets for chemoresistance with either biomarker and/or screening potential will have a significant impact for the treatment of this disease.

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Chapter 5

Genomic Copy Number Alterations in Serous Ovarian Cancer

Joe R. Delaney and Dwayne G. Stupack

Additional information is available at the end of the chapter

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Abstract

Precision medicine in cancer is the idea that the recognition and targeting of key genetic drivers of a patient's tumor can permit more effective and less toxic outcomes. Point mutations that alter protein function have been primary targets. Yet in ovarian cancer, unique genetic mutations have been identified only in adult granulosa cell tumors, with a number of other point mutations present in mucinous, clear cell and endometrioid carcinoma subtypes. By contrast, the serous subtype of ovarian cancer shows many fewer point mutations but cascading defects in DNA damage repair that leads to a network of gains and losses of entire genes called somatic copy number alterations. The shuffling and selection of the thousands of genes in serous ovarian cancer has made it a complex disease to understand, but patterns are beginning to emerge based on our understanding of key cellular protein networks that may provide a better basis for future implementation of precision medicine for this most prevalent subtype of disease.

Keywords: SCNA, aneuploidy, autophagy, beclin-1, p53

1. Introduction

When a patient asks an oncologist what tumor cells are, the frequent explanation is that the "Cancer cells are normal cells that accumulate genetic mutations, which causes them to grow out of control." Yet the idea of what a mutation is, and what it can do, varies. It has essentially become dogma that mutations be grouped into two broad categories. One class has been described as either *drivers*, which are key genetic changes that are known to potentiate tumor development. If a gene is not a driver, then it is typically considered a *passenger*,—a bystander mutation occurring due to the tumor-associated genomic instability. Passenger mutations are generally considered to be 'noise' in the system which do not influence tumor progression [1].

This categorization has now had clinical impact. Genes that are known as *drivers* are prioritized for diagnostic testing, and have become a focus for "molecular tumor boards" that review patient data in hospitals across the United States. These boards focus foremost on reviewing a molecular profiling of the tumor, rather than on histopathological features. Thus, tumors with similar genetic features may call for similar therapy regardless of whether they originate in the colon, breast or lung. The division of mutation into drivers and passengers fosters an environment where new mutations may be missed, because we are focused on the pre-established clinical screening protocols, because we both profile and act upon well characterized genetic problems. Even when they are reported, their impact may not be appreciated if they have not had a role as a driver assigned to them in prior peer-reviewed study.

The *driver* assignment comes from a breadth of work that focuses on a type of mutation called a somatic single-nucleotide variant (SNV). Driver SNVs are noted for their critical roles in tumor formation, frequently occur at precise locations within *oncogenes*, and can now be rapidly identified. Notable examples include K-Ras, where mutation of the glycine residue at position 12 (G12) inhibits GTPase activity, leaving the protein in an active, GTP-bound effector state. A second example is phosphoinositide 3'kinase, where mutation of the histidine residue at 1047 (H1047) similarly alters the ability of the protein to regulate activity. The gold standard for such driver mutations is their capacity to facilitate neoplastic disease in murine genetic models, most frequently by providing a dysregulated positive stimulus that drives mitosis and cell survival. Transcription factor mutations, such as the FOXL2 C243W mutation found in all adult type granulosa cell tumors, provide a good example of a key genetic driver.

A second class of drivers involve SNV-mediated inactivation of *tumor suppressor* genes, which act to ameliorate the effects of oncogenes, shunt tumors towards programmed cell death, and maintain the fidelity of DNA replication and repair. Tumor formation requires both oncogenic activation and the disruption of tumor suppressors. Mutation in TS genes do not require the same precision as those in oncogenes; SNV's occur across a swath of locations, any of which may be sufficient to disrupt tumor suppressor function. This chapter will focus on serous

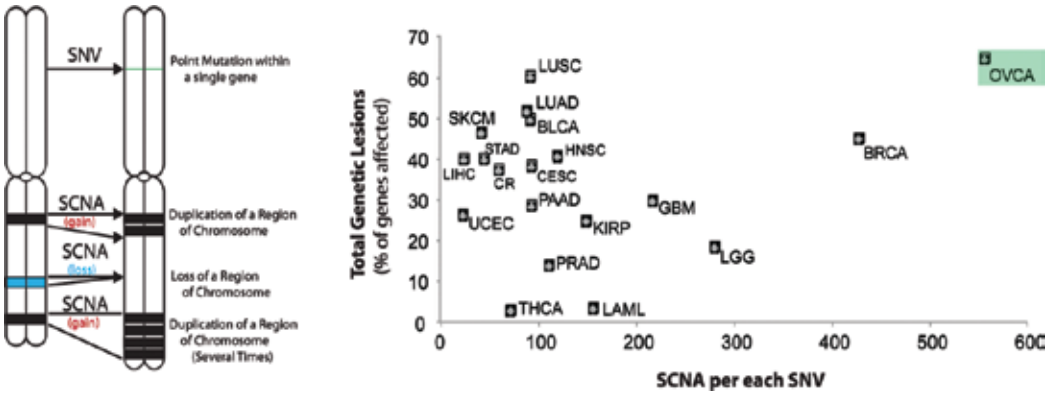

Figure 1. (Left) Possible changes in a single chromosome's architecture. One chromosome is shown; each copy of a chromosome can have different SCNA or SNV. (Right) A plot of the major cancers in the TCGA database, showing total genetic lesions (as percentage of genes) vs. the number of SCNA for each SNV. In each case SCNA are more abundant, with serous ovarian cancer (SOC, denoted as OVCA in the green box) bearing the greatest number.

ovarian cancer (SOC), a lethal tumor whose '*drivers*' are only beginning to be understood. The tumor suppressor gene *TP53* is mutated in more than 85% of serous ovarian cancer (SOC) cases [2], and disruption of DNA repair proteins is commonly identified. Yet most patients bear no SNV that results in oncogene activation.

However, SOC has a further characteristic related to its poor capacity to repair DNA. SOC has the highest ratio of somatic copy number alterations (SCNAs) to SNVs for any major cancer. SCNAs are a broad group of genetic changes that encompass a myriad of short insertions, short deletions, translocations and inversions (**Figure 1**, left panel). SCNAs contribute to the mutational landscape of cancer, expanding the scope of changes beyond the more 'simple' SNVs. The impact of this on SOC malignancy will be the focus of this chapter.

2. SCNA overview and incidence

A gene normally occurs in the human nucleus twice. This normal 2N "dosage" of copy number, which originates from zygote formation, consists of one paternal gene and one maternal gene. SCNAs, which alter this occur in two types: amplifications and deletions. An amplification occurs when a chromosomal region containing a gene is copied. That gene will no longer have the normal 2N copy number, but, depending upon the number of times it is copied, could be 3N, 4N, or in cases of massive amplification, up to 200 N and more. Contrasting with this expansive range, SCNAs that result from deletions most frequently reduce the copy number to 1N. Total gene loss (0N) can occur in rare cases, and is associated with a very small fraction of the overall number of deletions. Nonetheless, these rarer SCNA-derived genotypes will obviously impact function most, since the lack of any gene copy means that the encoded protein cannot be produced. SCNAs are the most common lesions in cancer, occurring much more commonly than SNVs (**Figure 1**, right panel).

SCNAs occur via a variety of mechanisms in cancer [3]. Entire chromosomes may be gained/lost during cell division, generating 3N or 1N copy number status for all genes on the chromosome. This occurs due to failed cell-division checkpoints resulting in chromosome missegregation. In contrast to such gains at the total chromosome level, tiny "focal" SCNAs may alter a single gene (or even part of a gene). The most common example of this is *CDKN2A*, a checkpoint protein which is fully deleted (0N) in 3% of SOC tumors. These focal deletions typically occur during repair of double-stranded DNA (dsDNA) breaks. During the attempted repair, short regions of homology can result in accidental deletion of DNA in between [4]. Focal amplifications occur through unknown mechanisms [5] and can form "double minute" chromosomes containing hundreds of copies of a gene, such as *ERBB2* or *EGFR* [6]. Finally, between the focal alterations and the whole chromosome losses, SCNAs can also encompass large regions of DNA through similar defects in dsDNA break repair. These intermediate sized SCNAs can contain many genes. However, they rarely contribute to a 0N copy numbers (loss on both chromosomes) since the regions affected frequently contain essential genes [7].

Within the Cancer Genome Atlas (TCGA) data sets, the presence of 3N and 1N gene copies dominate the SCNA genomic landscape. This is true across all tumors, including those tumors where SCNAs are highly prevalent, such as SOC [8]. SCNAs are prevalent in SOC. In

fact, only about one third of all genes in primary tumors have a normal 2N gene dosage. Roughly a quarter of the total genes in the tumors show an extra gene copy (to 3N) and just over a third lose a gene copy (to 1N). By contrast, only 0.7% loses both gene copies (0N), while 4.2% are amplified to 4N or greater. In practice, the focus on understanding tumor biology has been only on these last two cases (total deletion and gross amplification, respectively). This has a reasonable basis; the effects of total loss or gross amplification are easiest to study.

The common gene changes (i.e., 1N and 3N) have not been the subject of focused study. Many scientists assume that the deletion, or addition, of a single gene copy has limited effect. Recessive genetic alleles are not uncommon in nature, supporting the idea that the loss of a single gene copy can be compensated for. However, the loss of a single gene may not reflect the situation in ovarian cancer, where massive genetic alteration occurs, and compensation may not be possible if the same cellular pathway is repeatedly targeted by SCNAs (**Figure 2**).

More than 80% of genes affected by SCNAs show concordant alteration of mRNA levels [9, 10]. For ~70% of genes, this correlates with steady-state protein levels [11]. Thus, SCNAs offer a predictable, but not absolute, indication of protein expression. This is relevant to ovarian cancer, as SCNAs modify on average 67% of the SOC genome, whereas SNVs modify only

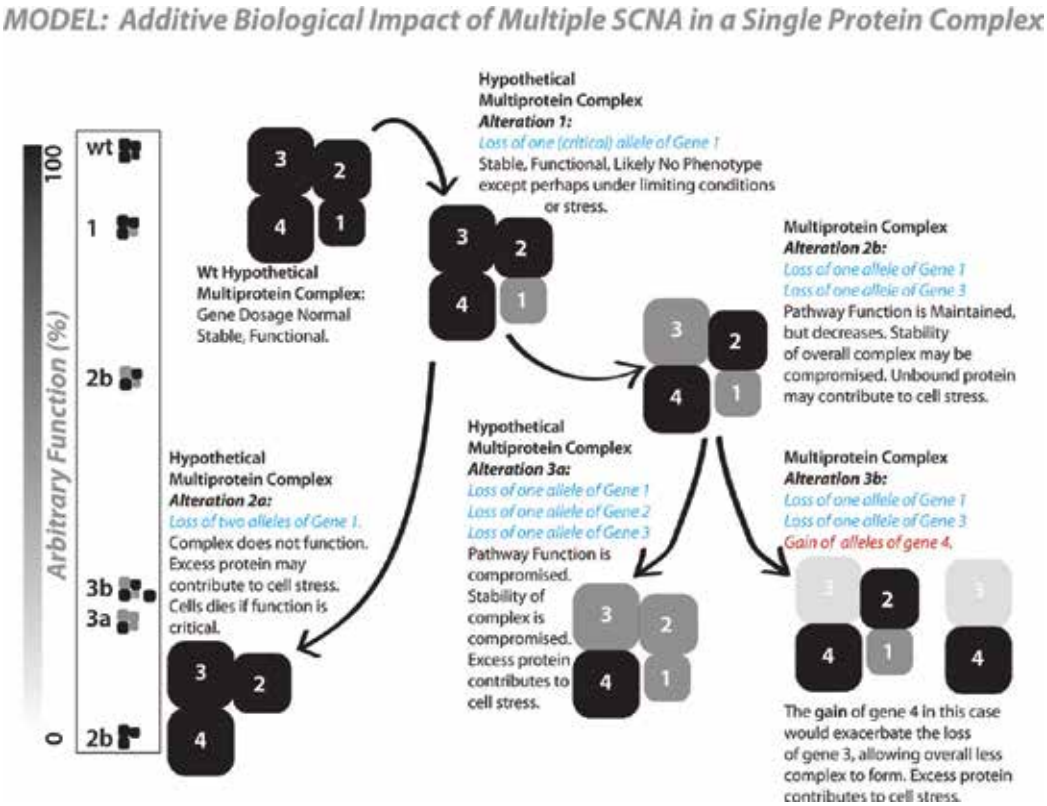

Figure 2. Model showing how SCNA changes resulting in differences in protein expression might impact overall function within a single multiprotein complex. The relative function of the complex as a % is shown at left. The right side shows how a sequence of SCNA changes within a pathway could cumulatively impact its function.

0.12% of the average SOC genome [12]. Less than 10% of SOC patients are mutated in a targetable driver gene [12, 13].

3. Ovarian cancer and copy number alterations

It seems self-evident that an understanding of "driver SCNAs" is absolutely essential to our capacity to target the biology of the disease. Genetic disorders such as Down's syndrome (trisomy 21) and Cri du Chat (5p monosomy) and DiGeorge Syndrome (loss of only 30–40 alleles on 22q11) clearly indicate the penetrative biology of multiple SCNA. More importantly, such lesions affect only ~2% of the genome, while SCNA in SOC affect 67% of genes. Other subtypes of ovarian cancer vary widely in their SCNA burden, but are typically much lower, and are associated with SNVs.

As most SCNA are "monoallelic" changes resulting in a 1N or 3N genotype, is there any reason to expect a phenotype, given our understanding of recessive alleles? Recurrent patterns in serous ovarian cancer suggest that frequently affected regions may be selected for as the tumor evolves. In high grade SOC, the most prevalent SNVs could have been predicted from literature preceding the genomics era. For decades, the mutation of *TP53*, the "guardian of the genome" has been appreciated due to its master control of multiple DNA repair pathways, cell cycle control, and metabolism. Interestingly, there is selection for SCNA deletion of the chromosome with the wild-type copy of p53, suggesting further suppression or misdirection of p53 furthers SOC development [14]. Inheritance studies have associated the *BRCA1/2* mutation with an increased risk of ovarian cancer, and not surprisingly these mutants contain opposite-chromosome deletions just like p53. *BRCA* genes are necessary to maintain the genome. Like p53, they play a coordinating role in facilitating homology-directed repair of DNA. However, single nucleotide variant mutation is not the most common mechanism of BRCA gene disruption. Only ~6% of patients display non-germline SNVs, while copy number deletions (to 1N) occur in more than 70% of tumors. PTEN, a tumor suppressor commonly mutated in many tumor types but not ovarian, was found as early as 2001 to have reduced expression due to shallow deletions across ~40% of samples [15].

Aside from very infrequent gene losses paired with mutations, there are also a few SCNAs which drive cancer through amplification of oncogenes. The stem-cell transcription factor *MYC* is the most amplified gene in the TCGA cohort (42% with at least a 4N copy number, and an additional 37% with 3N). Myc has been appreciated as a common SOC driver oncogene since 1990 [16]. Homozygous deletions in Rb were discovered around the same time [17, 18], and occur in 9% of tumors. *KRAS* amplifications and gene overexpression were discovered around the same time, but in a smaller minority (13%) of patients [19]. Her2, encoded by *ERBB2*, can be overexpressed but this appears to be a case unrelated to SCNA amplification, which occurs in only 3% of cases [20, 21]. Drug resistance can occur following increases in drug efflux genes, and one of the first identified was *MDR1* (*ABCB1*) [22]. Again, this is only found in a small minority of patients (4%). Comparative genomic hybridization in 2006 identified significantly amplified CCNE1 (cyclin E1) and MDM2 (a negative regulator of p53 from its E3 ubiquitin ligase activity) [23, 24]. The year these studies were published provides

a historical context to our knowledge. Were we missing a key driver for SOC? Despite the hundreds of genomes sequenced, few additional single-gene drivers were discovered in the recent "brute-force" landmark studies on SOC from either the TCGA [12] or the AOC [13].

There are plausible reasons for this. It may be that every SOC tumor is truly unique from a mutational standpoint: that those SNVs found in only one tumor nonetheless are driver genes, collaborating in ways that we understand poorly [25]. It is thus possible that drivers have already been sequenced and annotated by SCNA studies, but due to high "background" or "passenger" SCNAs it remains unclear which SCNAs are critical to the tumor's biology [8]. The implications of this are enormous, and would necessitate an unparalleled level of personalized therapies targeting such extremely rare mutations. A second reason that SNVs have not yielded common drivers may be that further sequencing of whole genomes and epigenomes will reveal additional drivers prevalent across patients which have remained undetected by exome sequencing.

The problem investigators consistently encounter is the ubiquitous heterogeneity in SOC. Heterogeneity exists at all levels of genetics, manifesting as *between-patient* heterogeneity, *between-tumor* (intra-patient) heterogeneity [14], heterogeneity in SNVs within a single tumor [12], heterogeneity in SCNAs [26], and heterogeneity in mRNA expression (correlating with protein expression) [11] or flux in SCNA status [10]. While such problems are not unique to SOC, they are magnified compared to many other tumor types because of the gross incidence of SCNA. Genetic and phenotypic heterogeneity remains the hardest issue to tackle [27, 28], and our own opinion mirrors that of several other groups working in this area: the analysis of affected pathways will offer new approaches to find hidden patterns of tumor suppressors and oncogenes within these heterogeneous data [8, 9, 29].

Fewer genomic studies have been performed on other types of ovarian cancer. Some limited data are available on SCNAs for Clear cell and endometrioid subtypes, which share the amplification of PIK3CA and the MYC-containing 8q24 region with SOC [30–32]. Larger SCNAs encompassing whole chromosome arms rather than smaller changes dominate the clear cell ovarian cancer SCNA landscape [32]. With the exception of 17p loss (containing *TP53*), focal *TPM3* amplification, and focal *ERBB2* amplification, SCNAs are infrequent in mucinous ovarian cancer, suggesting this histotype is SNV or epigenetic driven [30]. Generally, clear cell and endometrioid are intermediate in SCNA quantity between SOC and mucinous subtypes of ovarian cancer.

Despite the limited data on these non-serous subtypes, there is good reason to expect much more data is coming soon. The copy-number arrays employed in the Cancer Genome Atlas studies sell for less than $100USD per sample, which bests the current, but constantly decreasing, cost of whole-genome sequencing. Eight oncology treatment and research centers are participating in project GENIE, which has just released 19,000 new tumor datasets to the public and will continue to grow [33]. As sequencing becomes a normal part of the treatment strategy for patients, the number of samples will likely outpace scientists' ability to fully analyze and comprehend the complex data. Nonetheless, gathering these data is essential to progressing our understanding of the differences between cancer subtypes, which will facilitate the matching of pharmaceuticals to genotype. For now, the largest datasets exist in SOC, and will be the focus of the remainder of discussion.

4. The interplay of p53 mutation with copy number instability

Mutation in p53 has a long research history in many cancer types, and ovarian cancer is no exception. Ovarian cancer mutations within *TP53* have been observed since 1991 [34], and have been confirmed in every genetic study since. *TP53* has often been referred to as "the" primary tumor suppressor for its central role in responding to stresses: it can halt the cell cycle, divert metabolism, induce transcription of DNA damage response genes, or if the damage cannot be repaired, induce apoptosis or senescence [35–37]. For serous ovarian cancer, it has been used as a marker for false diagnosis as some studies presume that genuine serous ovarian cancers must contain mutant p53. Similarly, since the beginning of genomic copy number studies using comparative genomic hybridization, SCNAs have been labeled as a ubiquitous event in all types of epithelial ovarian cancer [31]. Not all p53 mutant tumors are high in SCNAs [38]. Nonetheless, tumors with higher than average SCNAs are much more likely to have a facilitating mutation in p53 [26, 39, 40]. This implies a basic premise: ovarian cancer tumors utilize the mutation in *TP53* to enable the proliferation of SCNAs. SCNAs can subsequently occur in additional tumor suppressors and oncogenes, which leads to SOC as we know it.

Mechanistically this could occur via the deletion or duplication of entire chromosomes or genomes, followed by many subsequent changes enabled by the extra copies of genes, or via chromosome missegregation event, leading one or more chromosomes to acquire massive damage [41]. Either possibility can explain the high frequency of chromothripsis, a highly-disorganized form of hundreds or thousands of SCNAS, in SOC. Mutant p53 enables such mechanisms of SCNA formation by preventing the death of the cell that bears them, as missegregation directly induces p53-dependent cell-cycle arrest followed by apoptosis [42]. In one well-controlled study, 'dominant negative' p53 reduced the cell cycle delay associated with trisomy in mammalian cells, yet it was rare that gain of any single chromosome in those cells resulted in any proliferative advantage [43]. Thus, partial or gained p53 function may contribute. Many p53 mutations maintain partial function, while mutations such as R273H (the most common variant of TP53 found in SOC) provide a gain of function by directly impairing Mre11/ATM-dependent DNA damage responses [44].

It is likely that mutation in *TP53* gene is an enabling event. Lineage tracing using millions of sub-clonal passenger mutations present in SOC tumors suggest that *TP53* mutation arises very early in the proliferation of pre-tumor cells [14]. While few studies focus on normal tissue, a publication on aged skin samples found that islands of cells had developed p53 mutations and achieved local proliferation. Exceptionally few copy number alterations were revealed, with the exception of deletions in *NOTCH1*, and these lesions did not progress to malignancy [45]. In murine models, too, SCNAs follow initiating mutational stimuli [46]. The findings support the idea that p53 is likely to become mutated prior to SCNA accumulation, and acts permissively to enable SCNA accumulation.

5. BRCA1/2 mutations and homologous repair defects

Though few SNVs in 'classic' tumor genes are found in SOC relative to other cancer types, BRCA1/2 mutations are among the most frequent at ~10% [12]. BRCA genes work in

coordination with dozens of other proteins to perform genome maintenance via homologous recombination [4]. The double-stranded break repair pathway begins with PARylation of the break site by PARP1, megabases of phosphorylation of H2AX and subsequent formation of Rad51 filaments. Brca1 & Brca2 bind Rad51 to stimulate strand invasion of sister chromatids during homology directed repair. While only ~10% of SOC are mutated in BRCA1 or BRCA2, it is noteworthy that 75% of patients have lost one of two alleles of BRCA1 and 57% have lost an allele of BRCA2. Very few tumors (~1.5%) have homozygous deletions in BRCA1 [12], suggesting a system of compromised (but not lost) function. In fact, mRNA expression level does not track linearly with such deletions. It remains somewhat unclear if these monoallelic deletions do have a phenotype under genotoxic stress in human cells.

Mutations in homologous repair coordinating factors are often found in serous, clear cell, endometrioid, and carcinosarcoma ovarian cancers [47, 48]. Specific mutation patterns are found within BRCA1/2 or otherwise homologous repair deficient cells. Without functional homologous repair, cells default to non-homologous end joining (NHEJ) to repair double-stranded DNA (dsDNA) lesions. NHEJ does not perfectly repair DNA, but rather often introduces small insertions or deletions along with single-nucleotide variants at the break site. These mutational marks are frequently found in non-serous ovarian cancers, yet are unlikely the main drivers of SCNA instability in serous ovarian cancer. However, NHEJ factors involved in repairing unresolved dsDNA breaks across different chromosomes, or creating translocations and other complex rearrangements, are compromised in 40% *ex vivo* ovarian cancer isolates [13, 49]. This can lead to resistance to PARP inhibitors and promote inappropriate translocation or "repair" events [50]. Such defects in NHEJ may explain why the majority of SOC initially respond to cisplatin-based chemotherapy. Complex dsDNA lesions incurred by cisplatin target NHEJ and promote mitotic catastrophe [51].

Genetic and epigenetic changes alter *BRCA1* and the homologous repair pathways in SOC. Gene breakage is commonly observed within *RB1*, *NF1*, *RAD51B*, and *PTEN* [13]. While suppression of homologous recombination may lead to initial disease formation, there is evidence of *BRCA1/2* reversion mutations in tumors which become chemoresistant. This phenomenon follows the strong selective effects of carboplatin and taxanes which require cellular DNA repair pathways to enable cell division. Clinically, these findings should be considered in the context of the search for patient populations for PARP inhibitors. A primary hypothesis for how PARP inhibitors like Olaparib and Niraparib work is by targeting a DNA repair pathway which compensates for homologous repair, thereby presenting synthetic lethality specifically in cancer cells [52]. In a Phase III trial of Niraparib, clinicians treated both BRCA1 or BRCA2 mutant tumors as well as patients who were not found to have mutations in homologous repair genes in their tumors. Unexpectedly, all groups responded to Niraparib therapy [53], although patients with mutated BRCA1/2 or otherwise were defective in homologous repair were further delayed in cancer progression. While this is certainly an exciting development in the treatment possibilities for SOC patients, some caution is warranted. Reversion mutations enabling resistance to Niraparib may actually confer resistance to subsequent chemotherapeutics normally used upon disease recurrence [54].

Loss in BRCA1 enables microsatellite instability in mouse models and in colorectal cancer [55], though not in ovarian cancer [56, 57]. Microsatellite instability directly leads to centrosome

amplification, but SCNA instability, which may explain why it is observed in only a small minority of ovarian cancer patients, and is not linked to BRCA mutation status. Nonetheless, BRCA genes are inactivated through allelic deletions and expression modulation in ovarian cancer. Inactivation of *TP53* also suppresses BRCA1 expression [58]. BRCA1 interacts with ATM, directing it to phosphorylate p53 to enable p21 induction and G1/S phase cell cycle arrest [59, 60]. Without these complementary functions, tumors spontaneously form in BRCA1−/− p53+/− mice [61]. Centrosome duplication correlates with BRCA1 deletion [60]. This complex is thus a critical factor determining proper chromosome segregation during mitosis, and upon centrosome duplication aneuploidy is assured to form upon cell division [62, 63]. This is facilitated by prior mutations of p53 that abolish its checkpoint control function, again stressing the role for early mutation of TP53 in SOC.

Coincident SCNA events enable subsequent SCNA catastrophe. BRCA1 is located within kilobases of the neighboring autophagy gene BECN1. Autophagy is a critical catabolic infrastructure that enables cellular survival, requiring only 10% or less of normal autophagy gene dose [64]. Monoallelic loss of *BECN1* promotes centrosome amplification and aneuploidy among apoptosis-resistant cells [65]. An activator of the BECN1–PIK3C3 autophagy initiating complex, UVRAG, displays a similar phenotype [66]. Remarkably, this centrosome amplification promotes cell migration independent of its function in aneuploidy via indirect, hyperactivation of Rac1 [67]. LC3B, a ubiquitin-related protein which marks autophagosomal membranes, also acts in microtubule quality control [68]. One allele of the LC3B gene, *MAP1LC3B*, is genetically deleted in over 75% of SOC, independent of changes to *BRCA1* and *BECN1*. These three aneuploidy-accelerating lesions are likely to play key roles in serous ovarian cancer tumor initiation: mutant p53 expression, along with *BRCA1* and *BECN1* loss. Additional tumor suppressors may synergize to foster genomic instability: *NF1* is on the same chromosome arm as *BRCA1* and *RB1* lies nearby to *BRCA2*. Cumulative haploinsufficiency associated with prime targets and nearby neighbors certainly contribute to SOC aneuploidy [9] (**Figure 3**).

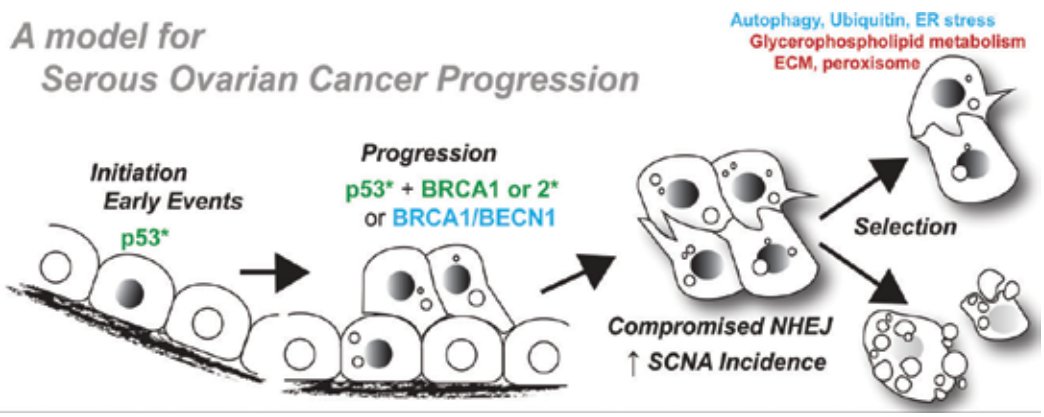

Figure 3. A model consistent with the ubiquitous mutation of p53 (SNV, indicated by green text) together with early SNV or haploid loss (indicated by light blue text) of repair enzymes, permitting a self-sustaining cascade of SCNAs and subsequent *in situ* selection for the observed SOC phenotypes.

In summary, *BRCA1/2* and homologous repair components are often suppressed by genetic deletions in SOC. This leads to further increases in SCNA formation and potentially independent metastatic phenotypes. However, SOC patients may benefit from the cancer's reliance on DNA repair pathways, as inhibition of PARPs prolongs progression free survival.

6. Pathways affected by SCNAs in serous ovarian cancer

Each cancer probably evolves at least 6–10 independent oncogene or tumor suppressor alterations [69] to circumvent natural homeostatic controls known as the "Hallmarks of Cancer" [70, 71]. These hallmarks include the evasion of regulated cell death, immortalization through telomere maintenance, defects in cell cycle control, immune system suppression, and enabling of metastatic capacity through physical and metabolic means. Traditionally, it has been assumed that single gene mutations are responsible for many of these oncogenic changes. Altered p53 function promotes escape to half of these hallmarks on its own, and mutations in strong oncogenes such as Ras family members, or growth factor receptor genes such as FGFRs, Her2 and even Met supplement many of the remaining hallmarks.

Individual gene amplifications can impact serous ovarian cancer. Aside from *TP53* and *BRCA1/2* mutations already discussed, tumors appear to be selected for specific chromosomal aberrations. Amplification of chromosomal region 8q, which contains the oncogenes *MYC* and *PTK2*, is a commonly found SCNV is SOC Myc overexpression promotes cell cycle progression, angiogenesis, and expression of target genes downstream of many other oncogenic factors such as NF-kB, β-catenin, and growth factor receptors [72]. Myc activation coordinately drives proliferation and promotes apoptosis, though since Myc-mediated apoptosis is p53-dependent, the pathway is averted. *PTK2*, the gene encoding focal adhesion kinase (FAK), enables metastatic phenotypes, cancer stem cell self-renewal, and neovascularization [73]. It is often co-amplified with *MYC*, as they both lie within cytoband 8q24. Myc overexpression is difficult to target therapeutically, although there are clinical trials underway for FAK inhibitors [74].

Recently, we analyzed single nucleotide and short 'in frame' deletion mutations across 120 validated oncogenes and tumor suppressors, finding that as many as 48% of serous ovarian primary tumors do not contain mutations in *any known* tumor suppressor or oncogene other than *TP53* [8]. Nonetheless, the average tumor has two-thirds of its genome altered by SCNAs. These findings support the notion that the actions of single oncogenes and tumor suppressors can only explain a portion of the genetics of ovarian cancer.

To analyze this, we developed new pathway network analytics tools to identify disrupted pathways in serous ovarian cancer in this unusually unstable genetic background. Despite the high levels of heterogeneity across patients, we found that coincident gene disruptions fell along surprisingly consistent patterns tumor-to-tumor, specifically suppressing or amplifying specific cellular pathways.

6.1. Autophagy

By far the most significantly suppressed pathway which stood out as unique in serous ovarian cancer and triple negative breast cancer was macroautophagy, which is most commonly

known, simply, as autophagy. The term autophagy ("*self-eating*") appropriately defines the process that cells use to recycle various macromolecular components, such as protein aggregates, lipids, and even entire organelles [75]. Autophagy is a primary method for the cellular catabolism, complementing turnover of proteins by the ubiquitin-proteosome system. Autophagy is described in terms of flux, which is the throughput of cellular '*detritus*' into autophagosomes, their transport to lysosomes, and subsequent enzymatic digestion. Ovarian cancer autophagy deletions impact the process primarily through deletions in *BECN1* (>75% of serous ovarian cancers) and in *MAP1LC3B* (>80% of serous ovarian cancers), though other genes are frequently affected. The average SOC tumor is 1 N across at least five different alleles; 95% of all serous ovarian cancers are deleted in BECN1 or *MAP1LC3B* and two others. The deficiency is as characteristic of SOC as p53 mutation. In addition to their roles in regulating chromosomal instability outlined above, *MAP1LC3B* and BECN1 (with the class three PI3 Kinase VPS34) play key roles in the formation of the early autophagosome, the phagophore, and recruitment autophagosome expansion proteins [76, 77].

Given this critical cellular function, we considered it counter-intuitive that cancer cells would delete a wide array of autophagy genes. In fact, KRAS mutant cancers have been described as "addicted" to autophagy, particularly in hypoxic or otherwise nutrient-stressed microenvironments [78]. This interpretation has been debated [79, 80], but the fact that autophagy has been established as a tumor suppressor system [81, 82], it is not exclusive of the possibility that specific tumor genotypes can promote addiction to autophagy [78]. Mono-allelic losses in the autophagy gene *BECN1* (homozygous deletions occur in only 0.9% of SOC cases) potentiate early development of tumors in mouse models [83, 84]. In this context, it is not at all counter-intuitive to consider that these gene losses likely synergize with defects in the BRCA1/2 pathway, the p53 pathway, and other initial SCNAs, thus producing the unique extreme level of aneuploidy associated with SOC. Moreover, the loss of gene copies does not completely "turn-off" autophagy. In fact, ovarian tumor cells, like other cells, require autophagy to provide clearing of protein aggregates, metabolic byproducts (especially in hypoxic environments), and possibly even to permit cell division, given their aneuploid state and relative chromosomal instability. This, in turn, may provide a second selection criteria for depressed autophagy. Autophagy is induced by missegregating chromosomes, chromosomal instability is a hallmark of SOC, and extreme induction of autophagy can promote cell death [85]. Therefore, it may be more appropriate to use the term "disrupted" rather than "suppressed" to define how ovarian cancer autophagy varies from that seen in normal somatic cells. The state renders SOC sensitive to agents that perturb autophagy by inhibiting the autophagic flux, or via the creation of proteotoxic stresses which must be resolved by autophagy (as discussed below).

6.2. Proteosome

Interestingly, a number of other proteostasis control pathways were suppressed in serous ovarian cancer, and foremost among these is complementary to autophagy, the ubiquitin-proteasome system. The core subunits, encoded by *PSMA1*, *PSMB1*, and *PSMC1*, are monoallelically deleted in 49, 62, and 41% of patients, respectively. Interestingly, the most interactive and deleted components of the proteasomal degradation pathway in ovarian cancer are enriched for cell cycle control related E3 ligases, including Park2, Fzr1, and Ube2d3. This suggests that not only is the core recycling process partly compromised by the core component

deletions, but that the pathway is redirected to allow for cell cycle progression proteins to persist and push the cell through division. The latter finding is perhaps to be expected, given that this has been established as a mechanism for tumor formation in many reviews [86–88]. Yet the proteasome may have a similar function to autophagy in suppressing aneuploidy. In a screen for mutations which are enabling for cell cycle progression in aneuploidy cells, ubiquitin-proteasomal degradation components were a top hit [89].

6.3. p53 Interactome

In addition to *TP53* gene mutation, serous ovarian cancer exhibits a number of p53-interacting components that are also suppressed by deletions. Among the top hits by HAPTRIG [8] include *CHEK2*, *BAX*, and *GADD45A/B* gene deletions, along with *CCNE1* and *ATR* amplification. Chk2 is a kinase which coordinates DNA repair and cell cycle arrest, in part by stabilizing p53. Bax is a pro-apoptotic Bcl-2 family member which associates with p53 to induce apoptosis [90]. The Gadd45 proteins mediate DNA damage signaling to p53 and act as tumor suppressors by leading to damage-induced senescence [91]. Conversely, an upregulated ATR network allows for potential enhancement of DNA repair pathways which lead to aneuploidy and may also lead to centrosome duplications [92]. This is further supported by a common suppression of Rad51 networks in SOC.

6.4. Metabolism

Metabolism is fundamentally disrupted in serous ovarian cancer. This may be predicted by the observation that patients with metabolic disruptions are at risk for disease, or have a predisposition to tumors to undergo metastatic growth to adipose tissue [93, 94]. A shift to glycolysis, the Warburg effect, is a general hallmark of cancer. Glycolytic shift is considered essential to provide the many constituent molecules required for cell division: nucleotides, lipids, and amino acids, moreso than simply ATP which is produced in higher quantities by oxidative phosphorylation [95]. A metabolic pathway found to be suppressed with almost equal magnitude to autophagy was the arginine and proline metabolism pathway, particularly through deletions in *SAT1* and *SAT2* and guanidinoacetate N-methyltransferase. Such deletions are predicted to reduce spermidine metabolism and polyamine formation, which is normally upregulated in tumors [96]. The reason for their ubiquitous suppression may lie in the increase in glutamate which would come from an inhibition of arginine biosynthesis, which can then be used in the TCA cycle [97].

6.5. Adipocytokine

Adipocytokine signaling and *fatty acid* metabolism was also altered, led by suppressed networks with the *CPT1B* gene and *ADH4,6,7*, and *1A*. Again, this result is unique and unexpected: *CPT1* isoforms are often upregulated in prostate cancer [98] as are ADH enzymes [99]. Dysregulation of ADH isoforms may enable acetaldehyde formation, which is oncogenic, or favor class I alcohol dehydrogenases, which are upregulated in cancerous ovarian tissue [100]. Conversely, one of the most upregulated metabolic pathways in serous ovarian cancer is glycerolipid metabolism. Upregulation is led by amplification of the *DGAT1* gene, encoding diglyceride acyltransferase, the committing step for synthesis of triglycerides and

an essential reaction for the formation of adipose tissue. The pathway is further reinforced by overexpression of *LPIN1* and *LPIN3* genes. While targeting metabolism has not historically been successful in cancer treatment, overexpression of these genes may act as early identifiers of ovarian cancer.

6.6. Peroxisome

An unusually altered pathway in serous ovarian cancer bridges metabolism, fatty acid oxidation and proteostasis disruption: *peroxisome* biogenesis. Peroxisomes are subcellular organelles whose primary function is to metabolize reactive-oxygen species and provide lipids to other organelles [101]. This pathway is amplified in serous ovarian cancer, and lung adenocarcinoma only [8]. *PEX5*, *PEX5L*, and *PEX19* are all commonly amplified. Pex5 and Pex19 bind to peroxisome enzymes in the cytosol and direct them to the peroxisome matrix [102, 103]. In fact, amplification of PEX5 is associated with poorer outcome in SOC. Peroxiredoxin-1 is also strongly amplified, and can be detected at increased levels in ovarian cancer patients' serum [104], and is also associated with lung cancer malignancy [105]. Upregulation of this pathway, and those associated with phospholipid metabolism may provide to a means to overcome oxidative stress, perform fatty acid beta-oxidation, and resist lipotoxicity associated with invasion of adipocyte-rich regions of the omentum.

While each of these pathways can help to define phenotypes associated with SOC, they also have the capacity to enable development of new classes of pathway-targeted therapeutics. It may be possible in future for SCNA-modified pathways to serve as targets the same way that SNVs do, now.

7. Potential for new treatments by targeting copy number alterations

SNVs have a proven track record of targetability using small molecules. Nonetheless, in the case of SOC, new cures are unlikely to be found unless somatic copy number alterations (SCNAs) are considered. Defining this interplay will be a difficult task. It remains unclear exactly which SCNAs are most critical to SOC proliferation and metastasis. The creation of cell line models will require new methods of whole-chromosome manipulation, even as attracting pharmaceutical company support will be harder due to limited experience which such targeting strategies, as well as conservative business approaches towards eventual clinical adaptation. Nonetheless, there are reasons to be optimistic that SCNA-targeted therapeutics can be effective and that some could enter the clinic in the near future.

Consider the abundance of SCNAs in advanced SOC relative to other cancer. The successful tumors have undergone selection. The phenotypes produced include well-known hallmarks of cancer: including cell cycle defects, heightened glucose uptake [106], spontaneous proliferative immortality [107], and dysregulated autophagy [108]. The same studies identify aneuploidy-associated characteristics which present vulnerabilities particular to these unstable cells. Perhaps the most promising vulnerability is an increased reliance on protein quality control processes such as ribosome biogenesis and maintenance factors and the cellular recycling process, autophagy. Aneuploid cells require these systems to function, and may result in a general

reliance on catabolic function due to the proteotoxic effect of protein-complex subunit imbalance. Early studies recognized a general, if partial, sensitivity of these cells to rapamycin [106].

Chromosome instability can incur resistance to taxanes, a common front line therapeutic for SOC [109]. Chromosomal instability endowed by docetaxel may in fact lead to subsequent additional chemoresistance [110], though it is clear that the resistant phenotype is at least initially offset by an increased sensitivity to carboplatin, the second primary chemotherapeutic co-administered with a taxane as standard of care. Although aneuploidy enables oncogenic characteristics, it offers targetable vulnerabilities as well.

The mapped SCNA patterns in SOC revealed a general fault in proteostasis control, centered on autophagy [8]. Yet these cells require autophagy to maintain viability. The delicate balance within SOC relative to normal tissue, appears to provide a therapeutic window for proteostasis-targeting agents. Since SOC cells are already severely disrupted in their proteostasis-regulatory mechanisms, further disturbance can greatly compromise survival even as normal cells readily process the insult. Given this premise, we developed the Combination of Autophagy Selective Therapeutics (COAST) method to effectively manage SOC in the lab [8]. The general approach involves directly stressing the proteostasis system, while inhibiting autophagy resolution (**Figure 4**).

Mice given the cocktail of five proteostasis drugs did not lose weight nor negatively alter their blood chemistry panel [111], tolerating these drugs for months of daily treatment [8]. In mouse models using recurrent human SOC cells, the proteostasis drugs out-performed platinum-taxane dual treatment. The results are consistent with previous approaches pursuing a "cyclops" hypothesis: that monoallelic deletions in cancer sensitize cancer cells to further disruption of that gene's function [112]. As normal cells bear a full complement of

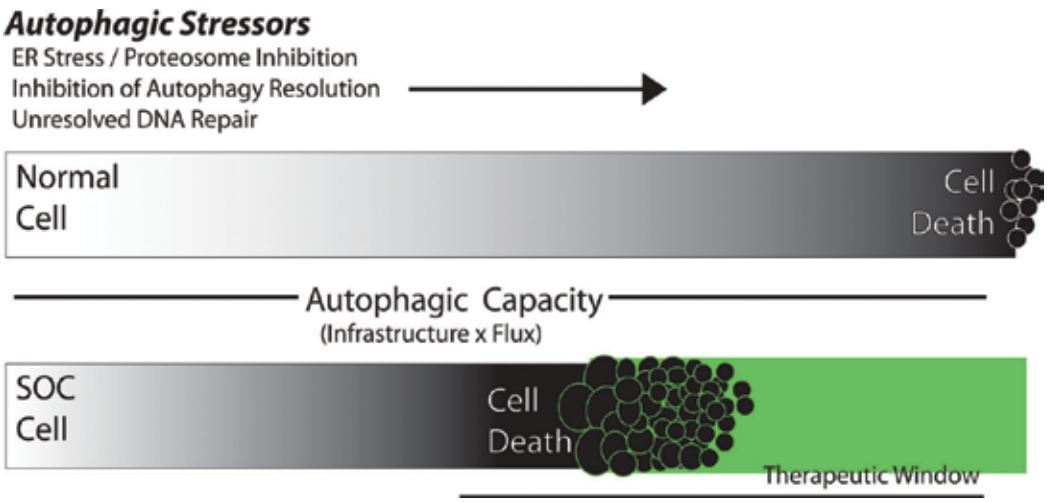

Figure 4. SCNA can compromise key cellular infrastructure. The figure shows how the disruption of autophagy capacity that is observed in SOC can render the cells more sensitive to agents that modulate autophagy. The lack of genetic infrastructure, combined with a constant requirement for autophagy, opens a therapeutic window for these agents. Nonetheless, the heterogeneity within tumors and between patients insures that no single agent, used alone, will provide a complete benefit.

all pathway genes, they are typically less sensitive to such stresses, which opens therapeutic windows for treatment.

Given that proteostasis pathways are only one type of disruption caused by SCNAs in SOC, what other SCNA-disrupted pathways might be targetable? Alluring targets may include the proteins downstream of the E3 ligases commonly deleted in SOC; inhibition of cell-cycle regulators may selectively target SOC cells even without any mutation or than copy number changes. Strong amplification of peroxisome transporters and the glycerophospholipid metabolism pathways suggest that metabolic targeting may be worthwhile. *GSK3B* controls signaling between varying development and stem cells pathways [113], and was marked as the most cumulatively impactful amplified gene in SOC by HAPTRIG [8]. Interestingly, inhibition of GSK3β in preclinical models of SOC showed strong tumor inhibition [114], and a number of drugs targeting GSK3β are in development for cancer, diabetes, and neurodegeneration [115]. Finally, the 8q24 region is the most commonly amplified genomic region in SOC, containing MYC and FAK. Inhibitors to FAK, such as defactinib, are already being tested in the clinic [116], and will hopefully provide some positive results in the near future.

A caveat of such designs is heterogeneity inherent in disease. Copy number instability is the result of SOC cells' extraordinary ability to create, tolerate, and expand genomic variation. Mathematical modeling of real tumor genetic data suggests that even small tumors with low mutation rates are statistically likely to contain multiple independent clones able to resist a particular drug treatment [117, 118]. Current SOC chemotherapeutics stimulate aneuploidy. Taxanes result in chromosome missegregation and platinum agents promote translocation events due to cross-strand DNA lesions. While it is absolutely true that common chemotherapeutics have limited combination potential due to dose limiting toxicities, that does not preclude the use of highly specific drugs to be used in combination or as maintenance therapy in SOC treatment regimens. Most likely, drugs independently targeting the many SCNA-disrupted pathways may be required to completely cure a patient.

While most patients are caught late in the evolution of their disease, it may not be "too late" to treat them. A genomic analysis in highly metastatic recurrent SOC patients found that the tumors likely form a metastasis-to-metastasis spread [14]. This may explain why round after round of different chemotherapy can extend the life of SOC patients [119]. This implies that current chemotherapy is quite effective at destroying a great majority of cells, and the challenge that remains is how to complement it. The COAST strategy studied in our lab functioned equally well or better for cisplatin resistant forms of SOC [8]. Autophagy has been widely implicated in the ability of quiescent cells to survive, including in SOC [120], and has been directly shown to enable growth of Doxil resistant disease [121]. However, given that one COAST agent, chloroquine is often prescribed for the same patient for decades in high-risk malaria areas, while another, nelfinavir, is a daily long-term HIV medication with no serious side-effects, the use of COAST is warranted based on the decades of use of COAST drugs, in humans, for diseases other than cancer. The side effects are well established to be below current chemotherapeutics carboplatin, paclitaxel, and Doxil [111]. The greatest health concern may lie in kidney cells, which are also exquisitely sensitive to autophagy drugs [122].

Since the drugs target different but complementary pathways, it is feasible to design clinical trials involving either simultaneous treatment or sequential treatment, enabling a greater

chance of minimized side effects. Compromised DNA repair feeds into this pathway, suggesting that the recent successes of the PARP inhibitors are not simply due to BRCA1 complementation. An expanded range of options must be aggressively explored in the near future if we are to understand how to exploit the SCNA genetics of ovarian cancer in a timely fashion.

Abbreviations

AOC	Australian Ovarian Cancer (study)
N	the number of copies of a given gene present in a cell (e.g., 3N)
SCNA	somatic copy number alteration
SNV	single nucleotide variant (a point mutation)
SOC	serous ovarian carcinoma
TCGA	the Cancer Genome Atlas
TP53	tumor protein 53 kDa gene (protein is p53)

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Chapter 6

Ubiquitin Signaling in Ovarian Cancer: From Potential to Challenges

Sumegha Mitra

Additional information is available at the end of the chapter

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Abstract

Ubiquitin proteasome system (UPS) is an emerging arena in cancer intervention. Dysregulation of various UPS components has been implicated with many cancers, and this knowledge is starting to be exploited for its role in cancer initiation, progression, and therapeutics. UPS regulates both protein turnover and non-proteolytic regulatory function of the proteins involved in cell cycle, signal transduction, DNA repair, histone modification, and transcription. In addition, chromosomal aberrations and genomic alterations often present in the cancer cell genomes lead to excess of conformationally challenged aggregation-prone proteins and proteotoxic stress that make cancer cells more dependent on UPS-mediated protein degradation than normal cells. This proposition is the basis of the clinical use of proteasome inhibitor, Bortezomib, to treat multiple myeloma and mantle cell lymphoma targeting cancer cells and mostly sparing the normal cells. This chapter provides an overview of various components of UPS which are implicated in cancer and regulate ubiquitin-mediated oncogenic signaling in ovarian cancer.

Keywords: ovarian cancer, mutant p53, ubiquitin, proteasomes, deubiquitinating enzymes

1. Introduction

Ovarian cancer is the most lethal gynecologic malignancy with a high case-to-fatality ratio [1]. According to American Cancer Society, approximately 22,440 new cases of ovarian cancer will be diagnosed in the year 2017 and about 14,080 women in the United States will die from this deadly disease [2]. About 90% of ovarian carcinomas are heterogeneous epithelial neoplasms with distinctive biology and clinicopathologic features at cellular and molecular

levels [1, 3]. The clinical management of ovarian cancer has addressed this heterogeneity and classified ovarian cancer into high-grade and low-grade serous, endometrioid, clear cell, and mucinous subtypes based on the histology, tissue of origin, prognosis, and genetic alterations that deregulate specific signaling pathways in these tumor cells [4, 5] (**Figure 1**). Of these, high-grade serous ovarian cancer (HGSOC) is the most prevalent and lethal subtype of ovarian cancer. It accounts for 70–80% of ovarian cancer deaths [1]. The low *five-year survival rate* of HGSOC patients is attributed to the late detection of extensively metastasized disease, especially to omentum, which is the primary site of ovarian cancer metastasis. Moreover, about 80–90% of HGSOC patients eventually develop chemo-resistant tumors, after an initial positive response to cytoreductive surgery and chemotherapy, which are important prognosticators of the survival of HGSOC patients [1, 3]. The initiation and development of HGSOC is known to proceed through the early acquisition of genetic alterations in the tumor suppressor gene *TP53* [3, 6]. About 96% of HGSOC patients carry gain-of-function (GOF) mutations in *TP53* gene [3]. It is believed that *TP53* mutations lead to the precursor lesions in fallopian tube fimbria, which develop into serous tubal intraepithelial carcinoma (STIC) and ultimately to HGSOC [7, 8]. The reduced risk of ovarian cancer in BRCA1 mutation carriers after salpingo-oophorectomy supports the theory of HGSOC origin from STIC [9]. Mutant p53 orchestrates a distinct pro-tumorigenic signaling network and confer chemo-resistance through transcription-dependent and independent mechanisms in cancer cells. A recent study in triple-negative breast cancer cells revealed the role of mutant p53-proteasome axis in regulating global effects on cancer cell's protein homeostasis, inhibiting tumor suppressive pathways or turning on the oncogenic signaling in cancer cells [10]. A growing number of evidences suggest the role of ubiquitin signaling in tumor progression and growth. This chapter discusses the role of ubiquitin-mediated signaling in ovarian cancer pathogenesis. The different components of ubiquitin proteasome system, which are involved in this regulation, will be highlighted.

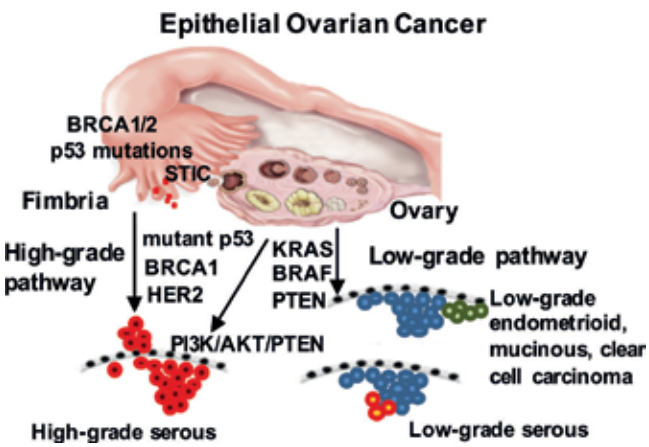

Figure 1. A schematic representation of molecular drivers of low- and high-grade ovarian cancer initiation and progression. Low-grade tumors are low malignant potential (LMP) tumors associated with KRAS or BRAF mutation and loss of PTEN. High-grade serous tumors frequently have mutated *TP53* gene as well as activated members of PI3K/Akt pathway. Highly invasive tumors originate from the fallopian tube precursor lesion, STIC, and spread to the ovary and other peritoneal surfaces. Genotoxic stresses in BRCA1/2 carriers predispose them to ovarian cancer.

1.1. Conceptual overview of ubiquitin modifications

Protein ubiquitination is a dynamic multifaceted posttranslational modification (PTM), which is involved in nearly all biological functions in a eukaryotic cell. Similar to phosphorylation, it functions as a signaling device and can be activated by extracellular stimuli, DNA damage, phosphorylation, ligand-dependent receptor activation, and signal transduction. Ubiquitin is a highly conserved 76-amino acid protein, which is expressed in all cell types. It has seven lysine (Lys or K) residues, K6, K11, K27, K29, K33, K48, and K63. Each lysine residue can result in a linkage-specific ubiquitin chain of certain topology [11, 12], which when bound to the target protein (substrate) dictates the fate of the protein (**Figure 2**). For example, the most predominant K48-linked polyubiquitin chains, which have a compact conformation, lead to the proteasomal degradation of the bound substrate. By contrast, the second most abundant K63-linked chains, which have an open conformation, are involved in non-proteolytic regulatory functions [13]. The K11-linked ubiquitin chains act as an additional proteasomal degradation signal, particularly in cell-cycle regulation [13]. The functions of the other lysine-specific ubiquitin chains remain less well characterized. K6-linked chains are shown to be upregulated with UV genotoxic stress and are known to be associated with BRCA1/BARD1 complex [14]. Similarly, K27 chains act to serve as scaffolds for protein recruitment such as p53-binding protein 1 in the DNA damage response. In addition, ubiquitin chain of mixed topology with different linkage at succeeding positions is also seen as in NF-κB signaling or in protein trafficking (**Figure 2F**) [13]. Moreover, branched ubiquitin chains of unknown function are generated when a single ubiquitin is modified with multiple molecules [12, 13]. These ubiquitin chains creating a multitude of signals with distinct cellular outcomes are referred to as "ubiquitin code" [13]. New layers of the ubiquitin code are emerging, based on findings that revealed the modification of ubiquitin chains with small ubiquitin-like (Ubl) modifier such as SUMO, phosphorylation, and acetylation [13].

Box 1. The discovery of ubiquitin-mediated protein degradation in the late 1970s by Drs. Avram Hershko, Aaron Ciechanover, and Irwin Rose was awarded 2004 Nobel Prize in Chemistry. Their study highlighted the role of protein ubiquitination in selective protein breakdown, regulating the cellular functions by modulating the levels of key enzymes, regulatory proteins and removal of abnormal proteins that arise by biosynthetic errors or post synthetic damages. Ubiquitin was first isolated from bovine thymus in 1975 by Goldstein et al. (PNAS, 1975;72:11-15) [88] and found to be covalently attached to histone 2A (Goldknopf and Busch, PNAS, 1977;74:864-868) [89]. Subsequently, Drs. Hershko, Ciechanover, and Rose in a series of biochemical studies discovered and characterized the ATP-dependent, ubiquitin-mediated protein degradation using the reticulocyte lysate system (PNAS, 1979;76:3107-3110) [90].

Ubiquitination is an orchestrated enzymatic reaction of E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and ubiquitin E3 ligase (E3). It is the most coordinated and conserved multistep process of covalently tagging a protein with mono- or polyubiquitin chain. The process begins with the ATP-dependent activation of ubiquitin by E1 ubiquitin-activating enzyme (E1s), which then transfers it to the active site cysteine of E2 ubiquitin-conjugating enzymes (E2s) forming a thioester linkage between ubiquitin and cysteine. Ubiquitin E3 ligases (E3s) have a central role in this process, as they recognize the specific protein substrates and facilitate the transfer of ubiquitin from the E2 onto the target protein [11, 12]. Deubiquitinating

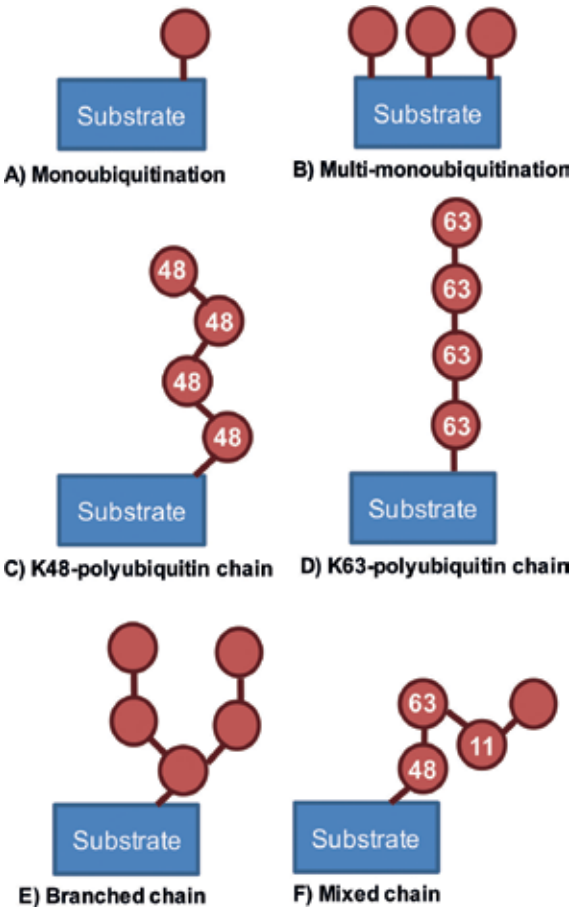

Figure 2. Linkage-specific ubiquitin chains of different topologies. Each circle represents one ubiquitin moiety. (A) Monoubiquitination, (B) multi-monoubiquitination, (C) K48-linked chain, (D) K63-linked chain, (E) branched chain, and (F) mixed chain.

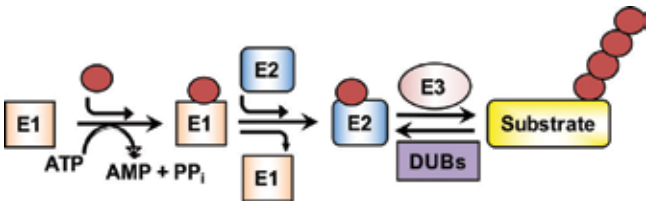

Figure 3. Enzymatic cascade of ubiquitin proteasome system. Ubiquitin is activated and conjugated to target protein by a conserved action of E1-ubiquitin-activating enzyme, E2-ubiquitin-conjugating enzyme, and E3 ubiquitin ligase.

enzymes (DUBs) are another class of enzymes, which removes or edits the ubiquitin chains attached to a protein, making this a highly reversible process and thus highlighting the dynamic regulation of ubiquitin signaling in the cell (**Figure 3**). These enzymes together with proteasomes, a cellular machinery involved in ubiquitin-mediated protein degradation, comprise the

ubiquitin proteasome system (UPS). UPS plays an indispensable role in regulating ubiquitin-mediated proteolytic and non-proteolytic regulatory signaling to control cellular homeostasis, protein stability, and a wide range of signaling pathways.

2. UPS components in ovarian cancer

Ovarian cancer is characterized by multiple genetic and epigenetic abnormalities and several major (about seven) activated signaling pathways, which are directly or indirectly implicated with UPS. Moreover, several UPS components, E1s, E2s, E3s, DUBs and proteasomes are known to be deregulated or mutated in cancer (**Table 1**), suggesting their role in cancer signaling and cancer progression. This section discusses each UPS component implicated in ovarian cancer and the role of key players of each component in regulating ovarian cancer signaling (**Figure 4**).

2.1. E3 ligases

E3 ligases (E3s) are the most heterogeneous class of enzymes in UPS as they facilitate ubiquitination with exquisite spatial, temporal, and substrate specificity. There are more than 600 E3s in a human genome, indicating the precise substrate specificity of E3s [15]. E3s can be classified into three main types, RING E3s, HECT E3s, and RBR E3s depending on the presence of type-specific domains and on the mechanism of ubiquitin transfer to the substrate protein. RING E3s are the most abundant type of ubiquitin ligases. They are characterized by the presence of zinc-binding domain called Really Interesting New Gene (RING) and U-box domain. RING E3s mediate a direct transfer of ubiquitin to substrate, functioning as a scaffold to orient the ubiquitin-charged E2, whereas E3s with homologous to the E6AP carboxyl

Gene.	Role	Effect	Cancer [references]
BRCA1	E3 ligase	Mutation, loss of tumor suppressor function	Ovarian and breast cancers [19, 20]
USP13	DUB	Amplification, oncogene	Ovarian cancer [41]
Mdm2	E3 ligase	Overexpression, loss of p53 tumor suppressor function	Ovarian cancer and various malignancies [63, 64]
USP7	DUB	Overexpression, oncogene	Ovarian cancer [42]
Skp2	E3 ligase	Overexpression, loss of tumor suppressor function of p27	Ovarian, breast, and prostate cancers [76–81]
UCHL1	DUB	Overexpression or methylation, role varies with cancer	Ovarian, breast, gastric, lymphoma, lung, Esophageal squamous cell carcinoma [44–48]
FBW7	E3 ligase	Mutation, loss of tumor suppressor function	Ovarian and endometrial cancer, leukemia [71]
VHL	E3 ligase	Mutation, loss of tumor suppressor function	Clear-cell carcinoma, lung cancer [49]

Table 1. Cancer-associated alterations in UPS.

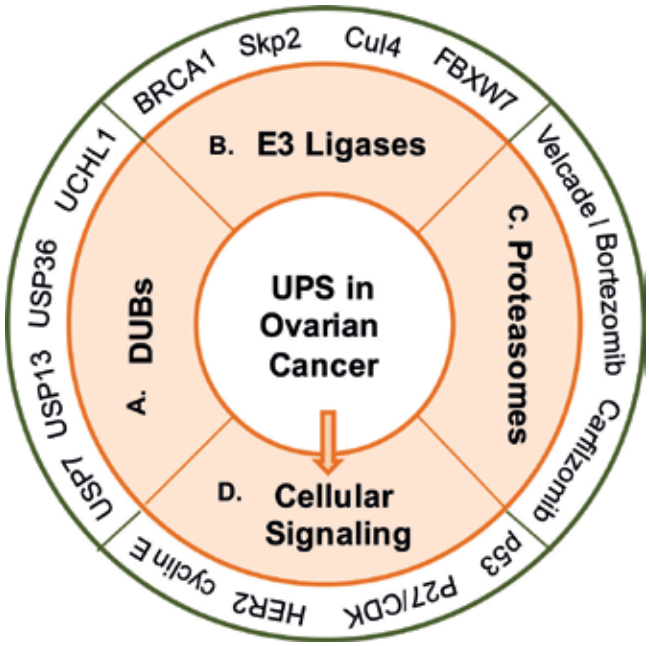

Figure 4. Key players of each UPS component involved in regulating ovarian cancer signaling. (A and B) DUBs and E3 ligases as candidate genes in ovarian cancer, (C) proteasomal activity and inhibitors in ovarian cancer, and (D) regulation of ovarian cancer oncogenic signaling by UPS.

terminus (HECT) domain transfer ubiquitin to the substrate in a two-step process—ubiquitin is first transferred to a catalytic cysteine on E3 and then to the substrate. Based on their N terminus extensions, HECTs are further classified into three subfamilies: Nedd4 family, HERC family, and other HECT that contain various domains. The RBR E3s are characterized by the presence of three RING domains, RING1 and RING2, separated by an in-between-RING (IBR) domain. RING1 recruits the ubiquitin-charged E2, RING2 possess catalytic cysteine. The IBR is called benign-catalytic domain as it lacks catalytic cysteine residue [15]. Given their cellular specificity and complexity, E3s are implicated in a number of pathophysiological conditions, which makes them an attractive therapeutic target in human diseases, including cancer [16].

2.1.1. BRCA1

The breast and ovarian cancer susceptibility gene, BRCA1, is a tumor suppressor gene [17]. Heterozygous mutations in BRCA1 gene predispose women to both familial and sporadic breast and ovarian cancers [18, 19]. Nonetheless, BRCA1 mutations are also associated with other cancers like stomach, pancreas, prostate, and colon [20]. BRCA1 acts as a hub protein, which participates in several different protein complexes to coordinate a diverse range of cellular functions including DNA repair, cell-cycle regulation, apoptosis, transcriptional regulation, and centrosome duplication to maintain genomic stability [17]. The structural analysis of BRCA1 protein suggested that it has a RING finger domain that harbors E3 ubiquitin ligase activity [14]. In addition, BRCA1 forms a heterodimer complex with BARD1, a protein with a RING finger domain [14]. BARD1 interaction stabilizes the proper conformation of BRCA1

RING domain for a potent E3 ligase activity and interaction with E2 UbcH5 [14, 21, 22]. BRCA1 E3 ligase substrate specificity is believed to depend on its phosphorylation-dependent binding to proteins containing phospho-SXXF motif such as CtIP, BACH1, and ABRA1 through a phospho-peptide recognition domain (BRCT) [23, 24]. The strong relation between BRCA1 tumor suppressor properties and E3 ligase activity is evident from the clustering of missense mutations that predispose to cancer in the Zn^{2+}-binding residues of BRCA1 RING finger domain crucial for its ubiquitin ligase activity [25]. The full range of function of BRCA1/BARD1 complex is not completely understood [18]. One of the most important functions of BRCA1 is to repair DNA double-strand breaks (DSBs). Following DNA damage, chromatin-associated histone H2AX phosphorylation by ATM and ATR at DNA damage site recruits an E3 ubiquitin ligase RFN8 and a phospho-module-binding mediator MDC1 at the damage site [17, 26–28]. RFN8 together with ubiquitin conjugase Ubc13, ubiquitinate histone H2A and H2B at chromatin lesions, which in turn translocate BRCA1 complex containing RAP80, a protein with ubiquitin-interacting motif (UIM), ABRA1, protein that interacts with BRCA1 BRCT domain and deubiquitinating enzyme, BRCC36 to Lys6- and Lys63-linked polyubiquitin chains at DSBs [17, 26, 29]. BRCA1 has also been implicated with the transcriptional activation of genes in response to DNA damage. The C-terminus of BRCA1 complexes with RNA polymerase II through RNA helicase, while N-terminus BRCA1/BRAD1 heterodimer binds to RNA polymerase II holoenzyme [30]. Identifying genes regulated by BRCA1 would shed a significant light on the transcriptional role of BRCA1. However, BRCA1 overexpression studies have shown induction in p53-responsive E3 ligase, mdm2, cell-cycle inhibitor, p21 and stress-response factor, and GADD45 in breast and small-cell lung cancer cell lines [31, 32]. Besides, BRCA1 also regulates G1/S, S-phase, and G2/M cell-cycle checkpoints through interactions with RAD3, ATM/ATR, and Chk1/Chk2 [26, 30, 33].

Over the last 10 years, significant information has been gained about the structure, function, and unique features of BRCA gene products, BRCA1 and BRCA2, which collectively contributes to the biological response to DNA damage through homologous recombination of DNA repair and regulation of cell-cycle checkpoints. BRCA1/2-deficient cancers, including ovarian cancer, are now recognized as the target for a class of drugs known as PARP (poly ADP-ribose polymerase) inhibitors [34]. PARP detects and initiates an immediate cellular response to metabolic or radiation-induced single-strand DNA breaks (SSB). It binds to DNA and synthesizes polymeric adenosine diphosphate ribose (poly ADP-ribose or PAR), which acts as signal to other DNA-repairing enzymes. PARP inhibition directly blocks the PARP enzymatic activity and subsequently leads to PARP accumulation on DNA, a process called PARP trapping, which converts an SSB into a double-strand DNA break through the collapse of replication fork [34]. BRCA-deficient tumor cells with impaired homologous recombination repair of double-strand DNA breaks are directed toward the error-prone repair process of non-homologous end joining which leads to genetic instability and cell death. Thus, BRCA1/2-deficient ovarian cancer cells with PARP inhibition undergo synthetically lethal cell death [34]. PARP inhibitor, Olaparib manufactured by AstraZeneca, is in phase I/II clinical trials for BRCA-deficient high-grade serous ovarian cancer [34]. Olaparib-treated ovarian cancer patients with BRCA1/2 mutation had a progression-free survival of 11.2 months compared to 4.3 months of patients receiving placebo [35]. In summary, BRCA is an ideal example of E3 ubiquitin ligase playing an essential role in ovarian cancer and its intervention.

2.1.2. Cullin-RING ligases: cullin 4

Cullin-RING ubiquitin ligases (CRLs), composed of CUL1, 2, 3, 4A, 4B, 5, and 7, are the largest family of E3s that ubiquitinate a wide array of substrates involved in cell-cycle, DNA-damage response, chromatin remodeling, and gene expression. Cullin (CUL) neddylation, a process of adding ubiquitin-like protein—NEDD8 to the cullin [36], is crucial for their activation. Neddylation is catalyzed by NEDD8-activating enzyme E1 (NAE), NEDD8-conjugating enzyme E2 (UBC12), and NEDD8-E3 ligase. The genome-wide analysis of human cancers revealed *CUL4A* amplification in 20% of the basal-like breast cancer subtype, characterized as "triple negative," and CUL4A levels were associated with aggressive growth and poor prognosis. Dysregulation of CUL4A in multiple tumor types leads to the hypothesis that CUL4A plays a role in promoting oncogenesis [36]. High CUL4A expression and activity in ovarian cancer is implicated with cancer cell proliferation and survival. NEDD8-activating enzyme inhibitor, MLN4924, which blocks cullin neddylation activation, is reported to induce cell-cycle arrest, apoptosis, and tumor cell growth in epithelial ovarian cancer cells. In addition, MLN4924 sensitized ovarian cancer cells to chemotherapeutic drug treatments [37].

The role of Skp2 and FBXW7 in ovarian cancer signaling is discussed in the next section.

2.2. Deubiquitinating enzymes

Reversibility is an important aspect of ubiquitin system, which is mediated by deubiquitinating enzymes or deubiquitinases (DUBs). DUBs are essential components of UPS that possess ubiquitin-isopeptidase activity and catalyze the removal of ubiquitin from the target proteins. Thus, DUBs play a crucial role in the regulation of ubiquitin-mediated regulatory and proteolytic signaling [11, 38]. DUBs activity affect the activation, recycling, localization, and turnover of multiple proteins, which in turn regulate cellular homeostasis, protein stability, and a wide range of signaling pathways [39]. DUBs also maintain ubiquitin homeostasis in the cell by generating free ubiquitin monomers, which is essential for ubiquitin-mediated regulation of cell function [38]. Consistent with this, an altered DUB expression or activity has been implicated with several diseases including cancer. Numerous DUBs have been characterized as oncogenes mediating cancer initiation and progression [11, 40]. Therefore, pharmacological interventions targeting DUB activity using small molecule inhibitors are being used as a rationale to search for novel anticancer drugs [11].

Box 2. About 98 DUBs are reported in human genome, which are mainly divided into five families based on their sequence and structural homology: Ubiquitin-specific protease (USP), ubiquitin carboxyl-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado Joseph disease proteases (MJD), and JAB1/MPN/Mov34 (JAMM) metallopeptidases. Most DUBs are cysteine proteases except JAMMs, which belong to catalytic class of metalloproteases. The recent discovery of DUBs with the selectivity of cleaving extended Lys-48-linked polyubiquitin chains belongs to new family of DUBs named Mindy. The DUB-substrate specificity somewhat depends on ubiquitin chain linkage and topology; however, by large, given the complexity of ubiquitin system, it remains unknown [38].

DUBs role is evident in several cancers including Fanconi anemia (USP1), prostate cancer (USP2), adenocarcinoma (USP4), non-small-cell lung carcinoma (USP7), glioblastoma (USP15), myeloma, and leukemia (USP9x) [11, 39]. Han et al. identified the role of USP13 as

the master regulator of ovarian cancer metabolism [41]. They reported the co-amplification of USP13 gene with PIK3CA (phosphatidylinositol-3-kinase catalytic subunit, α-isoform) in 29.3% of high-grade serous ovarian cancer patients and its association with poor clinical outcome. USP13 stabilized the protein levels of two key metabolic enzymes, ATP citrate lyase and oxoglutarate dehydrogenase, which in turn regulate the mitochondrial respiration, glutaminolysis, and fatty acid synthesis in ovarian cancer cells. USP13 inhibition suppressed ovarian tumor progression and sensitized the tumor cells to PI3K/AKT inhibitor [41]. Similarly, USP7 (also known as HAUSP, herpes virus-associated ubiquitin protease) plays a crucial role in ovarian cancer [42]. USP7 is a DUB for MDM2, which prevents MDM2 autoubiquitination, leading to its stabilization and consequent induction of p53 degradation. Treating an ovarian cancer xenograft model with a novel inhibitor of USP7, CDDO-Me suppressed tumor growth. CDDO-Me directly binds to USP7, which leads to a decrease in its substrate Mdm2, Mdmx protein levels [42]. USP4 overexpression is reported in invasive breast carcinoma, enhancing TGFβ signaling by stabilizing SMAD2/SMAD4 complex but not much is known about its role in ovarian cancer [11]. USP36 expression is increased in ovarian cancer cells compared to normal ovarian surface epithelium; however, further studies are needed to understand its role in ovarian cancer [43]. DUB UCHL1 (ubiquitin-carboxyl terminal hydrolase 1) plays a contradicting role in different cancers [11]; it is reported as a methylated tumor suppressor gene in ovarian cancer [44, 45], while it is overexpressed in lymphoma, esophageal squamous cell carcinoma, renal, lung cancers, and acts as an oncogene [46–48]. Under hypoxic conditions, UCHL1 is shown to deubiquitinate and stabilize HIF-1α and promote tumor metastasis [49, 50]. We for the first time identified the oncogenic overexpression of UCHL1 in high-grade serous ovarian cancer and association with poor clinical outcome (unpublished data). These studies suggest the emerging role of DUBs in ovarian cancer and the potential of DUB inhibitors in neo-adjuvant therapies for ovarian cancer.

2.3. Proteasomes

The efficient and selective degradation of cellular proteins is essential for protein quality control and maintenance of cellular homeostasis [51]. Impaired protein quality control and degradation is associated with many human diseases such as cancer, cardiovascular diseases, and aging-related pathophysiological conditions such as Alzheimer's and Parkinson's. UPS mediates targeted protein degradation under both normal and malignant conditions [52]. However, cancer cells are more dependent on UPS-mediated degradation to promote the degradation of tumor suppressors and various cell-cycle checkpoint proteins as well as to reduce proteotoxic stress accumulated due to genomic aberrations [53]. The 26S proteasome is a multi-subunit complex that contains one barrel-shaped 20S catalytic core particle (CP) and 19S regulatory particle (RP) that binds to one or both the ends of barrel-shaped CP. The active degradation of proteins is regulated by 20S CP harboring proteolytic active sites while 19S RP regulates substrate binding and target protein entry into 20S [52].

The amazing efficacy and clinical use of proteasome inhibitor Bortezomib (PS-341, Velcade) for the treatment of multiple myeloma and mantle cell lymphoma has encouraged researchers to explore the possibility of targeting other components of the UPS for cancer treatment [54]. However, Bortezomib has not demonstrated a significant activity against other solid

tumors [55]. This conundrum has spurred the development of next-generation proteasome inhibitors, including MLN9708 (Millennium Pharmaceuticals), Carfilzomib and ONX0912 (Onyx Pharmaceuticals, South San Francisco, CA), and CEP18770 (Cephalon, Frazer, PA) [56]. Although these compounds target the same 20S CP, they differ in targeted active site and enzyme kinetics, resulting in activity differences based on tumor type and tumor location. Bazzaro et al. reported elevated levels of ubiquitinated proteins and 19S and 20S proteasome subunits in both low-grade and high-grade ovarian carcinoma tissues and cell lines compared to benign ovarian tumors and immortalized normal ovarian surface epithelium controls. They reported an increased sensitivity to apoptosis in proteasome inhibitor, PS-341 treated cells, and a reduced growth of ES-2 ovarian carcinoma xenograft in immunodeficient mice [57]. In a similar study, proteasome inhibitor, MG132—a peptide aldehyde—showed an enhanced sensitivity of ovarian cancer cells, SKOV3 to cisplatin both *in vitro* and *in vivo* [58]. The effect of Bortezomib on ovarian cancer cells is also supported by the increased sensitivity of Bortezomib-treated chemoresistant ovarian cancer cells to TRAIL-induced apoptosis [59]. Together, these results indicate the essential role of proteasomes in mediating prosurvival signaling in cancer, which may also be due to altered proteasome composition resulting in an enhanced proteasomal activity [52].

3. UPS in ovarian cancer cellular signaling

Several important factors that are implicated in the molecular pathogenesis of ovarian cancer are known to be regulated by UPS, highlighting its significance in disease progression. Some of these factors are discussed subsequently.

3.1. Tumor suppressor p53 and Mdm2

Tumor suppressor protein p53 is a multifunctional sequence-specific transcription factor that plays a key role in cellular stress response. Abrogating p53 function is a key event in human cancers, leading to the deregulation of cell cycle, genetic instability, resistance to stress signals, and resulting in cancer development [60]. Due to its growth inhibitory properties, p53 is maintained at low levels in the normal cells. The E3 ubiquitin ligase Mdm2 promotes p53 ubiquitination and subsequent proteasomal degradation [61]. In addition, E4 ubiquitin ligase p300/CBP promotes polyubiquitination of p53 to accelerate its degradation by proteasomes [61]. Although Mdm2 is the predominant E3 ligase for p53, several other E3 ligases have been identified that can promote the degradation of p53, including C-terminus of HSP70-interacting protein (CHIP), murine double minute 4 (MdmX), and p53-induced protein with a RING H2 domain (Pirh2) [60]. In addition to proteolytic ubiquitination, p53 mono-ubiquitination mediates p53 nuclear export and activity [62]. Thus, UPS plays a crucial role in maintaining and regulating p53 functions.

Several cancers, including invasive breast cancer, pediatric rhabdomyosarcoma, and soft-tissue sarcoma, exploit Mdm2-p53 pathway to maintain low p53 levels under genotoxic or oxidative-stressed environment of cancer cell. Thus, Mdm2 gene amplification and overexpression

have been reported in many cancers [63]. In addition, the expression and activity of Usp7, a deubiquitinating enzyme for Mdm2, is increased in several cancers including breast and ovarian cancer, which prevents Mdm2 ubiquitination and promotes its stability. Reduced tumor growth was seen in an ovarian cancer xenograft model treated with Usp7 inhibitor [42]. On the other hand, when p53 acquires gain-of-function (GOF) mutations as in the case of nearly half of the cancers, it gains oncogenic functions and loses its wild-type tumor suppressor properties. Thus, in these cancer cells, several mechanisms stabilize mutant p53 through its activation or by inhibition of its degradation by disrupting Mdm2 and mutant p53 binding. Several splice variants of Mdm2 are reported in cancer, which lack a p53-binding domain and thus stabilizes mutant p53 expression [63]. In addition, GOF mutation-induced conformational changes in mutant p53 allow the binding of Hsp90 (heat shock protein 90) to mutant p53, which prevents Mdm2 binding and Mdm2-mediated degradation of mutant p53 [60]. It is now well established that elevated mutant p53 levels correlate with more aggressive tumors and poor prognosis. About 96% of high-grade serous ovarian cancer patients have GOF p53 mutations, which orchestrate a distinct pro-tumorigenic transcription and oncogenic programs. Knowledge of a UPS component responsible for mutant p53 stabilization, which could be chemically manipulated, will be useful in HGSOC. Nonetheless, Mdm2 is a great therapeutic target and prognostic factor for ovarian cancer with wild-type p53, such as clear-cell carcinomas [64].

3.2. Cyclin E

Genomic alterations in cell-cycle regulatory genes have been reported in almost every human carcinoma. Cyclins are the crucial regulators of cell-cycle progression [65]. A periodic increase in cyclin levels and their timed interplay with cyclin-dependent kinases (CDKs) is essential for the proper progression of cell cycle [65]. Their levels are regulated by a combination of transcription and ubiquitin-mediated degradation [18, 66]. About 30% of high-grade serous ovarian cancer patients have amplification of the CCNE1 gene, which encodes for G1/S-specific cyclin E. Cyclin E-CDK2 interactions commit the cell to S-phase genome duplication [3]. Aberrant accumulation and overabundance of cyclin E leads to premature entry of the cell into S-phase, resulting in chromosome instability and tumor formation [67]. Cyclin E amplification is likely to be an early event in the development of high-grade serous ovarian cancer [3]. This subclass of patients has no apparent defect in homologous recombination as seen in patients with BRCA1 and BRCA2 mutations with defect in DNA repair pathways [3]. The overexpression of cyclin E is an indicator of poor overall survival of ovarian cancer patients. Cyclin E protein levels are maintained by a multi-subunit SCF ubiquitin ligase, which mediates its ubiquitination and degradation [68]. Cyclin E auto-phosphorylation after its association with CDK2 is recognized by the SCF-associated F-box protein 7 (FBXW7), which binds to cyclin E and facilitates its ubiquitination and degradation [68, 69]. More than 30% of human cancers have a deleted FBXW7 gene located on chromosome 4q32. FBXW7 also regulates mTOR, Myc, and Notch1 degradation, depending upon the type of tumor [70, 71]. FBXW7 is known to be mutated in breast and ovarian cancer cell lines with high cyclin E levels [3]. The loss of cyclin E or CDK2 results in cell-cycle arrest or apoptosis in HGSOC cell lines [3], suggesting cyclin E inhibition as a novel therapeutic approach in ovarian cancer patients.

3.3. P27, a cyclin-dependent kinase inhibitor

Similar to cell-cycle regulatory proteins, cell-cycle inhibitors are frequently altered in cancer [72, 73]. $p27^{Kip1}$ inhibits cell-cycle G1 phase by interacting with CDK2/cyclin A or CDK2/cyclin E complexes [73, 74]. Low levels of $p27^{Kip1}$ protein are associated with tumor progression and growth resulting in poor prognosis of ovarian and breast cancer patients [74–76]. The evaluation of subcellular localization of $p27^{Kip1}$ in tissue microarray of late-stage ovarian cancer patients revealed that patients with nuclear-only expression of $p27^{Kip1}$ had a better overall survival than those with negative expression or cytoplasmic localization of the marker (p-value = 0.0002; n = 355) [77]. $p27^{Kip1}$ level is an important prognostic marker of malignant transformation. Genetically altered mice with $p27^{Kip1}$ haploinsufficiency are predisposed to cancer [78]. $p27^{Kip1}$ protein levels are regulated by SCF E3 ligase-associated protein Skp2. Skp2 binds to $p27^{Kip1}$ and mediates its ubiquitination and subsequent proteasomal degradation [79, 80]. Skp2 levels in different cancers correlate with tumor grade and inversely correlate with $p27^{Kip1}$ levels and cancer prognosis. Skp2 levels were upregulated in ovarian cancer patients and were associated with advanced FIGO stage III and IV and high grade of the tumor [81]. Skp2 levels were also associated with downregulation of both p27 and p21 in these patients, suggesting an important role of Skp2- $p27^{Kip1}$ pathway in ovarian cancer pathogenesis. A strong negative correlation between Skp2 levels and FOXO3a ($r = -0.743$; $p < 0.05$) in immunohistochemical analysis of ovarian cancer patients indicates that it is another potential target of Skp2 in ovarian cancer [82]. These findings and Skp2 overexpression or amplification in serous ovarian cancer characterize it as an oncogene and its inhibition a plausible approach in ovarian cancer management.

3.4. The epidermal growth factor receptor (also known as HER or ERBB) family

The EGFR family of receptor tyrosine kinases plays an important role in the pathogenesis of several cancers [83]. The four members: EGFR, HER2, HER3, and HER4 (or ERBB1–4), of EGFR family structurally consist of an extracellular ligand-binding domain, a single transmembrane-spanning region, and an intracellular tyrosine kinase domain. More than 30 ligands have been identified that bind to the EGFR family receptors, including EGF- and EGF-like ligands, transforming growth factor (TGF)-α, and heregulins (HRGs) [83]. The activated EGFR receptors undergo C-terminal phosphorylation of cytoplasmic tyrosine residues after receptor dimerization to mediate cell regulatory signaling. E3 ubiquitin ligase CBL binds to EGFR receptor at specific phosphotyrosine residues and mediates its ubiquitination subsequent internalization in clatherin-coated endosomes, which then lead to lysosome-mediated degradation of EGFR [84].

Amplifications and overexpression of various EGFR family members, including EGFR, Her2, and ErbB3, have been reported in epithelial ovarian cancer. Attenuated ubiquitination and HER2 gene amplification favor the formation of EGFR/HER2 heterodimers that recruit CBL to a lesser degree, thus stabilizing and recycling the receptor to cell surface [85]. BRCA1 mutations are known to be associated with an increased EGFR expression in serous ovarian cancer patients. EGFR expression was not only increased in BRCA1 mutated cancer tissues but was also high in BRCA1-mutated normal tissues compared to respective control tissues. These

results were confirmed by knocking down BRCA1 in ovarian cancer cells [86]. However, inhibitors targeting this pathway have little effect on cancer cells as a single agent due to the presence of alternative pathways affecting the cancer phenotype, particularly the activation of the PI3K/Akt/mTOR and mitogen-activated protein kinases (MAPKs) pathway [83], suggesting a combined use of EGFR and PI3K inhibitors in ovarian cancer [87].

4. Concluding remarks

It is now well known that UPS not only mediates protein degradation but is also involved in the extensive regulation of cellular functions and signaling. A large number of studies in various cancers have uncovered the diverse and intricate role of ubiquitin in oncogenic signaling. The alterations in the genes involved in UPS support its role in cancer development and progression. However, the lack of information on DUBs specificity and multiple targets of E3s raise a question on the use of DUBs or E3s inhibitors in cancer treatment. One possible way forward is to characterize the cancer-specific and tissue-specific expression of DUBs as certain DUBs are predominantly expressed in certain tissues and cancer, suggesting the cancer-specific use of a DUB inhibitor. Moreover, most DUBs studied thus far appear to regulate a small number of targets. It is also possible that only a fraction of ubiquitinated proteins are regulated by a specific DUB family. Similarly, the E3s can be manipulated in cancer if their role is characterized in cancer-specific aberrant molecular signaling. Moreover, further characterization of mutations in DUBs or E3s in cancer patients can be used for cancer screening. In addition, proteasomes carry a great potential in cancer treatment. Although Bortezomib did not show promising results against solid tumors, the advent of next-generation proteasome inhibitors opens new possibilities. Currently, five different types of next-generation proteasome inhibitors are in phase I or phase IIb clinical trials. Moreover, understanding the regulation of proteasomal activity by altered proteasome composition may open novel ways to target proteasomes in cancer.

Compared to breast cancer, ovarian cancer is a rare but far more lethal cancer. It is estimated that 69% of all patients with ovarian carcinoma will succumb to their disease as compared with 19% of those with breast cancer [1]. Ovarian cancer heterogeneity is represented by several genetic (BRCA1/2), epigenetic, and signaling (p53, CDK/p27, CCNE1) alterations, and various UPS components are implicated in these ovarian cancer-specific alterations. Several studies have established a link between UPS and ovarian cancer. However, further studies are needed to identify potential inhibitors for proteasome-based or E3s/DUBs-based therapies in ovarian cancer, which can be taken to clinical trials.

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Abbreviations

CUL4A	Cullin 4A gene
DUBs	deubiquitinating enzymes
E1	E1 ubiquitin-activating enzyme
E2	E2 ubiquitin-conjugating enzymes
E3	ubiquitin E3 ligases
EGFR	epidermal growth factor receptor
GOF	gain-of-function
HER2	human epidermal growth factor receptor 2
HGSOC	high-grade serous ovarian cance.
K	lysine
Lys	lysine
Mdm2	murine double minute 2
OSE	ovarian surface epithelium
PTM	posttranslational modification
STIC	serous tubal intraepithelial carcinoma

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Section 2

Diagnosis and Screening

Chapter 7

The Role of Circulating Biomarkers in the Early Diagnosis of Ovarian Cancer

Ece Gumusoglu and Tuba Gunel

Additional information is available at the end of the chapter

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Abstract

Ovarian cancer is the leading cause of gynecologic-related cancer death and epithelial ovarian cancer (EOC) is the most lethal sub-type. EOC is usually asymptomatic, and few screening tests are available. Diagnosis of ovarian cancer can be difficult because of the nonspecific symptoms. Despite the various diagnostic methods used, there is no reliable early diagnostic test and it needs to be developed. Specific biomarkers may have potential with the least possible invasive procedure. Biomarkers with a high sensitivity to ovarian cancer should be identified. Circulating biomarkers that are significant tools for non-invasive early diagnosis can be analyzed using circulating tumor cells, exosomes, and circulating nucleic acids. Protein, gene, metabolite, and miRNA-based biomarkers can be used for ovarian cancer diagnosis. As non-coding RNAs, MiRNAs may have an important role in ovarian cancer diagnosis due to their effects on mRNA expression levels. The most recent developments regarding the potential of circulating biomarkers to detect early ovarian cancer is presented in this chapter.

Keywords: ovarian cancer, biomarker, cell-free nucleic acids, early diagnosis, miRNA

1. Introduction

Ovarian cancer is a heterogeneous disease and the most important cause of gynecological cancer-induced deaths [1]. It is the fifth most important cause of cancer-related deaths among women in the world [2]. Different types of tumors may develop from each cell type. These tumors are epithelial tumors, germ cell tumors (originating from the ovary cell and follicular), and stromal tumors [3].

Molecular and cellular analyses of these tumor types may lead to earlier diagnosis of ovarian cancer and it is hoped better survival rates. Many factors play a role in the development of cancer, while genomic mutations and epigenetic changes are very important. For this reason, studies on mutations and epigenetic alterations may provide information about features such as early diagnosis, surveillance, and response to treatment.

2. Biomarkers used in the diagnosis of ovarian cancer

Tumor biomarkers are molecules that are produced by cancer cells or cells around them, which can be measured in body fluids or in the blood during the diagnosis, screening or treatment of cancer. Molecules that can be used as tumor biomarkers can be counted as cytoplasmic proteins, enzymes, hormones, surface antigens, receptors, oncofetal antigens (re-emerging proteins in cancer that is normally lost after birth), oncogenes or their products. An ideal tumor biomarker should be sensitive enough for early detection of small tumors while retaining the specificity of the identified cancer type. Unfortunately, however, today there is no known tumor biomarker carrying these features [4].

The features that should be found in an ideal tumor biomarker are given below [5]:

- It should have high specificity; it should be specific to only one type of tumor.
- Must have high sensitivity, should not be detected in cases of physiological or benign tumors.
- Levels should be proportional to tumor characteristics and size.
- The predictive and prognostic benefit of tumor biomarkers should be known.
- Half-life should be short, frequent and serial monitoring is possible.
- It should be cheap and easy to apply.
- Can be used as a screening test.
- Sample taking should be easy.

Potential biomarkers used in ovarian cancer are grouped as gene, protein, metabolite, and miRNA-based biomarkers according to their type [5].

The vast majority of ovarian tumors arise from the accumulation of genetic damage, but the specific genetic pathways that are involved in the development of epithelial, borderline, and malignant tumors are largely unknown. Considering the important relationship between genetic alterations and ovarian tumors, potential ovarian-cancer biomarkers can be found at gene-level (hereditary gene mutations, epigenetic changes, and gene expression) studies. The most common genes associated with epithelial ovarian cancer are shown in **Table 1** [6].

BRCA1, BRCA2, and Lynch syndrome genes show high penetrance and offer lifetime risks of 7–40% for ovarian cancer. Nowadays, the multigene panels used for clinical genetic testing

Gene	Gene full name	Protein class	Score[a]	No. of PMIDs[b]	No. of SNPs[c]
TP53	Tumor protein p53	Transcription factor	0.245958	144	2
CLDN7	Claudin 7	Cell junction protein	0.201099	5	0
ABO	ABO, alpha 1–3-N-acetylgalactosaminyltransferase and alpha 1–3-galactosyltransferase	Transferase	0.200549	3	0
SYNPO2	Synaptopodin 2	Cytoskeletal protein	0.200275	1	0
GPX6	Glutathione peroxidase 6	Oxidoreductase	0.200275	1	0
RSPO1	R-spondin 1		0.200275	1	0
WNT4	Wnt family member 4	Signaling molecule	0.200275	1	0
ATAD5	ATPase family, AAA domain containing 5	Nucleic acid binding	0.200275	1	0
EHMT2	Euchromatic histone lysine methyltransferase 2	Transferase; nucleic acid binding	0.2	1	0
MIR376C	MicroRNA 376c		0.2	1	0
BRCA1	BRCA1, DNA repair associated		0.02933	99	5
ERBB2	erb-b2 receptor tyrosine kinase 2		0.017792	57	0
BRCA2	BRCA2, DNA repair associated	Nucleic acid binding	0.01422	44	4
VEGFA	Vascular endothelial growth factor A	Signaling molecule	0.012847	39	0
MUC16	Mucin 16, cell-surface associated		0.009	25	0
EGFR	Epidermal growth factor receptor		0.008176	22	0
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	Transferase; kinase	0.007627	20	3
PGR	Progesterone receptor	Transcription factor; receptor; nucleic acid binding	0.007287	10	0
ERCC1	ERCC excision repair 1, endonuclease non-catalytic subunit	Nucleic acid binding	0.007077	18	0
EGF	Epidermal growth factor	Extracellular matrix protein; receptor	0.006528	16	0
ESR1	Estrogen receptor 1	Transcription factor; receptor; nucleic acid binding	0.006528	16	0
IGF2	Insulin like growth factor 2		0.006253	15	1
NBR1	NBR1, autophagy cargo receptor		0.006044	22	0
CDKN1A	Cyclin dependent kinase inhibitor 1A	Enzyme modulator	0.005704	13	0
TNF	Tumor necrosis factor	Signaling molecule	0.005704	13	0
ABCB1	ATP binding cassette subfamily B member 1		0.005495	20	3

Gene	Gene full name	Protein class	Score[a]	No. of PMIDs[b]	No. of SNPs[c]
MLH1	mutL homolog 1	Nucleic acid binding	0.005154	11	0
PTGS2	Prostaglandin-endoperoxide synthase 2	Oxidoreductase	0.005154	11	1
BRAF	B-Raf proto-oncogene, serine/threonine kinase	Transferase; kinase	0.00488	10	1
CDKN2A	Cyclin dependent kinase inhibitor 2A	Enzyme modulator	0.004605	9	0

[a]Gene-Disease Score.

[b]Total Number of PubMed ID (PMIDs) Supporting the Association.

[c]The Number of Associated Single Nucleotide Polymorphism (SNPs).

Table 1. The most common genes associated with epithelial ovarian cancer.

include the mild-penetrance genes (lifetime risks of 6–13%) such as BRIP1, RAD51C, and RAD51D. The common low-penetrance susceptibility genes make up the rest of the genetic risk. Besides, SNPs have approximately 1% risk which is shown by population-based genome-wide association studies (GWASs) [7]. Expression analyses of quantitative or semi-quantitatively specific genes in serum or tumor tissue can potentially contribute to tumor recognition. In the last decade, analysis of gene expression has gained momentum due to improvements in microarray technology. This is because microarray technology enables analysis of tens or hundreds of gene expressions in a single piece of tissue. Gene expression profiling has focused on three main topics: the separation of tumor tissue by normal ovarian tissue, the identification of different subtypes of ovarian cancer, and the determination of cancer according to possible responses to treatment.

DNA methylation and histone modification are epigenetic mechanisms that play important roles in gene regulation, tumor formation, and progression. Measuring the rate of methylation in specific genes in the promoter region helps early detection of cancer, detection of disease progression, and prediction of therapeutic response. Identification of specific genes that change with epigenetic regulation is one of the areas that are actively studied in ovarian cancer. In this chapter, we want to focus on circulating biomarkers and other types of biomarkers will not be discussed.

3. Tumor materials in circulation: liquid biopsy and their biomarker potentials

Non-invasive tumor diagnosis and screening has become an important area of study. Contrary to tissue biopsy, through detection of circulating tumor cells (CTCs), tumor nucleic acids ("circulating tumor DNA/RNA"), and exosomes, predictive and prognostic markers may potentially be developed which is far less invasive. Hence early and multiple evaluations of the disease can be made, including retrospective follow-up, identification of treatment effects and investigation of clonal development. Isolation and characterization of CTCs, exosomes,

and circulating tumor DNA (ctDNA) will improve cancer diagnosis, treatment, and imaging. Liquid biopsy can be performed "real-time" and at every stage of cancer. Although, it has some potential disadvantages such as; still is not certain to use in cancer diagnosis, difficulties in analysis of data obtaining from high-throughput screening and lack of data verification through clinical trials; it has significant potential for clinical cancer diagnosis in future [8].

3.1. Circulating tumor cells (CTCs)

Some cancer derived cells are detected in peripheral blood, and appear as solid tumor cells that have broken away into the circulation [9]. There are two main types of CTCs to explain this phenomenon. The majority are "Accidental CTCs", and these are CTCs that are passively pushed by external forces, such as tumor growth, mechanical forces during surgical operation or friction. The rest are CTCs which gain more plasticity and metastatic potential via the epithelial-mesenchymal transition (EMT) process [8]. These CTCs can stay in the non-divided form in the vein, can spread together, or settle into a new tissue to compose the metastatic deposit. Regardless of the CTC pathway, these cells carry important information about tumor composition, metastasis, drug sensitivity, and treatment.

CTCs have been demonstrated to have prognostic value among patients with breast, colorectal, gastric, lung, and pancreatic cancers in previous meta-analyses. However, the value of CTCs in ovarian cancer still remains controversial. Some studies did not observe any correlation between CTC status and prognosis. In contrast, other studies demonstrated an association Zhou et al. has shown that the prognostic value of CTCs was not associated with disease stage but with an elevated CA-125, both of which are known to correlate with prognosis either directly or indirectly. It has also been known that the CTC status was significant in respect to the overall survival (OS), progression-free survival (PFS), and disease-free survival (DFS) in ovarian cancer [10].

CTCs can be detected in both metastatic patients and patients with early, localized tumors. There is a significant potential for CTCs in the clinical management of cancers such as ovarian cancer. CTCs may enable real-time monitoring of treatment efficacy, identification of new therapy targets, and detecting and understanding drug resistance mechanisms [11]. CTC imaging and separation from leukocytes is dependent on reliable cell-surface markers. Based on the precipitation of CTCs in the low-speed centrifuge, the leukocyte fractions can be distinguished via physical features as well. Lee et al. used a nanoroughened microfluidic platform and detected CTCs in the sera of nearly all female participants (53/54, 98.1%) with ovarian cancer [12]. They also showed that although there is no relationship between CTC count and PFS in patients with newly diagnosed epithelial ovarian cancer (EOC), in patients with recurrent disease and chemoresistance; a relationship was found between CTC-cluster positivity and diminished OS [12]. It has been postulated that CTCs could result in metastatic progression and recurrence by way of epithelial-mesenchymal-transition (EMT) or development of stem-like features and hence a reduced OS. Therefore, researchers have tried to identify therapy-resistant tumor cells and to overcome treatment failure by analyzing CTCs transcriptional profiles [13]. In this study, the authors analyzed 15 single CTCs from 3 ovarian cancer patients and found them to be positive for stem cell (CD44, ALDH1A1, Nanog, Oct4) and EMT markers (N-cadherin, vimentin, Snai2, CD117, CD146) [13].

3.2. Circulating cell-free tumor DNA

Chang et al. were the first to examine the amount of cell-free DNA (cfDNA) in a patient's serum as a marker of disease presence in gynecologic malignancies [14, 15]. Cell free tumor DNAs (ctDNAs) circulate in the bloodstream and are derived from tumor cells. The presence of ctDNAs has been proven by detection of tumor-specific anomalies such as the presence of mutation in circulating tumor DNA (ctDNA), loss of heterozygosity of microsatellite, and methylation of CpG islands [16–18]. Similar to CTCs source; ctDNAs are released into the bloodstream in two ways: passively whereby ctDNAs from dead tumor cells and actively whereby ctDNAs are derived from live tumor cells spontaneously [8, 19]. ctDNA and apoptotic cell levels are lower in healthy individuals compared to cancer patients because chronic inflammation and excessive cell death cause accumulation of cell residues. cfDNA (cell-free DNA) is believed to originate from apoptotic cells content and found in elevated levels in cancer patients and related to higher tumor stage [20, 21].

The level of ctDNA is higher in the bloodstream of patients with solid tumors and metastatic disease compared to those without metastases [20, 21]. In patients with metastatic disease, the serum ctDNA level is higher (prevalence 86–100%) when compared to early-staged cancer types and patients with no radiographic evidence of disease (prevalence 49–78%) [20, 22]. Olsen et al. showed that in 86% of patients, ctDNA can be detected approximately 1 year before metastases while they are not observed in those clear of recurrence [23, 24] The anticipated short half-life of ctDNA of around 2 hours allows for an almost continuous analysis of tumor features including development, metastatic progression, and treatment efficacy. Thus, the identification of ctDNA has extraordinary potential as a potential biomarker for observing tumor load in the patient both prior and during treatment and in follow up [23].

Earlier studies in gynecological malignancies evaluated the presence of ctDNA at one time point using pelvic washings, ascites, serum, and plasma. Pereira et al. has demonstrated that serial estimation of ctDNA is a surveillance biomarker in gynecologic malignancies that is as sensitive and specific as the FDA-approved serum biomarker CA-125 [25]. Additionally, disease recurrence can be detected months earlier with ctDNA than CT checking [25]. Furthermore, the survival profiles of patients can be predicted with ctDNA level during the start of primary treatment, debulking surgery, and combined platinum/taxane doublet chemotherapy [25]. Both improved progression free and overall survival appear to be associated with undetectable levels of ctDNA [25] Additionally, ctDNA level maybe a stronger predictor than CA-125 of tumor size because of the longer half-life of CA-125 (9–44 days). It is also shown that in some patients, relapse of disease can be detected with ctDNA approximately 7 months before any CT scan changes [25]. Pereira et al. detected occult ovarian cancer cases by continuously monitoring the ctDNA even during apparent clinical remission [25]. These studies demonstrate that ctDNA could be used in early detection, it can act as a marker of disease stage as well as disease progression for gynecological cancers especially ovarian cancer.

Early diagnosis seems to be the best solution to reduce rates of ovarian cancer deaths unless highly effective drugs are developed with fewer side effects. Bettegowda et al. showed that for ctDNA detection in solid tumors, patients are treated at an earlier stage resulting in improved

survival [21]. Moreover, even in stage I patients (usually curable with surgery alone), detection of ctDNA level can be observed in around 47% of all patients [21]. Using ctDNA-level analysis, ovarian cancer can be detected in around 70% of all stage III patients [21].

3.3. Circulating cell-free tumor RNA (ctRNA)

Cancer cells have a very specific gene expression profile which differs from normal tissues. These tumor-specific gene transcripts can be detected in the circulation of cancer patients [26]. Despite the high amount of RNase present in the blood, circulating RNAs have been found to be surprisingly stable. This can be explained by the possibility that RNA is destructively protected by exosomes (such as microparticles, microvesicles, multivesiculas) that pass through the cell membrane into the bloodstream [26]. In addition, these mRNAs that are present in blood can be used as prognostic and predictive biomarkers [27]. Similar to ctDNA, ctRNA requires further study to assess the exact value as a biomarker in ovarian cancer.

3.3.1. Circulating microRNAs

MicroRNAs (miRNAs) are RNAs that do not encode proteins, at about 22 nucleotides in length, but they are involved with translation suppression, mRNA degradation, or sequencing specific gene regulation. Thus these molecules regulate various biological processes such as development, cell proliferation, differentiation, and apoptosis [28]. Approximately 3% of human genes encode miRNAs, while about 30% of genes encoding protein are regulated by miRNAs. These miRNAs vary according to the type of each cell, the stage of development, and differentiation of the cell. The release and biological functions of extracellular miRNAs are still not fully understood [29].

It has been shown that blood miRNAs in cancer patients have the similar importance as the miRNAs in tissues, and the relationship between solid tumors and miRNA expression profiles in the blood have been investigated [30, 31]. Circulating miRNAs are not bonded to the cell but are protected against endogenous RNase breakdown by binding to microvesicles, exosomes, microparticles, apoptotic bodies, and protein-miRNA complexes [32]. MiRNAs are resistant to severe conditions such as high temperature, low/high pH, long-term storage, and over-applied freezing/thawing [29]. Measurement of circulating miRNA level is difficult because it can be contaminated with cellular miRNAs of different hematopoietic origin [29]. The isolation and stabilization protocols of circulating miRNAs should be standardized and the cancer patient's plasma should be selectively distinguishable at the single molecule level [33]. MiRNA expression varies in tumor tissue with respect to normal tissue, and these changes can be detected in serum/plasma samples of cancer patients when compared to healthy individuals [34]. Further work is needed because of the low level of difference detected [29]; however miRNA has been shown to play an important role in cancer development as a new oncogene or tumor-suppressor gene class that varies according to the target gene [35].

In eukaryotic cells, there are several stages in miRNA biogenesis stages (transcription, pri-miRNA clipping, pre-miRNA transport, and pre-miRNA cloning) [36, 37]. MiRNA expression

levels vary from normal to ovarian cancer, with epigenetic changes, genetic changes (such as copy number changes), or differentiated expression of transcriptional factors, targeting miRNA genes. Transcriptional gene silencing in cancer cells is often associated with epigenetic defects [38, 39]. Studies have suggested that dysfunction or irregularity may occur in key proteins that are effective in miRNA biogenesis and may lead to tumor formation [39].

In recent years, many studies have been performed on the miRNA expression profile in EOC and it has been shown that there are significant differences in the miRNA expression profile compared to normal [35]. Iorio et al. compared 59 EOC operation samples with 15 normal ovarian species using a "custom" microarray and found 29 differently expressed miRNAs [35]. In EOC patients, miRNA expression profiles obtained from circulating tumor exosomes were compared with benign tumors and normal individuals and separated by different expression profiles. In this study, exosomes were separated by magnetic beads and anti-EPCAM antibodies, and miRNAs were analyzed by isolated microarray. As a result, there are several differentially expressed miRNAs in ovarian cancer samples [40]. In a study by Resnick et al., real-time PCR analysis of miRNA expression was performed on the serum collected from ovarian cancer patients and normal subjects, with different miRNAs expression found [41]. Patients with the three up-regulated miRNAs (miR-21, miR-92, and miR-93) were found to have a normal level of CA-125. Therefore, miRNA analysis may be complementary to other diagnostic methods [41].

It is clear that miRNAs play a crucial role in both normal and pathological processes due to their ability to regulate the expression of specific genes. However, no consensus has been reached as to the exact role/potential in diagnosis, metastasis, and prediction of response to treatment in EOC [28]. In addition, ovarian cancer is a heterogeneous disease, treatment and diagnostic options may vary from individual to individual; in this context, the tissue and origin specificity of miRNAs may be exploited and individualized treatment methods may be applied [42].

3.3.2. Circulating long non-coding RNAs

The Long Non-Coding RNAs (lncRNAs) are defined as >200 nucleotides in length and divided into five subclasses, which are intergenic, intronic, sense overlapping, *anti*-sense, and bidirectional lncRNAs [43]. LncRNAs are involved in various regulation processes which include protein-coding genes, functions at the level of splicing, chromatin remodeling, transcriptional control, and post-transcriptional processing after binding to DNA, RNA, or proteins [44]. These differ from tissue to tissue [45, 46] and lncRNAs play a role in growth, metabolism, and cancer metastasis [20, 47]. In several human cancer types, differentially expressed lncRNAs have been identified [48] which can be related to cancer metastasis and prognosis [49–51]. In addition, lncRNAs are specific for certain tumor origins such as the lymphatics, the cardiovascular or nervous system, circulating peripheral blood cells, or hematologic stem cells. Therefore, circulating lncRNAs may be informative about the tumor microenvironment [20, 52].

In ovarian cancer, lncRNAs have been shown to regulate several cancer processes such as development, metastasis, and relapse. Gao et al. [53] showed that a lncRNA named *HOST1*

(human ovarian cancer-specific transcript 1) plays a role in key biological pathways of EOC through the stimulation of tumor cell migration, invasion, and proliferation by inhibiting let-7b which is one of the most important miRNA involved in EOC [54]. In another study, Tong et al. showed that a lncRNA named RP11-190D6.2 regulates the WW domain-containing oxidoreductase (*WWOX*) expression by acting like an antisense transcript of this gene [55]. WWOX is linked with poor prognosis in several cancers, including EOC [56]. In addition, RP11-190D6.2 appears to play a role in the regulation of tumor metastasis, thus it can be counted as a potential biomarker and therapeutic target for EOC [55]. Zhou et al. compared several lncRNA expression profiles in a large number of OvCa patients from TCGA and found an eight-lncRNA signature predictive of overall survival [57]. Moreover, using lncRNA expression profiles, they could separate similarly aged patient into high-risk and low-risk groups, identify good or poor survival potential of patients, the eight-lncRNA signature maintained independent prognostic value, and was significantly correlated with the response to chemotherapy [57]. In a separate study [51], examining the expression profiles of lncRNAs and mRNAs in the high-throughput molecular profiles of OV patients; they found a correlation between lncRNA and malignant OV progression. Therefore; they suggest that two specific lncRNAs (RP11-284 N8.3.1 and AC104699.1.1) as may be candidate biomarkers for prognosis [51]. Clearly further study is required to understand their clinical application as a biomarker in EOC.

3.3.3. Circulating Piwi RNAs(piRNA)

Piwi RNAs (PiRNAs) are single-stranded, 26–31 nucleotide long RNAs which may inhibit transposons and target mRNAs through the formation of the miRNA silencer complex (RISC). Post-transcriptional regulation of piRNA (piRISC) happens in the cytoplasm [58]. The piRISC protects the integrity of the genome from alterations made by transposable elements (TE)—by silencing them; mRNA and lncRNA are other targets of piRNA complexes [58, 59]. piRNAs pathways play an important role to regulate some cancer-related pathways such as DNA hypomethylation and transposable element (TE) derepression. *L1* is a piRNA pathway gene that regulates these pathways, also overexpression of these genes (PIWIL1 and 2), have been shown in several tumor tissues [60]. Lim et al. showed that overexpression piRNA pathway genes and L1 elements may have a role in EOC [60]. They compared the EOC tissues and cell lines to benign and normal ovaries and found overexpression of PIWIL1 and MAEL, known as a cancer/testis gene [61] which are two genes of piRNA pathway which is a germ-line-specific RNA silencing mechanism. In situ analysis indicated that L1, PIWIL1, PIWIL2, and MAEL are up-regulated in cancerous cells, while MAEL and PIWIL2 genes are expressed in the stromal cells lining tumor tissues as well. PIWI, MAEL genes are essential for Drosophila and other vertebrates' germ-line stem-cell differentiation [60, 62]. These gene changes may promote a change in cell composition or identity in the tissue surrounding the cancer cells [60]. Also cancer stem cells may have potential as a biomarker for stem-cell definition [60, 63].

In addition, synthetic piRNAs may offer a new therapeutic approach through their use in silencing the expression of cancer-related genes. This approach has an advantage over other miRNA-based blocking methods because it does not require extra components for processing such as Dicer [59].

3.4. Exosomes and circulating microvesicles

Exosomes are multivesicular endosomal-derived extracellular vesicles (EVs) which are 30–120 nm size [64–67]. Exosomes can be distinguished from microvesicles which are heterogeneous in size (50–1500 nm) and result from the plasma membrane directly via a budding mechanism [68, 69]. Exosomes include several molecules such as proteins, metabolites, RNAs (mRNA, miRNA, long non-coding RNA), DNAs (mtDNA, ssDNA, dsDNA), and lipids and are used in cell communication [64, 70, 71]. Similar to circulating microvesicles, exosomes have also been shown to have specific functions and play an important role in coagulation, intercellular signaling, and the management of debris. Both circulating parts of the cell are found in different body and interstitial fluids [72, 73].

Tumor-derived exosomes are different from circulating healthy exosomes in terms of number of exosomes, content, and also cell-surface proteins [74]. Exosomes can be detected and isolated with several markers especially cell-surface proteins including those found only in the primary tissue. TGF β1, MAGE 3/6 proteins have a cell-surface biomarker feature special for ovarian cancer. These markers can be detected by filtration and ultracentrifugation methods in ovarian cancer plasma samples and can be used for prognosis/therapy monitoring of disease [74, 77]. Exosome contents are variable for cancer types as well. Taylor et al. indicated that several ovarian cancer specific exosomal miRNAs, (miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, miR-214), have been differentiated in serum samples by magnetic-activated cell-sorting (MACs) using anti-EpCAM array for diagnosis and screening of stage [40]. Exosomes are informative about tumor-specific features such as metastatic or benign form, stage, response to chemotherapies, and other drugs at that point in time via a possible blood sample [64].

Microvesicles have several common features with the primary cell such as membrane lipids, receptors, and diverse types of nucleic acids and proteins [75]. As in exosomes, microvesicles also have a potential to be biomarkers in several malignancies. Galindo-Hernandez et al. demonstrated that there were an increased number of microvesicles in breast cancer serum compared to healthy control samples [76]. It is also revealed that microvesicles derived from renal cancer stem cells include different miRNAs and mRNAs and these appear to play a function in tumor vascularization [75, 77, 78]. Microvesicles originated from tumor cells have been found in biological fluids in ovarian cancer. It has been shown that the number of microvesicles in malignant ovarian tumors is higher when compared to benign and nonmalignant pathologies (e.g., ovarian serous cysts, mucinous cystoadenomas, and fibromas) [79]. Ovarian cancer-induced ascites contains high levels of proteolytic enzymes such as matrix metalloproteinase (MMP-2, MMP-9) and urokinase-type plasminogen activator (uPA), which are the enzymes carried inside microvesicles [80–82]. Microvesicles may represent an ideal biomarker for ovarian cancer diagnosis and prognosis.

4. Biomarker detection technologies for ovarian cancer

High-throughput techniques of cellular transcriptome analysis mean that gene expression can be correlated with various aspects of disease in a variety of cancer types. This technology

used today in ovarian cancer research, such as expression microarrays and CGH, Real-time PCR, and Next-Generation Sequencing (NGS) allow genome-wide scanning and the discovery of altered genes involved in cancer.

4.1. Real-time PCR

Cell-free nucleic acids reflect both normal and tumor-derived nucleic acids released into the circulation through cellular necrosis and apoptosis. Stroun et al. have demonstrated with Reverse Transcription Quantitative PCR (RT-qPCR) that there is a consistent correlation between tumor load and quantity of cell-free DNA detected in a wide range of malignancies including ovarian cancer [83]. Several studies in OC with free DNA have also shown that miRNAs are abnormally expressed. Initial studies identifying tumor-derived miRNAs in the circulation of OC patients was published by Taylor et al. [40]. Zou et al. identified nine differentially expressed microRNAs (microRNA199a-5p, microRNA199a-3p, microRNA199-b3p, microRNA-645, microRNA-335, microR-NA-18b, and microRNA-141) through qRT-PCR expression analysis in SKOV3/DDP and A2780/DDP cells and these agreed with microRNA chip results [84].

4.2. Microarray

Microarrays together with clustering analysis have allowed genome-wide expression patterns in a lot of cancer types to be deciphered and compared. Wong et al. studied a group of genes (CLDN7, EPHA1, FOXM1, and FGF7), for the validation of the microarray findings; these were selected as these genes were associated with the alteration of crucial pathways involved in the regulation of cell cycle and cell proliferation [85]. Liu et al. [86] using the bioinformatics analyses of mRNA expression profiles retrieved from the Oncomine and Gene Expression Omnibus (GEO) Profiles online databases, they enriched two biological processes (cell cycle- and microtubule-related) and identified six genes (ALDH1A2, ADH1B, NELL2, HBB, ABCA8, and HBA1) that all were associated with ovarian cancer progression.

4.3. Next-generation sequencing

Clinical cancer next-generation sequencing (NGS) assays are dependent on many software subsystems and databases to deliver their results. The building of software systems for clinical use is a mandatory requirement of reliability and reproducibility imposed by diagnostic laboratory accreditation bodies such as Clinical Laboratory Improvement Amendments (CLIA), National Association of Testing Authorities (NATA), and the International Organization for Standardization (ISO 15189).

Pinto et al. [87] validated the use of next-generation sequencing (NGS) for the detection of BRCA1/BRCA2 point mutations in a diagnostic setting and also investigated the role of other genes associated with hereditary breast and ovarian cancer in Portuguese families. They obtained 100% sensitivity and specificity (total of 506 variants) for the detection of BRCA1/BRCA2 point mutations with their bioinformatics pipeline using a targeted enrichment approach when compared to the gold standard Sanger sequencing.

5. Conclusion

Ovarian cancer is one of the most significant and fatal gynecological cancer types worldwide. The earlier this disease can be detected, the better the success of treating it. There are several detection methods for ovarian cancer, but molecular diagnosis methods are more accurate, faster, and suitable for early detection. Recent developments have focused on identifying biological material with newer technological devices and these have become more precise, reliable, and more widely available over a short period of time. Although molecular markers, which are specific for ovarian cancer, have been extensively studied, they are still not used in a clinical setting. Clearly a greater understanding of their mechanisms and specificities are needed before they can be applied to early detection of OC.

Liquid biopsy using body fluids (e.g. blood, urine, saliva, and ascites) to isolate and characterize CTCs, exosomes, circulating tumor DNA, RNAs, and circulating free small RNAs is a new technique used in the detection and treatment of several diseases. Clearly further investigation is required but it is hoped that this may become a very important tool for early detection of ovarian cancer. In addition, these biomarkers may become an important part of the clinical strategies used in cancer diagnosis, treatment, and imaging. In this chapter, their roles in the early detection and management of ovarian cancer have been discussed. It is hoped that as our understanding of these markers increases, we will see an improvement in the rate of early cancer detection and ultimately increased survival.

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Chapter 8

The Past, Present and Future of Diagnostic Imaging in Ovarian Cancer

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Additional information is available at the end of the chapter

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Abstract

Ovarian cancers (OC) include a group of diseases with variable prognoses. While most conventional imaging techniques rely on the detection of tumour burden and distant spread to identify treatment plans, more emphasis is now being placed on screening for early detection and also for more accurate staging using molecular imaging techniques. It is generally accepted that there are some incremental benefits of using serum CA125 levels coupled with cross-sectional diagnostic imaging to aid in the diagnosis, staging and treatment planning of OC. This chapter provides a review of tests and diagnostic imaging modalities that aid in the detection and staging of OC with a particular focus on F18-Fluorodeoxyglucose positron emission tomography/computed tomography (F18-FDG PET/CT) imaging. This chapter also proposes a diagnostic algorithm for the management of ovarian cancer. F18-FDG PET/CT imaging can act as a catalyst for the development of personalised medicine by stimulating advancements in targeted therapy. In conclusion, diagnostic imaging with particular focus in molecular imaging has the potential for altering management plans, which can ultimately help improve the prognosis of ovarian cancer.

Keywords: diagnostic imaging, MRI, PET/CT, molecular imaging, adnexal mass

1. Introduction

This chapter regarding diagnostic imaging aims to guide multidisciplinary teams to decide on further investigation of ovarian cancer (OC), by proposing a diagnostic imaging algorithm (**Figure 1**) for the detection and staging of this disease [1, 2]. Furthermore, it provides a special focus on the evolving utility of molecular imaging, specifically PET/CT imaging in the management of ovarian cancer [3].

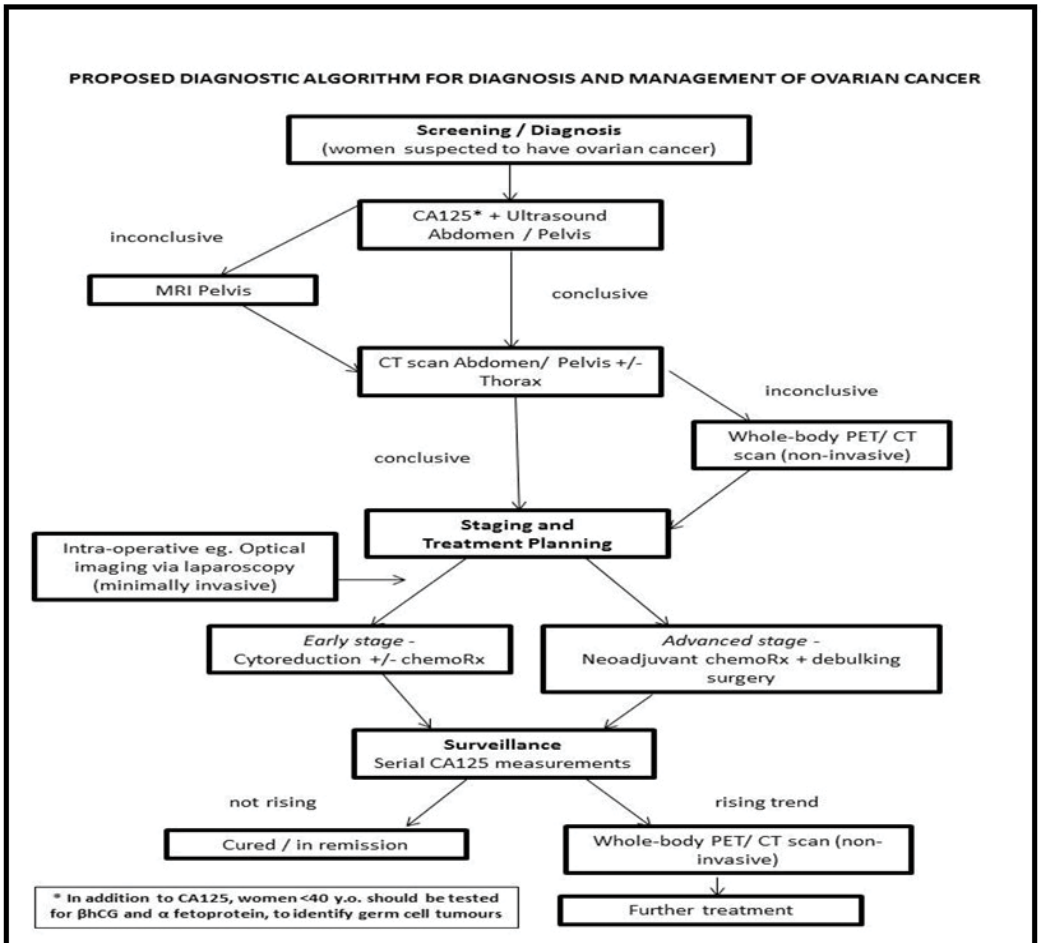

Figure 1. Diagnostic imaging algorithm for management of ovarian cancer (adapted from Suppiah et al. [1]).

Before illustrating diagnostic imaging methods for detecting OC, it is best to understand the embryology of the disease. Given that there is a remarkable morphologic and molecular heterogeneity in OC, therefore it has been postulated that ovarian cancers can be divided into Type I (indolent) and Type II (aggressive) tumours [4]. Type I is composed of low-grade serous, low-grade endometrioid, clear cell, mucinous and transitional carcinomas. These tumours are confined to the ovary at presentation and are relatively genetically stable and the majority display KRAS, BRAF and ERBB2 mutations [5]. Type II tumours include high-grade serous carcinoma (HGSC), undifferentiated carcinoma, and malignant mixed mesodermal tumours (carcinosarcoma), are highly aggressive, evolve rapidly and almost always present at an advanced stage. They display TP53 mutations in over 80% of cases and rarely harbour the mutations found in Type I tumours. Type I is suggested to be of Müllerian-type of tissues in origin, whereas Type II tumours are mesothelium in source and are suspected to originate from the Fallopian tubes [5].

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Currently there is no test which is entirely distinct and suitable to be used for population screening in women with low to moderate risk of developing OC. Serum tumour marker CA125 measurement has been studied, and include single cut-off points [6] and time-series algorithms [7]. Serum CA125 coupled with conventional imaging such as ultrasound scans have been used to stratify patients who may be at a higher risk of having OC. Magnetic resonance imaging (MRI) and Computed tomography (CT) imaging are useful in further characterising lesions and staging the disease respectively. Conversely, once diagnosed, intra-operative imaging such as optical imaging and hand-held spectroscopic devices can also guide in the detection of small cancers [8].

Furthermore, the advent of newer *in vitro* cancer models to assess for ovarian cancer specific biomarkers has paved the way for the development of potential novel therapeutics [9]. The study of micro-environmental cues in the regulation of miRNAs has also generated a growing need for advancement of *in vivo* functional tests that can help determine the phenotype and physiology of ovarian cancer. Functional imaging such as positron emission tomography/computed tomography (PET/CT) using radiopharmaceuticals, namely 18F-Fluorodeoxyglucose (FDG) enables non-invasive assessment of *in vivo* cellular metabolism.

2. Multimodality diagnostic imaging

Diagnostic tests to detect epithelial ovarian cancer include using serum tumour marker levels correlated with imaging findings. Diagnostic imaging modalities that are frequently used to detect, stage and monitor treatment of ovarian cancers include ultrasound, computed tomography, magnetic resonance imaging and positron emission computed tomography.

2.1. Serum tumour marker CA125

CA125 is a protein that is found in greater concentrations in ovarian cancer tumour cells than in other cells of the human body. Therefore, a simple blood test, using a sample taken from a peripheral vein, makes it possible for it to be used as a marker to detect the presence of ovarian cancer. Nevertheless, it is non-specific for ovarian cancer, as raised levels may also be found in other malignancies, e.g., breast, lung, colon and pancreatic cancer as well as in benign conditions such as in endometriosis, pelvic inflammatory disease and ovarian cysts [10]. CA125 has a high positive predictive value (PPV) of >95%, but a low negative predictive value (NPV) ranging from 50 to 60%, for the detection of OC [11]. Some studies have advocated the use of a single CA125 level measurement, frequently quoting the value 35 IU/ml as a cut-off point to indicate the presence of malignancy [12, 13]. Levels above this have a good positive predictive value, however many actual cancers may have lower levels of CA125 and can be missed [14].

The National Institute for Health and Care Excellence (NICE) clinical guidelines recommend further tests to be done if a CA125 level of >35 IU/ml is detected in a woman suspected to have ovarian cancer [2]. Conversely, when a cut-off of 30 IU/ml is used, the test has a sensitivity of 81% and specificity of 75% [15]. In general, one of the methods for assessment of treatment

response is by monitoring the CA125 levels. Moreover, longitudinal monitoring of CA125 levels can also provide additional information about survival in ovarian cancer [16].

2.2. Risk of malignancy index (RMI)

The risk of malignancy index (RMI) is a validated clinical tool that is used to assess the risk of having OC [2]. RMI combines three pre-surgical features which include serum CA125 levels using the unit IU/ml (CA125), menopausal status (M) and ultrasound score (U) for its assessment, using the formula: RMI = CA125 × M × U. The ultrasound result is scored 1 point for each of the following characteristics, namely the presence of multilocular cysts, solid areas, metastases, ascites and bilateral lesions. U = 0 (for an ultrasound score of 0), U = 1 (for an ultrasound score of 1), U = 3 (for an ultrasound score of 2–5). The menopausal status is scored as 1 = pre-menopausal and 3 = post-menopausal. The classification of 'post-menopausal' is a woman who has had no period for more than 1 year or a woman over 50 years of age and has had a hysterectomy [2].

According to the NICE guidelines, RMI scores are interpreted as low, moderate and high risk based on the total score [2]. Low-risk RMI is for scores <25 (noted in 40% of women, and the risk of cancer is <3%). Moderate-risk RMI is assigned for scores 25–250 (noted in 30% of women, and the risk of cancer is 20%), whereas scores >250 are associated with high-risk RMI (observed in 30% of women, and the risk of cancer is 75%). Women with moderate or intermediate risk are recommended to have an MRI for further evaluation of the ovarian lesion. Whereas, post-menopausal women with an RMI score of >250 should be referred to a cancer centre for further assessment and often undergo a staging computed tomography scan.

2.3. Ultrasound scan

Ultrasound (USS) is a safe, inexpensive and widely available diagnostic modality. Grey scale USS is a real-time imaging that detects the difference in the acoustic impedance (density × velocity of sound) of internal structures and can give excellent soft tissue detail for the evaluation of adnexal masses. Trans-abdominal scan (TAS) and transvaginal scan (TVS) are the first line diagnostic imaging modality for diagnosing OC. TAS utilises a low frequency (3.5–7 MHz) convex probe to characterise adnexal lesions that have grown beyond the pelvic brim. TVS uses a higher frequency (7.5–12 MHz) endocervical probe and gives better spatial resolution as it is placed closer to the ovaries; and is the first line modality of choice for small masses [16]. Nevertheless, a smaller field of view, leading to a possibility of overlooking a larger pelvic mass, is one of its limitations. Therefore, TAS is usually performed first followed by a TVS as a standard scan procedure.

In women suspected of having ovarian cancer, USS is indicated as a first line diagnostic imaging test. USS can diagnose the presence of an adnexal mass and help characterise it. The size, consistency, presence of loculations and solid component within a tumour; are some of the criteria used to characterise adnexal masses. A point to note is that an anechoic ovarian lesion detected in a postmenopausal woman should be considered as a physiological inclusion cyst, and not a pathological cyst if it was smaller than 10 mm and did not distort the ovary [17]. In certain occasions, the presence of bilateral lesions can be detected, and the pouch of Douglas is a common location for a left ovarian lesion (**Figure 2**).

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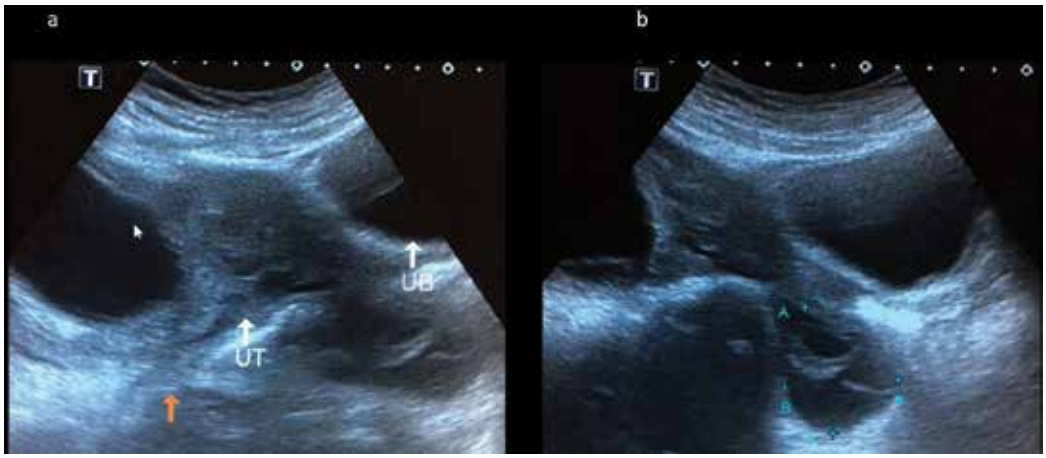

Figure 2. Grey scale ultrasound scans demonstrating suspicious bilateral large adnexal masses in a postmenopausal woman. The white arrow on the left indicates the uterus, the white arrow on the right indicates the urinary bladder and the orange arrow indicates the thick-walled right adnexal mass. Image (b) shows the dimensions of the left adnexal mass.

Figure 2a demonstrates a thick-walled, right adnexal lesion and **Figure 2b** demonstrates a multiseptated, left ovarian lesion in the pouch of Douglas.

2.4. Interpretation of ultrasound imaging

USS has improved specificity in detecting OC by the utility of simple ultrasound rules model [18]. In particular, by using the conventional technique of pattern recognition or subjective assessment by an experienced sonographer, the sensitivity and specificity were 83 and 90%. Whereas, by the technique of using the simple ultrasound rules (**Table 1**) was 92 and 96% respectively [19]. The rules comprised of five ultrasound features to predict a malignant tumour (M features) and five to predict a benign tumour (B features). These include features

Sonographic characteristics	Benign features (B features)	Malignant features (M features)
Tumour size	<100 mm	>100 mm
Loculations	Unilocular, smooth	Multilocular
Consistency	Cystic	Solid, mixed
Papillary projections	None/a few, thin	Multiple (at least 4), thick
Size of largest solid component	None/3–7 mm	Usually >7 mm
Wall	Thin, regular	Thick, irregular
Internal Doppler flow	None/minimal	Increased
Ascites	Absent	Present
Acoustic shadow	Present	Not applicable

Table 1. Sonographic characteristics of ovarian lesions based on simple ultrasound rules [18, 19].

of shape, size, solidity, and results of colour Doppler examination. Masses would be classified as malignant if one or more M features were present in the absence of a B feature. While if one or more B features were present in the absence of an M feature, the mass would be classified as benign. However, if both M features and B features were present, or if none of the features was present, the simple rules were considered inconclusive [20].

Colour Doppler can identify the presence of colour flow, within the papillary or solid components of an ovarian tumour and has good PPV for detecting malignancy. Nevertheless, the absence of colour flow in smaller lesions potentially causes falsely negative observations. False positive findings of flow can also occur in ovarian cysts in the luteal phase in premenopausal women [21].

The sensitivity and specificity of grey scale USS alone has been reported as 88% and 96% respectively; whereas, with the addition of colour Doppler as 83% and 97% respectively [22]. Although the introduction of power 3D Doppler has been able to increase the PPV of detecting malignancy; the availability of instruments and necessary expertise for interpretation has limited the use of this technique [23].

Several scoring systems have been suggested based on USS morphology of ovarian lesions to calculate and determine scores for malignancy [15, 24]. The PPV of these systems are small because the morphology of many benign lesions overlaps with that of malignant disease [21]. Rarely, certain OC are detected in large cysts, usually >7.5 cm in diameter; but do not exhibit an apparent complex morphology on ultrasound [25].

2.5. Limitations and future research in ultrasound

Recently, some experiments have been conducted to evaluate the role of contrast-enhanced USS to help further characterise ovarian tumours [26]. The meta-analysis of 10 studies revealed a pooled sensitivity of 0.89 and specificity of 0.91 respectively [27]. The limitation of ultrasound is the low sensitivity for detection of peritoneal metastasis [28]. Furthermore, screening low-risk population by transvaginal ultrasound may incidentally detect indeterminate lesions and lead to unnecessary biopsies [29]. Therefore, it should not be considered as a standalone investigation to be used to screen the general population for OC.

2.6. Computed tomography (CT) scan

Computed tomography (CT) scan utilises ionising radiation (photon beams of X-ray) to create cross sectional images of the internal organs. CT scans can give detailed information regarding tumour extent and metastatic disease (**Figure 3**). **Figure 3** is a multiplanar CT scan of a patient in axial, coronal and sagittal views, demonstrating a large ovarian cancer with intra-abdominal extension (white arrow) as well as gross ascites (black arrow) and thickened peritoneum consistent with metastasis (red arrow).

It is the preferred modality for the staging of OC and detection of recurrence because it is more widely available and less costly compared to magnetic resonance imaging (MRI) [30]. The Response Evaluation Criteria in Solid Tumours (RECIST) is often used in assessing treatment response in follow up CT scans and may be employed alone or in combination with CA125, for

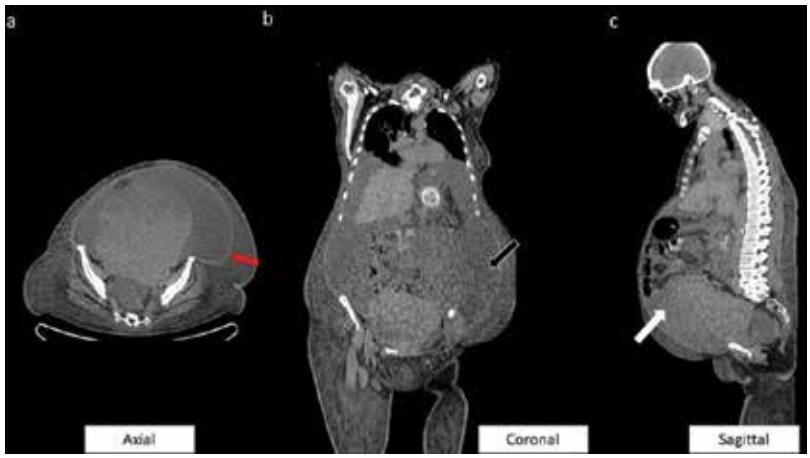

Figure 3. Multiplanar computed tomography scan demonstrating an ovarian cancer. The red arrow shows thickened peritoneum, black arrow shows gross ascites and white arrow shows a large adnexal mass.

evaluating the potential need to start or change the treatment regime [31]. Contrast-enhanced CT (CECT) studies have an added advantage compared to low dose non-enhanced CT scans, as they enable improved delineation of anatomical structures, and increased sensitivity for detection of pathological lesions [32]. Contrast-enhanced PET/CT is a more accurate imaging modality than PET using low dose CT for assessing OC recurrence [33].

Conventional CT has a limited and variable sensitivity of 40–93% and specificity of 50–98% for detection of recurrent disease [30]. Spiral CT can improve the detection of peritoneal lesions and implants, in particular in those with concurrent ascites. Obtaining a CT before secondary debulking may aid in surgical planning and to assess the feasibility of achieving maximum resectability [34].

Contrast-enhanced CT scans (CECT) can detect the involvement of specific intra-abdominal sites recognised to reduce the chances of optimal debulking. These sites include suprarenal aortic lymph nodes, disease in the root of the mesentery, portal triad disease, or bulky liver disease [35]. Conversely, multidetector CECT scans often underestimates the extent of liver surface disease and infra-renal para-aortic lymph node involvement [36]. The reliability of CT assessment is also related to improvements in imaging techniques as well as scanner equipment and this can vary across different centres [37].

2.7. Interpretation of computed tomography scans

CECT provides improved contrast resolution in delineating suspicious adnexal masses [38]. CECT is helpful in characterising benign and malignant ovarian tumours by observation of certain characteristic features (**Table 2**). It can also help differentiate OC subtypes, albeit with some overlapping features, especially the commoner subtypes such as serous tumours. Serous tumours are usually unilocular but with multiple papillary projections and often present bilaterally [39]. Furthermore, peritoneal carcinomatosis is also seen more frequently in serous adenocarcinomas [40].

CT scan characteristics	Benign	Malignant
Size	<4 cm	>4 cm
Consistency	Cystic	Mixed/Solid
Papillary projections	Absent/a few	Multiple
Wall	Thin, regular	Thick, irregular
Internal calcifications	Occasionally present	Infrequently present
Pelvic lymphadenopathy	Absent	Present
Laterality	Unilateral	Bilateral
Ascites	Absent	Present
Peritoneal involvement	Absent	Present
Distant organ metastasis	Absent	Present

Table 2. Computed tomography characteristics of adnexal masses (adapted from Suppiah et al. [38] and Jung et al. [9]).

2.8. Limitations and future research in computed tomography

The main limitation of a CT scan is its inability to detect deposits on bowel serosa, mesentery and omental regions that are smaller than 5 mm; especially in the absence of ascites. However, this can be solved by pre-surgical laparoscopic assessment. The detection of subdiaphragmatic peritoneal deposits are also difficult, but can be aided by multiplanar reformatting of contrast-enhanced scans.

2.9. Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) uses a high strength magnetic field and pulsed radiofrequency waves to generate images with excellent soft tissue detail. It does not utilise ionising radiation and is relatively safe to use. It commonly involves acquiring T1-weighted, T2-weighted, fat-saturation spin echo, and usually, includes post-gadolinium contrast-enhanced T1-weighted fat-saturation sequences for the pelvic region. This protocol includes a full abdominal scan in three planes for the staging of ovarian cancer [41].

The ability of MRI to correctly stage ovarian cancer is excellent, providing a sensitivity and specificity of 98 and 88% respectively, as compared to 92 and 89% respectively for CECT [41]. MRI and CT have been noted to be more sensitive than ultrasound for detection of peritoneal metastases. The accuracy of MRI (76%) is also better than CT (57%) for detection of lymph nodes [28]. The improved soft tissue resolution achieved by MRI is able to better delineate the presence of pathological lymph nodes, both within the pelvic cavity as well as extra-pelvic spread. However, its limitations are that it is rather costly, time-consuming and often difficult to interpret due to breathing and bowel movement artefacts.

Therefore, the clinical utility of MRI is limited to evaluation of indeterminate pelvic lesions. MRI can detect haemorrhagic lesions and enhancement in papillary projections, as well as

identify the fatty tissue components within individual adnexal tumours. It can delineate ovarian lesions from uterine or urinary bladder involvement. MRI is also useful in cases where CECT is relatively contraindicated such as in the pregnant woman, in a patient with, a history of dye allergy or where giving iodinated contrast material is contraindicated, e.g., in renal impairment. Hence, a non-contrast-enhanced MRI scan should be performed instead.

2.10. Interpretation of magnetic resonance imaging

MRI is able to differentiate simple ovarian cysts from malignant lesions with solid internal components. Simple cysts return a low signal in the case of T1-weighted images and a high signal in T2-weighted images, whereas malignant lesions are often heterogeneous and show marked enhancement of its solid components. MRI is best to delineate the local extent of the tumour as well as detect pelvic nodal metastases.

2.11. Limitations and future research in magnetic resonance imaging

The utility of whole-body diffusion-weighted imaging in magnetic resonance imaging (WB-DWI/MRI) has shown some promising results. Diffusion-weighted imaging measures the Brownian motion of extracellular water and thereby approximates tissue cellularity and fluid viscosity, hence malignant tumours that have increased cellularity will have restricted diffusion, thus giving lower apparent diffusion coefficient (ADC) values. Interestingly, DWI/ADC sequences have shown 94% accuracy for primary tumour characterisation which is comparable with the results of 18F-Fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) [42].

WB-DWI/MRI has also shown an improved accuracy of 91% for peritoneal staging compared with CT (75%) and 18F-FDG PET/CT (71%). It also has higher accuracy (87%) for detecting retroperitoneal lymphadenopathies compared to CT scans (71%) [42]. However, this study was limited by the relatively small number of cases, the discrepancy between the ratio of MRI and PET/CT cases performed as well as the relatively large number of patients who presented with advanced disease, potentially increasing the pre-test likelihood of detecting metastases.

2.12. Positron emission tomography-computed tomography (PET/CT) scan

Molecular imaging, namely Positron Emission Tomography-Computed Tomography (PET/CT) scans are indicated for the detection of recurrence of OC as even small volume disease can easily be detected. It is considered by the European Society of Medical Oncology (ESMO) as an appropriate imaging modality to help in the selection of patients for secondary debulking surgery. PET/CT can alter the management plan in metastatic ovarian cancers by detecting additional sites of disease not seen on CT scans, and identifying locations that are not amenable to cytoreduction [1].

PET/CT can assess tumour aggressiveness by demonstrating an elevated level of the injected radiopharmaceutical, e.g., 18F-Fluorodeoxyglucose (18F-FDG) that is trapped in tumour cells, as quantified by standardised uptake values (SUV) [43]. Interestingly, elevated maximum

standardised uptake values (SUVmax) are frequently detected in the ovaries in the luteal phase of the menstrual cycle. This is considered as normal physiological FDG metabolism and should not be mistaken for pathology. Therefore, PET/CT scans should be scheduled right after the menstruation to minimise this observed effect in premenopausal women.

PET/CT scans are performed using a hybrid PET/CT scanner commonly using 3D lutetium oxy-orthosilicate crystals as detectors for the PET component. It is recommended that the examination include a diagnostic contrast-enhanced computed tomography (CECT) scan by the administration of low osmolar iodinated contrast media. Apart from enabling attenuation correction, and anatomical localisation; CECT is essential for performing diagnostic clinical staging [1]. Patients are instructed to fast for a minimum of 6 h before scanning, and blood glucose is checked before the scan. Subsequently, 18F-FDG will be administered and subjects are kept in a dark room for approximately 60 min to allow for uptake time. Subjects are given approximately 100 mL (2 ml/kg body weight) of iodinated contrast media during the CECT scan. Immediately after the CT, PET images acquisition will be performed over the same anatomic regions. The attenuation corrected CECT images will then be fused with PET images. The combined images will be utilised for visual interpretation, tumour size and maximum standard uptake value (SUVmax) measurements.

2.13. Interpretation of positron emission tomography-computed tomography (PET/CT) scans

Abnormal FDG hypermetabolism is analysed on the PET/CT images, starting from a survey of the maximum intensity projection (MIP) 3D image of the PET component. Regions commonly evaluated to detect nodal spread and distant metastases include the pelvic, abdominal and inguinal lymph nodes; the uterus, urinary bladder, peritoneum, omentum, bowel, liver, lungs and bones. Adnexal lesions frequently have a variable FDG uptake irrespective of their histopathological origin. For instance, mucinous carcinomas do not demonstrate avid FDG uptake compared to serous tumours [44]. It is postulated that indolent (Type I) and aggressive (Type II) ovarian cancers may arise from different cell lines [5]. Thus, Type I tumours do not demonstrate significantly elevated SUVmax values.

The sensitivity, specificity, PPV and NPV of PET/CT in detecting OC metastases are 87, 100, 81 and 100% respectively [45]. Moreover, PET/CT has improved accuracy at detecting peritoneal seeding, sub-diaphragmatic involvement, distant organ metastasis, bowel invasion and extra-abdominal lymph node involvement which has led to a reduction in the rate of second look surgery [46]. A negative PET/CT has NPV of 90% for detection of recurrence within a two-year follow-up period [2]. PET/CT scan in axial, coronal and sagittal views was able to detect bowel invasion (red arrow) in an advanced ovarian cancer disease (white arrow) (**Figure 4**). Therefore, it can aid in the decision-making for primary debulking surgery followed by platinum-based chemotherapy as opposed to treatment using neoadjuvant chemotherapy.

PET/CT is also able to demonstrate the heterogeneity of ovarian cancers. (**Figure 5**) There is moderate FDG uptake noted in serous adenocarcinomas of the ovary as seen in **Figure 5a**. Endometrioid adenocarcinomas often have multiple cystic areas within and can be associated

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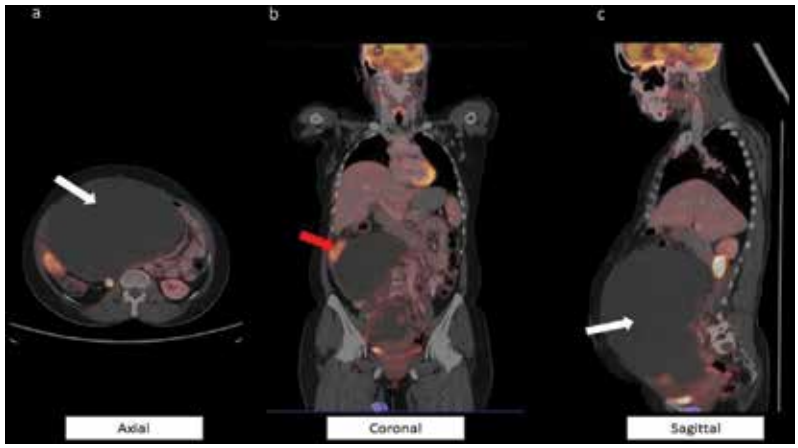

Figure 4. PET/CT scan demonstrating an advanced ovarian cancer. White arrows show a large adnexal tumour with heterogeneous FDG uptake. Red arrow shows bowel involvement.

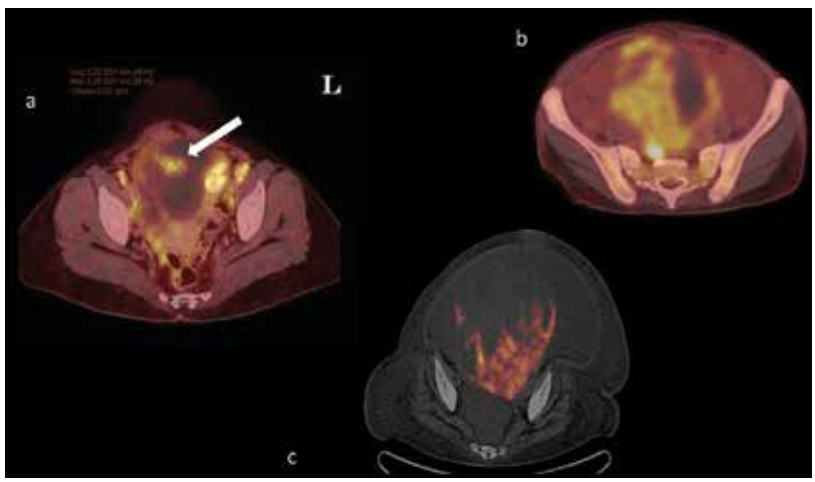

Figure 5. PET/CT scans in axial view demonstrating malignant ovarian tumours. White arrow shows markedly increased FDG uptake within the solid component of the tumour.

with internal calcifications as seen in **Figure 5b**. Mucinous adenocarcinomas often have low FDG uptake as seen in **Figure 5c** and represent a diagnostic caveat against dismissing them as benign lesions.

2.14. Limitations and future research in positron emission tomography-computed tomography (PET/CT) scans

The current theory postulates that high grade serous ovarian carcinoma (HGSC) originate from the fimbrial end of fallopian tubes [47]. It has sparked interest as to whether risk-reducing opportunistic salpingectomy could be performed to preserve fertility in a premenopausal

woman with high risk of developing ovarian cancer. There is a need to explore the role of PET/CT or rather MR/PET, which may be able to detect disease at an earlier stage especially when it is still localised to the fallopian tubes.

Apart from 18F-FDG, other tracers have also been studied to assess for recurrent or residual ovarian cancer. These include 11C-Choline which can help better delineate pelvic lesions [48]; as well as 16α-18F-fluoro-17β-estradiol (FES) which have the potential to evaluate the response to hormonal therapy for ovarian cancer [44]. Another tracer also in the experimental stage, is 3′-deoxy-3′-18F-fluorothymidine (FLT) that distributes rapidly in the extracellular fluid and is phosphorylated by thymidine kinase 1(TK-1) and becomes trapped in tumours with increased cellular proliferation activity. The role of FLT PET/CT may be in assessing and predicting response to an antitumour type of therapy, where it has been shown to be superior to 18F-FDG PET/CT [49].

2.15. Other research-based imaging techniques and work in progress

Positron Emission Tomography/Magnetic Resonance (PET/MR) is an emerging technique, which uses scanners that acquire MR and PET data either simultaneously or sequentially. Simultaneous acquisition devices, some called the mMR scanners, allow for concurrent imaging of the same body region. Alternatively, sequential scanning is done using two different scanners during one examination session, and the images are fused later. PET/MR acquisition protocol for assessment of a gynaecological tumour includes whole-body Dixon and a dedicated pelvic MRI exam that includes dynamic intravenous gadolinium administration [50]. It is suitable for assessment of the loco-regional extent of a pelvic tumour and evaluates the entire body for metastases, albeit having a very long scanning time of approximately 1.0–1.5 h [50].

Additionally, PET/MRI may be a more useful modality as compared to PET/CT for the detection of miliary disseminated metastases in cases of suspected OC recurrence [2]. As evident in **Figure 6** in which PET/MRI demonstrates FDG avid uptake in the para-aortic lymph nodes (**Figure 6**). Furthermore, PET/CT potentially gives high false negative results in the case of small volume disease which predisposes it to miss low-grade tumours and early adenocarcinomas [51]. Therefore, it is recommended to be used in conjunction with transvaginal ultrasound or MRI for characterisation of adnexal masses and the detection of OC.

PET/MRI ideally has added value in oncologic imaging due to its improved soft-tissue resolution. Furthermore, sophisticated sequences such as diffusion-weighted imaging, functional MRI, and MR spectroscopy can all be incorporated with molecular imaging, giving further information but with less radiation exposure. This can provide a significant reduction in radiation dose and exposure in patients who require follow-up imaging [3].

Some other imaging techniques are performed intra-operatively, namely the sentinel node procedure (SNP). SNP is sometimes conducted in patients with a high likelihood of having an OC in whom a median laparotomy and a frozen section analysis is planned. The concept of SNP is to determine whether the OC has spread to the very first lymph node (sentinel node). If the sentinel node is negative for cancer cells, then there is a high likelihood that the cancer has not spread to other lymph nodes [52]. Blue dye and radioactive colloid are injected into either the ovaries or the ovarian ligaments to perform the SNP [53].

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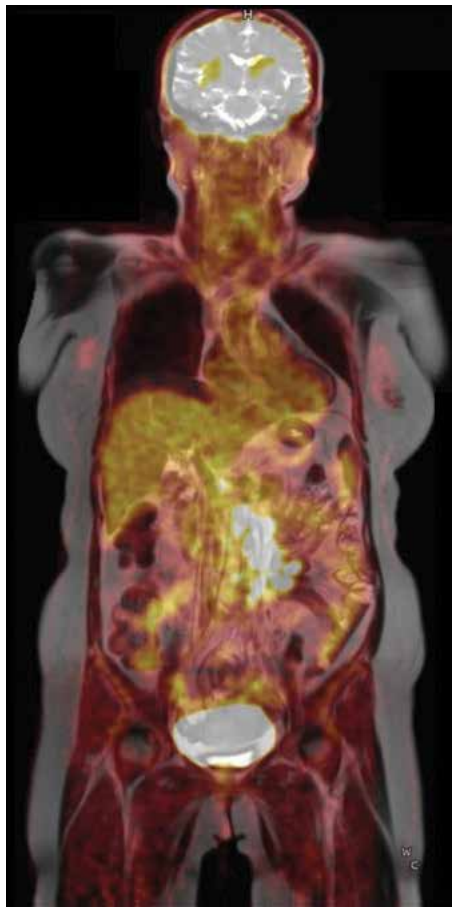

Figure 6. PET/MRI scan demonstrating recurrent ovarian cancer with para-aortic lymph nodes involvement. MRI gives good soft tissue resolution of the FDG avid lymph nodes noted at central abdomen.

After the incubation time, usually 10–15 min, the sentinel nodes can be visualised by either colorization (blue lymph nodes can be identified) and/or with a gamma probe that detects the radioactive tracer. The pathological examination of the sentinel node is an indication of the nodal status of the remaining nodes; when the sentinel node is negative, one can presume that the remaining nodes are also not involved. As a consequence, the patient may be spared from undergoing radical lymphadenectomy, and thus the morbidity associated with it.

Conventional diagnostic imaging modalities lack specificity and sensitivity in the detection of small primary and disseminated tumours in the peritoneal cavity. Using the knowledge that HER-2 receptors are overexpressed in ovarian tumours, a near infrared (NIR) optical imaging approach for detection of ovarian tumours using a HER-2 targeted nanoparticle-based imaging agent in an orthotopic mouse model of ovarian cancer has been conducted achieving improved detection of smaller lesions [54].

Furthermore, the overexpression of folate receptor-α (FR-α) in OC, has prompted the investigation of intra-operative tumour-specific fluorescence imaging. It has potential applications in

patients with OC for improved intra-operative staging and more radical cytoreductive surgery [55]. Additionally, optical coherence tomography (OCT) is another emerging high-resolution imaging technique that utilises an infrared light source directed to the tissues being examined. A novel prototype intra-operative OCT system combining positron detections; namely utilising Caesium (Tl204/Cs137) sources as well as 18F-FDG have shown potential for the development of a miniaturised laparoscopic probe to detect small volume disease of OC. This can offer simultaneous functional localisation and structural imaging for improved early cancer detection [56].

3. Conclusion

In summary, ovarian cancer is a heterogeneous spectrum of disease. Early detection and accurate staging using diagnostic imaging can help improve the prognosis of this condition. Prudent selection of the appropriate imaging modality can help expedite the correct treatment being instituted for the patients (**Table 3**). Molecular imaging, particularly 18F-FDG PET/CT can be a useful non-invasive biomarker to help stage the disease and detect recurrence based on the proposed diagnostic algorithm in this chapter.

Modality	Advantage	Disadvantage
Ultrasound	• Relatively cheap • Easily available • Does not involve ionising radiation	• Operator dependent • Unable to accurately stage the disease
CT scan	• Good for staging • Readily available	• Involves ionising radiation • Has pitfalls that lead to falsely negative findings
MRI	• Excellent soft tissue detail and able to characterise the pelvic lesion • Does not involve ionising radiation	• Relatively expensive • Longer scanning time • Requires specialised skills for interpretation
PET/CT	• Excellent for staging and detection of recurrence • Good for detection of extra-abdominal metastases	• Involves ionising radiation • Prone to false positive results • Requires specialised skills for interpretation
PET/MRI	• Excellent soft tissue detail and able to improve detection of nodal and peritoneal metastases • Slightly reduced radiation dose compared to pet/CT	• Expensive • Longer scanning time • Requires specialised skills for interpretation
Intra-operative devices	• Able to detect small volume disease • Able to delineate local extent of disease in a small region of interest	• Costly • Operator dependent • Requires specialised skills for interpretation

Table 3. Comparison of the diagnostic imaging modalities for the management of ovarian cancer.

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Chapter 9

Ascites in Advanced Ovarian Cancer

Katarina Cerne and Borut Kobal

Additional information is available at the end of the chapter

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Abstract

The presence of ascites is one of the general ovarian cancer (OC) symptoms detected at initial diagnosis and can be present at an early stage but is most often seen in advanced disease. In newly diagnosed OC patients, ascites is treated by the standard treatment for the underlying disease. However, once the chemoresistant and recurrent features of the disease develop, management of a large volume of ascites can be a major problem. By increasing abdominal pressure, ascites can cause severe symptoms; thus, palliation of symptomatic patients is the main goal. The elimination of fluid accumulation in OC patients with these symptoms will certainly improve their quality of life and may even prolong survival. Unfortunately, no standard treatment for OC-associated ascites exists. There are several traditional therapies for ascites, with limited effectiveness and significant adverse effects. Catumaxomab is the only medicine approved for intraperitoneal treatment of malignant ascites in patients with EpCAM-positive carcinomas. Advances in our understanding of malignant ascites aetiology and more effective treatment strategies for ascites and OC will help reduce the symptoms associated with ascites.

Keywords: advanced ovarian cancer, malignant ascites, aetiology, treatment, diagnosis

1. Introduction

Ascites is an abnormal accumulation of serous fluid (>50 mL) in the peritoneal cavity between the membrane lining the abdominal wall and the membrane covering the abdominal organs. Although ascites is most commonly observed in patients with cirrhosis, 7–10% of patients with ascites develop it secondary to malignancy. The commonest primary tumour associated with the development of ascites is ovarian cancer (OC) [1]. Large amounts of ascites in a patient with OC usually indicate the presence of peritoneal metastasis; therefore, ascites is found in the majority of patients (89%) with advanced disease (FIGO stages III and IV). However, the absence of ascites may not exclude malignant disease, since ascites is rarely

(17%) observed in the early disease (FIGO stages I and II) and is absent in nearly half of borderline tumours. Unlike in primary OC, recurrent disease is not strongly associated with ascites, which was found in 38% of patients with recurrent OC [2].

Throughout history, ascites has always been regarded as a poor prognostic sign. In the 1700s, Sir Thomas Spencer Wells wrote "surgeons stood and trembled on the brink of ovarian waters" [3]. Studies addressing the prognostic significance of ascites in patients with stage III or IV have shown a significantly poorer survival [4]. Ascites is also associated with pharmacoresistance [4]. Patch et al. showed that matched primary ascites (tumour cells isolated from ascites) share most genomic changes of acquired resistance with primary tumour samples across the whole genome [5].

In newly diagnosed ovarian cancer patients, ascites is treated by using the standard treatment for the underlying disease. However, once the chemoresistant and recurrent features of the disease develop, management of a large volume of ascites can be a major problem. Palliation of symptomatic patients is therefore the foremost goal, and elimination of fluid accumulation in patients with these symptoms will certainly improve their quality of life and may even prolong survival [6, 7]. An understanding of malignant ascites aetiology is of utmost importance if more effective treatment strategies for ascites and OC are to emerge in the future.

This chapter considers the aetiology and pathophysiology of malignant ascites in OC as well as current diagnostic modalities and explores the best form of management.

2. Mechanisms of malignant ascites formation

The word ascites originates from the ancient Greek askos, meaning a sac or bag. Celsus (c.30 BC–c.50 AD) postulated a link between ascites and renal disease, and he coined the term [8]. The peritoneal cavity, located between the parietal and visceral peritoneum, contains approximately 100 mL of serous fluids. Free fluid in the peritoneal cavity acts as a lubricant of the serosal surfaces and originates from the transduction of plasma through capillary membranes of the peritoneal serosa. Healthy women may normally have as much as 20 mL of free peritoneal fluid, depending on the phase of the menstrual cycle [9]. Under physiological conditions, transudation is balanced by efflux of the peritoneal fluid via lymphatic vessels. Tumour growth eventually disrupts the normal regulation of intraperitoneal fluid flow and the maintenance of a steady state in the peritoneal cavity by simultaneously causing a greater fluid inflow and a reduced outflow. Four major factors that contribute to the formation of ascites: two cause increased influx due to tumour-related factors and two cause decreased efflux due to lymphatic obstruction and mechanical obstruction by accumulation of tumour cells at the peritoneal surface (**Figure 1**) [1, 10]. The percentage of cases with a greater ascites volume increased as the stage of ovarian malignancy progressed [1].

2.1. Efflux from the peritoneal cavity into the blood

The peritoneal lymphatic system collects excess fluid, proteins, other macromolecules (>16 kDa) and cells and returns them to systemic circulation [11]. Decreased efflux from the peritoneal cavity due to lymphatic obstruction by tumour cells was first proposed as a hypothesis for ascites

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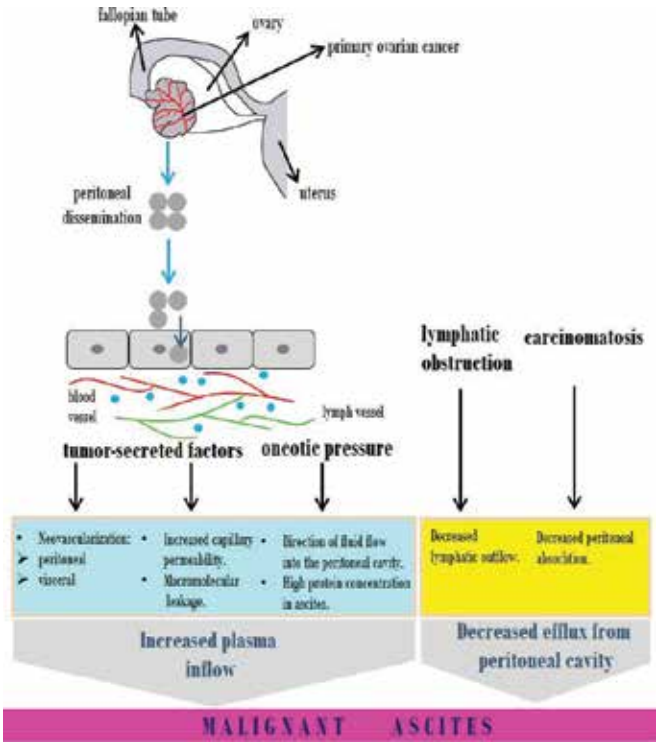

Figure 1. Aetiology of malignant ascites in ovarian cancer.

formation by Holm-Nielsen more than 60 years ago [12]. Published data using lymphoscintigraphy showed that patients with malignant ascites had no activity above the diaphragm after intraperitoneal injection of the isotope, in contrast to control patients with no ascites or cirrhotic ascites. Bronskill et al. [13] showed that OC patients with persistent, intractable ascites, who were approaching their terminal illness, had low peritoneal drainage rates (below 50 ml/h). This result generally indicates obstruction of the diaphragmatic plexus [13]. Initial events that lead to fluid accumulation were studied by Nagy et al. [14] who showed that in mice efflux the peritoneal cavity of ^{125}I-labelled human serum albumin and ^{51}Cr-labelled red blood cells is markedly reduced (fivefold) within 1 day of *i.p.* ovarian tumour cell line injection. A significant reduction preceding a detectable increase in tumour cell number was not attributable to the blockage of peritoneal lymphatics by tumour cells and by itself did not provoke peritoneal fluid accumulation. These results suggest a prominent role for nonobstructive mechanisms, including contraction of lymph vessels induced by secretion of tumour cell product(s). At later periods, the absorption of fluid from the peritoneal cavity might also be affected due to carcinomatosis [14].

2.2. Influx into the peritoneal cavity

Nagy et al. studied the influx of fluid into the peritoneal cavity of mice [14]. They found that after *i.p.* ovarian tumour cell line injection, influx of ^{125}I-labelled human serum albumin rose between days 5 and 7 to values 13- to 25-fold higher than control values, when the tumour cell number had increased >500-fold. By day 10, influx had increased sufficiently to exceed efflux,

resulting in net accumulation of fluid [14]. An increase in influx is a result of various factors: (1) increased capillary permeability, (2) angiogenesis, (3) increased area for filtration and (4) decreased oncotic pressure difference. In malignant ascites, various factors secreted by tumour cells are present, which increase vascular permeability and induce angiogenesis. An early step leading to angiogenesis is partial proteolysis of vascular basal lamina, resulting in hyperpermeability. Vascular endothelial growth factor (VEGF) is the most potent and specific angiogenic factor, secreted by a large variety of tumours, peritoneal mesothelial cells, monocyte/macrophages in malignant ascites and even tumour-infiltrating T cells [7]. Additionally, VEGF increases the permeability of vessels to plasma proteins, including albumin and fibrinogen, with a potency 10,000 times higher than histamine [15, 16]. Other factors that may also induce angiogenesis have been identified in malignant ascites and include basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor alpha and beta (TGF-alpha, TGF-beta), interleukin-8, placental growth factor (PIGF) and platelet-derived endothelial cell growth factor (PD-EGF) [11]. Influx into the peritoneal cavity after *i.p.* ovarian tumour cell line injection rose significantly when the surface area for filtration also increased; the size and number of vessels lining the peritoneal cavity increased as much as 15-fold [16]. The protein content of malignant ascites is greater than in peritoneal fluid of healthy women [11]. The oncotic pressure difference between plasma and ascites therefore decreases, and as a consequence, reabsorption decreases and interstitial fluid accumulation results [11]. Liver metastasis causing hepatic vein obstruction may be an important aetiology factor in some cases of malignant ascites [1].

3. Diagnosis

The absence of symptoms or the presence of symptoms that mimic other conditions often results in diagnostic delay with OC, and this worsens prognosis. Evaluation consists of physical examination, imaging [ultrasonography, computerized tomography (CT), magnetic resonance image (MRI)], serum tumour markers analysis and ascitic fluid analysis (visual inspection, biochemical analysis, cytology and tumour markers). Diagnostic laparoscopy is an additional investigation and may be useful in patients with whom simple investigations have failed to determine the cause of ascites (**Figure 2**) [6, 17–21].

3.1. Symptoms

The most common complaint in the presentation of OC is abdominal swelling or bloating [17]. These symptoms are commonly associated with the physical and surgical finding of ascites. As the amount of fluid increases, ascites can cause significant symptoms referable to the gastrointestinal and genitourinary tracts. Malignant ascites is associated with abdominal and pelvic pain, while liver disease tends to be relatively painless [6, 17].

3.2. Imaging

Transabdominal and transvaginal ultrasonographies are the most sensitive techniques for the detection of ascitic fluid (**Figure 3**) [19]. Uncomplicated ascites appears as ahomogeneous, freely mobile, anechoic collection in the peritoneal cavity that demonstrates deep acoustic enhancement. Generally, free ascites do not displace organs situated between them

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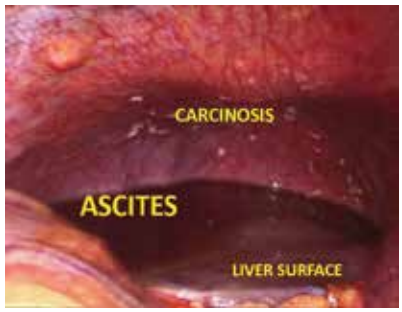

Figure 2. Diagnostic laparoscopy showing ascites and peritoneal carcinosis.

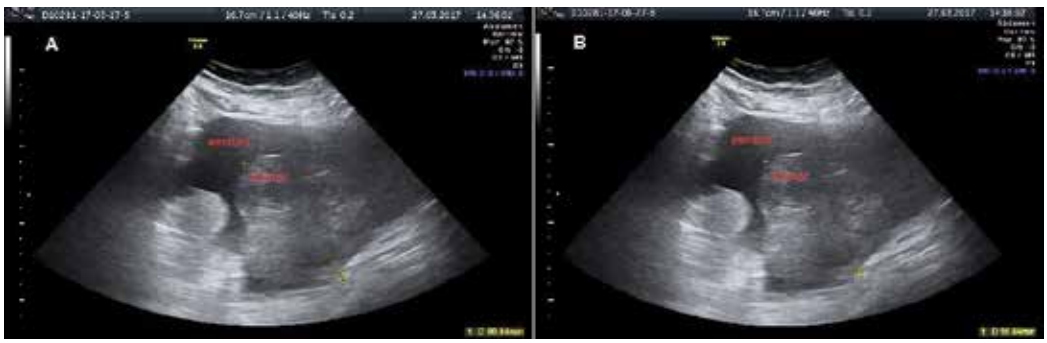

Figure 3. Ultrasound images of ascites. (A) Transabdominal ultrasound image demonstrates ascites and ovarian tumour. (B) Transvaginal ultrasound image demonstrates ascites and intestinal carcinosis.

(**Figure 3A**) [20]. Sometimes, bowel loops do not float freely but may be tethered along the posterior abdominal wall, plastered to organs or surrounded by loculated fluid collections (**Figure 3B**) [21]. When small amounts of ascitic fluid localise in the Morison pouch and the pouch of Douglas, CT scan demonstrated the best sensitivity [21, 22].

3.3. Ascitic fluid analysis

In patients with new-onset ascites of unknown origin, peritoneal fluid analysis may provide some information regarding the origin of the disease. However, it remains difficult to differentiate malignant ascites from other types [23].

On inspection, most ascitic fluids are transparent and tinged yellow. In the case of malignancy, it could also appear pink or red (when at least 10,000 red blood cells/μL are present). Any inflammatory condition can cause an elevated white blood cell count. In case of malignant ascites, lymphocytes usually predominate [24].

Conventional cytological examination shows high specificity, but its sensitivity is low (58–75%) [23]. The cellular components of malignant ascites contain a complex mixture of cell populations, including tumour cells and stromal cells [25]. Immunohistochemistry (ICH) staining and cytological diagnosis by using cell block (CB) sections prepared with the ascites cytological specimen are useful in delineation of the primary origin of the tumour cells. Since

multiple sections can be obtained by the CB method, this technique is particularly valuable when the ICH staining is required for a battery of markers. Typically, primary ovarian epithelial cancers are positive for ER/PR, PAX8, CK7 and negative for CK20 and CDX2. The reverse is true for gastrointestinal cancers. By using a combination of cytological conventional smears and CB methods, the primary site could be detected with 81% accuracy [26, 27].

A number of soluble factors are present in abundance in malignant ascites, but few have been validated for their biomarker potential [28].

4. Treatment

Many factors influence the optimal therapeutic interventions. The aim is palliation in a significant number of patients; only in a selected subgroup is it to improve survival [29].

4.1. Non-pharmacological treatment of ascites

Surgical treatment of malignant ascites involves a variety of different options, each with a certain degree of efficacy but not without risks [30]. Very few studies concern the benefits and harm of differing surgical interventions for intra-peritoneal fluid drainage. Numerous questions, such as how long should the drain stay in place, whether the volume of fluid drained should be replaced intravenously, whether the drain should be clamped to regulate the drainage of fluid and whether any particular vital observations should be regularly recorded, remain partially unanswered [31]. The most common surgical option for ascites drainage is abdominal paracentesis followed, in recent years, by the insertion of permanent tunnelled catheters (PleurX®) and peritoneal-venous shunts.

4.1.1. Paracentesis

Paracentesis (needle drainage of fluid) is an effective and widely used procedure for the management of treatment-resistant, recurrent malignant ascites [32]. It can provide good short-term symptomatic relief in up to 90% of hospital cases, although it may also be offered as a day-case procedure [33].

The procedure involves the placement of a fine tube into the peritoneal cavity to drain ascitic fluid. The procedure can be done all at once but, especially for large volume paracentesis, the catheter can remain in place for several hours and sometimes for days [31]. The volume of drained fluid can vary according to the patient's general conditions, from a few litres up to a maximum of 20 l [30]. Complications of the procedure may include peritonitis, sepsis, visceral injuries, bleeding and fluid leak. Moreover, especially for large volume drainage or repeated procedures, paracentesis may be associated with significantly higher incidence of hypotension and renal impairment [32, 34].

In general, intravenous fluid replacement is not routinely required for paracentesis with less than 5 l removed, but it depends on the patient's clinical condition [32]. However, some reports suggest the use of 5% dextrose infusion during the procedure to avoid severe hypotensive episodes [30, 34]. There is no evidence albumin infusion is of benefit during paracentesis for

malignant ascites, even though many studies focusing on cirrhosis related ascites have demonstrated great benefits of albumin infusion (6–8 g per litre of ascites removed) to maintain intravascular volume [30].

4.1.2. Peritoneal-venous shunting

Common peritoneal-venous shunts drain ascites from the peritoneal cavity into the superior vena cava and have a one-way valve that prevents reflux of blood [32, 34]. They are rarely used due to the high rate of complications such as occlusion, infection, coagulopathy and the widespread dissemination of malignant cells [35]. The only advantage compared to other techniques is related to saving electrolytes and proteins, preserving the body fluid balance [30, 32]. Two shunts are commonly used: the older LeVeen and the most recent Denver shunt, which require different pressures to open the valves [32, 36].

Contraindications of shunt positioning are as follows: congestive heart disease or renal failure due to the significant hemodilution and blood volume overload produced by the shunt, portal hypertension, and severe pleural effusion and clotting disorders [35].

A novel type of technique, automated low-flow ascites pump, drains ascites from the peritoneal cavity to the bladder. This novel device seems effective (even though tested only on liver disease patients) for symptom relief, although data about safety (especially linked to catheter dislodgement and infections) are only preliminary [37].

4.1.3. Catheter drainage

In cases of recurrent or refractory malignant ascites, when frequent paracentesis is required, patients may benefit from an indwelling catheter [32]. This device allows easy and self-drainage, eliminating the need for hospitalisation and frequent paracentesis. The most common permanent catheters are the tunnelled PleurX®, Tenckhoff, Port-a-Cath and cope-type loop catheters [30, 38]. Most authors prefer tunnelled catheters due to greater stability (higher long-term patency rate and success rate) and lower infection rate [30, 39]. Recent trials have suggested that untunnelled catheters have a 21–34% risk of developing peritonitis compared to 4.4% with tunnelled Tenckhoff and 2.5% for tunnelled PleurX® [30].

Catheter placement can be performed with ultrasound guidance or with CT guidance in cases of particular anatomical conditions or widespread carcinomatosis [30]. Antibiotic prophylaxis is recommended for catheter placement [39]. Patients should be instructed to drain the fluid frequently enough to avoid the development of tense ascites, usually once or twice per week. Intravenous fluid replacement and/or albumin supplementation is indicated according to clinical conditions and ascites volume [30, 39].

The safety and cost-effective profiles of tunnelled catheters for the management of recurrent malignant ascites have been demonstrated by several observational studies [38, 40].

4.2. Pharmacological treatment of ascites

If the patient's malignant disease is sensitive to chemotherapy, reduction of ascites production and relief of symptoms may be achieved. However, most patients with ascites have already been treated with several lines of treatment, and their disease has become refractory

to chemotherapy, and carcinomatosis may not be amenable to surgery. For such patients, no pharmacological therapy has been approved except catumaxomab in the EU. The effectiveness of other drugs to treat ascites has been explored in a few studies, with the majority of treatments having been studied in a small series.

4.2.1. Catumaxomab

Catumaxomab (Removab®; Fresenius Biotech) was approved in 2009 by the European Medicine Agency (EMA) for the intraperitoneal treatment of malignant ascites in adults with EpCAM-positive carcinomas, where standard therapy is not available or no longer feasible [41]. Catumoxomab is a trifunctional rat-mouse hybrid monoclonal antibody that is specifically directed against the epithelial cell adhesion molecule (EpCAM) and CD3 antigen (**Figure 4**). EpCAM (CD326) antigen is overexpressed in epithelial ovarian cancer of serous (68%), endometrioid (82%), clear cell (92%) and mucinous (49%) histological subtypes. EpCAM correlates with lower overall survival [42]. Over 80% of ovarian cancer patients have EpCAM over-expressed in tumour cells present in ascites [43]. EpCAM has been reported to initiate cell proliferation by upregulating the oncogene c-myc and to dampen antitumour immunity by blocking antigen presentation in dendritic cells [44, 45]. Mesothelial cells do not express EpCAM on their surface, so catumaxomab applied to the peritoneal cavity specifically targets epithelial tumour cells but not the normal tissue. CD3, as a second antigen, is expressed in mature T-cells as a component of the T-cell receptor. A third functional binding site in the Fc-region of catumaxomab enables the interaction with accessory immune cells (macrophages, dendritic cells and NK cells) via Fcγ receptors. Due to catumaxomab's binding properties, tumour cells and immune effector cells come in close proximity, and complex "crosstalk"

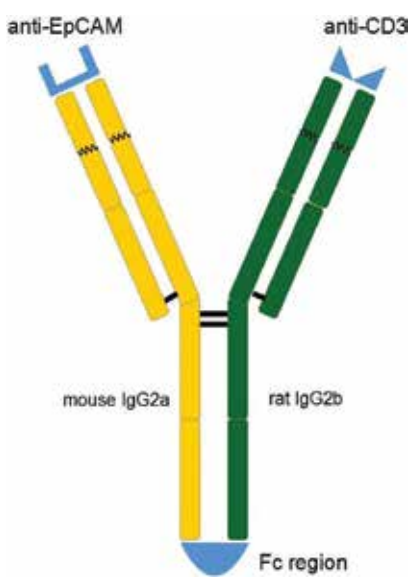

Figure 4. Schematic structure of catumaxomab.

between the T cell and accessory cell can occur, which includes cytokines and co-stimulatory signalling necessary for T-cell activation cascade, resulting in the killing of tumour cells [41].

The clinical efficacy of catumaxomab in the treatment of malignant ascites has been demonstrated in two clinical studies: a phase I/II study (STP-REM-01) and a pivotal phase II/III study (IP-REM-AC-01) [41]. In the first study treatment resulted in a significant reduction of the ascites flow rate from a median of 105 mL/h at baseline to 23 mL/h 1 day after the fourth infusion. Twenty-two of 23 patients did not require paracentesis between the last infusion and the end of the study. Tumour cell-count monitoring revealed a mean reduction up to 99.9% of EpCAM-positive malignant cells in ascites. In a pivotal study, 129 ovarian cancer patients with recurrent symptomatic malignant ascites were randomised to treatment with catumaxomab (as four 6-h *i.p.* infusions on days 0, 3, 7, and 10 at doses 10, 20, 50, and 150 μg, respectively) plus paracentesis or paracentesis alone (the control group). The median time to the next paracentesis was significantly longer for catumaxomab plus paracentesis than paracentesis alone: 77 versus 13 days ($P < 0.0001$) [41].

The safety profile of catumaxomab was established from five completed studies (STP-REM-01, IP-REM-AC-01, IP-REM-PC-01-DE, AGO-Ovar-2.10 and IP-REM-PK-01-EU) [41]. A total of 258 patients were treated with *i.p.* administration of catumaxomab and 207 (80%) patients completed treatment, underlining the good tolerability for the drug. Catumaxomab may cause symptoms related to local and systemic cytokine release: pyrexia, nausea and vomiting. In 48% of patients, abdominal pain was reported, which is considered in part a consequence of the *i.p.* route of administration. All mentioned adverse drug reactions (ADRs) are fully reversible. One hundred and twenty-seven (49%) patients had at least one ADR of grade 3/4, according to the Terminology Criteria for Adverse Events (CTCAE). Abdominal pain, pyrexia and vomiting were the most common symptomatic grade 3 ADRs. Grade 4 ADRs were isolated cases (1%), mostly related to the progression of the underlying malignant disease, such as ileus. In 1% of patients, symptoms of systemic inflammatory response syndrome (SIRS) were observed within 24 h after catumaxomab infusion, such as tachycardia, fever and dyspnea. These reactions resolve under symptomatic treatment. Conditions such as hypovolemia, hypoproteinaemia, hypotension, circulatory decompensation and acute renal impairment must be resolved before each infusion. Since patients with severe hepatic or renal impairment have not been investigated, treatment of these patients should only be considered after a thorough evaluation of benefit/risk. Catumaxomab is potentially immunogenic when administered to humans. In clinical studies, almost all patients (94%) developed human anti-mouse antibodies (HAMAs) or human anti-rat antibodies (HARAs) 1 month after the last infusion; however, patients who developed HAMAs 8 days after the fourth infusion showed a better clinical outcome as measured by puncture-free survival, compared with HAMA-negative patients, suggesting that HAMA development may be a biomarker for catumaxomab response. No hypersensitivity reactions were observed [41].

4.2.2. Other immunological approaches

Evidence to suggest that an immunological approach to the treatment of malignant ascites in OC may be effective and has been observed in small studies of intraperitoneal administration of triamcinolone (long acting corticosteroid), interferons and TNFα [10, 46].

Interferon alpha-2b (IFNα-2b), administered *i.p.* inserted with a 9-French catheter, was evaluated in a study by Sartory et al. Twelve of 41 patients had OC. A complete response (no fluid recurrence) within 30 days of treatment (six courses with an interval of 4 days with six or nine million units depending on a body weight) occurred in 65% of OC patients. The fluid reaccumulated after 11.4 days before and 70.5 days after the treatment. Adverse effects were flu-like symptoms, vomiting and infection with staphylococcus (two patients). If there is no response after the first three courses, the treatment should be stopped [47].

TNFα, installed inside the abdomen of advanced OC patients for 24–48 h (the procedure was repeated on day 8 at a dose of 0.08–0.014 mg/m^2), was evaluated in a study by Kaufmann et al. Production of ascites was supressed or reduced to a minimum for at least 4 weeks in 87% of patients. The treatment was not effective in patients with malignant ascites due to mucinous OC. Patients often suffer from flu-like symptoms, which can be reduced by taking indomethacin or paracetamol before the infusion [47].

4.2.3. Bevacizumab

Bevacizumab (Avastin®, Genentech, Inc., a member of the Roche Group) was approved as an *i.v.* infusion in 2014 by the Food and Drug Administration (FDA) and by EMA in combination with paclitaxel, topotecan, or pegylated liposomal doxorubicin, for the treatment of adult patients with recurrent epithelial ovarian cancer that is resistant to platinum-containing chemotherapy. Bevacizumab is a humanised monoclonal antibody directed against vascular endothelial growth factor (VEGF). Bevacizumab binds to VEGF and thereby prevents the binding of VEGF to its receptors, Flt-1 (VEGFR-1) and KDR (VEGFR-2), on the surface of endothelial cells. Neutralising the biological activity of VEGF regresses the vascularisation of tumours and inhibits the formation of a new tumour vasculature and thereby inhibits tumour growth. Interestingly, the delay of tumour growth induced by anti-VEGF antibody was mainly attributed to the blockage of ascites development and vascular permeability and to a lesser degree to the inhibition of VEGF-induced angiogenesis [7].

Approval of bevacizumab in the USA and EU was based on results of a phase III AURELIA study that involved 361 women with recurrent, platinum-resistant OC, who received either chemotherapy or bevacizumab in combination with chemotherapy. In the subgroup of patients with ascites at baseline, the absence of paracentesis after the first bevacizumab dose suggests that adding bevacizumab to chemotherapy improved the control of ascites [48]. Bevacizumab has been associated with serious (but rare) side effects, and the use of bevacizumab remains significantly more expensive than cytotoxic therapies. The identification of predictive clinical and biological factors that could be utilised to select patients with a greater likelihood of clinical benefit therefore remains a high priority. Using data from Phase III trial GOG218 (Gynaecologic Oncology Group), ascites as a prognostic factor and as a predictor of efficacy for bevacizumab in advanced OC was investigated. In multivariate survival analysis, ascites was prognostic of poor overall survival (OS) but not progression-free survival (PFS). In predictive analysis, patients without ascites treated with bevacizumab had no significant improvement in either PFS or OS, whereas patients with ascites treated with bevacizumab had significantly improved PFS ($p < 0.001$) and OS ($p = 0.014$). These findings support the

plausible biologic rational that patients with malignant ascites have cancer with a phenotype representative of the initiation phase of angiogenesis and are therefore more likely to respond to anti-VEGF therapy. If these findings could be validated through a similar analysis of data from one or more independent randomised phase III trials, the clinical determination of malignant ascites could be a simple and cost-effective way of selecting patients with the greatest probability of benefit from bevacizumab. However, it is possible that volume of ascites could be a more robust predictor of the degree of benefit from VEGF-targeted therapy [49].

Intraperitoneal administration of bevacizumab has also been explored, although only very few OC patients with malignant ascites have received this route of administration. In all patients, ascites resolved after a single *i.p.* dose (5 mg/kg) without re-accumulation or repeat paracentesis over a median observation period >2 months. Moreover, no grade 2–5 adverse events were observed [50]. To evaluate the great potential that preclinical data and clinical case reports have suggested for *i.p.* administration of bevacizumab, clinical trials should be undertaken regarding the safety of treatment, specifically for the palliation of ascites. Bevacizumab may have the potential advantage so that it could be used in patients with reduced performance [47].

4.2.4. Aflibercept (VEGF-TRAP)

Aflibercept (Zaltrap®, Sanofi-Aventis group) was approved for the treatment of adult metastatic colorectal cancer that is resistant or has progressed after an oxalipatin-containing regimen. Aflibercept, also known as VEGF trap (it binds to VEGF trapping it and inhibiting it) in the scientific literature, is a fusion protein, comprising a portion of human VEGF receptor Fit-1 (VEGFR-1) + KDR (VEGFR-2) extracellular domains fused to the Fc-portion of human IgG. Aflibercept binds to VEGF-A, VEGF-B and placental growth factor (PIGF). By acting as a ligand trap, aflibercept prevents binding of endogenous ligands to their cognate receptors and thereby blocks receptor-mediated signalling. VEGF-A acts via VEGFR-1 and VEGFR-2 present on the surface of endothelial cells. PIGF and VEGF-B bind only to VEGFR-1, which is also present on the surface of leucocytes. Excessive activation of receptors by VEGF-A can result in pathological neovascularisation and excessive vascular permeability. PIGF is also linked to pathological neovascularisation and recruitment of inflammatory cells into tumours [51]. In addition to the approved indication, aflibercept has demonstrated the ability to reduce the formation of ascites in patients with advanced epithelial OC [10, 52, 53]. In pre-clinical xenograft models, aflibercept inhibited tumour growth, angiogenesis, reduced blood vessel density and inhibited metastases [10]. The safety and efficacy of aflibercept, administrated *i.v.* at a dosage of 4 mg/kg every 2 weeks, was tested in two-phase II clinical trials in chemoresistant advanced OC patients with recurrent symptomatic ascites [52, 53]. In a randomised, double blind, placebo-controlled, parallel trial, 29 patients were treated. The mean time to paracentesis was significantly ($p = 0.0019$) longer in the aflibercept arm (55.1 days) than in the placebo arm (23.3 days). Two patients receiving aflibercept did not need paracentesis for a period of 6 months. The most common grade 3 or 4 adverse effects were dyspnea, fatigue or asthenia, and dehydration. The frequency of fatal gastrointestinal perforation was higher with aflibercept (three-bowel perforation) than with the placebo. In spite of the effectiveness of aflibercept in the reduction of malignantascites, the authors acknowledge that the limitation of this

treatment is the risk of significant morbidity associated with bowel perforation in patients with very advanced OC. Thus, the advantages of aflibercept over bevacizumab are unclear [53].

4.2.5. Matrix metalloproteinase inhibitors (MMPIs)

MMPs, mainly MMP9, play a role in the release of biologically active VEGF and, consequently, play a role in the formation of ascites. Batistamat, a potent reversible inhibitor of a broad spectrum of MMP, has been developed and has been shown to resolve ascites when given *i.p.* to mice ascites secondary to an ovarian carcinoma xenograft; treatment was accompanied by a 6.5-fold increase in survival [54]. Sixteen patients with OC (out of 23 patients) were included in a Phase I study of *i.p.* administration of batistamat after drainage of ascites. Patients acquired a predicted survival of 1 month or more. Of the 23 patients in the study, 16 did not require redrainage within 28 days of the initial treatment. Five of the 23 patients neither reaccumulated ascites nor died up to 112 days after dosing. Seven patients died without reaccumulating ascites. Adverse effects considered at least possibly related to the treatment occurred in 16 patients, the most common of which were fatigue, fever, vomiting and abdominal pain [46]. MMP inhibitors may warrant further study.

4.2.6. Intraperitoneal chemotherapy

Intraperitoneal chemotherapy is an effective way to palliate malignant ascites. By destroying the surface cancer, it induces a progressive fibrotic process, which will prevent the formation of fluid. If the sclerotic process is not complete, it may produce fluid loculation, which will interfere with uniform drug distribution, may cause obstructions and makes subsequent paracentesis difficult and risky [29]. Intraperitoneal therapy with cisplatin has been evaluated for the first-line treatment of optimal debulked OC patients with FIGO stage III. Despite a 16-month survival advantage, the catheter-related complications rate was 34%, and only 42% of women in the trial completed six cycles of chemotherapy [46].

The procedure called intraperitoneal hyperthermic chemotherapy (HIPEC) is an attempt to increase the cytotoxicity of selected cytotoxic drugs by a hyperthermic medium (40.5–43°C), thereby improving tissue penetration and reducing drug resistance. The primary objective is an increase of PFS and OS, not the control of ascites itself [47]. Finally, aggressive cytoreductive surgery combined with laparoscopic installation of HIPEC is reserved for selected patients with malignant ascites. In well-selected patients, results are encouraging, and this procedure not only controls ascites, but prolongation of OS is possible [29].

Laparoscopic installation of HIPEC has been recently reported as an option to treat resistant malignant ascites not suitable for surgery. The biggest series published, which also included patients with OC, was by Valle et al. [55], who achieved complete remission of ascites in 94% of 52 patients after 1 month of follow-up. There were no complications of the procedure, demonstrating the feasibility and safety of this technique [55].

4.2.7. Diuretics

Some patients with liver metastasis and malignant ascites have raised plasma renin concentrations, and these patients showed a good response to aldosterone competitive antagonist

spironolactone, which decreases reabsorption of water and sodium in the renal collecting duct. Packros et al. [56] found that 13 of 15 patients treated with increasing doses of spironolactone had a good response, with eight remaining free of ascites until death. Renin levels were raised in all of these patients [56].

5. Role of ascites in translational science

Ascites is often therapeutically removed from patients and is therefore an available source of valuable tumour material. Representing the local tumour environment, ascites is composed of cellular and acellular components. In addition to tumour cells present, either as single cells or as spheroids, the cellular component of ascites is composed of stromal cells, including fibroblasts, mesothelial cells, endothelial cells, adipocytes and inflammatory cells. Cells in ascites communicate with each other through acellular components, including cytokines, proteins, metabolites and exosomes. All these components work in coordination to create a tumour-friendly microenvironment. Better knowledge of the tumour microenvironment represented by ascites would thus certainly help to overcome the limitations of current anticancer treatments [23].

Targeting ascites components that cause immunosuppression of T-cells is an interesting future therapeutic option. T-cells present in ovarian tumour ascites do not respond properly to stimulation via the T-cell receptor. Since these T-cells were assayed in the absence of ascites, they gained their normal function, but when ascites was added to T-cells, this effect was rapidly reversed. This might explain why human tumours grow despite the presence of T-cells and other cells of immunological response [10].

In the study by Latifi et al. [57], it was demonstrated that cells in malignant ascites belong to two types of tumour cells, adherent cells (expressed mesenchymal features) and non-adherent cells with an epithelial phenotype, as expressed by EpCAM and cytokeratin 7. Patients with chemoresistant tumours had more tumorogenic, non-adherent cells in the ascites than non-tumorogenic adherent cells. Non-adherent cells featured increased mRNA expression of cancer stem cell-associated genes [10]. Since catumaxomab selectively kills epithelial tumour cells belonging to the non-adherent cell type, this might explain why it is beneficial for patients with OC.

Ascites is highly attractive as a source for biomarker discovery study. The concentrations of cancer-associated soluble factors are usually much higher in ascites than in serum [47, 58]. Moreover, investigation of the relationship between biomarker concentrations in ascites and serum in OC patients may help elucidate whether concentration changes in the local environment can be detected with a blood test [58].

6. Conclusions

The development of malignant ascites is probably dependent on a combination of factors, which disrupts the normal regulation of intraperitoneal fluid flow and the maintenance of a steady state in the peritoneal cavity. Each factor plays a greater or lesser role in each individual patient, so the results of available treatment alternatives are inconsistent. In advanced OC,

palliation of symptomatic patients is the foremost goal, and elimination of fluid accumulation in a patient with these symptoms will certainly improve the patient's quality of life and may even prolong survival. However, effective palliation of malignant ascites remains a difficult management issue. Present treatments have been developed, particularly for malignant ascites, with the primary aim of prolonging the time until a need for subsequent paracentesis. Further clinical trials are therefore necessary in order to investigate the influence on ascites-triggered intervention not only for symptomatic relief but also for the prolongation of both PFS and OS. For the use of targeted therapeutics in malignant ascites (catumaxomab, bevacizumab, aflibercept), it is mandatory to select patients carefully and to identify their risk factors so that the incidence of adverse effects can be minimised. The identification of predictive clinical and biological factors that could be utilised to select patients with a greater likelihood of clinical benefit remains a high priority. With advances in our understanding of malignant ascites pathophysiology, more effective treatment strategies for malignant ascites and ovarian cancer will emerge in the future.

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Chapter 10

Screening for Ovarian Cancer

Poonam Jani and Rema Iyer

Additional information is available at the end of the chapter

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Abstract

Ovarian cancer is often diagnosed at an advanced stage and is associated with poor survival. Screening aims at detection of early stage disease with a view of improving overall survival. Incidence of ovarian cancer is about 1–2% in the low-risk and 10–40% in the high-risk population. Transvaginal ultrasound (TVS) and serum CA125 levels have been used for early detection. Annual screening with TVS and serum CA125 levels (using a cut-off value) has not demonstrated detection of ovarian cancer at an early stage. Multimodal screening (MMS) using sequential CA125 levels (with interpretation of risk using Risk of Ovarian Cancer Algorithm—ROCA) and ultrasound as the second-line test have been shown to have improved sensitivity when compared to annual ultrasound in the detection of ovarian cancer. However, no impact on survival has been demonstrated, and therefore, screening cannot be recommended in the general or high-risk population. There is evidence now to suggest that high-grade serous cancers originate from the fallopian tube where precursor lesions have been identified. Newer screening strategies are likely to shift the focus to detecting these precursor lesions with novel techniques such as exfoliative cytology, circulating tumour DNA and use of microbubbles in ultrasound imaging.

Keywords: screening, transvaginal ultrasound, CA125, ovarian cancer, multimodal screening

1. Introduction

Ovarian cancer is the seventh most common cancer in women worldwide, accounting for 4% of cancers in women. Incidence of ovarian cancer is increasing, especially in Europe and Northern America, being the fifth most common cancer in European women [1]. Even though the life time risk of developing ovarian cancer is 1–2% in the general population, since it is often diagnosed at a later stage, ovarian cancer has the highest mortality rate associated with gynaecological cancers in the developed world [2]. Therefore, there is a need to introduce a screening programme for early detection of this disease.

Screening for any disease is aimed at detection of premalignant conditions or early stage disease. Cervical cancer screening is a successful programme as the progression from premalignant to malignant disease is well understood. However, until recently, precursor lesions were not recognised for ovarian cancer. Now, there is evidence to suggest that some of the high-grade serous cancers start as premalignant lesions in the fimbrial end of the fallopian tube as serous tubal intraepithelial carcinoma (STIC) [3]. Strategies to detect these premalignant lesions are likely to change the approach to ovarian cancer screening.

2. Role of pelvic ultrasound in ovarian cancer screening

Ultrasound has been used as a screening tool to detect early malignant lesions in the ovary and fallopian tube. Features such as presence of septa, papillary projections and solid areas are used to distinguish possible malignant lesions from benign ones. The use of colour flow Doppler to detect altered blood flow as a result of neo-vascularisation has also been explored in diagnosing ovarian neoplasms.

Transvaginal ultrasonography (TVS) has long been considered a useful modality for estimating morphological factors of carcinogenesis. Non-invasiveness and ease of implementation are amongst its benefits for screening, and women generally find TVS an acceptable modality for detection [4]. Factors often used for the assessment of ovarian masses include morphology and volume analysis, but more advanced methods such as Doppler and neuronal network analyses are being investigated for their efficacy.

There are many challenges to overcome in the utilisation of TVS as a screening modality. Variation in operator competence is one such challenge. The United Kingdom Collaborative Trial for Ovarian Cancer Screening (UKCTOCS) trial overcame this challenge by providing standardised training regimes to all sonographers. Although this could be a viable solution, there will always be variation in competence based on operator experience. For example, more experienced sonographers may be better at detecting borderline cysts than less experienced sonographers. The lack of standardised terms to describe ovarian sonographic features is another issue. The International Ovarian Tumour Analysis (IOTA) Group have created a set of recommendations to address this by setting definitions for morphological features such as ‘septum, solid, smooth, irregular’, and so on. [5].

Patient acceptability of screening modality is also an essential factor to consider. In a recent study, 72.7% of women (n = 651) reported no discomfort during TVS, 23.3% of women reported some pain or discomfort and 3.5% documented moderate to severe pain during the TVS procedure. Increasing pain was attributed to history of hysterectomy and a prolonged scanning time. Interestingly, those who experienced pain were noted less likely to return for a subsequent scan 1 year later [4].

Visualisation of the ovary is a further quality assurance factor to overcome in ovarian cancer screening. Decreasing follicular activity and ovarian shrinkage in postmenopausal women makes visualisation problematic. In a study involving TVS of 43,867 women (median age 60.6 years), factors affecting visualisation of ovaries in postmenopausal women included

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previous hysterectomy, unilateral oophorectomy, tubal ligation, increasing age and obesity. Interestingly, factors that increased visualisation of the ovaries included a history of infertility and increasing age at menopause [6].

One of the biggest challenges in ovarian screening lies in differentiating between benign and malignant macroscopic changes. Ovarian morphology varies greatly from patient to patient, and thus benign lesions can give rise to false positive results, leading to unnecessary interventions. Unilocular cysts and those with simple septations are often benign and self-resolving. Features increasing the risk of malignancy include identification of neo-angiogenesis, multiple loculations, presence of papillary structures and solid foci [7–9]. False positives can be reduced with serial ultrasonography [10] as many ovarian lesions resolve without intervention. Benign lesions such as cysts and non-malignant solid lesions are also prevalent in the older population. In a study involving histological and ultrasound characterisation of ovarian cysts from autopsy material from 52 postmenopausal women who had died from causes other than gynaecological cancers, 56% were found to have histologically benign ovarian masses. This evidence suggests that many women will have benign lesions and so ultrasound testing could potentiate unnecessary over-investigating and surgical interventions [11]. The malignant potential of inclusion cysts are yet to be determined, however, it has been proven that TVS is a valid system for detecting malignancy after initial assessment at 1 year. In a study assessing the malignant potential of inclusion cysts, of the 1234 patients carrying ovarian inclusion cysts and 22,914 patients with normal ovaries, 432 women were diagnosed with ovarian cancer, respectively. Overall, the study showed the wider potential of application of TVS as a screening modality [12].

A well-defined criteria or reliable method of quantification needs to be introduced in order to differentiate between benign and malignant cysts. The University of Kentucky has developed a morphological index (MI) score looking at ovarian volume and macroscopic features. In their study, malignancy correlated to an increase in MI score with serial imaging, whereas benign tumours correlated to a decreased or stable MI score [13]. There is scope, therefore, for more accurate quantification of malignant potential, using risk predictors and TVS-led assessment.

New strategies to aid in accurate detection of malignant tumours include neuronal networks and pattern recognition models [14]. These developments are still in their infancy; however, a multicentre study demonstrated that borderline tumours, struma ovarii, papillary cystadenofibromas and myomas proved most difficult to reliably differentiate using ultrasound even with logistic regression models [15].

Magnetic resonance imaging (MRI) is a further imaging modality to consider for screening, due to its detailed visualisation of the pelvis. As an option for screening, however, implications of cost, duration of test, contra-indications for the wider population including placement of metal work, all pose great hurdles to acceptability.

3. Tumour markers

Tumour markers are substances, mostly proteins produced by the tumour cells, which can be detected in the blood and other bodily secretions of the affected individual. These markers

can be produced by normal tissue as well but their levels are usually significantly elevated during a malignant process. Tumour markers are used for the early detection, to guide management and to assess treatment response in cancer.

CA125 is the most commonly used tumour marker for the detection of ovarian cancer. In 1981, Bast et al. developed OC125, a murine monoclonal antibody, which was found to react with ovarian carcinoma cells [16]. An immunoassay was then developed to detect the antigen CA125 in the serum of patients affected by non-mucinous ovarian cancer. CA125 levels were found to be elevated in 82% of women affected by non-mucinous epithelial ovarian cancer, and it was useful in monitoring the treatment response [17].

Elevated CA125 levels are seen in 50% of stage I and >90% of stage II–IV serous ovarian cancers [18]. However, the levels are usually not elevated with mucinous and borderline ovarian tumours. CA125 is also not very specific to ovarian cancer as the levels are increased in other malignancies such that of the gastrointestinal tract, breast and lung; and in benign gynaecological (e.g. endometriosis, fibroids, adenomyosis, benign masses and pregnancy) [18] and non-gynaecological conditions (e.g. heart failure, pancreatitis, hepatitis) [19].

A cut-off value of 35 U/ml is accepted as the upper limit of normal [17]. This cut-off value is acceptable in postmenopausal women, whereas, in premenopausal women, the cut-off value tends to be significantly higher at 50 U/ml [20]. Other factors have also been found to affect the CA125 level. A study on CA125 levels in healthy postmenopausal women observed varying levels with race (highest in Caucasian and lowest in African women), lower levels with previous hysterectomy, regular smoking and caffeine intake, and, higher levels with a previous (non-ovarian) cancer diagnosis. Age of the individual, age at menarche and menopause and previous ovarian cysts were also predictive of baseline levels in postmenopausal women [21].

The CA125 level can be elevated for up to 5 years prior to the diagnosis of ovarian cancer. This finding has been crucial for its application in screening asymptomatic women [22]. Given its low sensitivity and specificity, interpretation of CA125 level using a cut-off value has not been very useful in screening. However, sequential measurements of CA125 as a first-line test and transvaginal ultrasound as a second-line test in multimodal screening have been found to significantly improve its sensitivity and specificity [23].

Human epididymis protein 4 or HE4 is another tumour marker which is elevated in ovarian cancer but not with benign ovarian masses. It can therefore be used to distinguish between the two [24]. In a study using an algorithm combining both HE4 and CA125, 93.8% of epithelial ovarian cancers were accurately classified as high risk [25]. Other markers that have been tested include prolactin, transthyretin, CA72-4 and CA15-3. Combining these markers with CA125 has not shown to improve its efficacy in screening for ovarian cancer [26].

4. Screening population

There are two populations of women who are at risk of developing ovarian cancer—the general population whose life time risk is around 1–2% and the high-risk population (strong family history/gene mutations) whose risk can range from 10 to 46%. Most of the ovarian cancers are

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sporadic of which 90% occur in postmenopausal women, and for this reason, screening trials in the general population have been aimed at this cohort. In the high-risk population, however, even premenopausal women are at increased risk and are therefore included in screening studies.

5. Genetic predisposition to ovarian cancer

Approximately, 5–10% of ovarian cancers are attributed to genetic mutations. Mutations in the BRCA1 and BRCA 2 genes increase the risk of developing both breast and ovarian cancer. The life time risk of developing ovarian cancer (up to the age of 70 years) is 40% (95% CI, 35–46%) for carriers of BRCA1 mutation and 18% (95% CI, 13–23%) for BRCA2 mutation carriers [27]. A strong family history of breast and ovarian cancers could be an indicator of the presence of mutations in BRCA genes given their high penetrance [28]. The age of onset of ovarian cancer tends to be younger in BRCA carriers when compared to the general population. Median age at diagnosis is 63 years in the general population [29], 51.2 years for BRCA 1 and 57.5 years for BRCA2 mutation carriers [30].

Lynch syndrome or hereditary nonpolyposis colorectal cancer (HNPCC) is a syndrome secondary to mutations in the mismatch repair genes (MMR)—MLH1, MSH2, MSH6 and PMS2, which not only increases the risk of developing colorectal cancer but also ovarian and endometrial cancer in female carriers. The estimated cumulative risks of ovarian cancer by age 70 years for women with Lynch Syndrome is around 10% (range 6–14%) [31].

Traditionally, testing for gene mutations has been undertaken in individuals with a strong family history of ovarian cancer. Earlier studies looking at family history alone have shown that women with a first degree relative with ovarian cancer have a 4–5% life time risk of developing ovarian cancer. With two affected close relatives, the risk increases to around 10% and can become higher with even more relatives affected by ovarian cancer [32].

More recently there has been a different approach to screening for gene mutations. A randomised controlled trial looking at testing of the population regardless of family history in the Ashkenazi Jewish population reported a slightly higher incidence of BRCA mutations in the population screening group when compared with the family history group [33]. Such studies suggest that unselected testing of the population identifies 50% more carriers of genetic mutations than the traditional approach to screening based on family history alone.

Other than genetic factors, risk of ovarian cancer has also been found to increase with nulliparity, early menarche and late menopause, hormone replacement therapy and endometriosis. Factors suppressing ovulation such as use of the oral contraceptive pill, multiparity, longer periods of lactation have been associated with a decreased risk [34].

6. Symptom-based screening

Symptoms of ovarian cancer occur insidiously, with many patients presenting with non-gynaecological symptoms such as indigestion, abdominal bloating and early satiety,

leading to a cascade of trial therapies and investigations until a diagnosis is reached. Hence, there may be a time lapse from initial presentation to actual diagnosis of ovarian cancer. The National Institute for Health and Care Excellence (NICE) in the UK advises primary care physicians to conduct preliminary testing if a woman reports persistent or frequent symptoms of abdominal distension, early satiety and/or appetite loss, pelvic/abdominal pain or increased urinary urgency and/or frequency [35]. This has been followed-up by a nationwide campaign, encouraging patients to present if any of the aforementioned symptoms occur.

In an effort to trigger early detection in patients presenting non-specifically, Goff et al. developed a symptom index (SI) [36]. The presence of any one of six symptoms was considered a positive result, including bloating, increased abdominal size, pelvic or abdominal pain, difficulty eating and/or early satiety. In the detection of ovarian cancer, the specificity of the SI was higher in women over 50 (90%) when compared to women under 50 (86.7%) years of age [37]. The SI also had a better sensitivity for advanced stage disease (79.5%) when compared to early stage disease (56.7%). Similar data was noted in a further study, when considering the SI as an isolated screening tool [38]. Acceptability of symptom-based screening was assessed in a subsequent prospective study. Encouragingly, of the 1261 women involved, symptom-based screening yielded a mean acceptability score of 4.8/5 and 4.7/5 for TVS and CA125 utilisation, respectively [36]. A multivariate approach involving SI, CA125 and HE4 biomarkers has also been studied for suitability [38]. Use of all three variates combined yielded an overall sensitivity of 83.8% and specificity of 98.5%. The authors concluded that these combined tests could be beneficial as first-line screening tool to aid selection for second-line imaging.

Despite these results, the question still remains as to whether detection using a symptom-based approach increases survival rates. Overall, there has been conflicting data regarding the correlation between symptom onset, referral and diagnostic delays, stage at presentation and overall survival rates in ovarian cancer patients. Several studies have demonstrated no such association [39]. Moreover, a recent Australian study discovered no correlation between time of symptom onset and FIGO stage III and IV disease, and concluded that longer time to diagnosis does not affect survival in women, even with advanced stage ovarian cancer [40]. A large qualitative study noted no difference between duration of symptom onset or time to diagnosis amongst patients with early to more advanced disease. Interestingly, women with advanced disease were more likely to report disregarding their symptoms [41]. Overall, current evidence suggests that the most successful direction of symptom-based detection of ovarian cancer is with a multivariate approach, but further research is required to ascertain its applicability.

7. Trials in ovarian cancer screening

Ultrasound and serum CA125 testing are two main modalities that have been used in ovarian cancer screening. Ultrasound alone has been used in some of the studies. In some other studies, multimodal screening with a combination of serum CA125 and ultrasound have been used. Following are some of the larger trials in the general population.

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7.1. The University of Kentucky Ovarian Cancer Screening (UKOCS) trial

This trial was set up in 1987 to assess the efficacy of annual transvaginal ultrasonography (TVS) to detect ovarian cancer in asymptomatic women. All asymptomatic women: (1) 50 years or older and (2) 25 years or older with a family history of ovarian cancer in a first- or second-degree relative were eligible to participate in the trial. The control group for this study consisted of those women diagnosed with epithelial ovarian cancer entered in the University of Kentucky Tumor registry or statewide Kentucky Cancer registry between 1995 and 2001, who had not participated in screening [42].

A total of 37,293 women were screened over a period of 24 years between 1987 and 2011 with TVS. Women with an abnormal ultrasound at screening underwent repeat ultrasound in 4–6 weeks. If this scan was also abnormal, then further characterisation of the ovarian mass was performed with tumour indexing, colour Doppler and serum CA125 levels. Women underwent surgery if the second screen was also abnormal. However, if this screen was normal, then the scan was repeated in 6 months. As a result of screening, 47 invasive epithelial ovarian cancers and 15 epithelial ovarian tumours of low malignant potential were detected. An improved survival rate was noted in the screened group when compared to controls. The 5-year survival rate for all women with invasive epithelial ovarian cancer detected by screening as well as interval cancers was 74.8 ± 6.6% compared with 53.7 ± 2.3% for unscreened women with ovarian cancer from the same institution who had undergone treatment using the same protocol ($p < 0.001$) [43].

7.2. The Shizuoka Cohort Study of Ovarian Cancer Screening (SCSOCS) trial

A total of 82,487 asymptomatic postmenopausal women were enrolled into this study between 1985 and 1999 across 212 hospitals in Shizouka, Japan. They were randomised into an intervention group (n = 41,688) or a control group (n = 40,799) and were followed up for a mean period of 9.2 years. The women in the intervention group were screened with a pelvic ultrasound scan (USS) and a serum CA 125 test. If the USS was normal and if the CA125 was <35 U/ml, then they returned to yearly follow-up. If the scan suggested malignant disease and/or if the CA 125 was elevated, then the women were referred for surgery. However, if the scan was abnormal but suggestive of benign disease, it was repeated every 3–6 months. Also, if the CA125 was above a certain threshold with a normal scan, the women had a repeat scan in 6 months. There was no statistical difference between the number of ovarian cancers detected in the screening arm when compared to the control arm (27 vs. 32). However, there were a higher proportion of stage 1 ovarian cancers in the screened group when compared to the control group (63% vs. 38%) [44].

7.3. The Prostate Lung Colorectal and Ovarian (PLCO) Cancer Screening Randomised Controlled Trial

A total of 78,216 postmenopausal women aged 55–74 years were enrolled into this trial across 10 centres in the US. They were randomised to either annual screening (n = 39,105) or usual medical care (n = 39,111). Main outcome measure was mortality from ovarian/tubal/primary

peritoneal cancers. The women in the screening arm had annual transvaginal ultrasound scan and CA125 (using a 35 kU/L cut-off) for 3 years and CA125 alone for a further 2 years. Women with an abnormal screening result were managed by their physicians. The follow-up period was 13 years in total. A total of 212 women were diagnosed with ovarian cancer in the screening arm when compared to 176 in the no screening (usual care) arm. In the screening arm, there were 118 deaths when compared to 100 deaths in the usual care arm as a result of ovarian cancer (mortality RR, 1.18; 95% CI, 0.82–1.71). This trial concluded that screening with CA125 and transvaginal ultrasound did not reduce mortality from ovarian cancer [45].

7.4. Ovarian Cancer Screening Trials in the UK

In 1993, Jacobs et al. screened 22,000 asymptomatic postmenopausal women with serum CA125 using a cut-off value of 30 kU/L. A transvaginal ultrasound was performed if the CA125 level was ≥30 kU/L. Women were referred for a gynaecological opinion if the ovarian volume was ≥ 8.8 ml. Out of the 41 women who had a positive screening result, 11 had ovarian cancer. Of the 21,959 women with a negative screening result, eight subsequently developed ovarian cancer. This protocol achieved a specificity of 99.9% and a positive predictive value of 26.8% and an apparent sensitivity of 78.6% and 57.9% at the first year and second year of follow-up, respectively [23].

Jacobs et al. then conducted a randomised controlled trial to assess the feasibility of a multimodal approach using serum CA125 level and transvaginal ultrasound to screen for ovarian cancer [46]. A total of 21,935 postmenopausal women aged ≥45 years were randomised to either a screening group (n = 10,958) or a control group (n = 10,977). In the screening group, women were offered three annual screens using serum CA125 level as the first screening test. If the CA125 level was ≥30 kU/L, a transvaginal ultrasound scan was performed as a second test. If the ovarian volume was ≥8.8 ml on ultrasound, the women were referred for a gynaecological opinion. Twenty-nine women with a positive screening test had surgical intervention out of which six were found to have ovarian cancer and the remaining 23 had a false positive result. Therefore, the positive predictive value of screening was 20.7%. During the 8 year follow-up period, 10 more women in the screening group developed ovarian cancer bringing the total to 16 in the screened group. In the control group, 20 women were diagnosed with ovarian cancer. The median survival was better in the screening group when compared to the control group—72.9 months versus 41.8 months (p = 0.0112). There were nine deaths from ovarian cancer in the screened group when compared to 18 in the control group, which was not statistically significant (relative risk 2.0, 95% CI, 0.78–5.13; p = 0.083).

7.4.1. Risk of Ovarian Cancer Algorithm (ROCA)

The two UK studies discussed earlier used a cut-off value of CA125 of 30 kU/L for screening. Analysis of the serial serum CA125 data in women who subsequently developed ovarian cancer revealed a significant rise in the CA125 level after a 'change point'. In the unaffected women, however, the CA125 maintained a flat profile, fluctuating around the individual's baseline levels. The ROCA takes into account an individual woman's age, serial CA125 profile and estimates her risk of developing ovarian cancer based on known cases of ovarian cancer

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compared with the flat-profile model of known controls [47]. The ROCA calculates and updates the risk based on the most recent CA125 level. The risk is categorised as elevated, intermediate and normal. Women with an elevated risk are referred for an ultrasound, intermediate risk for repeat CA125 within a few months and normal risk for an annual CA125 test [48]. The ROCA has been used in subsequent screening trials.

7.4.2. The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS)

Between 2001 and 2005, 202,638 women were randomly assigned to a control arm (n = 101,359) and an intervention arm (n = 101,279). The intervention arm was further subdivided into a multimodal screening (MMS) arm with annual CA125 screening (interpreted using the ROC algorithm) followed by ultrasound as a second-line test (n = 50,640) or annual screening with ultrasound (USS) alone (n = 50,639). Randomisation into the control arm and the two-intervention arm was carried out in a 2:1:1 ratio. The main aim of the trial was to determine the impact of screening on mortality from ovarian cancer [49].

Women in the MMS arm had their serum CA125 tested at recruitment and their risk was interpreted using the ROC algorithm. They went on to have (1) ultrasound scan if their risk was elevated or (2) repeat CA125 in 12 weeks if their risk was intermediate and (3) annual CA125 screening if the risk was low.

Women in the USS arm had transvaginal ultrasound at recruitment. They had repeat scans if the initial scan was abnormal. Women with persistent abnormalities were referred for clinical evaluation and had surgery if indicated. This trial was conducted across 13 centres in the UK.

Analysis of the prevalence screen results revealed that the MMS strategy was superior to ultrasound alone for detection of ovarian cancer (sensitivity of 89.4% and specificity of 99.8% for multimodal screening group compared to sensitivity of 84.9% and specificity of 98.2% for ultrasound only group) [50].

During the follow-up period, a total of 1282 women were diagnosed with ovarian cancer (median follow-up—11.1 years). A total of 652 women in the screening arm were diagnosed with ovarian cancer, which included 338 women in the MMS group and 314 women in the USS group when compared to 630 in the no screening group. A total of 148 women in the MMS group, 154 women in the USS group (n = 302) and 347 women in the no screening died from ovarian cancer. There was no significant reduction in mortality from ovarian cancer demonstrated in the primary analysis. However, after exclusion of the prevalent cases, further analysis of the mortality data revealed a significant reduction in mortality in the MMS group when compared to the no screening group. An overall average reduction in mortality of 20% was observed in the MMS group, with a reduction of 8% in years 0–7 and 28% in years 7–14. However, the authors concluded that further follow-up was required to ascertain the benefits of screening [51].

7.5. Screening in the high-risk population

Screening studies in the high-risk population also adopted the following two strategies: (1) annual screening with transvaginal ultrasound and CA125 and (2) multimodal screening with 3–4

monthly measurement of serum CA125 as the first and transvaginal ultrasound as the second-line test based on the CA125 levels.

In a Dutch multicentre observational study, 880 BRCA1 or BRCA2 carriers who had annual screening with CA125 and transvaginal scan were followed-up between 1993 and 2005. There were 10 incident cancers diagnosed. Five out of these ten cancers were in women who had previously had a normal screening within the last 3–10 months preceding the diagnosis. Eight out of the ten incident cancers were stage III–IV. In this study, despite annual screening, a large majority of the cancers were interval cancers that were diagnosed at an advanced stage. This study concluded that annual screening with TVS and CA125 neither helped in early diagnosis nor reduced mortality in high-risk women from ovarian cancer [52].

In the UK, Stirling et al. conducted a study involving 1110 high-risk women who were screened in three cancer genetic centres with annual CA125 and transvaginal ultrasound, between 1991 and 2004 [53]. Thirteen ovarian cancers were detected (including one borderline tumour). Three of these were detected during the first screen and seven during annual follow-up. The remaining three were interval cancers out of which one was an incidental finding following prophylactic surgery 2 months after a normal screen and the remaining two presented with symptoms, 4 and 12 months after a normal screening, respectively. This study also concluded that annual screening with CA125 and TVS was not effective in early diagnosis of ovarian cancer to have an impact on prognosis. In addition, the false positive rate was high in pre-menopausal women leading to unnecessary surgical intervention.

7.5.1. United Kingdom Familial Ovarian Cancer Screening Study (UK FOCSS)

Between 2002 and 2008, 3563 high-risk women (≥10% estimated lifetime risk) aged 35 years or above were recruited into this multicentred study across 37 centres in the UK. The trial had two phases—1 and 2.

In Phase 1, women underwent screening with annual transvaginal ultrasound scan and serum CA125 measurement. For CA125, a cut-off of 35 IU/ml for premenopausal women and 30 IU/ml for postmenopausal women was used.

A total of 27 primary ovarian/fallopian tube/peritoneal cancers were diagnosed during the course of screening and a further 10 cancers developed after 365 days following the last screen (median 539 days, range, 382–1369) in Phase 1 of the study. Nine of the primary ovarian/fallopian tube cancers were diagnosed during the prevalent screen and 13 were incident, screen-detected cancers. The positive predictive value was 25.5% (95% CI, 14.3–40.0) and negative predictive value was 99.9% (95% CI, 99.8–100) for the incident screen. Of the 13 incident cancers, only four were stage I or II. There was a delay in surgical intervention in the prevalent and screen-detected cancers (median—79 days). This study concluded that annual screening was not adequate in high-risk women for early detection of ovarian/fallopian tube cancer.

Following from the results of Phase I, women underwent more frequent screening with CA125 testing in Phase 2 (2007–2012) of the study. Serum CA125 levels were measured every 4 months, and the risk of developing ovarian cancer was estimated using the Risk of Ovarian Cancer algorithm (ROCA). Ultrasound was used as a second-line screen depending on the

ROCA estimated risk [54]. If the risk was normal, TVS was performed annually and if it was abnormal, then TVS was performed within 2 months.

There were 13 screen-detected and 6 occult (diagnosed following risk reducing salpingo-oophorectomy) primary ovarian/fallopian tube cancers in women who had been screened in the preceding year. Five out of the 13 screen-detected cancers and five out of the six occult cancers were stage I–II. Of these 19 women, 18 underwent optimal cyto-reductive surgery, with zero residual disease. This protocol had a high sensitivity of 94.7%, high negative predictive value of 100% and a positive predictive value of 10.8% for the detection of ovarian/fallopian tube cancers within 1 year of screening. The conclusion from Phase II was that ROCA-based screening could be an option for high-risk women who declined risk-reducing surgery. However, there was no conclusive evidence to suggest an impact on survival.

7.5.2. Cancer Genetics Network and Gynecologic Oncology Group study

The Cancer Genetics Network (CGN) ROCA study in Australia and the Gynecologic Oncology Group (GOG) study-GOG-0199 in the US used the same protocol to screen women at increased risk of developing ovarian/fallopian tube cancer [55]. All women received an annual transvaginal scan and CA125 testing every 3 months. The ROCA was used to estimate the risk and an interval TVS was performed for an abnormal ROCA result.

A total of 3692 women were screened in the two studies combined. There were four prevalent cancers and six incident cancers detected as a result of screening. Nine additional cancers were detected following risk reducing surgery. Three out of the six incident cases were detected at CA125 levels <35 U/ml using ROCA. The specificity for referral for ultrasound was 92% and the positive predictive value was 4.6%. This study concluded that three monthly CA125 testing with result interpretation using ROCA had a high specificity in the detection of early stage ovarian cancer with half of the incident cancers being diagnosed at CA125 levels <35 U/ml. There was a high rate of complete cytoreduction following surgery for the incident cancers diagnosed during the study period. The authors concluded that this screening regime with three monthly CA125 measurements performed better than 6–12 monthly screening using an absolute CA125 cut-off of 35 U/ml; however, larger studies were required given the small number of incident cases.

Thus, screening studies in high-risk women have demonstrated that annual screening with CA125 using a cut-off value and TVS is likely to miss the cancers that develop during the interval period. More frequent testing with CA125 with result interpretation using the ROCA helps to estimate an individual's risk based on their baseline CA125 level, aiding detection of ovarian cancer at an early stage or advanced cancer with low volume disease that can be optimally cytoreduced surgically. However, there is still paucity of evidence with regards to a mortality benefit from screening. Therefore, screening cannot be recommended as an alternative to risk reducing surgery, which remains the definitive preventative strategy in high-risk women.

7.6. Future of ovarian cancer screening

Ovarian cancer is a heterogeneous group of cancers, which includes both epithelial and non-epithelial neoplasms. Within the epithelial cancers, there are both slow growing Type 1 cancers

that include mucinous, low-grade endometrioid, low-grade serous, clear cell and transitional cell carcinomas; and, the more aggressive, fast multiplying Type 2 cancers, which include high-grade serous carcinomas (HGSC), high-grade endometrioid, undifferentiated and carcinosarcomas [56]. Given their indolent nature, Type 1 tumours tend to be confined to the ovary at diagnosis, are easily detectable on ultrasound at an early stage and carry a better prognosis. Type 2 tumours, however, metastasise early in the natural history of the disease, are diagnosed at a late stage and carry a poor prognosis as a result. Traditional approach to screening using TVS and serum CA125 has not been effective in detecting these Type 2 cancers at an early stage. Detailed pathological examination of the fallopian tube from high-risk women who have undergone prophylactic salpingo-oophorectomy has revealed pre-cancer precursor lesions (serous tubal intraepithelial carcinoma or STIC) thereby, suggesting that a good majority of HGSC originate in the tube rather than in the ovary [3]. Majority of the incidental HGSCs in the low-risk population have also been shown to arise from STICs [57]. STIC lesions exhibit mutation in the TP53 gene which is likely to signal the early stages of carcinogenesis. Exfoliative cytology from the fimbrial end of the tube to detect these precursor lesions [58] and novel assays to detect TP53 mutations in circulating DNA are being explored [59, 60]. Angiogenesis is present early in the development of cancer. The use of microbubbles that are small enough to pass through capillaries is being explored to detect micro-vascularity in ovarian tumours on ultrasound [61].

A better understanding of tumourigenesis is opening up new avenues in ovarian cancer screening. Studies have shown that the target lesion is not always the ovary in 'ovarian cancer' and that STIC is the pre-malignant lesion in a good majority of HGSCs which include primary ovarian/fallopian tube/peritoneal cancers. The focus of future screening strategies will be used to detect low volume early disease either from the primary site of origin using exfoliative cytology or novel imaging modalities, or, in circulation using sensitive assays to detect low levels of tumour DNA and tumour markers.

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Section 3

Surgery in Ovarian Cancer

Chapter 11

Surgical Management of Ovarian Cancer

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Additional information is available at the end of the chapter

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Abstract

Advanced ovarian cancer remains a disease with a poor prognosis. Surgical therapy remains the cornerstone of treatment with essential contribution from chemotherapy. The combination therapy continues to offer the best treatment strategy. Complete cytoreductive surgery is still the most important prognostic marker. The role of primary debulking surgery in advanced ovarian cancer remains under investigation through high quality rigorous clinical trials. The current evidence regarding primary versus interval debulking surgery has drawn much criticism regarding patient recruitment and quality of surgery, both of which are key pillars in achieving complete cytoreduction. It is expected that greater centralisation and development of 'ovarian cancer surgery teams' will further enhance clinical outcomes.

Keywords: ovarian cancer, cytoreductive surgery, chemotherapy, complications, survival

1. Introduction

The surgical management of ovarian cancer has continued to evolve, particularly over the past 25 years.

The principles of cytoreductive surgery have been applied to not only the pelvic cavity but also within the abdominal and thoracic cavity. The 5 year survival for this cohort of patients has not significantly changed in the past 40 years. Presently clinical trials are examining the role of effective cytoreductive surgery (CRS) and combination chemotherapy (including anti-angiogenesis inhibitors, PARP inhibitors and immunotherapy) in optimising therapy. These are likely to yield encouraging results in the next decade or two.

Imaging, role of lymphadenectomy and the management of recurrent ovarian cancer are discussed elsewhere in this book. This chapter will describe the rationale and outcomes associated with first line (either primary or interval) surgery for ovarian, tubal or primary

peritoneal cancer. A technical description of the operative steps and intra-peritoneal chemotherapy are outside the scope of this chapter.

2. Peritoneal redistribution theory and carcinomatosis

Malignant spread of intraperitoneal tumours can occur via, local contiguous growth, non-contiguous spread along mesenteric planes, haematogenous, lymphatic or transcoelomic routes. Unlike the other routes, the transcoelomic pathway offers a rapid step change in facilitating metastasis form multiple sites from within the abdomen. The parietal peritoneum has both secretatory and absorptive functions. The omentum has an absorptive function. This has been exploited in fashioning omental flaps to minimise incidence of inguinal lymphocyst after lymphadenectomy or pelvic collection after exenteration.

The dynamics of peritoneal fluid is driven by secretion/adsorption by the peritoneum (in particular right diaphragmatic peritoneum), recesses formed by the peritoneal reflections, omental filtering, movement of diaphragm, negative pressure in the subdiaphragmatic region, motility of viscera on mesentry and the resultant fluctuation in pressure differential within the peritoneal cavity.

The 'redistribution phenomenon' was described by Sugarbaker in relation to pseudomyxoma peritonei [1]. In this process free floating malignant cells and other debris utilise the movement of peritoneal fluid (and the ascites produced) to become redistributed throughout the peritoneal cavity. This includes the lesser sac. The absorption and filtering of the peritoneal fluid by the greater and lesser omentum can result in debris and cells, including malignant cells, becoming adherent to the omentum. This may in time result in 'omental cake' noted in advanced ovarian cancer. Another major notable site of fluid absorption and disease conglomeration is the right hemidiaphragm. Gravitational distribution explains the deposits in the Pouch of Douglas, paracolic gutters and subhepatic recesses. The mobile organs such as small bowel are spared of deposits, early in the disease, whereas fixed retroperitoneal structures such as ascending/descending colon and gastric pylorus may be affected. This would necessitate resection of the organs.

3. Evolution of the concept of cytoreductive surgery

As early as in 1934, Meigs described that 'removal of as much tumour as possible' was beneficial for survival [2]. In 1968 the British gynaecological surgeon Hudson described a pioneering technique for the resection of ovarian cancer from the pelvis [3]. Although there have been modifications, the principles have remained the same and his procedure is recognised as the 'radical oophorectomy'. This was an important step in CRS. In fact it was the seminal work of Griffith's published in 1975 which demonstrated that CRS associated with smaller residual disease, can be linked to better survival in advanced ovarian cancer [4].

Benefits of CRS include removal of poorly vascularised tissues (removing the pharmacological sanctuary) and excising the chemoresistant clones. Therefore the resulting absent or minimal disease will have more favourable cell kinetics with regard to chemosensitivity [5].

As the concept of CRS became more widely embraced, the application became more aggressive. Disease on the diaphragm, large bowel, spleen or distal pancreas might have been considered unresectable are now readily resected. Patient selection for these ultraradical procedures is important. This was a concern for the doubters as it may be associated with greater risk of morbidity, delay in receiving chemotherapy as well as significant impact on the quality of life (QOL) [6–8]. Indeed it was felt that perhaps only those with smaller volume and earlier stage disease would benefit from aggressive CRS [5, 9]. However a structured approach to quality improvement through enhanced skills, team structure and commitment to CRS has been shown to improve extent of cytoreduction and hence overall median survival [10–12].

In an important meta-analysis, Bristow demonstrated that greater the volume reduction, greater the survival outcome [13]. In fact they revealed that for every 10% increase in nil residual disease, overall survival increased by 5.5% [13]. Similarly in a more recent meta-analysis of largely newer data in the platinum-taxane era, Chang et al. demonstrated that with each 10% increase in complete cytoreduction, the median overall survival improved by 2.3 months [14].

Three limitations to ultra-radical debulking surgery remain absence of grade A evidence confirming that radical surgery is more efficacious than standard surgery, morbidity/mortality associated with radical CRS and surgery in non-expert centres will only yield nil residual disease in a relatively small proportion of patients [15]. The first two arguments are unlikely to be resolved but one can certainly use big data to resolve treatment pathways for patients with advanced disease.

4. Management of early stage ovarian cancer

In early stage ovarian cancer, the disease is confined to the ovaries or the upper genital tract. Approximately 25% of ovarian cancer patients are diagnosed with stages 1 and 2. These women generally have an excellent prognosis, provided a full staging procedure has been performed. Proper staging allows identification of those who are truly early stage and those who might have more advanced disease. This will allow optimal recommendation regarding adjuvant chemotherapy for the apparent early stage patients [16]. The critical importance of proper staging is underlined by the long term (10 year) follow up data offered by the ICON 1 study [17]. In this study the 10 year survival varied between 56 and 78% depending on the completeness of staging [17].

Table 1 enumerates the steps in comprehensive surgical staging of suspected ovarian cancer. Even in unilateral ovarian cancer, the risk of contralateral lymph node metastasis only, is 3.5%; this is in addition to the 9.7% risk of metastasis on both sides and the 8.3% risk of ipsilateral metastasis [18]. Indeed the risk of para-aortic lymph node metastasis only is 7.1% and the risk

Midline laparotomy
Obtain peritoneal fluid for cytology
Careful examination of all peritoneal surfaces
Total abdominal hysterectomy and bilateral salpingo-oophorectomy
Frozen section of ovarian mass ± suspicious lesions
Infra-colic omentectomy
Appendicectomy (for mucinous tumours)
Peritoneal biopsy from diaphragmatic surface, four quadrants of the abdomen and pouch of Douglas
Pelvic and para-aortic lymphadenectomy

Table 1. Staging procedure for apparent early stage ovarian cancer.

of pelvic and para-aortic lymph node metastasis is 4.3% [18]. Therefore comprehensive staging should include bilateral pelvic and para-aortic lymphadenectomy. However, this may need to be tempered by the overall clinical status of the individual patient. The single exception to this, is early stage mucinous ovarian cancer in which the risk of lymph node metastasis is minor, that omission of lymphadenectomy can be a safe option [19]. The extent of lymphadenectomy appears to correlate with survival benefit, with better outcomes being associated with lymph node counts of greater than 10 per site [18, 20].

5. Role of primary debulking surgery and neoadjuvant chemotherapy

Abundant retrospective data supported the notion of cytoreductive surgery [11, 14, 21]. The standard treatment had been PDS followed by NACT. Where the initial PDS had not resulted in 'optimal debulking' i.e. the residual disease was >1 cm in size, a second look laparotomy for further debulking after 3 cycles of NACT had been the routine practice. Randomised studies have examined this aspect and the most recent study by Rose et al. lead to the abandonment of second look laparotomy [22]. **Table 2** lists the procedures required for the 'ultra-radical' cytoreductive surgery which is in addition to the essential staging procedure.

The EORTC group led by Vergote conducted the first randomised trial comparing PDS followed by adjuvant chemotherapy against IDS after 3 cycles of neoadjuvant chemotherapy [23]. The trial

In addition to the above, the following procedures may be required to obtain microscopic clearance of the disease.
Small bowel resection
Large bowel resection
Intestinal stoma formation
Supracolic omentectomy
Splenectomy ± distal pancreatectomy
Segmental or lobular liver resection
Cholecystectomy
Diaphragmatic peritoneal stripping ± segmental full-thickness resection of the diaphragm
Resection of suspicious or enlarged retroperitoneal lymph nodes
Resection of pleural disease ± hycardiophrenic lymph nodes

Table 2. Potential procedures in 'optimal' cytoreductive surgery.

recruited patients between 1998 and 2006. This was a time when both the surgical strategy with respect to second-look laparotomy and ultra-radical surgery as well as chemotherapy regimens was rapidly evolving.

In the EORTC 55971 study 670 patients were stratified and randomised to PDS or NACT followed by IDS groups. The selection criteria included PS score and 'severe disabling disease' but resectability of the disease by a surgeon was not a condition. The study design did not incorporate CT or laparoscopic scoring system in patient selection. At the time of surgery, in the PDS arm 61% of patients had tumour size >10 cm and 24.2% of the same in the NACT arm; the prevalence of these features at the time of randomisation was 39% in PDS and 42% in the NACT arms. This suggests that a significant proportion of patients appear to have rather aggressive disease. The median operation time was 165 min in PDS and 180 min in the NACT arms. The proportion of patients in whom microscopic clearance was achieved was 19.4% in PDS and 51.2% in the NACT arms. The splenectomy rates (5.8% in PDS, 4.0% NACT) and bowel resection rates (15.5% in PDS, 8.7% in NACT) in this study are far lower than those reported by other high volume centres at the same time [11]. The most frequent sites of residual disease were pelvis, diaphragm and abdominal peritoneum; most experienced teams would consider these sites technically resectable disease. In conjunction with other parameters of cytoreduction mentioned above, one would find it difficult to be certain that the benefits of upfront surgery could have been realised in these operatively unselected patients. The authors acknowledge that a drawback of NACT is fibrosis which might impede tumour resection [23].

The CHORUS group recruited patients with clinical/radiological stage III and IV disease between 2004 and 2010 [24]. Five-hundred and fifty-two women were randomised after stratification by tumour size, stage, PS score and tumour markers as well as prespecified chemotherapy regime (single agent carboplatin, carbotaxol or carboplatin with another agent). Patients received 6 cycles of chemotherapy in total with IDS performed after 3 cycles. The two groups were comparable with similar proportions diagnosed as stage IIIC or IV (89% in PDS and 87% in NACT arms). The median operation time was 120 minutes in both groups, which is a remarkable short time for debulking surgery in advanced ovarian cancer. This consistent with the rates of complete debulking achieved in arms, 17% in PDS and 39% in the NACT arms. Indeed the suboptimal debulking (residual disease >1 cm) was very high at 59% in the PDS and 27% in the NACT arms. These figures are out of kilt with data from high volume international centres. The median overall survival was similar in both groups, but lower than expected at 22.6 months for PDS and 24.2 months for NACT. Multivariate analysis did not identify a subgroup favouring one treatment over another. The size of the residual tumour was prognostic in both arms.

Overall the QOL parameters were comparable between the two groups, except at 6 months post-treatment, the NACT group had higher scores. As expected the PDS group experienced grade 3 and 4 adverse events more frequently than the NACT group with the exception of haemorrhage. Death was more frequent in the PDS (6%) compared to the NACT (<1%) group. The administration and toxicity of chemotherapy was comparable in both groups. The authors acknowledged that the older median age, significant prevalence of poorly differentiated tumour (77%) and high prevalence (19%) of poor performance (PS 2 or 3) status might have

contributed to the less than expected overall survival. Indeed only 56% of patients received combination chemotherapy in their first cycle if their PS was 2–3 but this increased to 72% if their PS was 0–1. This differential has been recognised in the design of the currently recruiting TRUST trial.

Indeed only 77% in the PDS and 79% in the NACT arms completed the allocated treatment strategy.

Both EORTC 55971 and CHORUS trials have been heavily criticised [23, 24]. Indeed one would have to question the rigour with which the peritoneal residual disease might have been assessed in these two studies. For instance the incidence of peritoneal disease within the omental bursa is in excess of 60% [25]. Adequate exploration of the omental bursa is an advanced technique requiring assessment of coeliac nodes, caudate lobe, supragastric lesser omentum and the recesses on the left lateral aspect. One cannot be certain if these steps had been implemented in the earlier RCTs. Thus leading to inaccurate estimation of residual disease. The potential issue of surgeon bias in estimating residual disease has been highlighted in a radiological referencing study [26].

Indeed three further RCTs are examining the question of primary debulking surgery versus neoadjuvant chemotherapy [27, 28]. In fact all three trials include physiological status of the patient in their inclusion criteria. This feature was lacking in the CHORUS trial; EORTC 55971 excluded patients with PS 3 or 'serious disabling disease'.

The Japanese studies (JCOG0602) and the Italian (SCORPION trial) studies have published the peri-operative outcomes [27, 28]. As expected both of these studies demonstrate fewer peri-operative complications, shorter length of stay and smaller blood loss after neoadjuvant chemotherapy compared to primary debulking surgery.

The Japanese multicentre study recruited 301 between 2006 and 2011. In total, 8 cycles of carboplatin and paclitaxol were administered. Those patients with a residual tumour of >1 cm following PDS were offered IDS after 4 cycles of adjuvant chemotherapy. This is despite the publication of GOG152 by Rose et al. in 2004 demonstrating no advantage to second look laparotomy after a maximal effort at PDS [22]. Another peculiarity of this study is the interval of more than 4 years between recruitment of the last patient and submission of the manuscript.

The 'optimal debulking' rate in this study, defined as residual tumour of <1 cm, was 82% in the NACT and 37% in PDS group; when all treated patients are taken into account, including the second-look laparotomy, then the optimal debulking rate changes to 71 and 63% in the NACT and PDS arms respectively [28]. The grade 3–4 complications were less frequent after NACT compared to PDS. Resection of 'abdominal organs' and 'distant metastasis' were more common after PDS. Curiously the duration of main surgery was longer in NACT (302 versus 240 min); this may be explained by the significantly higher rates of pelvic and para-aortic lymphadenectomy in the NACT arm (pelvic LND 72.3% versus 27.2%; para-aortic LND 49.2% versus 11.6%) [28].

In the phase 3 Italian study, Fagotti and colleagues set out to investigate the best strategy for managing patients with high tumour load [27]. Patients were recruited between 2011 and 2014.

This is the first prospective randomised study seeking to verify a finding of an exploratory analysis of EORTC 55971, which stated that for a subgroup of patients with large tumour volumes, NACT lead to fewer morbidites and significantly better overall survival [29]. Therefore the selection of patients for this SCORPION trial was guided by laparoscopic predictive index (PI) [30, 31].Those patients with PI of >8 and < 12 were deemed eligible. In this study 55 were randomly assigned to each arm. None of the patients were subjected to a 'second-look' laparotomy. The cytoreductive rate to nil macroscopic disease was 45.5% in PDS arm and 57.7% in NACT arm. There was no significant difference in terms of the distribution of the residual disease between the two arms—these were military disease on small bowel serosa, hepatic hilum and nodal disease above the superior mesenteric artery. Upper abdominal procedures were carried out in 100% in the PDS and 42.3% in NACT arms. The surgical complexity score was significantly higher in the PDS arm. As a result PDS was associated with longer operating times and higher blood loss; this was accompanied by a mortality rate of 3.6% in the PDS and none in the NACT arms. Three patients in the NACT arm were not submitted for surgery due to disease progression. Many of the generic and specific parameters of QOL measures were in favour of NACT; interestingly cognitive and social functioning showed longitudinal improvement with the PDS group only. The oncological outcomes are awaited. In due course, the findings of this study may complement those of the on-going TRUST trial.

The AGO initiated multicenter international trial, TRUST study (NCT02828618), aims to address many of the short comings identified in the EORTC 55971 and the CHORUS studies. These are addressed by applying selection criteria with regards to the patients, disease and surgical team characteristics. Unlike any of the earlier RCTs in evaluating the timing of surgery in advanced epithelial ovarian cancer, involvement in the TRUST trial will entail an audit of the participating centres prior to study engagement. This ensures that the surgeon(s), surgical team and the relevant infrastructure are in place to deliver the most optimal cytoreductive surgery. The study is expected to complete recruitment in 2023. The outcomes of interest include surgical complications at 28 days, clinical outcomes at 1 year, QOL as well as oncological measures at 5 years.

6. Predictors of optimal cytoreduction

The two most important prognostic characteristics in ovarian cancer are comprehensive staging and optimal cytoreductive surgery [21]. Disease morphology (as predicted by cross sectional imaging and/or laparoscopic assessment), physiological fitness of the patient and the skill set of the surgeon or surgical team are the three key domain determining the resectability.

Since we have long abandoned the concept of second look laparotomy, it is important that where risk of residual exists, we must seek alternatives to primary debulking surgery, otherwise the patient may be dealt with a treatment strategy which is overall suboptimal. Therefore can biochemical, molecular imaging or endoscopic assessment help predict optimal surgery? This subject is a vast area and it will be briefly reviewed here in the context of cytoreductive surgery for advanced ovarian cancer.

The reader should exercise caution in interpreting results from studies for two key reasons; each study will use a different protocol for the index test of interest and secondly the assessment of residual disease is not without bias [32].

Several studies have demonstrated that preoperatively raised level of CA125 and HE4 can predict suboptimal debulking [33–35]. Indeed higher platelet count, lower lymphocyte count or higher ratio of platelet to lymphocyte count can predict suboptimal debulking in advanced ovarian cancer [36, 37].

Functional imaging such as diffusion weighted MRI and PET-CT are rapidly evolving. The key aspect for imaging should be able to identify the rate limiting steps in relation to cytoreductive surgery. Diseases on small bowel mesentry and bowel serosa are consistently the rate limiting steps in delivering microscopic clearance of the disease. At present there is significant interest in diffusion weighted MRI and a prospective imaging study is underway to delineate the role of multiplanar MRI with CT as compared with standard CT alone in guiding decision making process (ISRCTN51246892). In a recent paper, the dwMRI appears to perform very well in predicting disease map [38]. The role of CT on its own in predicting resectability provides does not provide a consistent acceptable answer particularly as the current standard of 'optimal' debulking is nil visible disease [39–41].

Laparoscopic assessment can be useful adjunct in patient selection. One must acknowledge that a laparoscopic assessment can only provide information regarding the intraperitoneal disease. Fagotti and colleagues have accomplished a body of work regarding the role of laparoscopy in advanced ovarian cancer. Studies have consistently shown that small bowel and its mesentry are common site for the residual disease [27, 31, 42]. Petrillo et al. de on laparoscopic assessment, where unresectability is indicated by a predictive score of greater than 8 [31].

It is likely that a combination of metabolic analysis and morphologic characteristics will enhance non-invasive prediction of resectability in the medium future.

7. Optimising cytoreductive surgery

In the United Kingdom, the chief medical officer, Professor Dame Sally Davies recommended that 'the Royal College of Obstetricians and Gynaecologists should make sure that subspecialist training in gynaecological oncology equips doctors to perform optimal surgery for gynaecological cancers and reduce mortality from ovarian cancer' [43].

Both modifiable (technical skills, organisation of care) and constitutional (e.g. age, biology, functional status) factors need to be addressed if we are to make incremental improvements in the outcomes for our patients.

One approach to this could frame any death from ovarian cancer as an 'error'. With such an approach one can adopt a sophisticated approach to addressing the modifiable factors [44, 45]. Vincent and colleagues articulated seven levels at which an error should be tackled. These are

institutional (and national climate), organisation & management, work environment, team, individual staff member, task and patient [45]. How this model could be used for maximising optimal cytoreductive surgery is briefly illustrated here.

The statement by the CMO above creates a national sense of the overall aim. In addition, the European Society of Gynaecological Oncology (ESGO) published a benchmark regarding ovarian cancer surgery, which adds to this impetus, about the desirable end goal [46]. This expert consensus report recommends 10 indicators relating to structural, process and outcome metrics ranging from individual performance to team decision making process within a multidisciplinary setting. Such a suite of benchmarks can set the direction of travel.

Around the globe and even within the 'developed' nations the practice of patient selection, planning and delivery of upfront surgery varies a great deal. Therefore in terms of organisation and management, centralization of care delivery will help optimise outcomes [26, 47]. With regard to team and individual factors, training should target both and the end goal should be clear to both entities, that is, microscopic clearance of the disease. The delivery of surgery could be by a sufficiently trained gynaecological oncologist with the appropriate contribution from allied surgical oncologists. ESGO have organised or sponsored numerous workshops targeting technical skills and the philosophy to support primary debulking surgery. Such a concerted effort will help to redress one of the concerns about current training. The application of the current 'gold standard' evidence from two randomised controlled trials, (though heavily criticised) will adversely impact the training of the next generation of surgeons. Further, the timing of TRUST trial outcome and retirement of those trained prior to the wide acceptance of PDS, will change the landscape of practitioners.

Indeed individual and team training can be augmented by not only skills training in workshops, but through buddy operating during the transition phase. Such an endeavour could be supported by virtual platforms to maintain skills (e.g. video based feedback on specific subtasks in cytoreductive surgery) [48, 49]. This will augment task performance.

Patient selection is discussed elsewhere in this book and will not be discussed here. The factors will include demographic, biological (including molecular characteristics of the disease) and radiomics to mention a few [50].

Another important measure in improving the outcomes as part of CRS is to enhance chemotherapy with the use of Hyperthermic Intraperitoneal Chemotherapy (HIPEC). In a recent study, addition of HIPEC to CRS appears to prolong the overall survival by almost 12 months in patients undergoing interval debulking surgery [51]. This offers an exciting complement to maximal effort CRS. These findings will require independent verification prior to widespread adaptation. This topic will not be discussed further in this chapter.

8. Conclusions

At present the grade A evidence reveals no significant difference in the 5 year survival amongst patients receiving either primary or interval debulking surgery for ovarian cancer. It

must be borne in mind that the evidence base is not without significant criticism. It is likely that on-going surgical and medical trials in ovarian cancer will alter our management of this heterogeneous entity. Clinicians will appreciate that given the morbidity of cytoreductive surgery, development of this service requires appropriate governance structure.

How to optimise surgery: will involve better characterisation of the disease through molecular stratification, better selection of patients in terms of physiological fitness (so reduce complications), continued training of surgeons & teams, centralization of service for those who would be best suited for maximal effort surgery.

Conflict of interest

The author has no conflicts of interest to declare.

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Chapter 12

Patient Selection for Ovarian Cancer Debulking Surgery

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Additional information is available at the end of the chapter

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Abstract

Complete surgical cytoreduction is the most important adverse prognostic factor for survival in ovarian cancer. To achieve this, surgeons often have to perform radical and ultraradical procedures with associated significant postoperative morbidity and mortality. Adverse events are most pronounced in patients with borderline or suboptimal capacity to withstand the stress related to surgery. In frail, elderly, malnourished patients, surgeons face limitations to exercise maximum surgical effort; therefore, alternative treatment strategies are required. Neoadjuvant chemotherapy offers a safe and effective way to enhance recovery after delayed debulking surgery in patients who are not optimal candidates for primary debulking surgery.

Keywords: debulking surgery, ovarian cancer, neoadjuvant chemotherapy, nutrition, age

1. Introduction

Primary debulking or cytoreductive surgery followed by adjuvant chemotherapy has long been the mainstay of treatment for patients with advanced ovarian cancer. The goal of surgery is complete cytoreduction with no visible residual cancer as it is associated with better survival compared with residuals 0–1 cm or >1 cm [1–6]. During the past two decades, new surgical techniques were incorporated into the armamentarium of gynecologic oncologists to address disease located in the upper abdomen. Such paradigm shift in surgical philosophy has resulted in higher rate of complete cytoreduction, and this has translated into survival benefit [7]. Upper abdominal resection (the so-called ultraradical debulking) should only be performed if complete cytoreduction is achievable as even the presence of minimal residual disease will adversely affect the survival of patients [8].

For long, upfront surgery had been the standard approach for patients with advanced ovarian cancer; however, a new treatment strategy using neoadjuvant chemotherapy followed by delayed primary surgery has emerged two decades ago and been supported by retrospective

studies [9–11]. However, Bristow et al. in their meta-analysis demonstrated inferior outcomes for patients undergoing neoadjuvant chemotherapy, although this analysis was heavily biased by the retrospective nature of the studies included [12].

In 2010, Vergote et al. published a prospective, randomized, multi-institutional study on neoadjuvant chemotherapy followed by delayed primary surgery vs. upfront surgery followed by adjuvant chemotherapy. Although the study was heavily criticized by proponents of upfront surgery, it supported a new treatment paradigm by demonstrating equivalent survival with significantly reduced morbidity and mortality for patients undergoing neoadjuvant chemotherapy followed by delayed primary surgery [2]. Kehoe et al. in their prospective, randomized CHORUS trial corroborated these findings [4].

Since the publication of these trials, professional debate has been going on whether to offer primary surgery or neoadjuvant chemotherapy for patients with advanced ovarian cancer and what is the appropriate rate of upfront surgery in cancer centers [13–15]. There has been an apparent dichotomy between highly specialized, quaternary referral centers and smaller units with lower surgical volume and less generous resources. Unfortunately, most cancer centers fail to publish their denominator data, i.e., their referral pathways, the background ovarian cancer population of their catchment area, and the percentage of patients not taken to theater or not receiving any treatment, which brings a significant selection bias into these publications and scientific debates. This makes both the interpretation of the published data and their extrapolation to day-to-day practice difficult [16].

Both EORTC55971 and CHORUS trials have received extensive criticism. Indeed, significant recruitment bias was observed in both studies: patients with large tumor load, unresectable disease, and poor performance status were overrepresented in these studies, and, therefore, many clinicians have been reluctant to extrapolate the results into clinical practice. Furthermore, the rate of complete/optimal resection in these studies was low; in the EORTC55971 trial, <1 cm residual cancer was achieved in only 41.6% of the patients in the upfront surgery arm. Although it improved to 80.7% in the neoadjuvant chemotherapy arm, this surprisingly did not translate into survival benefit [2]. The CHORUS trial reported similar results at 41 and 73%, with no therapeutic advantage associated with such increase [4].

In view of this criticism, the survival results of two subsequent randomized trials, the SCORPION study from Italy and the JCOG0602 study from Japan, are highly awaited [17, 18]. Both studies confirmed significantly reduced morbidity and mortality associated with delayed primary surgery following neoadjuvant chemotherapy compared with upfront debulking surgery. In the Italian study, 91 and 90.4% of the patients with large-volume stage 3C and 4 ovarian cancer had <1 cm residual disease after surgery, in the upfront surgery and neoadjuvant chemotherapy arms, respectively. In the upfront surgery arm, 53% of the patients developed major postoperative complications compared with 6% of the neoadjuvant chemotherapy arm.

In the Japanese trial, 37% of the patients with primary debulking achieved residual disease <1 cm and 82% of those undergoing delayed surgery after neoadjuvant chemotherapy. Interestingly, one-third of the patients in the upfront surgery arm received an interval debulking

surgery, bearing in mind that the use of preoperative laparoscopy to select out unresectable cases was not permitted in the study protocol. Severe complications developed in 5% of the patients in the neoadjuvant chemotherapy arm vs. 15% of the patients in the upfront surgery arm. Survival data for both studies are awaited to confirm superiority of neoadjuvant chemotherapy for the patient cohorts represented in the study.

Despite all criticism, clinical uptake of neoadjuvant chemotherapy has increased worldwide; in the USA, it has increased from 9% in 2003 to 23% in 2013 [19]. Recently, in their joint clinical practice guideline, the Society of Gynecologic Oncology and the American Society of Clinical Oncology have promoted a more selective approach for patients with advanced ovarian cancer, recommending upfront surgery or neoadjuvant chemotherapy for patients with different clinical characteristics [20, 21].

In clinical practice, upfront surgery and neoadjuvant chemotherapy are not equivalent alternatives for all patients. The aim of this review is to aid the readers to find the most appropriate way to treat their patients by analyzing the factors affecting clinical outcome in ovarian cancer.

2. Selecting the right patient for the right treatment

There are numerous clinicopathological factors influencing the outcome of ovarian cancer patients:

- **Patient-related factors:** age, performance status, comorbidities, nutritional status
- **Cancer-related factors:** grade, stage, tumor extent and size, platinum resistance, molecular subtype
- **Treatment-related factors:** residual cancer after surgery, time from surgery to chemotherapy, complications during treatment
- **Institutional factors:** surgical philosophy and skills, available resources, availability of multidisciplinary team

2.1. Age

Age is an independent prognostic factor for survival for patients with advanced ovarian cancer, but age itself also has an impact on patients' ability to cope with stress related to major surgical interventions [22]. The reserves of the cardiovascular, renal, pulmonary, central nervous, and skeletomuscular systems progressively decline in the elderly, and their physiological response to stress is different. Due to altered physiology of the elderly, the pharmacokinetics and pharmacodynamics of medications especially anesthetics are altered.

Mahdi et al. in their review of postoperative outcome of ovarian cancer patients found that patients older than 70 but particularly those over 80 years of age more frequently developed chronic kidney failure, cardiorespiratory diseases, and neurological deficit [23]. Compared with patients <60 years of age, the odds ratio for 30-day mortality after surgery was 3.7, 3.1, and 9.3, for patient aged 60–69, 70–79, and ≥80, respectively. While 1% of the patients younger

than 60 died after operation, 9% of the over 80s suffered fatal complications postoperatively. There was no significant difference in the mortality of patients younger or older than 70 years who received neoadjuvant chemotherapy and underwent delayed primary surgery. Similarly, patients younger than 60 developed less postoperative complications than those aged 60–69, 70–79, and ≥80 (25% vs. 34%, 35%, and 39%, respectively).

Although old age alone is not a contraindication for debulking surgery, but it is an important surrogate to take into account when planning treatment for advanced ovarian cancer. Patients over 80 years with extensive disease requiring four-quadrant resection may benefit from alternative treatment approach, such as neoadjuvant chemotherapy with delayed debulking surgery or, if frail with multiple comorbidities, primary chemotherapy with no surgical intervention.

2.2. Nutritional status

Poor nutritional status has long been demonstrated to be an adverse prognostic factor for postoperative complications, due to reduced immunity and impaired repair capacity [24–28]. Cancer-related hypoalbuminemia is a multifactorial condition related to reduced protein intake, cancer-related systemic inflammation, and muscle protein depletion and is a marker for malnutrition [29]. Patients with advanced ovarian cancer often present with ascites, and in two-thirds of the patients, it is associated with cachexia, loss of muscle weight, and hypoproteinemia-hypoalbuminemia [30].

It has been demonstrated that ovarian cancer patients with hypoalbuminemia (defined as the serum albumin level less than 35 g/L) were 5–10 times more likely to develop severe complications after debulking surgery than those with a normal albumin level. The mortality rates for patients with a low and normal serum albumin level are 12 and 2.5%, respectively [22, 23, 31, 32]. Ovarian cancer patients with a low serum albumin level have a significantly higher anastomotic leakage rate than those with normal levels (18–21% vs. 0–3.4%), and the rate of wound-related complications, infections, and septicemia is also significantly higher [33, 34].

Global clinical assessment of ovarian cancer patients is paramount in diagnosing malnutrition; a single measurement of BMI is unreliable due to excess weight associated with ascites and generalized edema. A low serum albumin level is associated with malnutrition and is an easy test prior to surgery; it is a strong predictor for postoperative morbidity. As two-thirds of ovarian cancer patients are malnourished, it is important to explore the ways to improve nutrition and albumin levels prior to cytoreductive surgery.

Total parenteral nutrition (TPN) for 10 days prior to surgery reduced postoperative complications in gastrointestinal cancer patients with severe undernutrition (defined as the serum albumin level < 30 g/L or 15% weight loss during the past 6 months or BMI < 18) [35]. Geisler et al. demonstrated that in 50% of the severe malnourished ovarian cancer patients showed nutritional improvement on TPN. This translated into less postoperative complications compared with those not responding to TPN [36].

Neoadjuvant chemotherapy offers an alternative approach for patients with nutritional compromise. After two to three cycles of neoadjuvant chemotherapy, the serum albumin level shows

improvement, patients start gaining weight, and their performance status improves [37]. This allows the surgeon to exercise maximum surgical efforts during delayed cytoreduction.

2.3. Tumor extent

The relationship between tumor extent and tumor biology remains unclear. Eisenhauer et al. found that surgical resection counterweighed the presence of bulky upper abdominal disease and concluded that large tumor load did not indicate poor tumor biology [38]. Others, however, found that extensive disease cannot be "downgraded" by radical surgery and patients with high peritoneal cancer index will have poorer survival even if completely cytoreduced [39, 40]. Vergote et al. in their seminal EORTC55971 study found that patients with largest tumor diameter > 5 cm had better survival in the neoadjuvant chemotherapy arm [2]. It does not mean of course that maximum surgical effort should not be applied, as optimally cytoreduced patients consistently perform better than those with residual disease >1 cm [41]. In elderly or frail patients, however, even if it is technically resectable, four-quadrant disease distribution may render patients unsuitable for primary debulking surgery. Aletti et al. found in their study of patients with advanced ovarian cancer that those older than 75 years of age with high initial tumor load (or stage IV disease) plus poor performance or nutritional status were at significantly higher risk for postoperative complications with minimal survival benefit after undergoing complex radical surgery [42]. Delay in starting chemotherapy or dose delays occur more often in the elderly after primary debulking surgery, and this translates into poorer survival [43]. It is imperative, therefore, to find alternative treatment routes in elderly patients with large tumor volume and four-quadrant disease distribution. In such cases, neoadjuvant chemotherapy offers an effective and safe alternative.

2.4. Stage 4 disease

Patients with stage 4 ovarian cancer represent a heterogeneous group with extraperitoneal metastases. According to recent FIGO staging, stage 4A includes patients with pleural effusion and positive peritoneal cytology. For note, the false-negative rate of pleural cytology is high; in a literature review on the use of video-assisted thoracoscopy in ovarian cancer patients with pleural effusion, 23% of the patients with negative pleural cytology had macroscopic disease found in the pleural cavity [44]. The presence of microscopic disease has not been assessed and can potentially be even more frequent. Patients with plural effusion represent a high-risk subgroup for postoperative complications with potential prolonged recovery and delayed administration of chemotherapy, and, therefore, application of neoadjuvant chemotherapy is a considerable strategy.

Stage 4B includes all other types of extraperitoneal metastases including splenic, hepatic, or lung parenchymal disease and lymph node metastases outside the abdominal cavity including the mediastinum, groin, axilla, and neck.

There seems to be an agreement that patients with stage 4 disease by virtue of solitary splenic parenchymal metastasis can easily be cytoreduced (dependent on the peritoneal disease of

course), so can be those with liver metastases in favorable anatomical positions. Controlling the peritoneal disease by complete cytoreduction in stage 4 disease appears to be associated with survival benefit [6]. This effect can only be observed in patients with complete macroscopic cytoreduction within the peritoneal cavity but not in those with any residual disease [45].

On the other hand, those with extensive mediastinal, axillary, or supraclavicular nodes or multiple, unresectable hepatic metastases represent a disease with different biological behaviors, and complete cytoreduction is not achievable; therefore, alternative treatment strategies must be considered [20, 21]. Furthermore, approximately 5% of the patients will progress through chemotherapy so patients with unresectable extraperitoneal disease should not be exposed to primary radical peritoneal resection [4, 17].

In these patients, neoadjuvant chemotherapy offers a safe and effective treatment alternative. Firstly, patients with platinum-refractory disease will not receive an unnecessary peritoneal surgical debulking with the associated morbidity. Furthermore, neoadjuvant chemotherapy effectively eliminates pleural effusion and ascites, improves performance status and serum albumin level, and, therefore, provides the surgeon with an opportunity to exercise maximum surgical effort with acceptable morbidity [37, 46].

The EORTC55971 trial confirmed that neoadjuvant chemotherapy results in superior survival compared with primary debulking surgery in the management of patients with stage 4 disease [2]. In such clinical scenario, the joint ASCO/SGO guideline also recommends the use of neoadjuvant chemotherapy [20, 21].

3. Conclusions

With no doubt, the presence of any residual disease after cytoreductive surgery remains the most important adverse prognostic factor that clinicians have to control over. Therefore, complete macroscopic clearance of the peritoneal cavity should always be the aim of surgery. Preoperatively, patients should undergo holistic assessment by gynecologic oncologist with regard of the disease distribution, extent, stage, and resectability; the patients' physical and emotional capacity to cope with the burden of surgery; and their nutritional status. All efforts should be focused on optimizing patients to tolerate the maximal surgical effort with acceptable morbidity and mortality. While primary debulking surgery remains the standard approach for patients with stage 3 ovarian cancer with optimal age, performance status, and nutritional status, there is growing evidence that neoadjuvant chemotherapy offers a safe and effective alternative for patients with less favorable characteristics.

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Chapter 13

The Role of Lymphadenectomy in Ovarian Epithelial Cancer

Hans Nagar

Additional information is available at the end of the chapter

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Abstract

High-grade serous ovarian/tubal cancer commonly spreads via the peritoneal and lymphatic routes. This chapter discusses the anatomical lymphatic drainage of the ovary and tube with reference to spread from different epithelial ovarian cancer types. The role of lymph node surgery in apparent early stage curative disease will be discussed with reference to staging and directing the need for adjuvant chemotherapy. In advanced disease, the role of lymph node sampling versus systematic dissection surgery as part of cytoreduction is assessed. The result of two randomised controlled trials (RCTs) published on the subject will be analysed along with the ongoing Lymphadenectomy in Ovarian Neoplasia (LION) study. The chapter adopts an evidence-based approach to the role of lymph node surgery in women with epithelial ovarian/tubal cancer.

Keywords: epithelial ovarian/tubal cancer, high-grade serous, para aortic lymph node, pelvic lymph node, systematic dissection sampling, FIGO staging

1. Introduction

The modern of management of women with ovarian involves complete surgical cytoreduction of all visible disease [1]. It is therefore important to understand that approximately 70% of the women will also have lymphatic spread. Even in disease, apparently confined to one or both ovaries, there is evidence of nodal metastatic spread in up to 24% of women [2].

2. The lymphatic drainage of the ovaries

An understanding of the lymphatic drainage of the ovary and fallopian tube is important in the management of women with ovarian cancer. There are three main lymphatic pathways. The principal pathway is along the ovarian vessels through the infundibulopelvic ligament to the para aortic and para caval nodes surrounding the aorta and inferior vena cava (IVC). The second pathway occurs through the broad ligaments into the pelvic nodal region. Of note, spread to contralateral pelvic nodes in women with a unilateral cancer is reported in up to 30% of women [3]. Therefore, a bilateral pelvic node dissection (PND) is recommended even with unilateral apparent stage 1 tumours.

A third lesser route is through the uterine round ligament to the inguinal nodes. In addition, women with disease involving the rectum or sigmoid colon may have tumour spread to the mesocolic lymph nodes within the sigmoid mesentery.

3. Is histopathological type important?

Over the last decade, the understanding of the pathogenesis of epithelial ovarian cancer has changed. The most common histopathological subtype, high-grade serous cancer (approximately 70–80% of cases) appears to arise in the distal fallopian tube [4]. Most of these women present with disease spread to the transperitoneal surfaces and to the lymph system. The majority of this chapter will be concerned with the role of lymphadenectomy in this group of women.

Less common types of ovarian cancer include endometrioid, clear cell, low grade serous and mucinous tumours. These appear to have separate aetiologies with a different risk of lymphatic spread. The risk of nodal metastases appears to be lower in endometrioid and mucinous cancers. For example, a meta-analysis of 278 women with apparent early mucinous cancer of the ovary who underwent a full pelvic and para aortic nodal dissection reported an incidence of involved nodes of only 1.2% [5]. Most authors no longer recommend a lymphadenectomy in early mucinous cancers.

4. What are the methods of surgical assessment?

A definition of a pelvic node dissection (PND) is widely accepted in the gynaecological oncology literature [6]. PND includes bilateral removal of nodal tissue from the distal one-half of each common iliac artery, the anterior and medial aspect of the external iliac artery and vein to the level of the deep circumflex artery, and obturator fat pad anterior to the obturator nerve. The medial aspect of the dissection is the hypogastric artery. Enlarged nodes below the obturator nerve should also be removed. The obturator nerve should be identified and guarded prior to any sharp dissection. The nodes should be swept away from the nerve with careful attention paid to the area below the nerve to avoid damage to the numerous vessels present in this area. The ideal scenario is to remove the node in a single nodal unit to reduce

the risk of nodal fracture leading to possible tumour dissemination and port site metastases. A PND may be performed either as an open procedure or as part of a laparoscopic procedure. Laparoscopic surgery lends itself to PND due to the increased magnification and illumination of the surgical field and dissected nodes can be removed through an 11/12 mm suprapubic port or removed via the vagina if a hysterectomy is performed.

Para aortic assessment/dissection has in contrast to pelvic nodes not been well quantified. Pomel et al. [7] have published a proposed classification of para aortic node assessment which ranges from radiological assessment and palpation to a full systematic dissection of all nodal tissue including the dorsal surfaces of the vessel (**Table 1**).

Open para aortic dissection (type A1 to B1) requires a generous midline abdominal incision to the xiphisternum and a self-retaining retractor to allow access to the great vessels. The right side of the colon and small bowel are mobilised by incising the peritoneum at the level of the right common iliac artery extending medially and caudally to the fourth part of the duodenum and then incising the peritoneum along the right paracolic gutter to the hepatic flexure. This allows the surgeon to perform called 'Kocher manoeuvre' mobilising the bowel off both the right renal fascia and ureter and to be retracted out of the abdomen. Following this, the surgeon should identify the left ureter lying medially underneath the inferior mesenteric vein. The node dissection should not start until all the important anatomical structures have been identified including the inferior mesenteric artery (IMA).

Laparoscopic PA node dissection is well described in the literature and can be performed either via the conventional transperitoneal route or via an extra peritoneal route. Both routes require a high degree of laparoscopic training and is considered unlikely to replicate a systematic node dissection (Pommel type A) but rather an extensive node sampling (Pommel type B1–2).

Type	
A	**Systematic para aortic node dissection**
A1	Complete (includes infrarenal and suprarenal up to coeliac trunk to midpoint of common iliac vessels)
A2	Infrarenal (as above, but does not include suprarenal dissection)
A3	Infra inferior mesenteric artery (IMA) (as above but does not include dissection above IMA)
B	**Para aortic sampling**
B1	Extensive (incudes para aortic areas, but does not allow full visualisation of structures—adventicia of vessels. Renal vessels, anterior common vertebral ligament, psoas muscle and sacrum
B2	Minimal (includes limited para aortic areas, and does not allow visualisation of structures above)
C	**Non-excisional assessment**
C1	Palpation (direct) following full exposure of PA area
C2	Palpation (indirect), transperitoneal without any exposure
C3	Radiological assessment by PET/CT or MRI

Table 1. Proposed classification of para aortic node assessment (Pomel et al. [7]).

5. What is the role of lymphadenectomy in apparent early stage ovarian cancer?

A number of women will undergo surgery for an apparently benign ovarian cyst. Postoperatively, those women with confirmed malignancy can be offered staging including lymphadenectomy. Approximately, 30% of women with ovarian cancers apparently confined to the ovaries will be upstaged following further surgery including a pelvic/para aortic node dissection/sampling (Pommel type B1) with a gynaecological oncologist [8].

It is important to understand that lymph node status is not the only factor that determines the need for adjuvant chemotherapy. Many centres offer chemotherapy to women with stage Ic or above cancers, high-grade lesions and all clear cell cancers of the ovary [9].

However, node status is important for a number of reasons: it may influence whether or not chemotherapy is given, the number of cycles or types of chemotherapy and it may result in complete cytoreduction of the cancer. Node status also partially determines the true FIGO stage and prognosis.

The ACTION trial was a randomised controlled trial (RCT) of 448 women with stage IA, IB grades 2–3, all IC, IIA and all clear cell cancer stage I–IIA and compared the administration of adjuvant chemotherapy with a control arm. The main finding showed overall survival was significantly better with the administration of chemotherapy. A subset analysis revealed that stage I patients with complete surgical staging did not benefit from chemotherapy contrast to patients that underwent incomplete staging [10]. Long-term follow-up of this study has confirmed these results [11]. It has been surmised that patients that have not being staged harbour more advanced disease, and therefore have a poorer prognosis and chemotherapy does not compensate for incomplete staging.

In older women with complex masses or those felt to have a high risk of cancer, an intraoperative frozen section histopathological analysis may be performed. A study from the Gateshead Gynaecological Oncology Centre reported a with a sensitivity of 92%, specificity of 88%, positive predictive value of 82% and negative predictive value of 95% for frozen section analysis [12]. This is equally important in determining which women should not be exposed to unnecessary surgery such as a para aortic node dissection.

Laparoscopic staging is possible, though requires a high degree of specialist training. Several centres have reported on full laparoscopic staging and have found it feasible [13, 14]. Chi et al. performed a case control study comparing staging via laparoscopy or laparotomy in 80 women [13]. They found no difference in specimen sizes and lymph nodal counts. The laparoscopic group had levels of reduced blood loss and a reduced hospital stay. A laparoscopic nodal dissection/sampling should include both the pelvic and para aortic basins to the level of the renal vessels. A case series by Nezhat et al. [15] concluded that laparoscopic staging when performed by a gynaecological oncologist did not compromise survival.

Robotically assisted laparoscopic surgery is an evolution of minimal access surgery rather than a revolution. Perceived benefits include three-dimensional vision, control of the laparoscope by the operating surgeon, more precise instrument movement and a shortened learning

curve. Perhaps, the biggest advantage is the use of instruments that fully articulate at the end in the manner of a human wrist allowing fine delicate movements. This is particularly important in the obese patient, where the increased thickness of the anterior abdominal wall produces an increased torque effect leading to decrease manoeuvrability of standard laparoscopic instruments. Robotic platforms have been used in staging apparent ovarian cancer and appear comparable to laparoscopic surgery [16–19].

Maggioni et al. [20] reported a randomised controlled trial of 268 women with apparent stage 1 or 2 ovarian epithelial cancer. The women were randomised to either a random sampling of pelvic and PA nodal basin or systematic dissection (pommel type A) of the same areas. Positive nodes were found in 9% of the control group and in 22% of the SLD group. No significant difference was recorded in 5 years year overall survival (84.2 vs. 81.3%) or progression free survival (PFS) (78.3 vs. 71.3). The SLD group had a significantly longer operating time, blood loss and blood transfusion.

In view of the results of this study, SLD should not be offered over more limited dissection/sampling (pommel B) in women with apparent early ovarian cancer.

6. What is the role of lymphadenectomy in advanced ovarian cancer?

The goal of surgery in advanced ovarian cancer is to remove all visible disease including a removal of all enlarged lymph nodes. This requires intraoperative assessment of the bilateral pelvic nodes and the para aortic region (pommel type C1–B1).

Given that the nodal basin is considered by some to be relatively chemotherapy insensitive, this to the question whether removal of all involved microscopically and macroscopically involved nodes has a therapeutic benefit.

Panici et al. [21] reported a randomised controlled trial of 268 women with apparent stage IIIB, IIIC/IV cancer. The women were randomised to either resection bulky of pelvic and PA nodes or systematic dissection of the same areas. Positive nodes were found in 42% of the control group and in 42% of the SLD group. No significant difference was recorded in 5 years year overall survival (47 vs. 48.4%). A significant 7-month extension in progression free survival (PFS) was demonstrated (29.4 vs. 22.4 months). The SLD group had a significantly longer operating time, blood loss and blood transfusion. Subsequently, the authors have suggested that the study may be underpowered to detect an overall survival difference.

7. Common complications

7.1. Vascular injury

Working in close proximity to the large blood vessels poses a risk of major haemorrhage. Reducing this risk involves an appropriate surgical incision with a good operative exposure involving dissection/identification of anatomical structures. This allows easier identification

of vascular anomalies and reduces the risk of collateral damage to structures such as the kidney and ureter. Initial management includes pressure to the area and appropriate communication with the rest of the team including the anaesthetist. Small vascular injuries may be oversewn using a vascular needle and small monofilament suture, ideally avoiding constricting the vessel's diameter. Larger defects require the vascular clamp and the expertise of a vascular surgeon.

7.2. Lymphocyst formation

The incidence of lymphocyst after the para aortic/pelvic dissection maybe as high as 43% [22]. The vast majority of these will resolve spontaneously and do not require any intervention. Occasionally, a larger lymphocyst may require aspiration typically by interventional radiological drainage. Occasionally, chylous ascites develop in association with an aortic node dissection especially at the level of the renal vessels. This illustrates the importance clipping large lymphatic channels especially in this region. Management of how chylous ascites includes the low-fat diet, the administration of somatosatin and occasionally total parenteral nutrition.

7.3. Other complications

Other complications associated with lymph node dissection include postoperative ileus, damage to the duodenum, damage to relevant nerves and long-term lymphoedema.

8. Ongoing research into lymphadenectomy

8.1. Early stage ovarian/tubal cancer

Serous tubal intraepithelial carcinoma (STIC) is now considered the precursor lesion for high-grade serous cancer [4]. STIC may be an incidental finding in women undergoing a salpingectomy for benign reasons and the incidence is expected to rise in women undergoing risk reducing surgery for ovarian/tubal cancer. The management of women with STIC as an incidental finding it is unclear. It is apparent, the percentage of these women will have disseminated spread of high-grade serious cancer. Based on small series, authors have suggested comprehensive surgical staging including lymphadenectomy [23, 24]. This is relatively a new condition with larger case series publication expected over the next few years.

8.2. Advanced stage cancer

Following the Panici study reporting a significant difference in PFS, the role of a full systematic node dissection is the subject of two randomised controlled trials, the Lymphadenectomy in Ovarian Neoplasia (LION) and CURACO trials [21].

The Lymphadenectomy in Ovarian Neoplasia (LION) study is an AGO randomised controlled trial including women with FIGO stage IIB–IV ovarian epithelial cancer and complete

macroscopic resection of all disease. Around 640 women were randomised to either a full systematic lymph node (SLN) or no lymph node dissection and the study results are due in late 2017. The primary end point is overall survival (OS) and secondary endpoints include progression free survival (PFS) and quality of life (QOL).

The French CURACO trial is a randomised controlled trial including women with stage III–IV epithelial ovarian cancer with complete macroscopic resection. The women are being randomised to SLN versus no node dissection. The primary end point is progression free survival.

9. Conclusion

Spread to the lymphatic system is common in epithelial ovarian cancer is common and is an early event. Para aortic and bilateral pelvic node dissection sampling (Pommel type B1) should be included in surgical staging to determine chemotherapy use and to improve prognosis in ovarian cancer apparently confined to the ovary based on the results of the ACTION trial.

In women with advanced ovarian, the retroperitoneal lymph nodes should be assessed and bulky lymph nodes removed in an attempt to achieve complete cytoreduction. Systematic lymph node (SLN) of the para aortic nodes should not be routinely performed pending the results of the LION and CURACO studies.

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Chapter 14

Surgery for Recurrent Ovarian Cancer

Desmond PJ Barton

Additional information is available at the end of the chapter

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Abstract

Most patients with ovarian cancer (OC) have the epithelial subtype (EOC) and present with advanced stage disease. Despite improved surgical and medical management of primary disease, the majority of patients will develop recurrence and ultimately die of disease. The current surgical goal in primary EOC is complete surgical cytoreduction (CSC) as this significantly improves disease-specific survival and overall survival. CSC is a major independent prognostic factor in primary EOC. Recurrent ovarian cancer (ROC) can be diagnosed in the symptomatic or in the asymptomatic patient on clinical evidence, tumour marker results and/or imaging. There are data from cases series and retrospective series on the role of surgery in ROC but there is not yet level I evidence of secondary surgical cytoreduction improving overall survival. The published data emphasise that, as with primary disease, the surgical goal is CSC. In selecting patients for secondary cytoreductive surgery a number of predictive models have been proposed and tested. Patients with ROC who have undergone CSC have a better prognosis than those treated with chemotherapy alone or those in whom the surgical goal was not achieved. The counter-argument is that there is bias in the surgical reports—those patients not operated on chemotherapy alone, or who had incomplete cytoreduction and/or who had chemotherapy had less favourable disease-associated and patient-associated factors than those who had CSC. To address these concerns, there are currently three ongoing randomised controlled trials on surgery for ROC.

Keywords: ovarian cancer, recurrence, cytoreduction, surgery

1. Introduction

The hallmarks of cancer include (1) the potential for dissemination of cancer cells to adhere to distant sites and establish tumour growth—metastases and (2) the potential to recur following

primary or subsequent treatments. Frequently these develop together and herald relentless progression until the patient succumbs to disease. For all cancers, these processes show a greater propensity with higher stage (or TNM) of disease at presentation. Furthermore, it is known that certain types or subtypes of a given cancer have a greater or lesser tendency to metastasise and recur than others.

The typical clinical picture of ovarian cancer (OC) is presentation with advanced stage disease in the post menopausal woman and despite advances in medical and surgical treatments, most patients will die of disease. While arguably the goal of primary treatment is cure, this applies to those with early stage disease but not for all subtypes. Data from CRUK [1] show that there were 7378 new cases of OC and 4128 deaths from OC in 2014. These deaths were in most cases due to recurrent disease rather than primary disease. Survival is also associated with lower patient age and the overall 5-year survival is about 35%; the 5-year survival for stages III and IV disease is about 20 and <5%, respectively [1]. The majority of data on ovarian cancer is based on epithelial ovarian cancer (EOC) and this review predominantly deals with recurrent EOC.

2. Defining recurrent cancer

This is the detection of the cancer following a period of time after completion of primary treatment. The NCI Dictionary of Cancer terms [2], defines recurrent cancer as "Cancer that has recurred (come back) after a period of time during which the cancer could not be detected". This is vague and open to interpretation and in clinical practice requires more careful scrutiny:

1. How undetectable disease is defined at the end of primary treatment and how recurrence is defined?
2. How the recurrent disease is detected—clinically, by tumour marker(s), radiologically?
3. The time intervals in the follow-up of patients, the methods of surveillance and how often these are used.
4. Whether there is a clear distinction between persistence of disease following primary treatment and recurrence.

For example, a unit that regularly scans patients after primary treatment may detect evidence of recurrent disease sooner than a unit which relies on serial tumour markers. Indeed, 2 units may use imaging as part of surveillance but one unit may scan more often that another, or measure tumour markers more frequently than another. Complicating this further is that not all recurrences are associated with rising tumour markers and different modalities of imaging have differing sensitivities and specificities in detecting early or small volume recurrent disease. Compounding the understanding of the role of, and efficacy of, different managements for recurrent disease is tumour and patient heterogeneity [3]. As a consequence, caution needs to be given to the interpretation of data on the efficacy of different managements of recurrent cancer —including the role of surgery in recurrent ovarian cancer (ROC). Trial design and the endpoints of trials have important implications [3–5]. It is generally accepted though that overall survival (OS) is the most clinically relevant and the most clearly definable endpoint [3]. Modern imaging and tumour makers have replaced what was the common practice of second look laparotomy

(SLL) in OC, which is no longer recommended. Unlike most other recurrent gynaecological cancers where typically histologic confirmation of recurrence is required before treatment, this is the exception in cases of ROC.

Essentially all OC patients receive platinum-based chemotherapy as part of primary treatment and some concepts are used to help stratify and compare managements of recurrent cancer. These include (1) platinum-sensitive and platinum-resistant disease [6] and (2) platinum-free interval (the interval between date of last platinum dose and date of relapse, PFI) and (3) progression-free survival (PFS). The definition of platinum sensitive and platinum resistant is somewhat arbitrary, but clinically useful. There is an argument that surgical trials might instead focus on date of last treatment (treatment-free interval (TFI)), and date of last operation rather than response to platinum or PFI [7]. Platinum-sensitive OC is defined as disease that is undetected at completion of primary treatment with platinum and which is undetectable for at least 6 months after completion of platinum-based chemotherapy; platinum-resistant disease is ovarian cancer that is detected within 6 months of completion of platinum-based chemotherapy. Other terms used in reports on recurrent cancer are time to first subsequent treatment and intervention-free interval. It is not clear what impact the use of maintenance therapy as an extension of primary treatment will have on these definitions.

3. Determination of recurrent ovarian cancer

Recurrence is documented clinically, and/or by tumour marker levels and/or radiologically and in different clinical units the policy of post-treatment surveillance is variable. The clinical determination of relapse may be in an asymptomatic or symptomatic patient, and rarely OC patients may present acutely, for example, with bowel obstruction. Indeed, previously treated OC patients who develop bowel obstruction almost always have (recurrent) disease as the cause, even if this is not suspected on tumour marker levels or on imaging.

3.1. Clinical features

Recurrence may be suspected from the patient's history—symptoms include weight loss, weight gain (e.g. from ascites), leg swelling (unilateral or bilateral), dyspnoea, pelvic pressure symptoms and loss of appetite. More unusual symptoms relate to the paraneoplastic syndrome including features associated with hypercalcaemia, myositis, erythema nodosum and herpes zoster. Less commonly patients have haematuria, vaginal or rectal bleeding. The patient may of course be asymptomatic.

The clinical examination, which should include assessment of the lymph nodes, abdominal and pelvic examination and recto-vaginal examination, may be normal. If the patient presents more acutely, for example with dyspnoea or evidence of bowel obstruction, there are usually concerning clinical findings.

3.2. Blood results

Unless clinically indicated, the usual test off treatment is to measure the serum tumour marker(s). The evidence that this is useful clinically and contributes to more efficacious treatment and

improved prognosis has been challenged [8, 9]. With regard to the common EOC, recurrent disease may not be associated with high levels of CA 125, it may be associated with a normal level or with a rise within the normal range, and there are other non-cancer explanations for a rising level post-treatment. In a recent trial, it was concluded that treating recurrences (early) with chemotherapy based on rising tumour marker(s) was not associated with increased survival but was associated with a reduced quality of life [8–10]. It is important to note, however, that secondary cytoreductive surgery was not a standard of care in this trial. On the other hand, there is some evidence that early surgical intervention in asymptomatic patients might increase the rate of complete secondary cytoreductive surgery [11, 12]. This then is an argument for post-treatment surveillance by serial tumour marker estimations. With a rise in CA125 noted, the median time to clinical evidence of relapse is 2–6 months. There are no national guidelines in the UK regarding the post-treatment use of serial assessment of serum markers which is often to allay patient anxiety or as part of a trial protocol. Likewise in the USA, the national society, Society of Gynecologic Oncologists (SGO) [13], has not unequivocally endorsed routine post-treatment surveillance using serum tumour marker(s).

3.3. Imaging

In 2000, a collaboration of major cancer groups published criteria to help standardise radiologic interpretation of response to treatment of disease (cancer), which are known as Response Evaluation Criteria in Solid Tumours (RECIST) [14]. In the non-acute routine clinical follow-up, there is variation in the use of imaging, the modality used and the frequency of imaging. Patients on clinical trials typically will have regular imaging as part of the trial. There are no national guidelines in the UK. The National Comprehensive Cancer Network (NCCN) does not stipulate or recommend routine imaging after primary treatment of OC [15]. In most centres, imaging will be performed if there are symptoms (e.g. weight loss, abdominal distension) or signs (palpable pelvic mass). In the UK, the usual imaging will be a CT scan of chest abdomen and pelvis; in other centres FDG-PET may be performed instead of, or in addition to, CT. Practices also vary in the timing of imaging in relation to rising serum tumour marker(s)—including rising levels within the normal range, and levels that exceed the normal range. However, as noted above, early treatment of recurrence with chemotherapy is reportedly not in the patient's best interest whereas earlier surgical intervention may be [8, 9, 11, 12]. In the symptomatic patient with, for example, suspected bowel obstruction, a number of imaging tests will be performed in an effort to confirm the diagnosis, to determine the cause, and to aid in the management decisions.

When deciding on the management of a patient with ROC whose initial management has been in another institution, in many cases it is recommended that there be a review of histology and relevant imaging, and details of the prior surgery. The operative reports should be obtained rather than reliance on a brief summary in patient correspondence.

4. Surgical considerations in the patient with ROC

A general impression is that secondary cytoreductive surgery for ROC is more commonly routine practice in the USA and parts of Europe, and less so in the UK. This is evidenced by the

fact that most reports on the role or impact of such surgery have come from non-UK centres. Almost all reports on surgery for ROC refer to recurrent EOC and not to the non-epithelial types or borderline cancers. Furthermore, the reports on surgical management mostly focus on the first recurrence after primary treatment, rather than the second or third recurrence. The NCCN Guidelines [15] state that secondary cytoreduction can be considered in patients with recurrent ovarian cancer (1) (detected at) more than 6–12 months after completion of initial chemotherapy, (2) who do not have ascites and (3) who have an isolated recurrence (or few foci) of disease which can be completely resected.

In clinical practice, there are different scenarios in which the surgical option for ROC needs to be considered.

Broadly these may be described as:

1. Recurrent ovarian cancer with pelvic and/or abdominal disease (including retroperitoneal lymph nodes); the patient may asymptomatic or symptomatic.
2. Surgery and intraperitoneal chemotherapy (IP) or heated intraperitoneal chemotherapy (HIPEC) for recurrent cancer.
3. Recurrent ovarian cancer outside the pelvis and abdomen.
4. Recurrent ovarian cancer and bowel obstruction.
5. Further recurrence in patients previously operated on or treated for recurrence.
6. Recurrent non-epithelial ovarian cancer (borderline tumours are discussed elsewhere).

There are many published reports on the role and impact of secondary cytoreductive surgery in ROC. Many are from single institutions, often with small numbers, and with minimal quality of life data and, as yet, there are no published studies providing level I evidence on the impact of secondary cytoreductive surgery on overall survival in ROC. So although the best evidence at present is not yet confirmed in trials, there are three randomised controlled trials assessing the role of surgery in ROC, only one of which has just released preliminary data. These are DESKTOP III, SOCceR and GOG 213, in all of which an eligibility criterion is platinum-sensitive EOC [16–18].

a. DESKTOP III Trial: This follows on from the DESKTOP I and II trials and again the predictive model is the positive AGO score for complete secondary surgical cytoreduction. In this trial, two groups are compared—chemotherapy only group and cytoreductive surgery followed by chemotherapy group.
b. SOCceR Trial: This Dutch trial is of secondary CRS and chemotherapy compared to chemotherapy alone in recurrent disease. The primary endpoint is PFI.
c. GOG 213 Trial: In this trial after randomisation to cytoreductive surgery (CRS) patients are then randomised to one of four treatment arms, two of which contain bevacizumab.

Assessing surgery in ROC involves considering the can do/should do approaches and the best to worse scenario from surgery; allied considerations include the timing of surgery, the goal of surgery, morbidity and mortality from surgery and impact on quality of life issues (QoL). From

the patient's perspective when deciding on major surgery, the main considerations are whether there are symptoms or not, the impact of surgery on symptoms and on survival, morbidity and mortality from surgery, quality of life issues (QoL), and response to further chemotherapy or other agents. It is more often easy to decide who not to operate on electively for recurrent disease. This decision is based on disease-associated and patient-associated factors. The former include—disease-free interval, platinum-sensitive/platinum-resistant disease, histology, site or sites of recurrent disease, with and without ascites; the latter include whether the recurrence is symptomatic or asymptomatic, QoL and performance status. There are also surgeon-related factors which relate mostly to the surgical philosophy in the management of recurrent disease —in essence whether to operate on the asymptomatic patient or not, and whether to remove bulk disease only or to plan to achieve complete surgical cytoreduction (CSC) where at end of surgery there is no gross visible disease. As will be discussed, the evidence is very much in favour of CSC to maximise patient benefit as defined by overall survival. The surgeon and/or other members of the oncology team also need to discuss the treatment alternatives with the patient.

4.1. Patient selection criteria for secondary cytoreductive surgery

Major surgery for recurrent ovarian cancer is associated with morbidity and mortality—reportedly from minimal up to 88.8 and 5.5%, respectively [19]. Given the heterogeneity in the patient population and the variation in surgical practice, this perhaps is not surprising. However, it also attests to lack of appropriate reliable criteria for case selection. The goals for elective surgery for recurrent disease in the abdomen/pelvis are to (1) improve overall survival, (2) minimise surgical morbidity and (3) improve QoL. The data on QoL following secondary surgical cytoreduction are, however, sparse.

The rationale for surgery might be considered as an extension of the surgical philosophy in the management of primary ovarian cancer—that complete surgical cytoreduction and combination chemotherapy provides the best therapy to achieve increased overall survival. Furthermore, in the setting of recurrent disease and the known poorer response of ovarian cancer to second-line therapy compared to first-line therapy, one can argue that cytoreduction may have a more important role in recurrent cancer. Indeed, most of the evidence on clinical trials in the chemotherapy-only approach to ROC report median survival of about 18 months in platinum-sensitive disease and about 12 months in platinum-resistant disease [20]. Patients with ROC who undergo CSC have improved survival compared to those treated with chemotherapy alone, but selection bias is likely as those unfit for surgery, for example, will most often receive chemotherapy.

Repeatedly studies report that overall survival is improved with surgical cytoreduction in patients with platinum-sensitive disease but only in patients with CSC and in those with minimal residual disease. In essence the surgical goal in regard to cytoreduction for first recurrence is the same as for primary disease—complete resection. From these studies, a number of factors emerge which are associated with improved survival (**Table 1**). These factors are not dissimilar to those reported as important factors in improved outcome from chemotherapy for ROC [21, 22]. What is less clear from the reports is how much weight to

Primary disease
Initial FIGO stage (early versus late)
Residual disease after primary surgery (complete vs. incomplete)
Disease-free interval (platinum-sensitive, platinum-resistant)
Platinum-free interval
Recurrent disease
Performance status
Number of sites of recurrence
Ascites (present or absent (or <500 ml))
Serum CA 125
Tumour burden/largest tumour mass
Initial second-line chemotherapy before secondary surgery (yes/no)

Table 1. Prognostic factors for improved survival after cytoreductive surgery for ROC.

place on each factor in each individual case. Intuitively one would consider that long disease-free interval, good performance status (before elective surgery) and complete surgical cytoreduction would be favourable for improved survival. A number of predictive models been proposed to improve case selection for secondary complete cytoreductive surgery as these patients benefit most from surgery (**Tables 2** and **3**).

The original DESKTOP OVAR I trial which involved 25 institutions (Arbeitsgemeinschaft Gynaekologische Onkologie [AGO] Descriptive Evaluation of preoperative Selection (K) Criteria for Operability in recurrent ovarian cancer trial) reported that the main predictor for overall survival was complete surgical resection, which was achieved in 49.8% of patients [23]. Patients with non-epithelial ovarian cancer, those with low malignant potential tumours, and those undergoing palliative surgery (as opposed to cytoreductive surgery) were excluded [23]. In the subsequent DESKTOP I Trial [24], in patients with platinum-sensitive disease, the authors reported a median survival of 45 months compared to 19 months in those with complete and incomplete surgical resection, and those (in other studies) treated with chemotherapy alone. Of interest, they also reported that peritoneal carcinomatosis was not a negative factor if complete resection was achieved emphasising that carcinomatosis was not a contraindication to surgery and that complete resection despite the presence of carcinomatosis improved survival [24]. From this study, three prognostic factors for complete resection were identified: (1) good performance status (defined as) on the ECOG criteria [25] (European Cooperative Oncology Group), (2) complete resection at first surgery for primary disease and (3) ascites volume less than 500 ml. These were grouped as the AGO score and defined as positive if all three were present. These were subsequently validated in the DESKTOP II study [26]. It is of interest that imaging was relevant to their predictive model only for measuring volume of ascites and not for the number, size or anatomic location of tumour recurrences. Intuitively it might be considered that carcinomatosis in the setting of recurrent disease would be a contra-indication to secondary surgery and that resection of such disease would not improve overall survival. Laparoscopic assessment was not

AGO Score [23, 24]

1. Complete surgical cytoreduction at primary surgery
2. Absence of ascites at recurrence (<500 ml)
3. ECOG performance status ≤1

Tian Scoring System [33]

1. Initial stage
2. Residual disease after primary cytoreductive surgery
3. Progression-free interval
4. CA 125 at recurrence
5. Presence of ascites at recurrence
6. Performance status

SeC-Score [34]

1. CA 125 at recurrence
2. HE4 at recurrence
3. Presence of ascites
4. Residual tumour volume at completion of primary surgery

Minaguchi Proposal [37]

1. TFI >12 m (versus < 12 m)
2. No distant metastases (versus distant metastasis)
3. Single versus more than one site of recurrence
4. PS 0

Memorial Sloan Kettering Proposal [40, 41]

1. Time to recurrence (DFI)
2. Single or more than one site of recurrence
3. Presence or absence of carcinomatosis
    - DFI 6–12 m surgery for single site recurrence, possibly if more than one site
    - DFI 12–30 m surgery for one or more sites of disease; possible surgery if carcinomatosis
    - DFI > 30 m surgery for single site, multiple sites, and carcinomatosis

Table 2. Predictive models for complete surgical Cytoreduction in recurrent ovarian cancer (based on platinum-sensitive disease).

part of the protocol. There is some suggestion that open laparoscopy may help in case selection —Plotti et al. [27] reported 34 of 38 patients who had a laparoscopy suggesting suitability for surgery subsequently underwent complete secondary cytoreduction. Although there are some randomised data on the use of laparoscopy to determine complete surgical cytoreduction in primary EOC, there are no such data for recurrent disease [28, 29].

A subsequent analysis based on pooled data from an international collaborative cohort [30] reported a scoring system ranging from 0 to 8: progression-free interval < 23.1 months (2), ascites (1), multiple sites of recurrence (1), residual disease after secondary cytoreductive surgery

Primary disease
Early FIGO stage
Complete cytoreduction at primary surgery
Long DFI/PFI
Recurrent disease
Good performance status
No ascites
Number of sites of recurrence**
Maximum tumour dimension
CA125***

*Based on data from platinum-sensitive epithelial ovarian cancer.
**The fewer the better the outcome.
***Normal versus abnormal level.

Table 3. Predictive factors for complete surgical cytoreduction (CSC) in ROC*.

[none, 0.1–1 cm (2): >1 cm (4)]. Low and high-risk models were defined. The difference in median survival between the two groups (63.0 and 19.1 months) was highly significant, and they reported that complete surgical resection was the goal if survival gain was to be maximised. Their model, however, is arguably not straightforward. Note is made that the results of imaging had more influence on decision making (ascites and number of sites of disease) than in the AGO predictive model. In contrast, other studies have reported an improved outcome with single site versus multiple site recurrence [31] and with a DFI of 24 months or more [32].

Tian et al. [33] reported on another model in an attempt to better define those patients with recurrent disease most likely to benefit from cytoreductive surgery. Six criteria were identified—initial FIGO stage, residual disease after primary cytoreduction, progression-free interval, ECOG performance status, CA125 and ascites. They categorised patients into low and high-risk groups based on the score. Compared to other models they reported lower complete cytoreduction rates (53.4% in the low risk group and 20.1% in the high-risk group) than in DESKTOP I. Another group proposed another model which they defined as the SeC-score using four criteria [34]: pre-operative CA 125, pre-operative HE4, ascites and residual disease at primary surgery. They reported a sensitivity and specificity of 82 and 83%. This is one of the few reports to comment on the potential value of CA125, and in a previous study an elevated CA 125 was reported as a negative prognostic factor [35]. Angioloi et al. were the only group reporting on the newer tumour marker, HE4, and the only one in which performance status was not considered. Again in this model, as in the AGO model, the role of pre-operative imaging was essentially only to measure the volume of ascites. Frederick et al. [36] reported in a study on 62 patients with prior complete cytoreduction and platinum-sensitive disease that the only pre-operative factor predicting prolonged survival was a CA125 of less than 250 U/ml which was associated with complete surgical cytoreduction. A Japanese group proposed another model using four criteria [37]—treatment-free interval > 12 months, single site disease, absence of distant metastasis(es) and performance status of 0. Depending on the number of favourable factors, the outcome in terms of complete resection, and overall survival were significantly different.

A number of studies have assessed the two most used predictive models—that proposed by Harter (AGO) and that proposed by Tian [23, 33]. Janco et al. [38] reported that although a positive AGO score was predictive of complete SCR in 79% of patients, in 64.4% of AGO negative cases complete SCR could also be achieved—and as such the AGO score was not an independent factor associated with improved survival. Similar findings of complete cytoreduction—high positive predictive value and high false negative rates—were reported for both models in a population based study on Dutch patients [39]. In this study, 48% of patients had had chemotherapy before surgical cytoreduction but this did not impact on their results. Following on from an earlier proposal for surgical resection in ROC [40], the Memorial Sloan Kettering group compared their scoring system to the AGO and the Tian models in identifying those patients likely to benefit from secondary cytoreductive surgery—that is, those patients in whom complete surgical resection is more likely to be achieved. They proposed to offer secondary cytoreductive surgery to those with: (1) a disease-free interval of less than 6 months, if there was single site disease, (2) disease-free interval of 12–30 months, even if multiple sites of disease provided there was no carcinomatosis and (3) those with carcinomatosis, if the disease-free interval was more than 30 months. These selection criteria might be considered to be counter-intuitive and are different to those of previous reports, but their assessment of the impact of carcinomatosis, is similar to that of the DESKTOP I study, albeit in the context of a longer DFI. They reported [41] that their model was more predictive of complete resection than either the AGO or Tain model. A study from two French centres [42], where initial laparoscopic assessment was common, both the AGO and Tian models were used to evaluate patients; they reported high positive predictive values for complete cytoreduction (80.6 and 74%, respectively, for each model) yet high false negative values (65.4 and 71.4%, respectively).

It can been seen than that although various models have been proposed with some common criteria, the more commonly used AGO and Tian models are associated with significant false negative predictions. It is of no surprise that the factors associated with improved survival in ROC and factors associated with increased rate of CSC in ROC, are similar (**Tables 1** and **3**). Perhaps surprising is that in most series pre-operative CA125 is not considered relevant. Most studies do not report on or recommend an initial laparoscopic assessment, a procedure not without risks, limitations and the associated logistic problems of planning operating lists. Other than Eisenkop's early reports [43, 44], it is also surprising that in most other later models determining and evaluating criteria for surgery of ROC, tumour volume or size of recurrence were not considered relevant. An exception is the report by Onda et al. [45] in which size of recurrent disease or tumour burden was an important factor in case selection. While much emphasis has been given to the importance of complete resection in primary EOC and the positive impact on survival, some reports have emphasised that initial tumour burden in primary disease limits the gains from such surgery—the argument again about surgical skill and tumour biology [46–48]. If indeed tumour burden is important in primary disease, arguably it should be of similar if not more importance in recurrent disease, where chemotherapy is less effective. Furthermore, it is quite clear that patients treated for primary EOC by gynaecological oncologists who achieve CSC have an improved outcome when the cancer recurs, compared to patients in whom primary surgery was incomplete. The positive effects of optimum treatment of primary EOC, continue through recurrent disease. Quite evidently, the characteristics of primary

disease and its management (e.g. complete versus incomplete surgical cytoreduction) have a major impact on the surgical decision making for recurrent disease.

Most recently the preliminary results of one of the RCTs on secondary cytoreductive surgery for recurrent ovarian cancer, DESKTOP III, have been reported in an abstract at the 2017 meeting of ASCO [49]. These were that (1) complete resection was achieved in 67% of patients, (2) there was an increase in PFI (14 months versus 19.6 months), (3) an increase in time to first subsequent treatment (TFST) (13.9 months and 21 months) and (4) data on OS are immature.

5. SCS in platinum-sensitive recurrent ovarian cancer

There are now numerous reports on secondary cytoreductive surgery (SCS) for recurrent ovarian cancer, with the focus on the epithelial subtype. They consistently show a benefit in overall survival—that is in ROC, complete surgical cytoreduction (with or without subsequent chemotherapy) is superior to chemotherapy only in these patients. The counter-argument is that the cases selected for surgery have more favourable features than those treated with chemotherapy alone. But as with primary disease, there is a subgroup who will not undergo surgery and be treated with chemotherapy alone, or rarely palliative care only. These treatment options should not be seen as competing for patients or as an either/or dilemma but as part of the multi-disciplinary team decision as to what is the best management for a particular patient.

The initial report by Berek et al. [50] on ROC showed a survival benefit where the surgical result was optimal (<1.5 cm residual) compared to suboptimal. In a later small study on 36 patients Eisenkop, and a subsequent study by the same authors on 106 patients [43, 44] reported a survival benefit from cytoreduction which was compromised by prior second-line chemotherapy before secondary cytoreductive surgery and where the tumour burden (maximum tumour diameter) was large (>10 cm). Their reports are unusual in that most other reports do not consider either factor as important in case selection for SCS. They also reported that the key surgical factor improving overall survival was complete cytoreduction. Other reports have found the same association and reported [51] that chemotherapy before surgical cytoreduction had a negative impact on surgery.

A common intraoperative finding in recurrent disease is carcinomatosis, which is most problematic where there is extensive involvement of the small bowel serosa and/or mesentery and often results in incomplete surgical cytoreduction. However, the DESKTOP I and II trials reported that even with carcinomatosis, if complete surgical clearance is achieved, carcinomatosis is not a negative prognostic factor in recurrent disease. Indeed, Chi et al. also consider that carcinomatosis is not a contra-indication to secondary cytoreductive surgery if the disease-free interval is 30 months or more as there is patient benefit if CSC is achieved [40, 41]. In a retrospective review of patients with ROC treated in the CALYPSO trial [52], complete surgical cytoreduction was associated with improved survival compared to patients treated with chemotherapy alone; however, as patients who had less favourable features and who did not have complete cytoreduction derived notably less benefit from surgery, then, as noted by the authors,

there is likely to be a significant selection bias in the surgical studies on ROC [52]. Most reports have not addressed quality of life (QoL) issues, but in one report [27], no difference was found to be in QoL in patients with ROC who had chemotherapy alone and those who had surgery and chemotherapy.

6. SCS in platinum-resistant recurrent ovarian cancer

This subgroup of patients has a poor prognosis and more recently bevacizumab has been used as part of second-line treatment. With the associated operative morbidity and possible negative impact on QoL of major surgery in these patients, there has been understandable reluctance both from surgeons and patients to undertake surgery. Where there has been initial suboptimal cytoreduction the surgical goal of complete CSC is rarely achieved, if one extrapolates from the results of Rose et al. [53] in primary disease. A key finding in that study was the training and skill of the surgeon who performed the primary surgery—a gynaecological oncologist whose goal was complete cytoreduction, or a non-specialist surgeon. Case selection for surgery in ROC is also influenced by the patient's performance status, the number of and sites of metastasis and in these cases obtaining the operative report from the initial surgery is often instructive. The practice in the UK is more towards non-surgical management of recurrent disease in platinum-resistant cases. A more common clinical situation is the patient with persistent but stable disease after primary treatment, in whom the disease progresses. In these patients, elective surgery with the goal of achieving complete clearance of disease is most unlikely to be achieved if the original surgery by a gynaecological oncologist was suboptimal and in such cases the recommended treatment is second-line chemotherapy.

Nevertheless, there are some patients who were disease free at completion of treatment for primary disease and have recurrent disease at one or a few sites within 6 months of completing treatment and in whom secondary cytoreductive surgery may be an option [41, 54, 55] and may enhance the otherwise limited response to chemotherapy. Whether or not there is a role for initial laparoscopic assessment is unclear and practices vary. Treatment alternatives must be discussed including palliative care [15]. In other clinical situations, a decision may be made to operate on a patient to remove a large mass that is symptomatic even if CSC cannot be achieved or warranted.

A less common EOC is the low grade serous carcinoma, which typically is less chemosensitive and runs a more indolent course than the high grade serous carcinoma. Often in recurrent disease, there is calcification which can render surgical resection more difficult. Given these usual clinical features there more often is recourse to secondary cytoreductive surgery [56]. This is an individual decision and the pace of growth of the tumour site(s) and whether or not the patient is symptomatic are important considerations.

7. Chemotherapy or surgery as initial treatment for ROC

In an early study [43], a less favourable outcome from secondary surgical cytoreduction was reported if this was preceded by second-line chemotherapy. This was not found in a later

study [56] on a small number of patients. However, if second-line chemotherapy has been given and there has been disease progression, in general there would be a greater reluctance to operate. This sequence of management of initial chemotherapy has been proposed as a means to case select for secondary cytoreduction as only those showing a response should undergone surgery. Bulky disease has been considered an adverse factor in those undergoing surgery for ROC, but only in a few reports; Eisenkop et al. [43, 44] reported on patients with tumour mass more than and less than 10 cm and Onda et al. reported [45] a poorer outcome from surgery with tumour masses greater than 6 cm. Perhaps not surprising that amongst all patients treated initially with chemotherapy for ROC, those who do better are those who also have more favourable factors for surgery—such as longer DFI, good performance status and small volume disease. As with surgery, predictive models for response and outcome for patients treated with chemotherapy for ROC have been described. In the model proposed by Lee et al. [22], CA125 level (≤ 100 IU/l or > 100 IU/l) was assessed as was largest tumour size (<5 cm or >5 cm) but the role of secondary cytoreductive surgery was not assessed. Different managements of ROC may be appropriate in a particular patient but in patients with favourable factors, secondary cytoreductive surgery (with or without chemotherapy) results in a better outcome (overall survival) than chemotherapy alone [24, 30, 33], although level I evidence on overall survival benefit is awaited [49]. In a large retrospective study on ROC in which patients were treated with chemotherapy alone or with cytoreductive surgery and chemotherapy, the latter group had improved overall survival, but only in those with no residual disease or smaller volume residual disease [57].

8. Surgery and IP/HIPEC chemotherapy for recurrent ovarian cancer

The Cochrane review on the use of intraperitoneal (ip) chemotherapy for primary OC [58] concluded that this treatment prolonged PFS and OS. While there is evidence of a survival benefit for IP chemotherapy/HIPEC after cytoreductive surgery in primary disease, there are fewer reports on its use and efficacy in recurrent disease [59]. No mention was made of this type of treatment in the Cochrane review on recurrent ovarian cancer [60] nor in the review by the Fifth Ovarian cancer Consensus Conference of the Gynecologic Cancer InterGroup [7].

Boisen et al. [61] reported on a retrospective study of 25 patients treated with iv/ip chemotherapy but without secondary cytoreductive surgery. The study period was over 6 years on a selected group of patients and 10 of 25 had an improved treatment-free interval. In a feasibility study of ip chemotherapy in 56 patients with platinum-sensitive recurrent disease all of whom had had prior secondary cytoreductive surgery (67.9% to <1 cm), 79% tolerated 6 cycles of ip platinum. No difference in outcome was noted related to the completeness of secondary surgery and the median overall survival was 51 months; no clinical factors associated with improved PFS or OS were identified [62]. The data from other studies report that the main indicators for response to ip chemotherapy are (1) volume of residual disease and (2) platinum-sensitive disease [63, 64]. Fujiwara, in contrast reported responses in patients with suboptimal surgical resection [65].

Ansaloni et al. [66] provided one of the first reports on HIPEC following cytoreductive surgery in 30 patients with recurrent disease. In this small study, HIPEC was considered safe and there

was a trend to improved survival with complete cytoreduction and HIPEC. A more recent study [67] reported a survival benefit in what they described as randomised trial on the use of HIPEC in recurrent ovarian cancer. However, there were a number of deficiencies in study design and questions were raised about the validity of the results and the efficacy of HIPEC as reported in that study [68]. In another retrospective review [69], Cripe et al. reported on 32 patients that CRS and HIPEC were feasible. However, they also noted 65.6% grade 3 or 4 toxicity (morbidity) and that troublesome pleural effusions were associated with diaphragmatic stripping and/or resection. As a number of chemotherapeutic agents were used with varying dwell times and temperatures, it is unclear what regimen to recommend. As with primary disease, a key component in the use of HIPEC is complete cytoreduction or minimal residual disease (<5 mm deposits). A recent report on a retrospective cases series from China on 46 patients with advanced (n = 16) or recurrent (n = 30) ovarian cancer reported a survival benefit with HIPEC but only when there was complete surgical cytoreduction [70]. However, the adjuvant treatment included iv/ip chemotherapy and it is not clear what contribution HIPEC and ip chemotherapy made to improved survival. In contrast, in a study on secondary cytoreductive surgery in EOC, 50 patients underwent surgery only and 29 also had HIPEC, although there were no deaths in the latter group and two in the former group, the addition on HIPEC did not confer an advantage on median disease-free survival [71]. Data were not presented, however, on overall survival or disease-specific survival. In a larger retrospective multi-centre Italian study on 226 patients with primary ovarian cancer and 285 with ROC treated over 16 years, HIPEC was of benefit in patients with ROC who had had complete surgical resection and platinum-sensitive disease [72]. In a large French study of HIPEC in primary and recurrent ovarian cancer, no difference was noted in overall survival between patients with platinum-sensitive and platinum-resistant disease and the main prognostic factor for survival and DFI was the extent of disease, or tumour burden, as measured on the peritoneal cancer index [73]. In the studies showing benefit of CSC and HIPEC, it is still unclear what, if any, additional benefit HIPEC can achieve over CSC. There is still ongoing debate about the role of HIPEC, with the view that HIPEC should be offered only in clinical trials [74]. In fact a number of trials of ip chemo and HIPEC in recurrent ovarian cancer are recruiting [75].

9. Recurrent ovarian cancer outside the abdomen and pelvis

With the improvement in overall survival in ovarian cancer, and better understanding of cancer genetics, targeted therapies and improved surgery, it is now more common to see patients with unusual or atypical sites of recurrent disease [76]. Sites include breast, brain, bone (including vertebral spine), chest wall, skin (other than port site metastasis) and lymph nodes such as the axillary nodes [77–79]. Given the unusual location of metastasis it is important to exclude other sites of disease and commonly PET-CT is used. Biopsy is often necessary to exclude another cancer. In contrast, histologic confirmation of recurrent OC in the pelvis and/or abdomen is not usual clinical practice. Management of the recurrence will include general supportive measures such as pain relief, radiotherapy (e.g. with vertebral metastasis) and chemotherapy, trial drugs and specialised surgery, for example, neurosurgery. The surgery may be indicated for symptom relief and may be considered necessary, even life-saving, in the

presence of metastatic disease at other sites. In assessing the role of specialised surgery for recurrent metastatic ovarian cancer, factors to be considered include—morbidity of surgery, likelihood of resecting disease, likelihood of palliating symptoms by surgical resection, and the patient's prognosis, with and without surgery. There is also some evidence that patients treated with IP chemotherapy and then subsequently with bevacizumab have a greater propensity to develop unusual sites of metastastic recurrence [80]. Patients with a BRCA mutation compared to those who do not have a BRCA mutation more often develop unusual sites of recurrence.

10. Recurrent ovarian cancer and bowel obstruction

Most patients with EOC present with advanced stage disease and most will develop recurrence. A common presentation of recurrent disease is relapsing and remitting bowel obstruction, the course of which is more often chronic than acute [81, 82]. Invariably the development of bowel obstruction indicates recurrent (or progressive) disease, even if the tumour markers are not elevated and there is no radiological evidence of disease. The management is conservative, at least initially with fasting, intravenous fluids and pharmacological manipulation [81, 82]. Involvement of the palliative care team is important. Surgical intervention is associated with significant morbidity and mortality and not all patients, perhaps only about two-thirds benefit from surgery in terms of resumption of adequate oral intake. Despite this common problem in recurrent ovarian cancer, QoL data on surgical and non-surgical intervention are notably absent from most reports.

Surgical intervention includes—placement of a gastrostomy tube [83], by pass procedures, but most often formation of a diverting stoma. As the disease is often more extensive in the pelvis with serosal and mesenteric disease, more often an ileostomy is raised rather than a colostomy, although often when a loop ileostomy is performed it is necessary to defunction the large bowel by raising a mucous fistula. If a recto-vaginal fistula develops from extensive pelvic disease, a colostomy may provide successful palliation but typically to a limited extent. That is, the patient will continue to have other problems related to the pelvic disease—including pelvic pain, discharge and vaginal or rectal bleeding. It is important to discuss with the patient the likely palliative benefit of surgery, as it is to discuss the outcomes from the surgical and non-surgical management of bowel obstruction.

11. Surgery for second recurrence and beyond

There are fewer reports on the role of surgery for second, third, etc. relapse of EOC. Intuitively the factors that are important in surgical decision making for first recurrence should also be important in surgical decision making in patients with second and subsequent recurrence. It is clear too that if surgery is contemplated for such relapses the patients are highly selected and more often than not surgical intervention will be for palliation (e.g. bowel obstruction) rather than for complete cytoreduction. More usually in clinical practice patients with second and

subsequent relapse will be treated with chemotherapy or other drug therapy. The paucity of cases and reports on tertiary cytoreduction emphasises the uncommon clinical scenario of a patient with second relapse of EOC undergoing surgery. In a multi-centre retrospective review of 406 patients [84], based over a 16-year period, it was reported that residual tumour after secondary and tertiary surgery was an important prognostic factor and surgical outcome was compromised by ascites and upper abdominal disease. Avras et al. [85] reported that the surgical goal, as with first recurrence, should be complete cytoreduction as this improved overall survival. The usual factors to be considered for surgery in recurrent disease with the goal of complete cytoreduction, such as disease-free interval, were reported but they also found an association with increased size of recurrent disease and reduced benefit from surgery. Another report highlighted the importance of case selection and maximixing cytoreduction [86]. No QoL data were presented in these papers.

12. Recurrent non-epithelial ovarian cancer

Most reports on ROC almost exclusively deal with epithelial ovarian cancer. Even with the EOC, the subgroup of mucinous cancers, which are less chemosensitive than their serous counterparts, arguably should more often be treated with surgery for first recurrence than with chemotherapy. The recent Gynecologic Cancer Intergroup (GCIG) report provided little guidance [87]. Two reports describe a very poor outcome when mucinous ovarian cancers relapse and caution about surgical intervention [88, 89]. It remains unclear whether recurrent mucinous cancer should be managed as recurrent pseudomyxoma peritoneii with extensive peritoneal resection and HIPEC.

There are fewer reports on the less common OC subtypes. Granulosa cell tumours, which have limited chemosensitivity compared to EOC typically have an indolent course. Whereas primary disease is often of low stage, recurrent disease is characterised by multi-site relapse which presents different surgical challenges if complete cytoreduction is the goal [90]. Given their more indolent behaviour there may be an argument for targeting symptomatic masses rather than CSC. For germ cell tumours, most of the information is extrapolated from data on male patients. Germ cell tumours are rare in females and the immature teratoma, defined by the presence of immature cancerous tissue, most often immature neural tissue, typically is managed by chemotherapy after initial surgery. Two conditions described in the literature on germ cell tumours are the “growing teratoma syndrome” and “chemotherapeutic retroconversion” are generally considered to be the same as histologically the tissue found is mature teratoma [91]. In the former, after successful chemotherapy, there is recurrent disease but of mature not immature teratoma; in the latter, chemotherapy given to immature teratoma resulted in subsequent mature elements only. This is important to recognise as otherwise disease-progression or recurrence (of original immature disease) is diagnosed. If further immature teratoma is diagnosed after primary treatment this is associated with a less favourable prognosis and pathological confirmation of recurrence as mature or immature is necessary to appropriately manage. Typically treatment of recurrent disease is conservative surgery and further chemotherapy [92]. The specific considerations are the young age of patients and fertility preservation, chemosensitivity and the growing

teratoma syndrome. The more usual indication for surgery is to remove a symptomatic mass or a growing mass that is causing pressure symptoms (the growing teratoma syndrome). In such cases, the focus of surgery in the typical young patient, with fertility preservation necessary, is not complete cytoreduction but resection of the symptomatic mass. A less common clinical problem is of peritoneal disease with mature glial tissue—gliomatosis peritoneii, which most often has a very indolent course. Typically the initial primary surgery has been fertility preserving. With relapsed disease, which may be in the pelvis or disseminated, including involvement of the retroperitoneal lymph nodes, it is important to determine whether the relapsed disease is mature or immature teratoma, and although both pathologies may be present the more common is mature teratoma [93]. For gliomatosis peritoneii, which is of different grades, surgery should be in symptomatic patients only, the goal is palliation and not complete cytoreduction, which is most often not feasible. When secondary surgery is undertaken for recurrent disease the reproductive organs should be preserved if possible (including the uterus). The surgical goal is cytoreduction with fertility preservation, and it is reasonable to leave small volume disease on the one remaining ovary.

13. Conclusion

Most patients with OC present with late stage disease and most are destined to develop recurrence and to die of disease. Consideration needs to be given as to how recurrence is diagnosed and whether the patient is asymptomatic or symptomatic. The majority of data on ROC is from studies on EOC, but the role of secondary surgery is influenced by the histologic subtypes of OC. Patients treated with second-line chemotherapy tend to have less favourable features than those treated initially with surgery. In non-randomised studies, where there is likely selection bias, usually showed a benefit in overall survival from secondary cytoreductive surgery compared to chemotherapy alone. Consistently non-randomised studies report that the benefit of surgery in terms of DFI and survival is seen only in patients with complete surgical cytoreduction. Only one of three current randomised trials has reported preliminary data which show a benefit from surgery and data on overall survival are awaited. As complete surgical cytoreduction at primary surgery is an important factor in improved outcome from primary treatment and from secondary treatment, patients with primary OC should be managed in specialist units where complete cytoreduction is achieved in the majority of patients. There may be a benefit from ip chemotherapy or HIPEC following cytoreductive surgery for ROC but level one evidence is needed.

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Section 4

Chemotherapy and Other Treatment Options in Ovarian Cancer

Chapter 15

Chemotherapy for Primary and Recurrent Epithelial Ovarian Cancer

Nora Naqos

Additional information is available at the end of the chapter

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Abstract

Epithelial ovarian cancer is the second most common gynecological cancer. It causes more deaths despite advances in treatment over the last few decades. Following explorative surgery and after histological assessment, the tumor can be formally "staged" according to the size, extent and location of the cancer. Staging during surgery determines the appropriate treatment regimen and the long-term outcome (prognosis). Recommendations for treatment after surgery are dependent on the stage of the cancer. Chemotherapy is recommended after surgery for stage III or IV ovarian cancer; certain tumor factors determine its use in stage I or II disease.

Keywords: chemotherapy, ovarian cancer, recurrence

1. Introduction

Epithelial ovarian cancer is the second most common gynecological cancer. It causes more deaths despite advances in treatment over the last few decades.

Epithelial ovarian cancer is the most common histological subtype diagnosed, accounting for 80% of cases [1]. It arises from the coelomic epithelium, 75% are serous cystadenocarcinoma, other types are less frequent and include endometrioid, mucinous, Brenner transitional cell, clear cell and unclassified carcinomas. Germ cell and sex cord-stromal tumors represent the other 20% [1].

2. Staging

Following explorative surgery and after histological assessment, the tumor can be formally "staged" according to the size, extent and location of the cancer. Staging during surgery determines the appropriate treatment regimen and the long-term outcome (prognosis).

Recommendations for treatment after surgery are dependent on the stage of the cancer. Chemotherapy is recommended after surgery for stage III or IV ovarian cancer; certain tumor factors determine its use in stage I or II disease.

3. Chemotherapy in advanced and metastatic ovarian cancer

3.1. History of chemotherapy

Twenty years ago, patients with advanced ovarian cancer were treated most commonly with the alkylating agents such as cyclophosphamide, chlorambucil, thiotepa and melphalan, all as monotherapy. These drugs have resulted in overall objective response rates between 33 and 65% and complete clinical responses in nearly 20% of patients [2].

In 1970, cisplatin was established by Wiltshaw and Kroner [3] as one of the most active agents for ovarian cancer, with a reported overall response rate of 26.5% in 34 patients who were resistant to alkylating agents. Moreover, Young et al. [2] obtained objective responses (one was complete) in 29% of 25 patients refractory to alkylating agents.

The North Thames Cooperative Group reported in 1985 the results of the first randomized comparison of first-line cisplatin and an alkylating agent cyclophosphamide in women with advanced ovarian cancer, it demonstrated significantly longer survival and response duration rates in patients receiving platinum therapy [4].

3.2. Which platinum: carboplatin or cisplatin?

The meta-analysis of the advanced ovarian cancer trialists group and two trials comparing cisplatinum with cyclophosphamide and carboplatin + cyclophosphamide showed that cisplatin and carboplatin have the same activity in ovarian cancer [1].

3.3. What is the effective dose of platinum?

A retrospective review reported a significant correlation between the dose intensity of cisplatin and response rates and survival [4]. Data from 10 trials focusing on platinum agents in approximately 2000 patients showed improvements in outcomes with doses up to 25 mg/m^2/week [5]. When the dose is increased above this there is increasing toxicity but without any clinical benefit observed [5]. In respect to carboplatin, clinicians use AUC from 5 to 7.5 [1].

3.4. What drug should be combined with platinum (the role of taxane)?

3.4.1. Anthracycline

Five meta-analyses from 10 trials in 1702 patients compared cyclophosphamide plus cisplatin with cyclophosphamide, cisplatin and doxorubicine (C A P), a modest but significant improvement in survival was seen for the regimen using doxorubicine (overall hazard ratio 0.85, P 1/4 0.003) [5] . Most investigators in the United States abandoned the use of anthracycline in 1986 due to cardiotoxicity that may outweigh the clinical benefit [5].

3.4.2. Paclitaxel

A significant development in the treatment of ovarian cancer was the discovery of the taxane class of cytotoxics. Two randomized controlled trials of first-line cisplatin based dual therapy showed additional clinical benefit when cyclophosphamide was replaced by paclitaxel [6, 7].

The Gynecological Oncology Group (GOG) 111 trial studied 386 women with stage III suboptimally debulked or stage IV disease [6]. Whereas the intergroup OV10 trial had wider selection criteria and assessed 675 women with FIGO stage IIb, IIc, III or IV disease with or without successful debulking surgery [7].

In GOG 111, patients received paclitaxel at 135 mg/m^2 over 24 h with cisplatin at 75 mg/m^2 or cyclophosphamide at 750 mg/m^2 every 3 weeks for a total of 6 courses. The same drugs were studied in OV10 and paclitaxel was given at 175 mg/m^2 over 3 h. The median follow-up intervals were 38.5 and 37 months in the OV10 and GOG 111 studies, respectively; the combination of platinum and paclitaxel is more effective with respect to OS and PFS. Hence the chemotherapy regimen is based on this combination.

3.5. Carboplatin as a substitute for cisplatin

Regimens containing carboplatin and paclitaxel were generally better tolerated than cisplatin plus paclitaxel in three major studies in which the two doublets showed similar efficacity.

The Dutch/Danish study [8], treated 208 patients and Arbeitsgemeinschaft Gyneco-oncology (AGO) study [9] examined 798 patients (3 weekly paclitaxel at 175 or 185 mg/m^2 given over 3 h plus cisplatin at 75 mg/m^2 with carboplatin AUC 5 or 6 plus the same dose of paclitaxel). Patients in both studies had stage IIb, IV and were followed up for a median of 37 months [8]. The GOG 158 trial compared 792 eligible patients with optimal stage III disease given paclitaxel 135 mg/m^2 over 24 h added to cisplatin at 75 mg/m^2 with paclitaxel 175 mg/m^2 over 3 hrs added to carboplatin AUC 7.5 [10].

The final results from AGO, GOG 158 and Dutch/Danish study noted little difference between treatments in the median PFS (the median overall survival was similar between treatment arms in each study), toxicities were mainly as expected, paclitaxel plus carboplatin were better tolerated [8–10].

4. Neoadjuvant chemotherapy

Neoadjuvant chemotherapy (NAC) followed by interval debulking surgery (IDS) has been proposed in the management of advanced Epithelial ovarian cancer in order to increase the rate of complete optimal surgery with less surgical morbidity [11–13]. Reserved initially for unresectable disease or for patients in bad and poor general condition, the use of NAC and IDS has increased over the past two decades and frequently the first debulking is now realized only after several cycles of chemotherapy [11, 13]. Vergote et al. [11] in a large phase III randomized trial including patients with advanced stages IIIc-IV reported the non-inferiority of interval surgery after 3 cycles of NAC compared to upfront surgery [11] s The hazard ratio for death (intention-to-treat analysis) in the group assigned to neoadjuvant chemotherapy followed by interval debulking was 0.98 (90% confidence interval [CI], 0.84–1.13; P = 0.01 for non-inferiority) [11].

However, in clinical practice, optimal surgical timing and selection criteria for neoadjuvant chemotherapy and interval surgery remain controversial. Retrospective studies and meta-analyses observed a large survival advantage for patients receiving initial and complete removal of all macroscopic tumors prior to chemotherapy [14]. Moreover, the quality of surgery was heterogeneous in the EORTC trial among participating centres with variations in surgical aggressiveness and rates of complete resection, residual tumor of 1 cm or less was achieved in 42% of patients in the primary cytoreduction arm and in 81% of patients in the NACT arm. In the intent to treat analysis, the NACT arm was non inferior to the primary surgery arm with respect to the primary outcome of overall survival [14]. This argument explains the comparatively low survival observed for those treated with upfront surgery in this study. Furthermore, retrospective data have also suggested that NAC and IDS compared to primary surgery may increase the risk of developing platinum-resistant disease and less sensitive recurrent disease [15]. A minimum of 6 cycles of treatment is recommended including at least 3 cycles of adjuvant therapy after interval debulking surgery [16].

5. Targeted therapy

The addition of bevacizumab (a humanized monoclonal antibody to VEGF) to first-line chemotherapy based on platinum-taxane in advanced ovarian cancer demonstrated a significant improvement of PFS. This was evaluated in GOG-218 [17] a phase 3 trial in which they randomly assigned patients with newly diagnosed stage III (incompletely resectable) or stage IV epithelial ovarian cancer who had debulking surgery to receive one of these three treatments:

1. Cycles 1–6: carboplatin, AUC 6 Paclitaxel, 175 mg/m^2 Placebo (starting in cycle 2) every 3 wk. Cycles 7–22: placebo every 3 weeks.
2. Cycles 1–6: carboplatin, AUC 6 Paclitaxel, 175 mg/m^2 Bevacizumab, 15 mg/kg (starting in cycle 2) every 3 weeks the Cycles from 7 to 22 patients received Placebo every 3 weeks.
3. Cycles 1–6: carboplatin, AUC 6 Paclitaxel, 175 mg/m^2 Bevacizumab, 15 mg/kg (starting in cycle 2) every 3 weeks the Cycles from 7 to 22 patients received Bevacizumab at 15 mg/kg every 3 weeks.

The median progression-free survival was 10.3 months in the control group, 11.2 in the bevacizumab-initiation group, and 14.1 in the bevacizumab-throughout group [18].

The administration of bevacizumab during and up to 10 months after paclitaxel and carboplatin chemotherapy prolongs the median progression-free survival by about 4 months in patients with advanced epithelial ovarian cancer [18].

Similar results were obtained in the ICON-7 trial [19] where a total of 1528 women from 11 countries were studied, 70% had stage IIIC or IV ovarian cancer. In this study patients were randomly assigned to carboplatin and paclitaxel (175 mg/m^2) every 3 weeks for 6 cycles, or to this regimen plus bevacizumab (7.5 mg/Kg), given every 3 weeks for 5 or 6 cycles and continued for 12 more cycles or until disease progression. The PFS at 36 months was 20.3 months with chemotherapy alone, as compared with 21.8 months with chemotherapy plus bevacizumab. In the updated analyses, PFS at 42 months was 22.4 months without bevacizumab versus 24.1 months with bevacizumab (P = 0.04); in patients at high risk for disease progression, the benefit was greater with bevacizumab than without it, with PFS at 42 months of 18.1 months with bevacizumab, versus 14.5 months with standard chemotherapy, with median overall survival of 36.6 and 28.8 months, respectively [19].

These observations suggest that effectiveness of anti-angiogenic therapy may be greater in more advanced disease. However this was not supported by other studies testing the impact of different anti-angiogenesis factors added to chemotherapy in advanced ovarian cancer [18]. Both pazopanib [20] and nindetanib [18] showed a significant increase in PFS in patients with small tumors. The PFS benefit of the addition of nindetanib to first-line chemotherapy resulted in a more pronounced effect in the non-high-risk subgroup (stage II or stage III and residual ≤1 c m) with 27.1 vs. 20.8 months. In contrast, there was no significant benefit noted for high risk patients (FIGO IV or stage III with residual tumors). Pazopanib as maintenance therapy after first line chemotherapy showed a significant advantage with respect to PFS compared to the control group 17.9 vs. 12.3 months, HR 0,77, P = 0.0021) [18].

6. Intraperitoneal chemotherapy

The peritoneal cavity is the most common route of ovarian cancer spread.

The rational for giving chemotherapy directly into the peritoneal cavity is supported by preclinical, pharmacodynamics and pharmacocinetic data [21]. Compared with intravenous (IV) treatment, intraperitoneal (IP) administration allows an increase in drug concentration inside the abdominal cavity.

In the majority of patients, epithelial ovarian cancer is confined to the peritoneal cavity at initial diagnosis and in recurrence [22]. As a result ovarian cancer is a good target for intraperitoneal therapy.

The hypothesis of improved effectiveness is explained by the increasing concentration of the cytotoxic agent in the tumor microenvironment. Analysis of intratumoral drug concentrations demonstrates that higher drug exposure is observed for lesions 2–3 mm or smaller

when intraperitoneal administration is performed compared with intravenous infusion [23]. Moreover, avascular tumors are more exposed to higher drug concentrations with intraperitoneal rather than intravenous administration [24].

A meta-analysis of five clinical trials confirmed a benefit in OS for intraperitoneal chemotherapy [25]. This led to a National Cancer Institute alert in 1996 recommending that intraperitoneal chemotherapy should be considered in patients with small volume (<1 cm) or no residual disease after surgery [16]. However, this has not been adopted as a standard care of in the majority of institutions and countries due to its great toxicity [16].

7. Adjuvant chemotherapy in early stage disease

After surgery, there is still a risk that cancer cells remain and may return or spread to other organs of the body. Adjuvant chemotherapy is administered after surgery to destroy these cells and improve the chance of curing ovarian cancer and to decrease the risk of the death due to ovarian cancer.

A recent Cochrane meta-analyses of five prospective clinical trials (4 of 10 with platinum-based chemotherapy) demonstrated that chemotherapy is more beneficial than observation in patients with adequately staged early-stage ovarian cancer [26]. Patients who received adjuvant chemotherapy had better OS [hazard ratio (HR) 0.71; 95% confidence interval (CI) 0.53–0.93] and PFS (HR 0.67; 95% CI 0.53–0.84) than patients who did not receive adjuvant treatment [26].

Two-thirds of the patients included in the two major studies were suboptimally staged, in optimally staged patients, benefit for chemotherapy cannot be excluded, Long-term follow-up of the ICON 1 trial confirms the benefit of adjuvant chemotherapy, particularly in those patients at higher risk of recurrence (stage 1B/C grade 2/3, any grade 3 or clear-cell histology) [26].

Therefore, adjuvant chemotherapy should be recommended not only to suboptimally staged patients but also to those optimally staged at higher risk of recurrence [16].

8. Recurrent ovarian cancer

Recurrent ovarian cancer can be diagnosed by the appearance of new symptoms, radiologic evidence of recurrent disease or a rising CA-125 level in an asymptomatic patient.

In the past, treatment for recurrent ovarian cancer was given based on rising levels of tumor markers alone even without symptoms. However, a phase III randomized study (OV05-EORTC 55955) demonstrated no survival benefit of starting chemotherapy based on the increasing level of CA-125 alone and that quality of life may be improved by awaiting the appearance of symptoms or signs of ovarian cancer recurrence.

In this study treatment was delayed by a median of 4.8 months with no benefice on OS (HR 1.01; 95% CI 0.82–1.25; P = 0.91) [27]. Similarly, third-line treatment was started 4.6 months

earlier in the patients who had regular CA 125 monitoring. Quality of life was lower in the early treatment group [27].

The choice of chemotherapy agents in recurrent disease is based on the response to first line treatment, the current symptoms; the time elapsed from last chemotherapy and the side effects of previous drugs administered.

The prognosis and the response to second-line therapy and subsequent lines depends in great part on the progression-free interval after the last dose of the preceding line of chemotherapy.

We define:

- Platinum-refractory disease when the progression occurs during treatment or within 4 weeks after the last dose.
- Platinum-resistant disease as a progression within 6 months of platinum-based therapy;
- Partially platinum-sensitive disease when the progression occurs between 6 and 12 months;
- Platinum-sensitive patients progressing with an interval of more than 12 months (GCIG Consensus) [28].
- For patients with platinum-sensitive recurrent ovarian cancer: carboplatin-doublet should be the treatment of choice [16].

A meta-analysis including four randomized trials confirmed an improvement in PFS with a HR of 0, 68 (95% CI 0.57–0.81) and OS with a HR of 0.8 (95% CI 0.64–1.0) [29].The phase III Calypso [30] trial compared two doublets, taxol and carboplatin vs. carboplatin with pegylated lipososmal doxorubicin (PLD). The PFS with the second regimen (11.3 months) was not inferior to the taxane-carboplatin (9.4 months, $P < 0.001$, HR = 0.82) [3]. However the PLD regimen was better tolerated because of the minimal incidence of neuropathy, alopecia, and arthralgia and with less hypersensitivity reactions [3].

Again, the selection between the different options of platinum-based doublets should be based on the previous toxicity profile and convenience of administration [16].

Bevacizumab (Avastin) has also been studied as a treatment option in patients with recurrent ovarian cancer. The phase III OCEANS [31], study performed in women with platinum-sensitive recurrent ovarian cancer compared gemcitabine plus carboplatin with or without bevacizumab for 10 cycles followed by bevacizumab alone until disease progression or toxicity as compared to placebo. Chemotherapy with bevacizumab improved PFS, 12 months with bevacizumab vs. 8 months in the placebo group, as well as the response rate (79 vs. 57%, $P < 0.001$) [31].

Regimens based on non-platinum combinations are another option for patients with platinum-sensitive disease. In a phase III randomized trial OVA 301 [32], PLD alone was compared with PLD combined with the Mariane-derived alkaloid trabectidin (Yondelis), this combination regimen improved the PFS [32], Median PFS was 7.3 months with trabectedin/PLD v 5.8 months with PLD (hazard ratio, 0.79; 95% CI, 0.65–0.96; $P = 0.0190$). Overall response rate (ORR) was 27.6% for trabectedin/PLD vs. 18.8% for PLD ($P = 0.0080$) [32].

It has been hypothesized that this benefit is due to the restoration of 'platinum-sensitivity' by prolonging the platinum- free interval. This is now being explored in two prospective randomized trials [16].

8.1. Maintenance therapy in platinum-sensitive recurrent ovarian cancer

Many clinical trials have evaluated the role of drugs aimed at prolonging the second remission. One of these is the OCEANS trial that demonstrated the role of bevacizumab as noted above in combination with chemotherapy and as maintenance therapy [31]. Chemotherapy with bevacizumab improved PFS, 12 months with bevacizumab vs. 8 months in the placebo group, as well as the response rate (79 vs. 57%, $P < 0.001$) [31].

In women with the BRCA mutation, the Poly-ADP ribose polymerase (PARP) inhibitor: rucaparib, olaparib is an active drug; a trial assessed olaparib in women with recurrent advanced ovarian cancer; the overall response rate was 34% (complete response, 2% and partial response, 32%) [33].

The FDA approved olaparib for patients with advanced ovarian cancer who have received treatment with 3 or more lines of chemotherapy and have germline BRCA mutation [34].

8.2. Platinum-resistant recurrent ovarian cancer

8.2.1. Treatment selection

In platinum-resistant recurrent cancer, patients should be treated with non-platinum based chemotherapy. The treatment aims to palliate symptoms, optimizing quality of life and prolonging life. In general in this case, response rates are low and the prognosis is poor.

We should use non cross-resistant agents and avoid toxicities based on side effects that have developed from previous therapies, in general higher response rates and PFS rates longer than 2–3 months are obtained with the use of combination regimens. But combination drugs is associated with higher toxicity without any improvement in OS compared with the use of single agent therapy [16]. In fact, for the platinum-resistant cancer, a treatment based on single agent is preferable since it may offer a balance between efficacy and toxicity.

8.2.1.1. Taxane

Many drugs have documented activity in platinum-resistant disease. In phase II and III trials, the use of single agent paclitaxel has permitted objective responses in 22–30% of patients [35].

8.2.1.2. Pegylated liposomal doxorubicin

A phase III trial compared PLD with topotecan [36] in women with recurrent ovarian cancer, patients were stratified prior to being randomized according to the platinum sensitivity of their tumor. Similar results were obtained for each of these regimens with respect to the overall RR (20 vs. 17%), time to progression (22 vs. 20 weeks) and median OS (60 vs. 56.7 weeks) [36]. PLD has resulted in a significant OS benefit with longer follow up, mainly for patients

with platinum-sensitive disease, and PLD was found to be significantly superior to topotecan (P = 0.0.08) [36].

Compared with topotecan, PLD caused lower rates of neutropenia, thrombocytopenia and was associated with higher rates of hand foot syndrome and stomatitis [36].

8.2.1.3. Topotecan

Topotecan has similar efficacy to paclitaxel and PLD in the treatment of platinum-resistant recurrent ovarian cancer [36, 37]. Its use is usually associated with some degree of myelosuppression especially neutropenia.

Monochemotherapy is therefore the standard of early "platinum-resistant" relapse of ovarian cancers [16].

The use of bevacizumab was demonstrated in the AURELIA trial that showed an improved PFS in patients with platinum-resistant ovarian cancer treated with bevacizumab in combination with single agent chemotherapy when compared to treatment with chemotherapy alone (5.7 vs. 4 months) [38].

9. Summary

Chemotherapy is the first systemic treatment of epithelial ovarian cancer at all disease stages. It is based on platinum with paclitaxel in the adjuvant and neoadjuvant setting. In advanced stage cases chemotherapy with bevacizumab improved the response.

Most cases of newly diagnosed ovarian cancer will respond to initial therapy, but 80% or more will ultimately relapse and further chemotherapy may be indicated. Newer strategies involving gene testing such as BRCA has proven to be an important addition to the treatment strategy. The choice of treatment for recurrent disease is based on the duration of response to prior therapy, previous treatment toxicity and quality of life.

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Chapter 16

Ethnic Differences in Susceptibility to the Effects of Platinum-Based Chemotherapy

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Additional information is available at the end of the chapter

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Abstract

There is substantial interindividual variability in the efficacy and tolerability of anticancer drugs. Such differences can be greater between individuals of different ethnicities. The clinical studies demonstrate that individuals from Asia (East Asia) are more susceptible to the effects of platinum-containing chemotherapies than their Western counterparts. To determine whether population-related genomics (i.e., frequencies of DNA polymorphisms) contribute to differences in patient outcomes, polymorphisms in 109 genes involved mainly in xenobiotic metabolism, DNA repair, the cell cycle, and apoptosis were tested in Russian (Caucasians) and Yakut (North Asians) ovarian cancer patients receiving cisplatin-based chemotherapy. Totally, 232 polymorphisms were genotyped in individual DNA samples using conventional PCR and arrayed primer extension technology. Single nucleotide polymorphisms (SNPs) in more than 30 genes were found to be associated with one or more of clinical end points (i.e., tumor response, progression-free survival, overall survival, and side effects). However, all associations between SNPs and clinical outcomes were specific for each of ethnic group studied. These findings let us to propose the existence of distinctive ethnic-related characteristics in molecular mechanisms determining the sensitivity of patients to platinum drug effects.

Keywords: cisplatin, DNA polymorphisms, ethnic diversity, chemotherapy, ovarian cancer

1. Introduction

There is substantial interindividual variability in the efficacy and tolerability of pharmaceuticals, including anticancer drugs. Such differences can be greater between individuals of different

ethnicities [1]. Currently, pharmacoethnicity, or ethnic diversity in drug effectiveness and/or toxicity, is an increasingly recognized factor for accounting interindividual variations in drug response [2]. Although the reasons underlying ethnic diversity in drug response are likely multifactorial [3], the results of numerous population studies suggest that they may be attributed, at least in part, to the interpopulation differences in frequencies of DNA polymorphisms – inherited variations at the DNA sequence level [4–6]. In terms of F_{ST}, the most commonly used measure of population differentiation; the proportion of such differences is 5–13% of total genetic diversity depending on the type of polymorphic markers chosen [6]. The opponents of ethnic-/race-based explorations in pharmacogenomics often consider these portions of variation as non-essential in the context of considerably larger proportions of within population variation which represents the average difference between members of the same population and accounts for 87–95% of total variance [7, 8]. Nevertheless, significant differences in the population prevalence of functionally impaired allelic variants of genes may create a potential for ethnic differences in responses to drugs that are detoxified (transported or targeted) by the proteins that are encoded by those genes [9–11]. A prominent example how population-based genetic differences can affect the drug response is the significantly greater risk for Stevens-Johnson syndrome and toxic epidermal necrolysis among East/Southeast Asian carbamazepine users, particularly Han-Chinese, Thais, and Malaysians, that has been associated with *HLA-B*1502* allele [12]. The relationship was not evident in non-Asian patients as well as in Japanese and Koreans due to infrequency of the allele in these populations. Another example is the lower average warfarin requirements of Asians linked to the higher frequency of AA genotype at SNP rs9923231 upstream of *VKORC1* gene among them [13]. Finally, considering potential pharmacoethnicity of anticancer drugs, one of the most illustrative examples, although not associated with germline variations, is the higher response rate to EGFR inhibitors (e.g., gefitinib) of Asian (East Asian) lung cancer patients compared to Caucasians that correlates to higher frequencies of activating *EGFR* mutations in East Asians [14].

Keeping all that in mind, we carried out a comparative study aimed to explore the genetic bases of differences between Asian and Caucasian cancer patients in their sensitivity to the effects of platinum-containing chemotherapy. Platinum-based drugs are among the most widely used cytotoxic agents for the treatment of many types of cancer [15]. The first information about lesser tolerance of Asian patients to standard, approved for Europeans, doses of platinum-containing regimens came from Japanese physicians [16]. In both individual small studies and some common arm trials conducted in Japan and by Southwest Oncology Group, the higher frequency of toxicity, particularly hematologic toxicity, was registered in Asian patients than non-Asians (mostly Caucasians) [1, 17]. Moreover, it was also found that the incidence of toxicity was still higher among Asians even after appropriate dose reduction [1]. Although some comparative pharmacogenetic studies have been conducted, the reasons underlying the higher sensitivity/toxicity of Asians to the systemic platinum-containing therapy are not yet well understood [18–20]. To assess the effect of population genomics on difference in patient response, we comparatively explored the results of cisplatin-based chemotherapy in Russian (Caucasians) and Yakut (North Asians) ovarian cancer patients. Principal component analysis, performed by us using genotype data of a common set of 125,000 genome-wide SNPs, demonstrated significant differences between gene pools of Asian and non-Asian populations (**Figure 1**).

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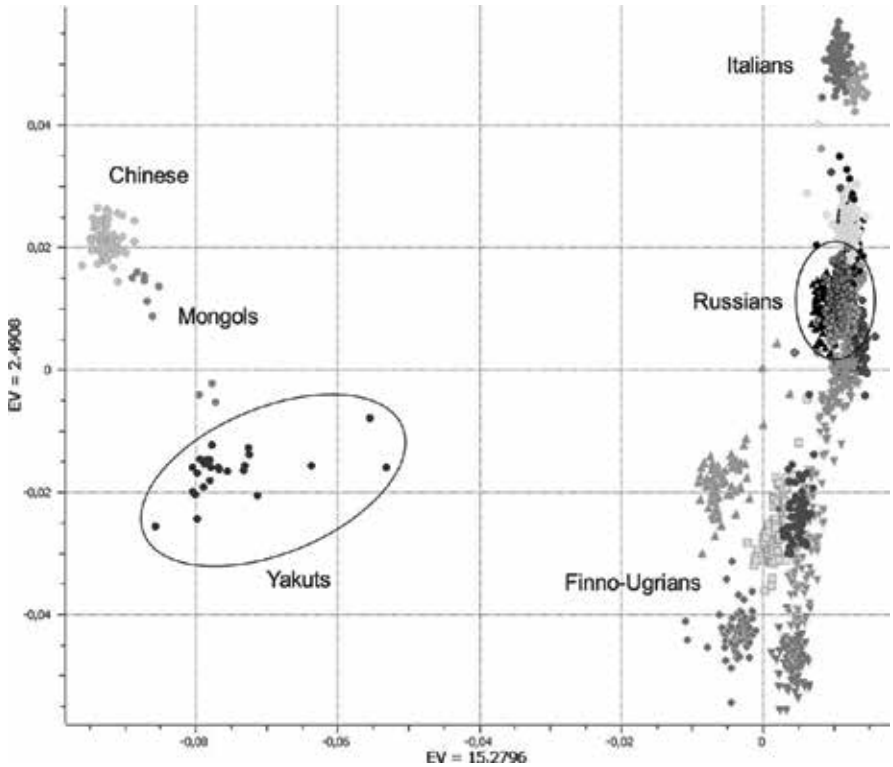

Figure 1. Genetic structure of Eurasian populations (based on 125,000 autosomal SNPs). The first two PCs are shown. Each individual is represented by a sign and the label corresponding to their self-identified population origin.

The estimates, obtained using the same set of polymorphic markers, showed that a portion of variation accounted for population-related differences, F_{ST}, in allele frequency between Russians and Yakuts was as high as 0.08, creating the potential for searching a causative polymorphism(s) with corresponding prevalence in population frequency. In the current study such candidates were searched among 232 polymorphisms from 109 genes involved mainly in xenobiotic metabolism, DNA repair, the cell cycle, and apoptosis.

2. Materials and methods

2.1. Patients

Ovarian cancer patients were identified and treated between 2003 and 2007 years at the N. N. Blokhin Cancer Research Centre and the Yakutsk Republic Cancer Clinic. Once identified, patients were invited to participate and were enrolled after they signed an informed consent. Detailed procedures of patient enrolment and data collection have been described previously [21, 22]. Briefly, unrelated Russian and Yakut women with morphologically confirmed epithelial ovarian carcinoma, who had received no previous chemotherapy or radiation therapy, were recruited. The upper age limit was 65 years. Exclusion criteria were serious concomitant

diseases (diabetes, uncontrolled hypertension, myocardial infarction within the last 6 months, etc.), and clinically significant hearing impairment (grade 2 or higher). To ascertain ethnicity, women completed a questionnaire about their ancestry; only self-described Russian and Yakut patients with no history of interethnic marriages in the past two generations were recruited. Before the initiation of chemotherapy, venous blood samples were obtained for genetic testing. The chemotherapy regimen was intravenous cisplatin (100 mg/m^2) plus cyclophosphamide (600 mg/m^2) on day 1, every 3 weeks, for a maximum of 6 cycles. Intraperitoneal chemotherapy and radiotherapy were not allowed. Toxicity of the treatment was described according to standard National Cancer Institute Common Toxicity Criteria version 2.0 [23]. All patients were assessed for the maximal grades of nephrotoxicity, ototoxicity, neurotoxicity, emesis, neutropenia, anemia, and thrombocytopenia.

The tumor response was assessed every 2 cycles. After the completion of chemotherapy, the patients were followed-up for disease relapse and survival. Patients with progressive disease were treated with second-line chemotherapy, mostly taxane based. The study protocol and informed consent form were approved by the Ethics Committee of the N. N. Blokhin Cancer Research Centre.

2.2. Genotyping

DNA was isolated from the venous blood samples (leukocytes) using a conventional approach including proteinase K treatment with subsequent phenol-chloroform extraction [24]. Some polymorphisms (**Table 1**) were genotyped using a polymerase chain reaction restriction fragment length polymorphism (RFLP)-based technique or determined directly through evaluation of their PCR product lengths.

Other polymorphisms were genotyped using a microarray "DNA repair single nucleotide polymorphism detection test" (version 2, Asper Biotech, Tartu, Estonia). The microarray genotypes 228 SNPs in 106 genes involved in[1] DNA repair, cell cycle control, apoptosis, and xenobiotic metabolism. Most of manually genotyped loci were also in the list of the microarray's polymorphisms and served as controls of genotyping efficiency. To check into account the potential mistakes in genotyping with the microarray [25], all polymorphisms, which were associated with any clinical endpoints, were additionally tested using the RFLP method.

2.3. Statistical analysis

A permutation exact test, a two-sided Fisher exact test, and a χ^2 test were used to determine the relationship between the variables and alleles/genotypes tested. Correlations between survival and genotype or genetic polymorphism were assessed using the Kaplan-Meier product limit method and the log-rank test. The significance of associations was set at $P < 0.05$ [26]. The statistical analyses were performed using the Statistica software (version 6.0, StatSoft, Inc., Tulsa, OK, USA) or the IBM SPSS Statistics software package (version 19, SPSS, Inc., IBM Company, IBM Corporation, Armonk, NY, USA), GraphPadInStat (version 3.00, GraphPad Software, San Diego, CA, USA), and the PowerMarker software (version 3.0) [27].

[1]Alternative variant – "genes which are involved in".

Polymorphism	#rs ID	Genotypes (No. patients)*			P
GSTA1 − 69 C/T	rs3957357	CC (43/60)	CT (49/22)	TT (12/5)	0.0007
GSTM1 gene deletion		0/0 (47/28)	+/0** (57/59)	NA	0.0754
GSTM3 AGG deletion	rs1799735	AGG/AGG (83/75)	AGG/− (16/12)	−/− (5/0)	0.1054
GSTM3 $Val^{224}Ile$	rs7483	Val/Val (39/30)	Val/Ile (57/37)	Ile/Ile (8/20)	<0.0001
GSTP1 $Ile^{105}Val$	rs1695	Ile/Ile (41/67)	Ile/Val (53/17)	Val/Val (10/3)	<0.0001
GSTP1 $Ala^{114}Val$	rs1138272	Ala/Ala (80/84)	Ala/Val (24/3)	—***	0.0001
GSTT1 gene deletion		0/0 (18/22)	+/0** (86/65)	NA	0.2123
ERCC1 19007 T/C	rs11615	TT (43/53)	TC (46/29)	CC (15/5)	0.0146
ERCC1 8092 C/A	rs3212986	CC (61/52)	CA (37/31)	AA (6/4)	0.9351
ERCC2 $Asp^{312}Asn$	rs1799793	Asp/Asp (34/66)	Asp/Asn (50/19)	Asn/Asn (20/2)	<0.0001
ERCC2 $Lys^{751}Gln$	rs13181	Lys/Lys (28/67)	Lys/Gln (54/18)	Gln/Gln (22/2)	<0.0001
XRCC1 $Arg^{194}Trp$	rs1799782	Arg/Arg (94/69)	Arg/Trp (10/18)	—	0.0398
XRCC1 $Arg^{280}His$	rs25489	Arg/Arg (95/80)	Arg/His (9/6)	His/His (0/1)	0.5007
XRCC1 $Arg^{399}Gln$	rs25487	Arg/Arg (49/40)	Arg/Gln (45/39)	Gln/Gln (10/8)	0.9752
TP53 $Arg^{72}Pro$	rs1042522	Arg/Arg (52/47)	Arg/Pro (40/35)	Pro/Pro (12/5)	0.3733
CYP2E1 96bp insertion		−/− (100/74)	−/ins (4/13)	—	0.0097
CYP2E1 − 1053 C/T	rs2031920	CC (102/68)	CT (2/18)	—	<0.0001
CYP2E1 7632 T/A	rs6413432	TT (91/67)	TA (12/20)	AA (1/0)	0.0753
CYP2E1 9896 C/G	rs2070676	CC (82/78)	CG (20/9)	GG (2/0)	0.0908

*The first value in parentheses means the number of patients with corresponding genotype in Russian group, the second one – in Yakut group.

**The genotype was defined as positive if at least one copy of gene was present.

***The corresponding genotype was not occurred in populations.

NA – not available (not determined with the genotyping method used).

Table 1. Genotype frequencies in Russian and Yakut patients.

3. Results and discussion

During 2003–2007 years, 104 Russian patients and 87 Yakut patients were enrolled in the study. The median age of patients was 52 and 51 years, respectively. The majority of patients in both groups had stage III disease (72 and 53 women, respectively). Stages I, II, and IV were detected in 14, 6, and 10 Russian patients and 1, 8, and 25 Yakut patients. A total of 21 Russian patients and 11 Yakut patients were receiving adjuvant chemotherapy and were not eligible for evaluation of tumor response (i.e., they had no residual disease after surgery). Overall response rates, comprising complete and partial responders, were 85% in the Russian group and 58% in the Yakut group.

The median progression-free survival (PFS) in the Russian group was 12 months, and the median overall survival (OS) was 55 months. In the Yakut group, both intervals were shorter —8 and 29 months, respectively. However, being adjusted for disease stage, the values became similar to those for the Russian group.

More contrast results were obtained in analysis of occurrence of adverse events. To assess the association between genotype and the toxicity of the treatment used, patients were classified as having good or poor tolerance to treatment (grades 3–4 of neutropenia, grades 2–4 of anemia, grades 2–4 of neuropathy, grades 3–4 of emesis, all grade of thrombocytopenia, nephrotoxicity, and ototoxicity were considered as clinically significant toxicities). Comparison of the frequencies of side effects registered in Russian and Yakut patients confirmed higher toxicity of platinum-based regimens for patients of Asian origin than for Europeans. Particularly, Yakut patients suffered more frequently than Russians from nephrotoxicity and severe emesis (P = 0.027 and P = 0.061, respectively), which both were known to be the most common adverse events observed in regimens using cisplatin [28].

Genetic testing of the patients from our groups was performed in two stages. At first, we explored the associations between outcomes of a cisplatin-cyclophosphamide regimen in Russian and Yakut ovarian cancer patients and some most common polymorphisms in several genes [21, 22], among the tested genes, were glutathione S-transferase (GST) genes (*GSTA1, GSTM1, GSTM3, GSTP1* and *GSTT1*), DNA repair genes (*ERCC1, ERCC2* and *XRCC1*) as well as *TP53* and *CYP2E1* genes (**Table 1**). GST and DNA repair genes have been described as important for cisplatin metabolism and activity [29, 30]. GSTs can directly limit the amount of reactive cisplatin species available for interaction with DNA, by catalyzing their binding to tripeptide glutathione. The resulting cisplatin-glutathione conjugates can be further easily excreted from the cell by the GS-X pump transporters [31]. DNA repair proteins remove platinum-DNA adducts, the persistence of which underpins the antitumor potential of platinum drugs. In contrast to GST and the DNA repair proteins, TP53 protein does not seem to directly affect cisplatin metabolism or transformation. At the same time, it has a crucial role in mediating cellular responses to DNA damage, initiating programmed cell death when the effective DNA repair is impossible [29, 30, 32]. There is also no evidence that cisplatin is metabolized by CYP2E1. However, CYP2E1 is a significant potential source of catalytic iron and can serve as a site for generating reactive oxygen species in the presence of cisplatin [33]. It has been proposed that this mechanism underlies cisplatin-induced nephrotoxicity and hepatotoxicity [34, 35].

One of the most significant associations found in the first part of the study was the correlation between the survival time intervals and *GSTP1* Ile105Val polymorphism registered in Russian subjects (P = 0.004 and P = 0.016 for PFS and OS, respectively). Russian ovarian cancer patients with a homozygous Ile/Ile genotype had longer PFS and OS than those ones who carried Ile/Val and Val/Val genotypes. However, the association was not observed in Yakut patients. PFS and OS of Yakut women with Ile/Ile genotype did not differ from the corresponding time intervals of patients with 1 or 2 Val alleles. In the Yakut group, PFS correlated with *CYP2E1* 7632 T/A polymorphism (P = 0.015), being longer in patients with a homozygous TT genotype than in patients with the heterozygous TA genotype.

Analysis of genotype distribution in the population groups for toxicities revealed that occurrence of nephrotoxicity and severe emesis in Yakut patients correlated with *GSTT1* and

CYP2E1 genotypes, respectively. Patients with a homozygous *GSTT1* gene deletion (*GSTT1* null) suffered more frequently from nephrotoxicity than carriers of functional *GSTT1* variants (OR = 3.31, 95% CI 1.15–9.54, P = 0.028). Patients who had a 96 bp insertion in the promoter region of the *CYP2E1* were more prone to severe emesis (OR = 4.69, 95% CI 1.31–16.77, P = 0.027). Grade 3 or 4 emesis was also associated with another *CYP2E1* polymorphism—a single nucleotide substitution (9896 C/G) in intron 7. Patients with a heterozygous CG genotype had a higher risk of severe emesis than patients with the homozygous CC genotype (OR = 16.96, 95% CI 2.01–143.16, P = 0.002). In contrast, in Russian patients, *CYP2E1* polymorphisms were not associated with any clinical outcomes and distribution of *GSTT1* genotypes correlated with severity of emesis. The risk of severe emesis was higher in Russian patients with the *GSTT1*-null genotype than in patients with a functional *GSTT1* variant (OR = 4.06, 95% CI 1.40–11.78; P = 0.014). As for the nephrotoxicity in Russian subjects, it was associated with *ERCC1* 19007 T/C or 8092 C/A polymorphisms, and cases of renal dysfunction were more prevalent among patients with the heterozygous genotypes of each locus. Other found genotype-clinical end point associations were also discordant in Russians and Yakuts (**Table 2**).

When genotype distributions in both groups were compared significant differences in population genotype frequencies were noted for 10 of 19 polymorphisms studied but only 5 of them were associated with clinical outcomes (**Tables 1** and **2**).

Evaluation of the direction/strength of the associations showed that they were not correlated with the differences in population frequencies of corresponding genotypes. For example, the absence of correlation between the *GSTP1* Ile105Val polymorphism and survival in Yakut patients was not simply due to the higher prevalence of Ile/Ile genotype among them because carriers of Ile/Ile genotype did not differ in PFS or OS from those who had genotypes with 1 or 2 Val alleles. Similar results were obtained for the *GSTA1*-69 C/T polymorphism. Its CT and TT genotypes were associated with a risk of anemia in Yakuts; yet the greater frequencies of the CT and TT genotypes in Russians did not result in a higher risk of anemia. Similarly, risk genotypes of *ERCC1* 19007 T/C and ERCC2 Asp312Asn polymorphisms (i.e., heterozygous genotypes in Russians) were not rare among Yakut patients but they did not demonstrate correlations with the corresponding side effects (**Tables 1** and **2**). It seems that only in the case of *CYP2E1* 96 bp insertion polymorphism an effect of allele frequency differences could be proposed but due to small number of individuals with the insertion containing genotypes in Russians the suggestion requires verification in a larger sample.

Taken in the context of other data, the obtained results generally supported the role of ethnicity as an additional reason for differences in the outcomes of clinical trials in which the same treatment is used. At the same time, the results of genetic testing suggested that a single genotypic difference was unlikely to account for the observed ethnic variation in toxicity and survival. Moreover, they also suggested that assessing traditionally tested common polymorphisms in GST and DNA repair genes is not enough for relevant description of lesser tolerability of Asians (North/East Asians) to the effects of platinum-containing chemotherapies and further studies involving more polymorphic markers are required.

In the second part of our study, we systematically investigated the associations between patients' outcomes and SNPs in more than 100 genes using the microarray "DNA repair single nucleotide polymorphism detection test" (version 2) [36, 37]. Like similar genotyping panels, the list of genes tested comprised candidate genes involved in key pathways of cellular

Gene name	#rs ID	Clinical outcomes**															
		PFS		OS		Anemia		Neutropenia		Thrombocitopenia		Nephrotoxicity		Ototoxicity		Emesis	
		R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
GSTA1	rs3957357						+										
GSTM1	gene deletion					+				+							
GSTM3	rs1799735					+				+							
GSTM3	rs7483																
GSTP1	rs1695	+		+													
GSTP1	rs1138272																
GSTT1	gene deletion												+			+	
ERCC1	rs11615											+					
ERCC1	rs3212986											+					
ERCC2	rs1799793									+							
ERCC2	rs13181																
XRCC1	rs1799782																
XRCC1	rs25489																
XRCC1	rs25487							+									
TP53	rs1042522							+									
CYP2E1	96bp insertion																+
CYP2E1	rs2031920																
CYP2E1	rs6413432		+														
CYP2E1	rs2070676																+

*The registered associations are indicated by "+" in the corresponding cells.

**Tumor response and ototoxicity were not included in the table as there were no associations between them and polymorphisms tested.

Table 2. The associations between polymorphisms and clinical outcomes observed in Russian (R) and Yakut (Y) patient groups (the first stage of the study)*.

response to different drugs [38, 39], including many genes that are related to the cisplatin pathway (platinum pathway) [40]. A total of 213 SNPs from 228 genotyped SNPs were new (i.e., they did not include SNPs from the first stage) and 27 SNPs were associated with one or more of the assessed clinical end points (**Table 3**).

Increasing number of polymorphisms yielded an association with tumor response. To assess the association, patients who achieved a complete remission were compared with those without it (i.e., patients with partial response, stable and progressive disease). In the Russian group, a significant difference in complete response was observed according to polymorphism in the *ADH1C* gene (A/G, rs698) (P = 0.0002). The proportion of patients who achieved a complete response was higher among carriers of homozygous genotypes AA and GG compared with patients with a heterozygous variant AG. In Yakut patients, the occurrence of complete response was correlated with an allelic status of SNP in *CDKN1B* gene (T/C, rs34330), particularly with an allele C. There were no cases of complete remission among patients who carried a homozygous genotype TT at rs34330. The SNP was also associated with PFS in Yakut subjects (P = 0.0051); patients with the genotype TT had shorter PFS than patients with CC and CT genotypes. The protein encoded by *CDKN1B* gene participates in regulation of the cell cycle by binding and inhibiting activation of cyclin E-CDK2 or cyclin D-CDK4 complexes, and thus blocking the transition of the cell into the S-phase. C > T substitution at rs34330 locus results in decreasing the levels of mRNA and CDKN1B protein [41]. Reduced level of *CDKN1B* expression has been associated with a poor outcome in various cancers [42, 43]. In contrast, the *ADH1C* SNP (rs698) has been associated with a risk of alcoholism and ethanol-related cancers [44], but our study was the first to demonstrate an association with a chemotherapy outcome (i.e., tumor response) but the mechanism is not obvious.

Genotypes of seven SNPs were associated with differences in PFS in the Russian group (**Table 3**). The most significant SNPs were rs1142345 (A/G) in *TPMT* ($P = 3 \times 10^{-8}$) and rs4986998 (C/T) and rs1800566 (C/T) in *NQO1* genes ($P = 2 \times 10^{-6}$ and $P = 9 \times 10^{-11}$, respectively). The longer PFS was seen in patients with the most frequent genotypes AA and CC. SNPs in *NQO1* demonstrated similar correlations with OS.

Thiopurine S-methyltransferase (TPMT) is a cytosolic methylating enzyme with unknown physiological role [45]. However, this enzyme is known to be able to catalyze the S-methylation of some aromatic and heterocyclic compounds, particularly thio-compounds (e.g., 6-mercaptopurine and 6-thioguanine). Discussing the associations revealed between SNPs in *TPMT* and cisplatin ototoxicity, Ross et al. [46] have hypothesized that TPMT can affect cisplatin-induced hearing impairment through inactivation of cisplatin-purine compounds that form cytotoxic DNA cross-links, and cause cell death. As might be expected from that data, those patients in our study who had the loss-of-function genotype AG at rs1142345 should demonstrate a better outcome as a result of decreased inactivation of cisplatin-purine compounds, but such a correlation was not observed. Moreover, patients with an AG genotype had even shorter PFS than those who carried the functionally normal AA genotype.

The tested SNPs in *NQO1* gene are characteristic polymorphic variants affecting functional ability of the corresponding protein — a cytosolic flavoenzyme NAD(P)H: quinone oxidoreductase 1 (NQO1). *NQO1* polymorphic status has been associated with anticancer chemotherapy

Gene name	#rs ID	Clinical outcomes																	
		Response		PFS		OS		Anemia		Neutropenia		Thrombocitopenia		Nephrotoxicity		Ototoxicity		Emesis	
		R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
ADH1C	rs698	+																	
ALDH2	rs4646777			+															
APEX1	rs1048945															+			
CCNH	rs2266690							+											
COMT	rs4633			+															
CDKN1B	rs34330		+		+														
CYP1A1	rs4646903						+												
CYP1A2	rs2470890						+												
DRD2	rs1079597			+															
EPHX1	rs1051740							+						+					
EPHX1	rs2234922																+		
ERCC5	rs1047768									+									
ERCC5	rs17655									+									
GRPR	rs4986946			+															
GSTA4	rs405729							+											
LIG3	rs1052536									+									
MSH3	rs26279											+							
MSH6	rs1042821																	+	
MUTYH	rs3219484									+									
MUTYH	rs3219489									+									
NAT2	rs1801280							+											

Gene name	#rs ID	Clinical outcomes																	
		Response		PFS		OS		Anemia		Neutropenia		Thrombocitopenia		Nephrotoxicity		Ototoxicity		Emesis	
		R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y
NBN	rs1063045																		+
NQO1	rs1800566			+		+													
NQO1	rs4986998			+		+													
RAD52	rs11226									+									
TPMT	rs1142345			+															

*The designations are the same as in **Table 2**.

Table 3. The associations between polymorphisms and clinical outcomes observed in Russian (R) and Yakut (Y) patient groups (the second stage of the study)*.

outcome (i.e., individuals with CT and TT genotypes at rs1800566 showed reduced survival compared to CC homozygotes) [47, 48]. The same correlations were observed in our study. Taking into account the key role of NQO1 in preventing the formation of reactive semiquinone radicals and generating reactive oxygen species (ROS) via redox cycling, one can propose that the worse survival of carriers of CT and TT genotypes is at least the consequence of a chronically elevated level of ROS, which results in enhanced ROS-mediated DNA damage, increased genetic instability, and further cancer progression [47].

In addition to *CDKN1B*, SNPs in *CYP1A1* (T/C, rs4646903) and *CYP1A2* (C/T, rs2470890) were found to also be associated with survival, particularly with OS, in Yakut patients (P = 0.007 and P = 0.0072, respectively). In each case the longer OS occurred in patients with homozygous genotypes TT. In contrast to TPMT and NQO1, the role of CYP1A1 and CYP1A2 in cisplatin metabolism or toxicity is difficult to discern. At the same time, it cannot be excluded that the observed associations are related to metabolic pathways of drugs used in the second and subsequent lines of the chemotherapy (taxanes and anthracyclines).

Totally, 16 SNPs were associated with the side effects of chemotherapy. Thirteen such SNPs were revealed in the Russian group and three SNPs in Yakuts (**Table 3**). A total of 6 of 13 SNPs were associated with an incidence of severe neutropenia in Russian patients. A strong association was estimated for SNP rs1052536 in *LIG3*. Carriers of its homozygous genotype CC had more than 20-fold higher risk of grade 3 or 4 neutropenia than patients with other genotypes (OR = 23.211, 95% CI =2.976–181.02, $P = 2 \times 10^{-6}$). However, the most significant was SNP rs3219484 in *MUTYH*. The SNP was represented by only two genotypes, and patients who carried a heterozygous genotype AG had very low (actually unobserved) risk of developing severe neutropenia (OR = 0.013, 95% CI 0.000–0.220, $P = 4 \times 10^{-8}$). *MUTYH* encodes a DNA glycosylase involved in repair of oxidatively damaged DNA, in particular by excising adenines misincorporated opposite 7,8-dihydro-8-oxoguanines. Such mispairs are promutagenic, and if left unrepaired before the next round of replication, they can give rise to CG → AT transversion mutations [49]. The observed association may reflect the substantial contribution of oxidative stress (i.e., ROS) into cisplatin-induced cytotoxicity. On the other hand, an associative grouping of *MUTYH* SNPs together with SNPs from other DNA repair genes, particularly *RAD52* and *ERCC5*, may also indicate a role of MUTYH in the repair of cisplatin-produced DNA lesions (e.g., participation in detection of the lesions) [50].

Another side effect for which multiple associations were found in the Russian group was anemia. SNPs in *NAT2*, *GSTA4*, *CCNH*, and *EPHX1* genes were associated with the toxicity (**Table 3**). The most significant was SNP rs1801280 in *NAT2*. Cases of anemia occurred more frequently among patients with the heterozygous CT genotypes (78.4%) compared with the homozygous variants (OR = 5.945, 95% CI 2.351–15.031, P = 0.00009). This association is of particular interest because there is no information about a role of NAT2 in the metabolism or toxicity of cisplatin or cyclophosphamide (the second drug in our chemotherapy regimen) [51]. One can propose that the association found is due to linkage between the SNP with a functional SNP(s) in other gene(s). Unlike *NAT2*, three other genes (i.e., *GSTA4*, *CCNH*, and *EPHX1*) are more relevant to the effects of intracellular processing of cisplatin. The cyclin encoded by *CCNH* is a part of a TFIIH complex that is an essential component of a nucleotide excision repair pathway, widely accepted as a main player in removing platinum-DNA adducts from DNA molecules [52].

GSTA4 plays an important role for the detoxification of 4-hydroxynonenal [53], a toxic product of lipid peroxidation, increasing immensely under oxidative stress conditions (e.g., overproduction of ROS), including cisplatin treatment [54, 55]. The role of epoxide hydrolases (EPXHs) in cisplatin-induced toxicity appears to be also related to the effects of oxidative stress. However, in contrast to GSTA4, EPXHs role is inhibitory and results from the abilities of epoxide hydrolases to metabolize epoxyeicosatrienoic acids (EETs) possessing multiple functions, particularly anti-inflammatory effects. The data about relationships between EET hydrolysis and cisplatin toxicity have been mainly obtained from the studies of cisplatin nephrotoxicity [56, 57]. It has been shown that the anti-inflammatory effect of EETs substantially depends on EPHX2, a cytosolic partner of EPHX1. EPHX1 also accepts EETs, although generally to a much lesser extent than EPHX2 [58]. Therefore, a role for EPHX1 in cisplatin toxicity should not be excluded, particularly because of its high expression in kidneys. The association between the SNP rs1051740 in *EPHX1* and nephrotoxicity of the regimen used supports this suggestion.

Three SNPs in *APEX1* (rs1048945), *MSH3* (rs26279), and *MSH6* (rs1042821) were associated with ototoxicity, thrombocytopenia, and emesis in Russian patients (**Table 3**). *APEX1* gene encodes apurinic/apyrimidinic endodeoxyribonuclease 1 playing an essential role in the DNA base excision repair pathway, where it removes apurinic/apyrimidinic sites produced during the repair of bases modified by ROS, alkylating agents, or ionizing radiation [59]. High *APEX1* expression has been associated with a poor outcome for chemoradiotherapy, poor complete response rate, shorter local relapse-free interval, poorer survival, and high angiogenesis [59]. At the same time, a role for APEX1 in protection against toxicity, particularly neurotoxicity, induced by ionizing radiation, and cisplatin treatment, has also been demonstrated [60–62]. ROS and oxidative DNA damage induced by them were shown to be important components of the deleterious effects of cisplatin on neuronal cells. Taking into account the proposed role of ROS in the mechanism of cisplatin-induced hearing loss [63], a contribution of APEX1 can also be hypothesized.

The protein products of *MSH3* and *MSH6* are essential components of the DNA mismatch repair system (MMR). The presence of MMR is thought to be important in mediating cisplatin and carboplatin cytotoxicity, whereas its deficiency, by contrast, may contribute to desensitization of cancer cells to the drugs [64, 65]. In our study, SNPs in *MSH3* and *MSH6* were not associated with tumor response or survival. Nevertheless, patients with minor alleles of the SNPs rs26279 and rs1042821 were at higher risk of thrombocytopenia and emesis, respectively.

Only one gene from the list above was also present among the genes whose polymorphisms were associated with the adverse reactions in Yakut patients, namely, *EPXH1* gene. However, it was associated with a different side effect (i.e., ototoxicity). Furthermore, the corresponding polymorphisms were also different (A/G, rs2234922 and C/G, rs2260863) (**Table 3**). Higher risk of ototoxicity was observed in patients with the most frequent genotypes AA and CC (OR = 26.26, 95% CI 1.502–458.98, P = 0.0005). Although the role of EPXHs in cisplatin toxicity has been mainly associated with their effects on cisplatin-induced kidney injury, the observed link between the *EPHX1* polymorphism and cisplatin ototoxicity can be due to similarity of mechanisms underlying cisplatin-induced hearing impairment and renal dysfunction [66].

The second gene whose allelic variants were associated with a side effect of chemotherapy in Yakut patients (i.e., severe emesis) was *NBN* (G/A, rs1063045). The risk factor for the development

of severe emesis in patients was their heterozygous status at the rs1063045 locus. The protein encoded by *NBN* gene is an important component of the system repairing DNA double-strand breaks that can be induced by different environmental and endogenous agents, including cisplatin. The existing data suggest connections between polymorphic variants of the *NBN* gene and the results of cisplatin-based chemotherapy [67].

Intergroup comparison of genotypes generated with the microarrays revealed substantial differences in population frequencies of alleles and genotypes for many polymorphic markers. More than half of all markers differed significantly in the occurrence of their allelic variants in Russian and Yakut patients.

The proportion of significant genotype frequency differences resembled the results obtained in the first part of the study where a smaller number of polymorphic markers was involved (**Table 2**). Furthermore, the results of the comparisons of population-related associative spectra were also the same: there were no identical correlations for any of significant polymorphisms. All associations between the polymorphic markers and clinical outcomes were specific for each of the ethnic group studied.

These findings are generally compatible with the results of the HapMap project studying of the toxicity of platinum compounds (i.e., cisplatin and carboplatin) to lymphoblastoid cell lines from three groups of racially different individuals [19]. One can propose that the failure to detect common associations/commonly associated polymorphisms in our two groups was due to distinctive ethnic-related characteristics in the molecular mechanisms determining the sensitivity of patients to platinum drugs. Hence the difference in platinum drug sensitivity might not exclusively depend on the difference in variant frequencies of given polymorphisms. Another, but not exclusive, explanation of the findings could be a limitation of the number of polymorphisms tested and a possible omission of other potentially important markers. The latest may be mainly due to the misunderstanding of molecular phenotype(s) of the particular drug(s) [68]. The more relevant is the molecular phenotype, the higher is the potential to optimize the use of a particular drug. For some drugs, such as fluorouracil, irinotecan, and mercaptopurine, some relevant variants (i.e., *DPYD*2A*, *DPYD* 2846T/A, and *TYMS* 2R/3R; *UGT1A1*28* and *UGT1A1*6*; *TPMT *2*, *TPMT *3A*, and *TPMT*3C*) have been established but for other ones, including platinum-containing agents, they are less apparent [68, 69].

The importance of DNA repair, particularly nucleotide excision repair, for platinum cytotoxicity is widely accepted [64]. However, the overall contribution of even the most common genetic variants to predictions of response to platinum-based therapy is not yet well established [70, 71]. In principle, the situation with other "canonical" pathways affecting mainly cisplatin pharmacokinetics could be described the same way [72]. Therefore, the role of additional mechanisms that are not directly related to cisplatin cellular processing has also been proposed [73]. The results of our study overrepresented with the associations with polymorphisms in genes for different metabolic enzymes (*TPMT, NQO1, EPXH1,* etc.) supports the suggestion (the associations would remain significant even if they were adjusted with the Bonferroni method). The abundance of associations with genes involved in processing of

ROS or ROS-mediated lesions is of particular interest. First, it can point to the higher potential of ROS in total cisplatin-related cytotoxicity [66, 73]. Second, it has been proposed that populations from different geographic regions possess a difference in efficiency of coupling mitochondrial oxidation with phosphorylation, with more heat production and lower ROS generation in North/Northeastern Asians [74, 75]. Consequently, we can expect in Asians lower ability to utilize extra ROS and higher sensitivity to effects of platinum-based drugs. However, because of the relatively small sample sizes and limited number of markers tested, further studies are required to confirm this hypothesis.

In summary, comprehensive exploration of genotypes of polymorphisms in more than 100 genes in ovarian cancer patients from Russian and Yakut ethnic groups, receiving cisplatin-based chemotherapy, revealed pronounced differences in associative spectra between them. Taken in the context of absence of correlations between the associations and polymorphic genotype frequencies, the differences suggest a potential for distinct ethnic-related molecular mechanisms determining the sensitivity of patients to platinum drug effects. The mechanisms are thought to be associated with activity of different metabolic enzymes, including those involved in processing the reactive oxygen species. These genetic findings and differential responses to platinum-based chemotherapy between ethnic groups suggest that future genetic testing may be invaluable not only in predicting chemotherapy response but also in deciding the most appropriate chemotherapy regimen. It may be possible to identify in detail the susceptibility differences to chemotherapy sensitivity at the molecular level and harness this for therapeutic gains.

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Chapter 17

Novel Systemic Treatments in High Grade Ovarian Cancer

Amit Samani, Charleen Chan and Jonathan Krell

Additional information is available at the end of the chapter

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Abstract

Most patients with ovarian cancer present at an advanced stage and are never cured. To improve outcomes a variety of novel systemic strategies are being developed. Traditional cytotoxic chemotherapy is being optimised, anti-angiogenic strategies are already in the clinic and several PARP inhibitors have gained regulatory approval. In addition, immunotherapy is showing promise and novel targeted strategies including against folate receptor alpha are also generating excitement. As our therapeutic choice increases, a challenge will be how to best utilize the options available. Here we discuss recently established and other emerging therapies with a focus on key concepts rather than detailed synopses of trial designs and outcomes.

Keywords: ovarian cancer, PARP inhibitors, immunotherapy, antiangiogenic therapy

1. Introduction

Over a quarter of a million women are diagnosed with epithelial ovarian cancer (EOC[1]) each year and it is responsible for around 140,000 deaths worldwide. There is no effective screening program so the majority present with advanced disease. Despite improved surgical technique most patients are never cured. Novel systemic treatments are needed both to prolong overall survival *with* the disease but also increase the fraction of patients in whom cure is achieved. A variety of distinct but complementary approaches are discussed here.

[1]In this chapter, EOC refers also to primary peritoneal and fallopian tube carcinoma. Definitions of platinum-sensitive, resistant and refractory are as per the relevant citation.

2. Chemotherapy in ovarian cancer

Despite the emergence of alternate antineoplastic strategies, chemotherapy remains the backbone of EOC treatment. Although EOC is chemosensitive, with most patients responding initially, the majority will eventually relapse and subsequent responses are poorer. Efforts are being made to try and enhance the efficacy of 'traditional' cytotoxic chemotherapy. These include manipulation of dosing schedules, efforts to understand resistance and discovery of novel agents. These strategies are discussed in this subsection.

2.1. Dose-dense chemotherapy

Dose densification refers to the administration of an agent more frequently than in the 'standard' regimen. It can imply dose intensification (i.e. increasing the net mg/m^2/week) but some authors use it to describe splitting the standard scheduled dose into weekly fragments while maintaining the same (rather than increased) dose intensity [1].

The rationale for dose-dense treatment stems from the Norton-Simon hypothesis (**Figure 1**).

The rationale for dose densification extends beyond the Norton-Simon hypothesis. Firstly, the pharmacokinetics of a dose-dense approach may reduce toxicity. For example, paclitaxel-induced myelosuppression is dependent on the time during which the plasma level exceeds 50 nM [3]. This is considerably shorter for 80 mg/m^2 weekly compared to 240 mg/m^2 q3w [4]. Secondly, weekly paclitaxel may confer an additional anti-angiogenic effect compared to q3w scheduling [5].

Weekly paclitaxel was initially studied in the recurrent setting. Notably in one trial patients resistant to the q3w regimen achieved an objective response rate (ORR) of 25% with the weekly regimen possibly due to the additional anti-angiogenic effect of this schedule [6].

Weekly paclitaxel has also been studied in the adjuvant setting (**Table 1**).

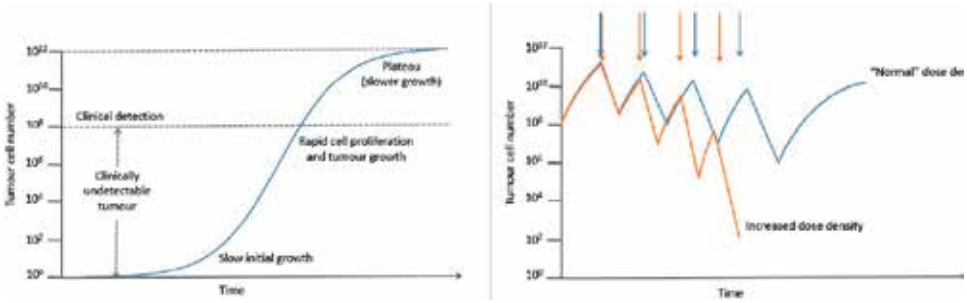

Figure 1. The Norton-Simon hypothesis assumes a Gompertzian model of tumour growth (left). This was combined with their observation that after treatment, smaller tumours regress faster than larger ones. Crucial to their mathematical model is the fact that 'log-kill' is not constant for a given dose of therapy but instead depends on tumour size, being greater for smaller tumours. Their model predicts that a dose-dense approach is more likely to eradicate a tumour [2].

Study	Eligibility	Treatment	Efficacy (months)	Safety (grade ≥ 3, P < 0.001)
JGOG 3016 [10]	Stage II-IV	Carbo q3w + either taxol q3w **or** weekly[1]	PFS 28.2 vs. 17.5 OS 100.5 vs. 62.2	Anaemia 69% vs. 44% Discontinuation due to tox. 60% vs. 43%
GOG 0262 [11]	Incompletely resected III or IV	As above + uncontrolled bevacizumab[2]	PFS 14.7 vs. 14.0 (not significant)	Anaemia 36% vs. 16% Neutropenia 72% vs. 83%
MITO-7 [12]	Stage IC-IV	Carobplatin/paclitaxel either q3w **or** weekly[3]	PFS 18.3 vs. 17.3 (not significant)	Neutropenia 42% vs. 50% Thrombocytopenia 1% vs. 7%

[1]Carboplatin AUC 6, paclitaxel 180 mg/m^2 (q3w) or 80 mg/m^2 (weekly).
[2]Carboplatin AUC 6, paclitaxel 175 mg/m^2 (q3w) or 80 mg/m^2 (weekly). 84% of patients received bevacizumab.
[3]Carboplatin AUC 6, 175 mg/m^2 (q3w) or carboplatin AUC 2, paclitaxel 60 mg/m^2 (weekly).

Table 1. Comparison of phase III trials testing weekly paclitaxel in the adjuvant setting. All values given as weekly vs. q3w.

In JGOG 3016 patients derived both PFS and OS benefit from the dose-dense approach, whereas in GOG 0262, there was no PFS difference in the intention to treat (ITT) population [7–9]. The two trials, however, had key differences. Patients in GOG 0262 were allowed bevacizumab (BEV) in an uncontrolled fashion. Since weekly paclitaxel has an anti-angiogenic effect, this may have been negated by the addition of BEV in 85% of the trial population. Consistent with this, in those who didn't receive BEV, weekly paclitaxel improved PFS (14.2 vs. 10.3 months). Pharmacogenomic differences in the two trial populations may also have been important. There are consequently unanswered questions about dose-dense chemotherapy which may be answered by two phase III trials yet to report. In the 3-arm ICON 8 trial (NCT01654146), q3w carboplatin/paclitaxel is compared to 2 dose-dense regimens without BEV. In ICON 8B (NCT01654146), bevacizumab use is allowed but is controlled and pre-specified.

2.2. Understanding resistance to facilitate chemosensitization

EOC is initially chemosensitive so efforts to understand resistance could improve outcomes. Acquired resistance is secondary to diverse mechanisms which includes alterations to DNA repair and/or response to DNA damage. Mk-1775 is an anti-Wee1 tyrosine kinase inhibitor (TKI) that may sensitize cells to chemotherapy by abrogating the G2 checkpoint (crucial in P53 deficient cells) causing premature entry into mitosis [10]. It has shown promising results in several phase II trials [11]. In a different approach, the 2-arm PiSARRO trial (NCT02098343) involves the addition of APR-246 (capable of restoring mutant P53 to wild-type confirmation) to platinum-based therapy with the aim of restoring the apoptotic-response to chemotherapy-induced DNA damage. There are many other pre-clinical and early clinical efforts aiming to reverse chemoresistance including efforts to target primary resistance by targeting cancer stem cells and epithelial to mesenchymal transition [12].

2.3. Novel chemotherapeutic agents

Lurbinectedin is a recently discovered marine-derived antineoplastic agent that has a multi-modal mechanism of action similar to trabectedin. It showed promising results in a phase II

trial in platinum-resistant EOC and is being investigated in a phase III trial against either PLD or topotecan [13]. It has also shown *in vitro* synergy with cisplatin raising hopes of clinical application to reverse platinum resistance [14]. Trabectedin itself is undergoing phase III testing in patients with platinum partially-sensitive disease (NCT01379989).

3. Antiangiogenic strategies in ovarian cancer

Key mediators of physiological angiogenesis include products of the vascular endothelial growth factor (VEGF) gene family including VEGF-A (often abbreviated to VEGF), VEGF-B, C and D and placental growth factor. The receptor family includes VEGFR-1, 2 and 3. Different combinations of ligand-receptor interaction result in diverse outcomes such as promotion of survival, proliferation of endothelium, increased permeability and lymphangiogenesis. The binding of VEGF-A to VEGFR-2 is most important in endothelial proliferation and the regulation of permeability [15].

In physiology VEGF is important for the cyclical angiogenesis that takes place in the female reproductive tract [16]. Many tumour cell lines overexpress VEGF and in one series over 97% of human ovarian lines had overexpression [17]. Clinically, expression levels have been found to be an independent prognostic factor in several studies [18] and have also been found to correlate with peritoneal dissemination and ascites formation [19].

Given the role of VEGF in physiology as well as pre-clinical and observational data supporting a role for VEGF in cancer, several VEGF-directed therapies exist.

3.1. Bevacizumab

Bevacizumab (BEV) is a humanized monoclonal antibody able to bind all VEGF-A isoforms [20]. It is the most extensively studied of the antiangiogenic agents in EOC. Two phase III studies (GOG-218 and ICON7) tested adjuvant BEV. In GOG-218 [21] patients received 6 cycles of carboplatin/paclitaxel q3w and either 1) placebo (cycles 2–22), 2) BEV induction (cycles 2–6) then placebo maintenance (7–22) or 3) BEV induction (cycles 2–6) then maintenance (7–22). BEV was given at 15 mg/kg. The median PFS was 14.1 months in the BEV throughout arm compared to 11.2 months in the induction-only arm and 10.3 months for the control. Overall survival was not significantly different. 22.9% developed grade ≥ 2 hypertension in the BEV throughout arm vs. 7.2% in the control arm. In ICON7 [22], high-risk patients were given carboplatin/paclitaxel q3w with either placebo or bevacizumab (7.5 mg/kg) for cycles 2–18. Median PFS was 19.0 months in the BEV arm vs. 17.3 months (HR 0.81, $p < 0.01$). Among patients with incompletely resected IIIC or IV disease the median PFS was 15.9 vs. 10.5 months in the control arm. Bleeding (39 vs. 11%), hypertension (18 vs. 2%), thromboembolism (7 vs. 3%) and GI perforations (10 vs. 3 patients) were higher with BEV. Mean global QoL score was higher, at 54 weeks, in the control arm (76.1 vs. 69.7 points - EORTC questionnaire) [23]. Recent exploratory analysis of a 'high-risk' subgroup revealed significantly increased OS (restricted means) in the BEV group of 39.3 vs. 34.5 months [24].

There were similarities and differences between these trials. Both suggested greater benefit in a subpopulation with higher stage and suboptimal debulking. They also agreed that QoL was not improved with BEV. Conversely, different doses and durations of treatment were used and overall survival data also differed, perhaps confounded by the 40% crossover in GOG 218. BEV received regulatory approval from the EMA using 15 mg/kg [25] although ESMO guidelines supported the 7.5 mg/kg dose used in ICON7, which is also prescribed in the UK currently [26]. Analysis of both trials showed greatest separation of the PFS curves at the end of BEV treatment (12 or 15 months), raising questions about extending maintenance duration. This is being investigated in the phase III BOOST study (NCT01462890).

Bev has also been studied for recurrence. In AURELIA [27], patients with platinum-resistant disease and ≤2 prior lines of chemotherapy were given single agent investigator-choice chemotherapy either alone or with BEV continued until progression/toxicity. Median PFS was higher in the BEV arm, 6.7 vs. 3.4 months with an ORR of 27.3 vs. 11.1%. Of the 113 patients with baseline ascites 17% required paracentesis in the control arm vs. 2% in the BEV arm and PROMs for GI symptoms were better with BEV [28]. OS was not significantly different in the context of 40% crossover but a recent exploratory analysis suggestive a survival advantage in those who received BEV during or after the study [29]. Adverse events were consistent with previous studies. BEV has been granted FDA and EMA approval for this indication.

In the OCEANS study [30], the addition of BEV to carboplatin/gemcitabine in patients with platinum-sensitive disease resulted in a median PFS of 12.4 months vs. 8.4 months. OS was not significantly (38% crossover). Hypertension, proteinuria and non-CNS bleeding were significantly more common in the BEV arm. BEV was also tested in the platinum-sensitive setting with carboplatin/paclitaxel, in the factorial GOG-213 trial [31]. Median OS with BEV was 42.2 months compared to 37.3 months without ($p = 0.056$). BEV has EMA regulatory approval in this setting.

3.2. VEGFR tyrosine kinase inhibitor (TKI) therapy

Whereas BEV binds directly to VEGF, VEGFR TKIs affect signalling via competitive inhibition of the intracellular kinase domain. They have the advantage of being orally bioavailable and multitargeted. Conversely, plasma concentration is unpredictable and off-target effects narrow the therapeutic window.

Cediranib inhibits VEGR-1,2 and 3 and c-Kit. ICON 6 [32] randomised patients with recurrent platinum-sensitive disease to chemotherapy plus: placebo concurrently + maintenance (Arm A), cediranib concurrently + placebo maintenance (Arm B) or cediranib concurrently + maintenance (Arm C). Median PFS was 11 months in Arm C vs. 8.7 months in Arm A ($p < 0.0001$). Recent OS data [33] by restricted means showed 34.2 months vs. 29.4 months in Arms C and A respectively (95% CI for the difference: −0.1-9.8). During chemotherapy grade ≥ 3 fatigue (16 vs. 8%), diarrhoea (10 vs. 2%), hypertension (12 vs. 3%), febrile neutropenia (7 vs. 3%) and thrombosis (3 vs. 1%) were higher with cediranib. 48% discontinued treatment due to toxic effects in Arm C compared to 17% in Arm A and 37% in B. Although recent analysis showed no detriment in

QOL at 1 year [34], filing for regulatory approval for cediranib had been previously withdrawn. Nonetheless cediranib maintenance is undergoing investigation in ICON9 (see below).

Pazopanib inhibits VEGR1,2 and 3, c-Kit and PDGFR. The AGO-OVAR 16 study [35] evaluated first-line maintenance pazopanib. PFS was 17.9 months for pazopanib compared to 12.3 months for control. Grade 3/4 adverse events were significantly higher for pazopanib including hypertension (30.8%), neutropenia (9.9%) and diarrhoea (8.2%). Discontinuation due to AEs occurred in 33% in the pazopanib arm compared to 5.6% in the placebo arm. Regulatory approval filing was withdrawn due to perceived imbalance in benefit–risk ratio.

Other VEGFR TKIs have been studied in ovarian cancer [35]. Nintedanib was given in the first-line setting with chemotherapy and then maintenance. Again, a PFS benefit was seen but no significant OS advantage [36]. Other multitargeted VEGFR TKIs such as sunitinib and sorafenib have also been studied with similar outcomes. As a class the TKIs appear to have some effect however their multi-targeted nature and unpredictable bioavailability means that their perceived risk:benefit ratio has not led to any regulatory approvals as yet.

3.3. Other antiangiogenic strategies

The Ang-Tie pathway is distinct from the VEGF axis, involved in vascular remodelling. Trebananib is peptide-Fc fusion protein that binds Angiopoietin 1 and 2 and prevents interaction with Tie on endothelium. Although promising results were seen in phase II [37], a phase III trial (TRINOVA-2) [38] failed to meet its PFS endpoint and a third terminated early for futility (NCT01493505).

3.4. Combination therapy

Vascular disrupting agents (VDAs), in contrast to inhibiting formation of new vessels, target existing tumour vasculature. The VDA's combretastatin and fosbretabulin disrupt the endothelial cytoskeleton (by binding tubulin) aiming to cause endothelial detachment and eventual vessel obstruction. Tumour vasculature lacks pericytes and smooth muscle making them selectively susceptible. Fosbretabulin is being examined for synergy with bevacizumab and chemotherapy in platinum-resistant disease in a phase II/III trial (NCT02641639).

There is pre-clinical rationale for the combination of VEGF-targeted therapy with poly (ADP-ribose) polymerase inhibitors (PARPi); anti-VEGF induced hypoxia can impair DNA repair and sensitize otherwise insensitive cells to PARPi. In a phase II trial of olaparib and cediranib [39] PFS with the combination was prolonged (17.7 vs. 9.0 months) and, consistent with pre-clinical rationale, the difference was most marked in BRCA wild-type patients. Grade 3/4 toxicity however was 70% with the combination vs. 7% for olaparib monotherapy. The combination is currently undergoing phase III testing (ICON 9). The combination of bevacizumab and olaparib in first-line maintenance is also being studied (NCT02477644).

Combining VEGF blockade and immunotherapy also has pre-clinical rationale (see below). Combinations of anti-angiogenesis and chemotherapy have been discussed in the paragraphs above. Of note, an early phase trial of pazopanib with carboplatin/paclitaxel was terminated early because of toxicity (GI perforations and myelotoxicity).

3.5. Predictive biomarkers in anti-angiogenic therapy

Given the relatively modest median PFS benefits and lack of OS benefit in some trials combined with toxicity and economic considerations, biomarkers for patient selection are needed. None have yet been validated for routine use although many have been suggested. Studies have been retrospective and focussed on different markers including gene-expression signatures, serum and tissue proteomic biomarkers. There have been some intriguing results including a 63-gene signature that identifies an immune subgroup that may be harmed by bevacizumab treatment [40]. Prospective validation is needed for this and other candidate markers.

4. PARP inhibitor therapy

DNA constantly undergoes single and double-strand breaks (SSBs/DSBs). SSBs are repaired predominantly by base excision repair (BER). PARPs are nuclear proteins with diverse functions including in BER and chromatin remodelling. PARP-1 is the most abundant member which upon binding to SSBs activates its ADP-ribosyltransferase catalytic domain allowing PARylation and recruitment of DNA repair effectors [41]. DSBs are mostly repaired by homologous recombination (HR) or non-homologous end joining (NHEJ), the latter being error-prone [42]. HR involves a number of key proteins including BRCA1, BRCA2, RAD51 and PALB2. A detailed discussion is beyond the scope of this chapter but the process of HR is reviewed here [43]

4.1. Homologous recombination repair in ovarian cancer

The Australian Ovarian Cancer Study Group screened 1001 patients with stage I-IV ovarian cancer for point mutations or large deletions in BRCA genes. 14.4% of patients overall had a germline mutation (including 17.1% with serous histology) [44]. A similar frequency was found in The Cancer Genome Atlas (TCGA) [45] although globally the prevalence varies between ethnic groups. In addition to germline mutations, BRCA genes can be somatically mutated, epigenetically silenced or the protein inactivated through post-translational mechanisms, e.g. EMSY amplification [46]. Various series have found somatic mutations of BRCA in 3–6% of EOC [47]. In contrast to somatic mutations, epigenetic silencing by promoter methylation is a dynamic process and may be harder to quantify. Studies report prevalence in the region of 5–30% of ovarian cancers.

However, BRCA1 and 2 are just two of many proteins involved in HR. TCGA undertook exomic analysis of 316 ovarian cancers as well as studies of promoter methylation, RNA expression and copy number changes [45]. Pathway analysis demonstrated that 51% of tumours had either mutations or silencing of components in the HR pathways. (**Figure 2**).

4.2. PARP inhibitors in ovarian cancer

HR deficiency (HRD) in EOC provides a target that can be exploited therapeutically. It was noted that cells with non-functioning PARP develop increased nuclear foci of Rad51 implying an increased burden of lesions being repaired by HR in these cells [48]. Farmer et al. [49] tested

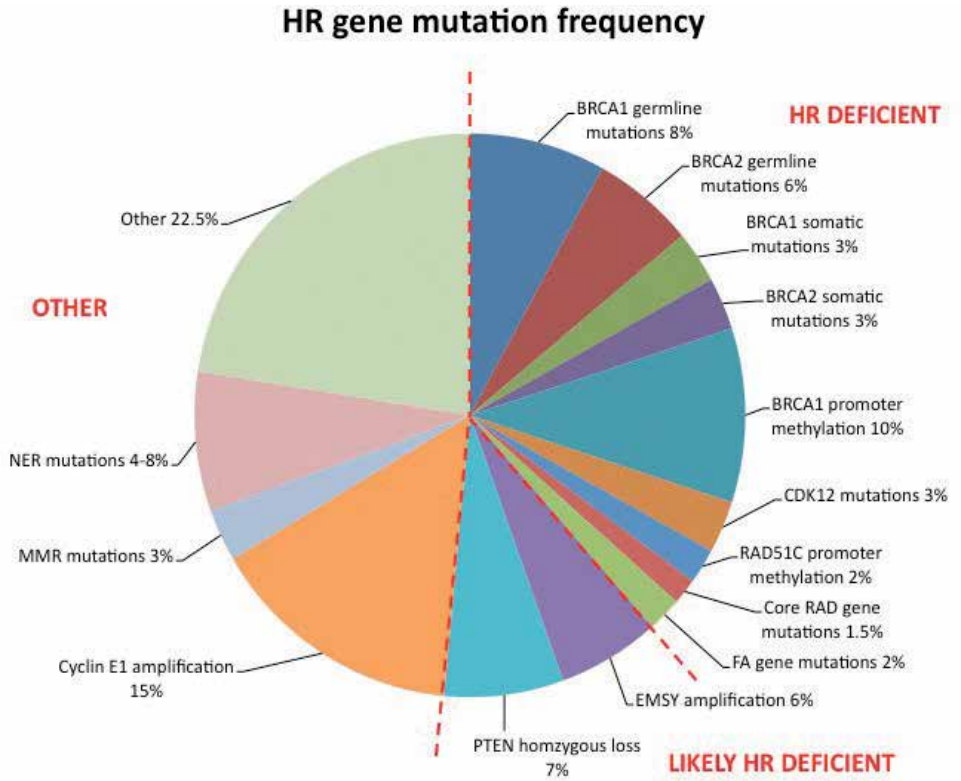

Figure 2. Distribution of HR gene mutations in EOC. Adapted from Ref. [47].

the hypothesis that BRCA 1/2 dysfunction would hypersensitize cells to PARP inhibition and were able to demonstrate this in BRCA deficient cell lines. This example of 'synthetic lethality' whereby either defect alone is tolerable but the combination is fatal has been exploited in the generation of a family of drugs, the PARP inhibitors. (**Figure 3**).

Following this, further work began on designing a PARP inhibitor (PARPi) suitable for clinical use. Early agents mimicked the substrate-enzyme interaction between NAD^+ and the catalytic domain of PARP1/2 and further optimization led to the design of Compound 47, that would be developed as Olaparib [50]. Since Olaparib, several agents have been developed (discussed later) designed to inhibit PARP 1/2 catalytic activity.

In addition to catalytic inhibition, a distinct antitumour mechanism of PARPi, 'PARP-trapping' has been described. Trapped PARP-DNA complexes were more cytotoxic than unrepaired SSBs in PARP deficient cells and different PARP inhibitors had different PARP-trapping potency which was not correlated with their catalytic inhibitory properties [51].

4.3. Olaparib

Olaparib is an orally bioavailable small molecule with a nicotinamide moiety that competes with NAD^+ for binding to PARP. The MTD for olaparib was established from early phase

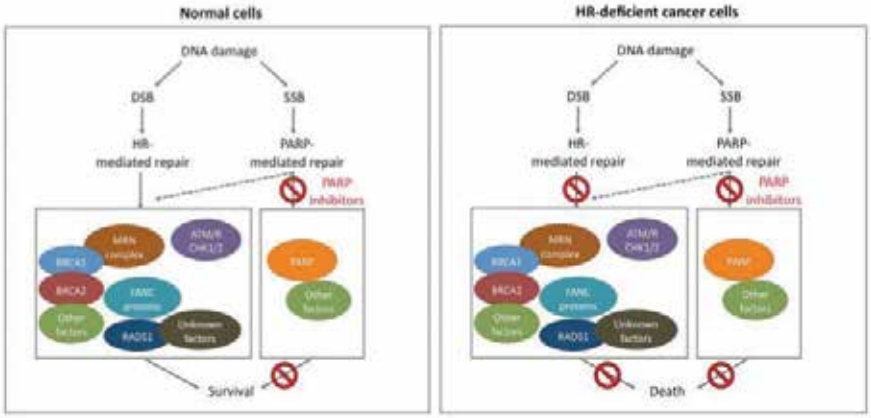

Figure 3. Schematic of synthetic lethality of PARP inhibition in HR deficient cells.

trials at 400 mg BD. Objective responses were seen mainly in patients with germline BRCA mutations (gBRCAm) [52] Further support for the efficacy of olaparib in in the gBRCAm population came from a proof-of-concept phase II where the ORR in the 400 mg BD cohort was 33% including some complete responses (CRs) [53]. Of note, one heavily pre-treated patient developed acute myeloid leukaemia (AML) 9 months after cessation.

A further phase II study gave 193 heavily pre-treated EOC platinum-resistant/unsuitable patients with gBRCA mutations olaparib at a dose of 400 mg BD [54]. The ORR was 31%. AEs were similar to those seen in earlier trials with a grade 3/4 rate of 54% including anaemia (17%) and fatigue (6%). Two patients developed leukaemia and one myelodysplastic syndrome, all were heavily pre-treated (25, 26 and 34 cycles each). These results (along with other applicant-submitted data) earnt olaparib FDA approval as monotherapy for patients with gBRCA mutations after three prior lines. The recent phase III SOLO3 study randomised patients with gBRCA mutations who have received at least 2 prior lines of platinum-based therapy and who are deemed at least partially platinum-sensitive to either Olaparib 300 mg BD or single agent chemotherapy of investigators choice [55]. Results are awaited. While the previous formulation of Olaparib required 16 capsules a day, the current tablet formulation requires only four raising hopes that some of the gastrointestinal toxicity will be mitigated.

In the aforementioned studies olaparib was given as monotherapy for treatment of 'active' disease. In contrast, Study 19 randomised patients with recurrent platinum-sensitive cancer with at least 2 prior lines to *maintenance* olaparib or placebo post-chemotherapy [56]. In a predefined subset analysis of patients with known germline or somatic BRCA mutation (most retrospectively determined), median PFS in the gBRCAm group was 11.3 vs. 4.3 months with Olaparib and placebo respectively (HR 0.18). OS was not significantly different (23% crossover). The findings led to EMA approval. SOLO2 was a phase III double-blind placebo-controlled study in patients with recurrent platinum-sensitive EOC who had received at least 2 prior chemotherapy lines. Patients either got maintenance olaparib 300 mg BD or placebo. Investigator-assessed median PFS was 19.1 vs. 5.5 months (HR 0.30). Median PFS2 was also

improved from not reached vs. 18.4 months (HR 0.5) and OS data are immature. Although nausea (76% vs. 33%) and vomitting (37% vs. 19%) were higher in the olaparib arms, grade 3/4 events were infrequent (2.6% for both). Grade 3/4 anaemia occurred in 20%. Patient-reported outcomes showed no detriment for olaparib [57].

The phase III SOLO1 has completed accrual and randomised patients with BRCAm following first-line platinum-based chemotherapy to either Olaparib 300 mg BD or placebo.

4.4. Niraparib

Niraparib is a potent PARP1 and PARP2 inhibitor whose pharmacokinetics allows once daily dosing. A phase I dose escalation trial established the MTD as 300 mg/day. Dose-limiting toxicities included fatigue, reversible pneumonitis (in the context of recent chest wall irradiation) and reversible grade 4 thrombocytopenia. Of the 20 patients with gBRCA mutations and evaluable tumours the ORR (at doses between 80 and 400 mg) was 40% [58].

The pivotal phase III NOVA trial enrolled patients with platinum-sensitive disease who had received at least two prior lines of chemotherapy and who had measurable disease of <2 cm post-treatment [59]. Patients were randomised to niraparib 300 mg or placebo as maintenance till PD or unacceptable toxicity. Patients were stratified into gBRCA mutations vs. those without. Those without gBRCA mutations were further stratified into those with or without a positive HRD score (see below) and a predefined cut-off. PFS in the gBRCA mutated group was 21 vs. 5.5 months in the niraparib and control arms respectively (HR 0.30) and 12.9 vs. 3.8 months (HR 0.45) in the HRD positive cohort.

QUADRA is an ongoing single-arm phase II trial in patients pre-treated with 3–4 lines of chemotherapy and who were platinum sensitive at first recurrence regardless of BRCA mutation status. Patients who entered the trial underwent testing for homologous recombination deficiency (HRD) using a validated commercial assay. This assesses tumour samples for three SNP array-based 'signatures' of genomic instability (loss of heterozygosity, telomeric allelic imbalance and large scale transition) to derive an overall 'HRD score' that should predict sensitivity to PARP inhibition [NCT02354586].

PRIMA is an ongoing phase III of niraparib maintenance after 1st line chemotherapy. Unlike SOLO1, patients are enrolled on the basis of HRD score rather than gBRCA mutation status.

4.5. Rucaparib

Rucaparib is another orally bioavailable PARPi with both catalytic inhibitory and PARP-trapping activity, the potency of the latter being equivalent to olaparib [60].

Rucaparib was granted accelerated FDA approval largely based on composite data from 2 phase II studies. 106 patients with gBRCA mutations who had received at least 2 prior lines of chemotherapy received continuous rucaparib at 600 mg BD [61]. The confirmed ORR by RECIST was 54%. Toxicity at ≥ grade 3 included anaemia (27%), fatigue (15%), transient AST/ALT elevation (13%), vomiting (6%) and nausea (4%).

Part 1 of the ARIEL2 trial (from which the gBRCA mutation data was pooled in the above analysis) enrolled 206 patients who had been received at least 1 prior platinum containing chemotherapy regimen and who had progressed after at least 6 months after their most recent course [62]. Patients were prospectively divided into three subgroups based on their HRD status: 1) germline or somatic BRCA mutations 2) BRCA wild-type and LOH-high 3) BRCA wild-type and LOH-low. LOH was assessed using a next generation sequencing assay and a cut-off of 14% was assigned using microarray and survival data from TCGA. Based on this pre-specified score, PFS was 12.8 months, 5.7 months and 5.2 months in the BRCA mutated, BRCA wild-type/LOH-high and BRCA wild-type/LOH-low subgroups. Although median PFS was similar in the latter groups, the HR for PFS was significantly in favour of the LOH-high subgroup (0.62 95% CI 0.42–0.90), and ORR by RECIST (29% vs. 10%) and 1 year survival (28% vs. 10%) were also better for the LOH-high subgroup. Of note, LOH exists on a continuum and exploratory post-hoc analysis revealed that a cut-off of 16% provided better discrimination between the two subgroups [63]. Also importantly, there were patients in the LOH-negative group with very good partial and even complete responses (by ca125). In this single arm phase II study, it is not possible to exclude the possibility that LOH-high tumours simply have a better prognosis and that LOH is a prognostic rather than predictive marker. In order to address this question (in a maintenance setting at least) the NGS assay is being prospectively applied in the phase III Ariel 3 study which is investigating maintenance rucaparib in platinum-sensitive ovarian cancer. The phase III Ariel 4 study is will compare rucaparib as an active treatment vs. standard of care chemotherapy in platinum-sensitive disease after at least 2 prior lines.

4.6. Veliparib

Another orally bioavailable PARP inhibitor, veliparib is far less potent at PARP-trapping than the previously mentioned agents although it is a more potent catalytic inhibitor than niraparib and has been shown to cross the blood–brain barrier [51]. In a phase I trial 40% of the 28 BRCAm positive evaluable patients had an ORR at the MTD (400 mg BD). Commonest toxicities were nausea, vomiting and lymphopenia and 2 patients had grade 2 seizures [NCT01472783].

In a phase II trial in patients with gBRCAm who had been treated with 3 or fewer prior regimens (median 2) and of whom 60% were platinum resistant, the ORR was 26% (35% in the platinum-sensitive cohort). Grade 3 fatigue, nausea and neutropenia occurred in 6%, 4% and 2% respectively with no other grade 3 toxicities. Veliparib is currently being explored in phase III trial concurrently with carboplatin/paclitaxel and then continued as maintenance (NCT02470585, see below).

4.7. Talazoparib

Talazoparib is a novel PARPi that traps PARP approximately 100-fold more efficiently than olaparib and rucaparib and exhibits cytotoxicity at nanomolar (compared to micromolar) concentrations) [60]. At an MTD of 1 mg/kg, 5/12 patients with BRCAm ovarian cancer achieved

an ORR with a 24% and 18% rate of G3 anaemia and thrombocytopenia respectively [64]. Given its unique potency for trapping, there is hope that it may have efficacy as a second line agent for patients who have progressed on a previous PARPi [65].

4.8. Combination therapy with PARP inhibitors

PARPi were originally developed as potential chemo/radiosensitizers. There is obvious rationale in combining PARPi with other agents, especially in tumours that are HR proficient. When combining PARPi with chemotherapy, rational combination necessitates consideration of the mechanism of action of the chemotherapy plus the relative catalytic inhibitory/trapping properties of the PARPi. For example, PARPi combination with topo-1 inhibitors is synergistic primarily because of catalytic PARP inhibition whereas synergy with alkylating agents relies on trapping too [66]. Several PARPi/chemotherapy combinations are in trials, reviewed here [67]. Synergistic toxicity (e.g. myelotoxicity) will have to be borne in mind. PARPi/VEGFR targeting combinations have previously been discussed. Other targeted combinations include PI3K/MTOR pathway inhibitors, HSP90 and CHK1/2 inhibitors [67]. Finally, talazoparib had immunomodulatory effects in a pre-clinical mouse model; studies looking at immunotherapy with PARPi are underway (NCT0257172).

4.9. Resistance to PARP inhibitors

Several putative mechanisms of resistance have been described. These include a secondary mutation in BRCA which either restores the correct open reading frame (i.e. where the original mutation caused a frameshift) or which fully reverts the original mutation to wild-type. This also causes platinum resistance and in one study of platinum resistance in BRCAm patients, 46% had acquired a secondary BRCA mutation [68]. Other mechanisms include upregulation of P-glycoprotein and loss of 53BP1 (which usually promotes NHEJ and prevents HR). Knowledge of the specific resistance mechanism may have clinical relevance as some (e.g. secondary mutations) cause platinum resistance too whereas others do not. Also, 53BP1 loss causes resistance in BRCA1 but not BRAC2 deficient tumours.

5. Immunotherapy in ovarian cancer

In 2003 Zhang and colleagues showed that the presence or absence of tumour-infiltrating lymphocytes (TILs) in EOC is an independent prognostic factor (in multivariate analysis) for PFS and OS. Of 174 patients, those with TILs had a median overall survival of 50.3 months compared to 18.0 months in the 72 patients without [69]. Tumour-associated antigens discovered in EOC include mesothelin, Her2, NY-ESO and ca125 amongst others [70].

Around 50% of EOC has genomic/epigenetic changes in genes implicated in HRD [45]. Therefore there is a subset of EOC with a higher mutational burden possibly more likely to benefit from immunotherapy. Analysis of TCGA data showed a significantly higher predicted neoantigen load in HRD vs. HR proficient tumours [71]. In addition, BRCA1/2 status and neoantigen load

were independent predictors of OS in multivariate analysis and BRCA mutated tumours had an increased TIL burden and PD-L1 expression. Lastly, tumour burden/volume is an important factor in predicting the response to immunotherapy [72]. Ovarian cancer is unusual as patients presenting *de novo* with bulky disease can be treated with radical surgery to no residual disease. Although the majority relapse, there is a window of time where disease remains undetectable. Given the data that exists on enhanced effectiveness of immunotherapy in patients with a low overall tumour burden, this may present a window of opportunity to maximise effectiveness of this therapeutic approach.

5.1. Checkpoint blockade

Co-inhibitory checkpoints usually act to minimize collateral tissue damage during immune-activation. Upregulation of these checkpoints can subvert anti-tumour immunity. The binding of CTLA-4 to B7.1/B7.2 is one such inhibitory interaction that can be prevented by the anti CTLA-4 monoclonal antibody ipilimumab.

In a phase I study including 2 patients with ovarian cancer, one patient had a 43% reduction in ca125 levels while the other developed a plateau in ca125 levels despite rapidly rising levels before treatment [73]. In a follow-up study of 9 patients one developed a radiologic PR with complete resolution of mesenteric lymphadenopathy. Three others achieved radiographic and ca125-defined stable disease of 2, 4 and >6 months duration. In a phase II study of 40 patients with recurrent platinum-sensitive EOC (NCT01611558), 50% developed at least G3 toxicity and the ORR was 10.9% by RECIST. A phase II trial testing a combination of nivolumab and ipilimumab for recurrent ovarian cancer is currently underway (NCT02498600).

A trial using another CTLA4 antagonist, tremelimumab, is currently enrolling patients for phase I trials in combination with olaparib (NCT02571725, NCT02485990).

Another inhibitory checkpoint interaction is between PD-1 (on T-cells) and PD-L1 (that may be upregulated on tumour cells and their microenvironment). Avelumab, a fully humanised IgG1 anti-PD-L1 antibody, was tested in a phase Ib trial in 124 patients with platinum resistant/refractory disease after a median of 4 lines of therapy [73, 74]. The drug was well tolerated with a grade 3/4 adverse event rate of 6.4%. ORR in this heavily pre-treated population was 9.7% and the relationship between germline BRCA status and probability of response is being investigated. Avelumab is currently being tested in two randomised phase III trials. The three-arm JAVELIN Ovarian 200 study (NCT02580058)I is recruiting patients with their first platinum resistant/refractory relapse and randomising to either Avelumab or PLD alone or in combination. In JAVELIN Ovarian 100 (NCT02718417) patients with previously untreated III/IV ovarian cancer are randomised to carboplatin and paclitaxel followed by placebo or avelumab maintenance or carboplatin and paclitaxel with concurrent *and* maintenance avelumab.

Atezolizumab is also a fully humanized IgG1 anti-PD-L1 antibody. In the phase III ATALANTE trial (NCT02891824) patients with platinum-sensitive relapse are being randomised to platinum-based chemotherapy with concurrent and maintenance bevacizumab + placebo (control arm) or bevacizumab + avelumab (experimental arm). The combination of bevacizumab and avelumab is a rational one based on evidence that endogenous VEGF signalling has a variety

of immunomodulatory effects. VEGF-A has been postulated to suppress dendritic cell maturation, increase the presence of immunosuppressive CD34+ haematopoetic progenitor cells in the tumour microenvironment and inhibit T-cell maturation [75]. Another trial combining atezolizumab with bevacizumab (NCT02839707) in a phase II/III setting is randomising platinum resistant patients between 3 arms each containing PLD with either bevacizumab alone (control), atezolizumab alone or bevacizumab and atezolizumab.

Instead of targeting PD-L1, pembrolizumab is a humanized anti PD-1 antibody. Keynote-028 included 26 EOC patients. 1 patient had a CR and 2 had PR by RECIST. The median duration of response was not reached (range 24.9+ to 26.5+) [76]. There are currently several ongoing phase I/II trials with pembrolizumab both as monotherapy and in combination with chemotherapy, niraparib and various small molecule inhibitors in the frontline and recurrent settings (NCT02865811, NCT02520154, NCT02440425, NCT02674061).

Nivolumab, a PD-1 blocking antibody, was given to 20 patients with platinum resistant EOC. 40% of patients developed G3/4 toxicity (lymphopenia, anaemia, hypoalbuminaemia, maculopapular rash, fever, ALT increase). Three patients (15%) had an OR including 2 CRs. One of these was in a patient with clear cell carcinoma (often chemoresistant) and this response was ongoing at the time of study reporting [77]. As with the pembrolizumab data, although the ORR was modest, there was evidence of durable responses in both studies. Nivolumab is being studied in several ongoing trials including in combination with ipilimumab for (NCT02498600), in combination with bevacizumab (NCT02873962) and with a vaccine against the tumour-associated antigen WT1 (NCT02737787).

5.2. Adoptive T-cell therapy

Adoptive T-cell therapy (ATT) involves the direct administration of various types of anti-tumour T-cells to the patient. Given the prognostic value of TILs (see above), TIL-based ATT seems logical. In one study, 13 patients who had no residual disease after surgery and adjuvant therapy were treated with TIL infusion. A matched control group was followed up concurrently [78]. In this small study 3 year OS was 100% in the TIL group vs. 67.5% in the control group. TIL-based trials are ongoing (NCT02482090, NCT01883297). Another ATT approach involves using chimeric antigen receptor (CAR) T-cells that have been engineered to express a CAR with an extracellular single chain variable fragment incorporating immunoglobulin heavy and light chains capable of targeting any extracellular target (not just those complexed with MHC). There are currently over 20 trials registered on ClinicaTrials.gov testing CAR-T-cell-based therapy in ovarian cancer against targets including Her2, mesothelin, folate receptor-α (FRα) and NY-ESO-1.

5.3. Other approaches

The field of immunotherapy is advancing rapidly and various other approaches are in early phase trials. Vaccine based therapy has yielded objective responses demonstrating proof-of-concept, for example using a dendritic cell whole-tumour based approach [79]. Although clinical trials for vaccines have been disappointing, various techniques for optimisation are leading to renewed enthusiasm [80]. Another approach used a tri-functional antibody, catumaxomab,

which binds to epithelial cell adhesion molecule (EpCAM), CD3 (found on T-cells) and has an Fc portion that is recognised by various cells including macrophages. This allows immune cells to colocalize with tumour and cause cytotoxicity. EpCAM positive cells are found in 70–100% of malignant effusions and in a phase II study intraperitoneal (IP) administration of catumaxomab significantly improved the puncture free interval in heavily pre-treated patients [81]. It was given EMA approval for IP administration but the manufacturer withdrew this for commercial reasons in July 2017. One of the problems of 'targeted' immune therapy such as this is toxicity with systemic administration. Consequently, IP administration may be the only viable route with some therapies.

5.4. Combinations

Combination immune therapy PARP inhibitors, VEGF therapy and chemotherapy have already been mentioned. In addition, checkpoint inhibition has recently been combined with epacadostat, an inhibitor of 2,3-dioxygenase (IDO). IDO activation in tumours is associated with immune escape via T-cell dysfunction. Combining epacadostat and pembrolizumab has shown efficacy in patients with EOC although randomised trials are needed to ascertain the effect of epacadostat over and above pembolizumab monotherapy [82].

6. Other novel agents

The aforementioned systemic strategies are of most relevance because they are either already in (or close to) the clinic. There are however various other strategies being explored, some of which have already been trialled in clinical studies. One approach involves targeting folate receptor and, specifically, the α isoform (FRα). This receptor is absent from normal ovarian epithelium but expressed on the majority of EOC [83]. The receptor has been targeted by various classes of therapy including folate-drug conjugates, small molecule FRα inhibitors, monoclonal antibodies, vaccines and oncolytic viruses. The phase III trial of vintafolide (folate conjugated with a derivative of vinblastine) in combination with PLD (NCT01170650) was discontinued for futility. Further trials of folate-drug conjugates are ongoing [84]. Farletuzumab, a monoclonal antibody that causes antibody and complement- dependant cellular cytotoxicity is being investigated in combination with platinum-based chemotherapy in patients with relapsed EOC and low ca125 following promising sub-group analysis from a previous phase III trial (NCT02289950). Phase I results for ONX-0801, a FRα-targeted thymidylate synthase inhibitor that accumulates in EOC cells generated a PR in 5/11 patients at the MTD with 4/4 FRα expressing tumours showing response [85].

Aside from FRα targeting therapy, there are multiple other targeted strategies in EOC in pre-clinical and early clinical phases. Cell cycle targeting with WEE-1 inhibition has been discussed but other strategies including CHK1/2 inhibition with prexasertib (which yielded a PR in 5/13 patients in cohort 1 of a recent phase II trial [86]) are being explored. PI3k/AKT/mTOR, Her2 and molecules in the apoptotic machinery are amongst a plethora of other avenues being explored. As our understanding of the molecular basis of EOC progresses, future

therapies are likely to employ biomarker or other selection criteria within trial protocols. For example, clear cell ovarian carcinoma is known to harbour mutations in the PI3K/AKT/mTOR pathway and the GOG-0268 trial of temsirolimus in addition to carboplatin/paclitaxel as first-line therapy was restricted to the clear cell population for this reason. Beyond the 'traditional' histological subtyping of EOC, analysis of TCGA data and recent advances in bioinformatics as led different groups to propose various molecular classifications of high grade serous EOC. Once such classification proposes four subtypes; mesenchymal, immunoreactive, differentiated and proliferative. Prospectively defined subgroup analysis of future trials using such novel molecular classifications may allow us to tailor therapy to maximise efficacy.

7. Conclusion

Several distinct strategies have been discussed. PARP inhibition have probably had the biggest clinical impact however mature OS data is awaited from many trials and further work is required to understand resistance and the potential role of combination therapy and sequencing of PARPi. Anti-angiogenic strategies have had a modest impact overall but research into patient selection may identify a subset who have more marked benefit. Similarly, with immunotherapy, the majority of patients do not show objective response but a subset has durable benefit. It seems, therefore that future success will depend on improved patient selection for trials, possibly through continued progress in understanding the molecular landscape of EOC. While progress has been made, there is a long way to go and the next few years should see continued incremental benefit in this difficult to treat disease.

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