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Targeted therapy in ovarian cancer: novel agents and predictive
biomarkers

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Abstract

Ovarian cancer is the second most common gynecological malignancy and the leading cause of death from gynecological cancer. Most women present with advanced stage disease, having a poor prognosis even with adequate treatment. Cure is unlikely for advanced disease. Despite the high rate of initial response to chemotherapy, the majority of women will develop recurrent disease, and, thus, new therapeutic options are required. Molecularly directed therapy has been developing rapidly for ovarian cancer, either as single therapy or in association with chemotherapy. Bevacizumab, an anti-VEGF antibody, is among the most promising agents as in phase III clinical trials it appeared to improve survival. PARP inhibitors may also have an important role for patients with BRCA1/BRCA2 mutations. This article will review the various targeted approaches under investigation in ovarian cancer, its current developments, clinical benefits, safety and also searching for predictive biomarkers of response to treatment.

Introduction

Ovarian cancer is the second most common gynecological malignancy and is by far the most lethal gynecological cancer. For the year 2010, it is estimated that 21,880 new cases will be diagnosed and that 13,850 patients will die from ovarian cancer [1]. The overall prognosis of this malignancy is poor, with a five-year overall survival of 45% for all stages [1].

Most ovarian cancers are of epithelial origin and its staging is performed according to the FIGO staging system, which differentiates early stage tumors (stage I-II) from advanced disease (III-IV). The later corresponds to 75% of patients and carries a poor prognosis. Most women present with advanced disease due to the unspecificity of symptoms and the often asymptomatic course in the early stages of this malignancy. The current therapeutic approach consists on the combination of maximal cytoreductive surgery followed by first-line chemotherapy based on platinum compounds (carboplatin or cisplatin) plus paclitaxel. Intraperitoneal drug administration and neoadjuvant chemotherapy are clinically acceptable variations of treatment regimens as they may benefit some patients, but it should be selected on an individualized basis.

Managing recurrent ovarian cancer

There are different criteria for defining relapse in ovarian cancer. These include

continuous rise in CA-125 level, CA-125>100 U/ml, and /or radiographic (usually CT) or symptomatic evidence of recurrence.

Response rate is expected in over 70% of women who receive standard platinum and paclitaxel as first-line treatment [2]. However, the majority of patients treated for advanced disease will relapse, requiring a second and, possibly, more treatments [3, 4]. The most beneficial sequence of treatments has not been established. Recurrent patients can respond to platinum re-treatment and the response rate is directly related to the length of time elapsed since the last course of platinum chemotherapy to the documented recurrence disease [5]. The second-line treatment has been studied on the basis of improved progression-free survival (PFS) or overall survival (OS). Given that recurrent disease is currently incurable, palliation of symptoms and extending survival with minimum drug toxicity are the goals of management. For platinum-sensitive disease (relapse more than six months after completing first-line chemotherapy), a platinum based therapy continues to be the main regimen, often in combination with a drug like paclitaxel [6], gemcitabine [7] or pegylated liposomal doxorubicin (PLD) [8]. More recently, PLD in association with trabectedin has been approved for platinum-sensitive cases [9]. Expected response rates vary from 30% to 40% or even higher, especially for those with a treatment free interval larger than 24 months [10, 11]. On the opposite, platinum-resistant disease (remission-free period less than six months) is typically treated with a single non-platinum agent, such as PLD, gemcitabine, paclitaxel, or topotecan and the reported response rate for each of these drugs is in the 10-20% range [12]. Thus, the current scenery of the treatment of ovarian epithelial cancer (EOC) demands new approaches which might contribute to improve patients' survival. Targeted therapies appear to be promising and several clinical trials are ongoing or have been published over the last few years. Among these, compounds targeting tumor-related angiogenesis have provided the more promising results and as such they constitute the main focus of this review.

Angiogenesis as therapeutic target

The growth of malignant tumors and metastatic capacity are both highly dependent upon angiogenesis. Any increase in tumor burden must be accompanied by an increase in vessel formation to adequately supply the tumor mass. In addition, these new vessels are leaky because of the fragmented basement membranes, creating an easy access into circulation and, thus, facilitating metastization. Interestingly, studies evaluating the vessel count in EOC concluded that the degree of neovascularization is of prognostic significance in both early-stage and advanced disease [13, 14].

Ovarian tumors, like many other malignancies, overexpresses proangiogenic factors, including vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), angiopoietin, platelet-derived growth factors (PDGFs), and pro-angiogenic cytokines such as tumor necrosis factor alpha and interleukins 6 and 8 [15]. Among the markers of increased angiogenesis, VEGF is the most investigated in ovarian cancer. VEGFs constitute a family of growth factors that includes VEGF-A, -B, -C, -D and placental growth factor (PGF). VEGF-A is generally referred to as VEGF. VEGFs bind to a family of receptors (VEGFR-1, -2 and -3) with intrinsic tyrosine kinase activity. VEGFR-2 is believed to be the major mediator of the proangiogenic effects of VEGF-A [16]. VEGF activation promotes endothelial cell proliferation and migration for the formation of new blood vessels and increases permeability of existing blood vessels to allow for the leakage of multiple plasma proteins, including those playing a role in angiogenesis [17]. There is evidence that VEGF can recruit bone-marrow derived endothelial progenitor cell into sites of neovascularization [18]. Evidence based on measurement of serum VEGF levels suggests that VEGF may be a useful serological biomarker for clinical diagnosis, prognosis, follow-up of tumor metastasis and monitoring the efficacy of therapy [19, 20]. There is still some controversy about the concept of VEGF as an independent prognostic factor for EOC. Whereas some agree with this statement [21, 22], others believe that VEGF prognostic significance is related to its correlation with FIGO stage [23].

Based on VEGF pathway inhibition, it is possible to adopt different blocking strategies: inhibition of the VEGF ligand itself (e.g., Bevacizumab), inhibition of

the VEGF receptor (e.g., Tyrosine Kinase Inhibitors - TKIs) or neutralizing VEGF by administrating soluble receptors (e.g., VEGF Trap).

In fact, angiogenic stimulation is not limited to VEGF. Endothelial cells express PDGF receptor (PDGFR) which is activated by its ligand – PDGF - for recruitment of pericytes and maturation of the microvasculature. PDGF secretion by tumor cells may also recruit stromal cells that further support angiogenesis through the release of VEGF [18]. FGF produced by tumor cells also promotes angiogenesis via endothelial cell proliferation, migration and differentiation [24]. PDGF and FGF are implicated in resistance to VEGF/VEGFR agents, explained by the fact that while an anti-VEGF agent is active, other proangiogenic factors that are overexpressed may restore angiogenesis [25]. Therefore, using a multi-target antiangiogenic strategy is a desirable option to counteract this resistance.

Targeting VEGF ligand – Bevacizumab

Bevacizumab (Avastin®) is an intravenously administered humanized monoclonal IgG antibody directed against human VEGF-A, and this drug acts by binding to VEGF-A isoforms preventing them to activate its receptors. It is currently approved for treating various advanced solid tumors: colorectal, breast, renal cell carcinoma, non-squamous non-small cell lung cancers, and glioblastoma.

Phase II and III clinical trials results suggest that bevacizumab is a promising strategy in recurrent or persistent ovarian cancer, either as single agent or in combination with chemotherapy, as detailed below.

Phase II clinical trials

Two important studies, involving patients with relapsed ovarian cancer treated with bevacizumab as single agent (15 mg/kg every 21 days) were the first to demonstrate evidence of activity for a targeted agent in ovarian cancer. The Gynecology Oncology Group (GOG) 170 D study reported by Burger and colleagues [26] enrolled 62 patients with either platinum-sensitive or platinum-resistant disease with no more than 2 prior chemotherapy regimens. Cannistra et al [27] recruited 44 patients only platinum-resistant who failed the topotecan

or liposomal doxorubicin therapy and could have received up to three prior chemotherapy regimens. Burger et al. [26] documented an objective response rate of 21% and 52% stable disease. At 6 months, 40% of patients were progression-free, median PFS was 4.7 months and OS 16.9 months. Cannistra et al. [27] demonstrated a response rate of 16% and 61.4% with stable disease as their best response. The median PFS was 4.4 months and the median OS 10.7 months. These results were encouraging since they were achieved by bevacizumab alone, contrasting with the lack of effectiveness in breast, colon or lung cancer.

Regarding adverse events, Burger et al. reported two cases of deep vein thrombosis (3.2%), one case of grade 4 proteinuria (1.6%) and six cases of grade 3 hypertension (9.7%) [26]. The toxicity profile was different in Cannistra's study. They had five patients (11.4%) with gastrointestinal (GI) perforation, one of which was fatal, and other three patients developed arterial thromboembolic disease [27]. Three deaths (myocardial infarction, GI perforation and hypertensive encephalopathy) were suspected to be related with bevacizumab. Those who experienced GI perforation had receive three prior chemotherapy regimens compared with the rest of the group who had receive two prior chemotherapy regimens and none experienced perforation. Thus, higher GI perforation incidence in this study might be related with the fact that its population had more advanced disease [27].

Other trials suggested that bevacizumab in combination with chemotherapy (carboplatin, paclitaxel, cyclophosphamide or topotecan) is effective in advanced ovarian cancer. Two studies with chemotherapy-naïve patients with advanced ovarian carcinoma, concluded that carboplatin plus paclitaxel and bevacizumab (PCB) as first line therapy is safe and effective, with similar results [28, 29]. Micha et al. submitted 20 patients to six cycles of PCB and found 80% response rate; 23.3% and 25% of cycles were associated with grade 3 and 4 neutropenia, respectively. Two additional patients developed deep vein thrombosis that required hospitalization, although neither case appeared to be related to bevacizumab. No GI perforation, severe thrombocytopenia or anemia were reported [28]. Penson et al. recruited 62 patients. The study differs from the previous because patients maintained bevacizumab for a year after six to

eight cycles of PCB. Their results were the following: 76% of best overall response, median PFS duration of 29.8 months and 58% of patients were free from progression at 36 months. Treatment was associated with some important toxicities: two cases of pulmonary embolism, two GI perforation (grade 1 and 4), four cases of neuropathy and 14 patients developed neutropenia, all occurring during the chemotherapy phase of treatment. No grade 4 toxicities were seen during maintenance therapy with bevacizumab [29].

Metronomic chemotherapy (MC), which is intended to prevent tumor angiogenesis, is based on more frequent and low-dose drug administrations compared with conventional chemotherapy. This model of treatment suppresses tumor growth in experimental models [30, 31] and was encouraged by studies in metastatic breast cancer where MC was associated with clinical improvement and drop in VEGF levels with minimal toxicity [32]. To assess the efficacy of MC in ovarian cancer, two phase II trial were developed. Combination of bevacizumab and metronomic chemotherapy with cyclophosphamide was applied by Chura et al. [33], which conducted a trial of 15 heavily pre-treated patients with bevacizumab in a lower dose than the previous trials (10 mg/kg) plus cyclophosphamide. A response rate of 53.3% and a median duration of progression free survival of 3.9 months were found. Despite being heavily pre-treated and having confirmed intra-abdominal cancer, no GI perforations were reported. In another study, Garcia et al. [34] enrolled 70 patients with platinum-sensitive and platinum-resistant disease and administered the same treatment regimen. They reported a 24% response rate, 63% stable disease and 56% of patients were free from disease at 6 months. The median time to progression and median survival time were 7.2 months and 16.9 months, respectively. However, GI perforation and thromboembolism occurred (4% of cases each), along with three treatment-related deaths. The levels of angiogenesis markers (VEGF, E-selectin, and thrombospondin-1) of less than half of participants were measured and were not associated with clinical outcome. Table 1 summarizes all phase II trials mentioned above.

Phase III clinical trials

The first two phase III studies designed to study addition of bevacizumab to standard chemotherapy (carboplatin/paclitaxel) in front-line treatment, GOG-218 and ICON-7, have already reported their preliminary results. Both have PFS as the primary endpoint. GOG-218 [35] is a three-arm, placebo-controlled, randomized trial involving 1,873 stage III or IV patients. The 3 treatment arms are: placebo plus chemotherapy followed by placebo maintenance (Arm 1), bevacizumab plus chemotherapy followed by placebo maintenance (Arm 2), and bevacizumab plus chemotherapy followed by bevacizumab maintenance (Arm 3). Dosage of bevacizumab used was 15 mg/kg. PFS was defined according to either Response Evaluation Criteria in Solid Tumors (RECIST), or CA-125 levels defining progressive disease according to Gynecologic Cancer Intergroup (GCIIG) criteria. Using both criteria simultaneously, a significant improvement in PFS for maintenance bevacizumab group compared with control group was detected (PFS 14.1 months vs. 10.3 months for Arm III vs. Arm I, $p < 0.0001$), but no significant benefit of bevacizumab plus chemotherapy without maintenance ($p = 0.08$). The PFS difference censoring CA-125 was 18 vs. 12 months for Arm III vs. Arm I ($p < 0.0001$), allowing for the same conclusion about the benefit of bevacizumab maintenance. The toxicity profile was favorable, with 23% of grade 2 or greater hypertension. Across the 3 arms, GI perforation/fistula events were observed in less than 3% of patients. The low rate of GI perforation may be explained by the fact that patients with a history of small bowel obstruction were excluded. On the other hand, the ICON-7 [36] study is an open-label randomized trial which recruited 1,528 patients with high-risk stage I-II or stage IIb to IV disease. It allocated patients into two different arms: standard carboplatin/paclitaxel (Arm 1) and carboplatin/paclitaxel plus low dose bevacizumab (7.5 mg/kg) followed by bevacizumab (Arm 2). Their PFS was measured according to RECIST criteria and the results differed significantly (18.3 months vs. 16 months for Arm 2 vs. Arm 1, $p = 0.001$). The toxicity profile was similar to that of GOG-218 with 1.3% GI perforation (compared with 0.4% in the control arm) and 18.3% grade 3 or greater hypertension. Table 2 compares the results of GOG-218 and ICON-7.

When comparing patients' profiles, several characteristics distinguish these two phase III trials (see figure 1). GOG-218 enrolled poorer-prognosis patients (only advanced disease was accepted) and only 34% had optimal debulking stage III disease (compared with 73% in ICON7). The dosage of bevacizumab in ICON-7 was half of that of GOG-218 and the duration of exposure was shorter. Both trials suggest an advantage of bevacizumab maintenance in ovarian cancer, but the absolute benefit in terms of PFS is modest, and neither trial showed an overall survival benefit. Thus, more phase III trials using bevacizumab are required and those that are ongoing are depicted in Tables 3 and 4, concerning first-line or relapsed ovarian cancer treatment, respectively.

Toxicity

The adverse events associated with bevacizumab in ovarian cancer trials are similar to those described in other solid tumors, mainly comprising hypertension, proteinuria, thrombosis and GI perforation [37]. Gastrointestinal perforation is a potentially life-threatening complication of major concern. It is estimated to occur in up to 2.4% of patients treated with bevacizumab in colorectal cancer [38]. Cannistra's study was stopped prematurely due to the higher than expected incidence of GI perforation [27]. The recent GOG-218 and ICON-7 trials provided relevant information in this regard because the rate of potentially fatal GI perforation was lower than suggested by earlier phase II trials. The confirmation of safety of bevacizumab is obviously critical for any further investigation on this drug. Retrospective reviews of small studies prior to GOG-218 and ICON-7 trials results indicated that incidence of GI perforation may be slightly higher in pretreated patients and when a history of bowel obstruction exists [39, 40].

VEGF Trap

VEGF trap (Aflibercept) is a fusion protein containing the VEGF binding domains of both VEGFR-1 and 2 linked through the Fc region of a human IgG1. Aflibercept binds VEGF-A and neutralizes all VEGF-A isoforms plus PGF. The clinical applicability of this drug in age-related macular degeneration is already in phase III trials. Experiments in ovarian models suggested that VEGF Trap

may reduce malignant ascites and tumor burden [41, 42]. In Tew et al. study [43], 162 platinum-resistant patients who failed the subsequent topotecan or liposomal doxorubicin therapy were treated at dosage levels of 2 mg/kg or 4 mg/kg every 2 weeks. Five partial responses in a sample size of 45 patients (11%) were obtained. Grade 3–4 adverse events included: hypertension (9%), proteinuria (4%), encephalopathy (2%) and renal failure (2%). Two cases of GI perforation and one of pulmonary embolism were considered drug-related. In another study, Columbo et al. [44] reported the results of VEGF Trap in patients with advanced ovarian cancer and symptomatic ascites requiring frequent paracentesis. Aflibercept 4mg/kg was administered every 2 weeks. Primary endpoint was repeat paracentesis response rate (RPRR), defined as at least a doubling of time to the first paracentesis compared to a baseline average. Eight out of ten evaluable patients achieved a RPRR response as per protocol. Grade 3-4 adverse events included bowel obstruction (40%) nausea, vomiting (30%), anorexia, edema, general health deterioration (20%) and 1 case of bowel perforation. Thus, further data on survival rates is needed to back up VEGF Trap use. Finally, a phase I/II trial assessing the efficacy of VEGF Trap associated with docetaxel in recurrent ovarian cancer, as well as other phase II/III trials investigating VEGF Trap for recurrent malignant ascites are ongoing.

VEGFR TKIs

In contrast to bevacizumab anti-VEGF action, small molecule TKIs bind to the intracellular component of VEGFRs and therefore block downstream signal transduction pathways activated by VEGFs and other ligands of the VEGF family. These small molecule inhibitors are also active against other tyrosine kinases, thus playing a multi-target role that may contribute to its antitumor effects.

Several VEGFR TKIs are under investigation in patients with recurrent ovarian cancer, either as monotherapy or in combination therapy. Given the convenience of oral administration, it is likely that these drugs will have an important role as maintenance therapy in advanced ovarian cancer.

Cediranib (AZD2171) is a highly potent inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFR and c-KIT. Hirte and co-workers [45] recruited 60 patients

with recurrent disease who had received no more than one prior chemotherapy regimen. Evaluation of platinum-sensitive and resistant patients was done separately. The median time to progression (TTP) and median survival time for all patients were 4.1 months and 11.9 months, respectively. Matulonis et al. [46] used Cediranib in the same context for 46 patients but up to two prior chemotherapy regimens were permitted. Partial responses were observed in 17% of patients, and 13% exhibited stable disease. PFS was 5.2 months. Both trials had to reduce the initial 45 mg daily dose to 30 mg, due to toxicity. ICON 6, a double blind placebo controlled phase III trial in recurrent platinum-sensitive patients, is currently testing this agent in association with standard initial chemotherapy. Patients are randomized to one of three groups: carboplatin/paclitaxel, carboplatin/paclitaxel/cediranib or carboplatin/paclitaxel/cediranib, with cediranib maintenance.

Sorafenib is a tyrosine kinase inhibitor targeting BRAF and other receptor kinases (VEGFR, PDGFR, FLT3, c-KIT), approved for the treatment of advanced inoperable hepatocellular carcinoma and advanced renal cell cancer. Either acting as single agent or associated with gemcitabine, available data demonstrated significant disease stabilization, but at a cost of increased toxicity, including grade 3 and 4 hand-foot syndrome [47, 48]. The combination therapy tested in a phase II trial with two anti-VEGF agents, sorafenib and bevacizumab, was not well tolerated requiring dose reductions in the majority of patients with advanced solid tumors. Despite the toxicity, partial responses were seen in six (43%) of 13 patients with ovarian cancer [49]. Biomarker analyses in Matei's trial included measurement of extracellular signal-regulated kinase (ERK) and BRAF expression in tumors and phosphorylation of ERK (pERK) in peripheral-blood lymphocytes (PBLs) before and after 1 month of treatment. ERK and BRAF were expressed in all tumors and exploratory analyses indicated that pERK in post-treatment PBL specimens was associated with PFS [48]. Front-line treatment with sorafenib in association with carboplatin/paclitaxel in advanced ovarian cancer is being tested in a phase II study.

Single-agent use of sunitinib demonstrated modest activity in recurrent platinum-sensitive ovarian cancer, but only at the 50 mg intermittent dose

schedule, suggesting that dose and schedule may be vital considerations in further evaluation of this agent [50].

Pazopanib inhibits VEGFR, PDGFR, and c-KIT. As single therapy in recurrent ovarian cancer, with CA-125 decrease as parameter of response, it showed a biochemical response in eleven of 36 patients (31%) and 18% of overall response in patients with measurable disease [51].

BIBF-1120 is a triple angiokinase inhibitor, targeting VEGFR, PDGFR and FGFR tyrosine kinases. A sustained inhibition characterizes this novel agent [52]. Results from a randomized, placebo-controlled study using maintenance therapy with BIBF 1120 following chemotherapy in relapsed ovarian cancer, reported a 9 month PFS rate of 15.6% for BIBF 1120 and 2.9% for placebo. This suggests that maintenance therapy with BIBF 1120 could delay disease progression in patients who previously responded to chemotherapy [53].

Clinical trials with imatinib, a PDGFR and c-KIT inhibitor had disappointing results, with almost negligible activity as a single agent [54, 55].

Polyadenosine Diphosphate-Ribose Polymerase (PARP) Inhibitors

The defects in DNA repair pathway in a specific group of ovarian cancer patients (those with BRCA1 or BRCA2 mutations), underlie the successful results obtained in the clinical trials involving PARP Inhibitors. Mutations in either of those genes confer a lifetime risk of breast cancer between 60 and 85%, and a lifetime risk for ovarian cancer between 15 and 40% [56]. BRCA1 and BRCA 2 proteins are critical for genome integrity maintenance since they participate in DNA double-strand break (DSBs) repair via homologous recombination (HR), an error free pathway. Poly (ADP-ribose) polymerase (PARP) is a key nuclear enzyme involved in the repair of DNA single-strand breaks (SSBs) using the base excision repair pathway. PARP inhibitors generate DNA SSBs, which may lead to DSBs [57-59]. Non-cancerous cells from patients inheriting BRCA 1/ 2 mutations retain a single wild-type copy of the BRCA 1/2 gene, allowing for sufficient HR mediated DNA repair to take place in the presence of PARP inhibition. In contrast, the BRCA1/2-mutated cancer cells, with both alleles inactivated, have to repair DSBs by preferential use of an error-prone mechanism, leading to excessive accumulation of DNA

damage. This difference between tumor and normal cells explains why PARP inhibitors can be lethal for tumor cells while normal cells are spared [59-61]. The concept of synthetic lethality is applied here. It is defined as the situation where two defective pathways acting individually have no effect, but in combination lead to cell death [61]. Preliminary observations from two phase II trials of Olaparib (AZD2281), aimed at advanced hereditary ovarian cancer, are encouraging. Audeh et al. [62] studied a group of chemo-refractory ovarian cancer patients and reported an objective response rate and clinical benefit rate (objective response rate and/or confirmed $\geq 50\%$ decline in CA125) of 33% and 57%, respectively, at 400 mg twice daily dose. Lower activity was observed with 100 mg twice daily. Toxicities were generally mild. Grade 3 toxicity occurred infrequently, and comprised primarily nausea (7%) and leukopenia (5%). Fong et al. [63] reported 40% response rate (complete or partial responses and/or CA125 responses). There was a significant association between the clinical benefit rate and platinum-sensitivity.

PARP inhibitors were initially thought to benefit only the small group of women carrying germline mutations of BRCA1 or BRCA2. However some tumors display the so called "BRCAness", characterized by a phenotypic similarity of sporadic cancers with those carrying BRCA mutations, in the absence of germline mutation [64]. This phenomenon may occur due to aberrant promoter methylation [65]. Baldwin et al. analyzed a group of sporadic ovarian cancers and found BRCA1 promoter methylation in 15% of cases [66]. "BRCAness" may thus allow for the use of PARP Inhibitors in some sporadic ovarian cancer, increasing the number of women diagnosed with ovarian cancer which might benefit from this drug.

HER Inhibitors

The human epidermal growth factor receptor (HER, EGFR or ErbB) family consists of four members of tyrosine kinase receptors: HER-1 (commonly termed EGFR), HER-2/Neu, HER-3, and HER-4. A number of ligands, the EGF-related peptide growth factors, bind specifically to the extracellular domain of HER leading to dimerization of the receptor with any of the four members of the HER family molecules, forming homo- and heterodimers. Subsequently,

intracellular proteins involved in signaling pathways are activated, resulting in modulation of gene transcription. HER-2 and HER-3 are functionally incomplete receptors - HER-2 lacks ligand-binding activity whereas HER-3 has a defective kinase activity - but they are able to form heterodimeric complexes with other HER receptors generating potent cellular signals. Abnormal activation of the HER pathway is responsible for malignant transformation and tumor growth through the inhibition of apoptosis, cellular proliferation, promotion of angiogenesis, and metastasis. This abnormal activation may be initiated by one or several mechanisms, including increased production of growth factors, overexpression of receptors in cells, as well as mutation of genes encoding for these receptors or certain downstream enzymes [67-69]. There are two main strategies for targeting the HER family: monoclonal antibodies (e.g., trastuzumab, cetuximab) or tyrosine kinase inhibitors-TKIs (e.g., gefitinib, erlotinib).

Data concerning EGFR and HER-2 overexpression and their association with prognosis in ovarian cancer is controversial. EGFR overexpression rate varies from 9–62% [70], depending on the antibody, the assay and the cutoff standard. Expression of HER-2 has traditionally been evaluated by immunohistochemistry with inconsistent prognostic results for EOC [71-73]. Owing to these disparate results, fluorescence *in situ* hybridization (FISH) analysis of HER-2 amplification has been applied to a series of EOCs in an attempt to lessen the difficulty in quantifying immunohistochemical staining. Recently, GOG analyzed primary tumors from 133 women with suboptimally-resected and advanced stage EOC using FISH, and concluded that HER-2 amplification is rare and has no predictive or prognostic value [74].

Thus, it not surprising that already concluded phase II trials evaluating anti-HER therapies have provided disappointing results. Reports on cetuximab (Erbix®) in unselected HER status ovarian cancer, acting as single-agent or in combination with carboplatin, with or without paclitaxel, did not meet satisfactory outcomes [75-77]. We know already from the colorectal cancer that *KRAS* mutations may predict unresponsiveness to cetuximab. Hence, it would be interesting in future studies to search for those mutation and compare the response to cetuximab in groups of tumors with wild-type or mutant *KRAS*.

Schielder et al. analyzed 12 different serum markers before and during cetuximab monotherapy and found no correlation between PFS and marker changes, but high levels of baseline markers were associated with earlier disease progression [75]. Unsatisfactory results were also seen with pertuzumab as single agent in heavily pretreated patients [78]. Other study in which pertuzumab was administered with gemcitabine in a placebo controlled randomized phase II study. The objective response rate was 13.8% in gemcitabine + pertuzumab regimen, compared to 4.6% in patients receiving gemcitabine + placebo. Moreover, low HER-3 mRNA expression may predict pertuzumab clinical benefit [79]. Trastuzumab (Herceptin®), an anti-HER-2 antibody widely used in breast cancer, was tested as a single agent in GOG 160 phase II study, obtaining an overall response rate of 7.3% in the 41 eligible patients with HER-2 overexpression (1 CR and 2 PR). Furthermore, there was no relationship between the serum level of the extracellular domain of HER2 and patient's outcome. This study showed a relatively low frequency of HER-2 amplification in unselected ovarian cancers (11,4%), assessed by immunohistochemistry [80].

Because TKIs have the convenience of oral administration, Gefitinib (ZD1839, Iressa) was given in monotherapy to unscreened patients but none reached complete response and only 37% had stable disease. A decrease in both EGFR and p-EGFR was observed following gefitinib therapy in >50% of patients, providing a proof of effective targeting in a clinical setting, despite the lack of clinical benefit [81]. The GOG 170C phase II trial also tested gefitinib alone and reported that one of 27 (4%) patients had partial response and four of 27 (15%) patients were free from progression at 6 months. Because mutations in the tyrosine kinase domain region of EGFR have been associated with sensitivity to gefitinib in non-small cell lung cancer (NSCLC), exons 18 to 21 of EGRF were sequenced. Interestingly, the only tumor harboring a mutation in catalytic domain corresponded to the patient which experienced an objective response [82]. The combination of gefitinib with tamoxifen was found to be ineffective in advanced ovarian cancer since there were no tumor responses [83]. Another small molecule inhibitor, erlotinib (Tarceva®) demonstrated limited activity for recurrent ovarian cancer, with 6% partial response and 44% stable disease [84].

Erlotinib has also been tested in combination with bevacizumab. The objective response rates were 15% (2/13 patients) and 54% (7/13 patients) had stable disease. Due to lack of improvement over bevacizumab therapy alone and two incidents of fatal gastric perforation, the erlotinib plus bevacizumab study was stopped [85].

Estrogen Receptor

Since ovarian cancers commonly express estrogen receptors (ER), trials have been carried out to whether they might constitute therapeutic targets. The hormonal agent tamoxifen is routinely used to treat breast cancer in women whose tumors express ER. The recent Cochrane review of tamoxifen in ovarian cancer was unable to provide evidence-based recommendations as comparative studies assessing the effectiveness of tamoxifen are not available. The small phase II trials that were reported thus far showed an overall objective response rate of 9.6% (range: 0-52%), whereas 31.9% (range: 0-83%) of patients achieved disease stabilization [86]. Thus, questions such tamoxifen improving survival and symptom control or whether hormone receptor status is useful in selecting patients for tamoxifen treatment remain unanswered. Concerning the aromatase inhibitor letrozole (Femara®), phase II trials results indicated disease stabilization rather than improvement [87-90]. In a study comprising 42 patients with ER+ relapsed ovarian cancer, Smyth et al. reported 9% partial remission and 42% stable disease, according to radiological response criteria. Using CA-125 levels as a response marker, they observed 17% of responders (decrease > 50%) and 26% of patients had stable disease (no doubling of CA-125) at 6 months. In addition, the CA-125 response was more likely in cancers with the highest level of ER expression [87].

Other potencial targets

Oregovomab, a monoclonal antibody against CA-125, was tested in a phase III trial. This monoimmunotherapy after front-line therapy did not demonstrate clinical benefit compared to the placebo group [91]. The folate receptor α -FR is overexpressed in 90% of ovarian cancers [92]. Farletuzumab (MORAb-003) is in a phase III trial comparing the efficacy and safety of intravenous carboplatin

and taxanes with and without farletuzumab in individuals with first platinum-sensitive relapse. Angiopoietin is involved in an angiogenic pathway parallel to VEGF pathway. AMG 386 is a peptide-Fc fusion protein that inhibits angiogenesis by neutralizing the interaction between the Tie2 receptor and angiopoietin 1 and 2. A recent phase II showed increased PFS with the AMG 386 and paclitaxel association compared to paclitaxel alone [93].

Conclusions

Contrarily to other common malignancies (e.g., breast, colon, and lung cancers), targeted therapy in epithelial ovarian cancer is still at its inception. Most phase II trials testing bevacizumab indicated that VEGF targeting might be an efficient strategy for ovarian cancer treatment. The results from the latest phase III trials GOG-218 and ICON-7, however, showed only a modest benefit in progression-free survival. Importantly, the potentially fatal GI perforation events observed in previous studies were lower in phase III trials (<3%), although we should be aware that the eventual risk factor for perforation (bowel obstruction) was an exclusion criteria in GOG-218 trial. This study revealed that treatment of ovarian cancer with carboplatin, paclitaxel and bevacizumab followed by bevacizumab maintenance may be an effective first-line treatment option. However, the optimal dosage of bevacizumab and its duration is still unclear. More consistent judgments about bevacizumab use can be made when OS results are matured and other phase III trials report their preliminary results. Moreover, the RECIST criteria traditionally used to evaluate objective response rate might not be the best for assessing antiangiogenic agents, as these agents appear to slow tumor growth and do not cause tumor shrinkage. The search for adequate response biomarkers is also a critical issue as current studies only rely on the unspecific CA-125 measurement. Notwithstanding, even before assessing therapeutic response, physicians need biomarkers that will identify which patients will or will not benefit from the addition of the novel agent. This requires a better understanding of ovarian cancer tumorigenesis and a broad search for key genetic and epigenetic alterations. In this regard, BRCA1/BRCA2 mutations or the “BRCAness” status might constitute an example of this effort if further

studies are able to prove the efficacy of PARP inhibitors in defined subgroups of patients.

Given the multiplicity and redundancy of aberrant pathways involved in ovarian cancer, it is unlikely that inhibition of a single cascade will be highly effective, thus contributing to resistance to VEGF-targeted therapy. Combining multiple antiangiogenic agents may be, then, a solution. An additional question needs to be answered, i.e., whether it is most effective to inhibit different signaling pathways (horizontal blockade) or different molecules within the same pathway (vertical blockade).

Finally, the cost-effectiveness of adding targeted therapy to ovarian cancer treatment has to be thoroughly analyzed but only when a significant impact on survival has been proven and when more definite answers to the questions raised above are provided by additional research in this field.

Table 1. Reported phase II trials of bevacizumab in ovarian cancer

Study	Patients characteristics	Intervention	Results	Common grade ≥ 3 adverse effects
Burger et al. (2007) [26]	62 patients with persistent or recurrent of EOC or PPC 1-2 CT regimens 42% were platinum resistant	Single agent bevacizumab (15 mg/kg q21)	CR: 3% PR: 18% SD: 52% PFS: 4.7 mos 6mPFS: 40% OS: 16.9 mos	Hypertension: 9.7% GI events: 6.5% TED: 3.2% Proteinuria: 1.6%
Cannistra et al. (2007) [27]^a	44 patients with recurrent platinum-resistant EOC or PPC after discontinuing topotecan or liposomal doxorubicin. 2-3 CT regimens	Single agent bevacizumab (15 mg/kg q21)	CR: 0% PR: 16% SD: 61.4% PFS: 4.4 mos 6mPFS: 27.8% OS: 10.7 mos	GIP: 11.4% Small intestinal obstruction: 9.1% Hypertension: 9.1% TED: 6.8%
Micha et al. (2007) [28]	20 patients with Stage III or IV EOC, PPC and FTC 85% optimally cytoreduced	First line carboplatin + paclitaxel + bevacizumab (15 mg/kg q21)	CR: 30% PR: 50% SD: 5%	Neutropenia: 18 pts TED: 2 pts ^b Hypertension: 2 pts Neuropathy: 1pt
Penson et al. (2010) [29]	62 patients with advanced EOC, PPC, FTC and UC 82% optimally cytoreduced	First line carboplatin + paclitaxel + bevacizumab (15 mg/kg q21) with maintenance of bevacizumab for a year	CR: 21% PR: 55% SD: 21% PFS: 29.8 mos 36mPFS: 58% OS: NR	<u>CT phase</u> Neutropenia: 14 pts Metabolic: 8 pts Hypertension: 6 pts Thrombocytopenia: 4pts Neuropathy: 4pts TED: 2 pts GIP: 2 pts (grade 1,4) <u>Maintenance phase</u> Hypertension: 5 pts
Chura et al. (2007) [33]	15 patients with recurrent ovarian cancer Median number of previous chemotherapy regimens = 8	Bevacizumab (10 mg/kg q14) + cyclophosphamide	CR: 13.3% PR: 40% SD: 20% PFS: 3.9	Pancreatitis: 1 pt Diarrhea: 1 pt
Garcia et al. (2008) [34]	70 patients with recurrent EOC and PPC 1-3 prior CT regimens 40% were platinum-resistant	Bevacizumab (10 mg/kg q14) + cyclophosphamide	CR: 0% PR: 24% SD: 63% OS: 16.9 mos TTP: 7.2 mos 6mPFS: 56%	Lymphopenia: 14 pts Hypertension: 11 pts GI obstruction: 6 pts Proteinuria: 3 pts GIP: 3 pts (grade 2,4,5) TED: 3 pts

^a Study stopped prematurely due to high rate of GIP

^bTED cases were not directly attributed to bevacizumab

Abbreviations: CR, complete response; CT, chemotherapy; EOC, epithelial ovarian cancer; FTC, fallopian tube cancer; GIP, gastrointestinal perforation; mos, months; NR, not reported; PFS, progression-free survival (6m, 6 months; 36m, 36 months); PPC, primary peritoneal cancer; PR, partial response; pt(s), patient(s); q14, every 14 days; q21, every 21 days; SD, stable disease; TED, thromboembolic disease (either arterial or venous); TTP, time to progression; UC, uterine carcinoma.

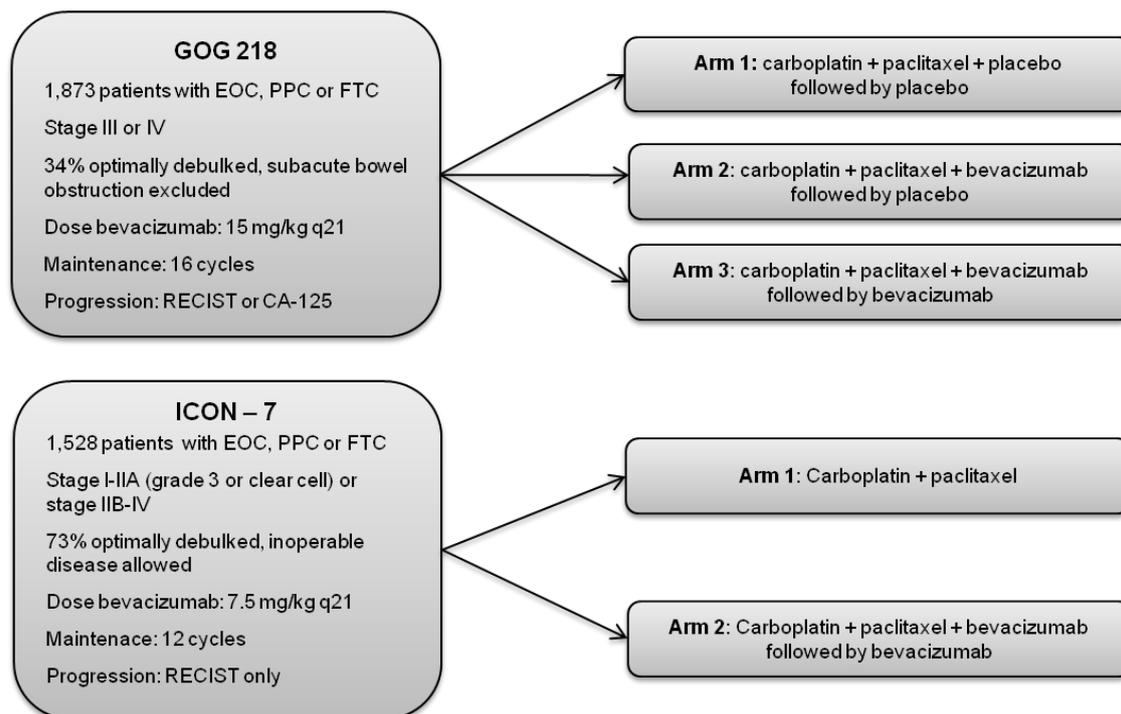


Figure 1. Differences between two phase III trials of first line treatment with chemotherapy alone or in combination with bevacizumab: GOG 128 and ICON – 7 (see table 2 for results). Abbreviations: EOC, epithelial ovarian cancer; FTC, fallopian tube cancer; PPC, primary peritoneal cancer; q21, every 21 days; RECIST, Response Evaluation Criteria in Solid Tumors.

Table 2. Results of two phase III trials of first line treatment with chemotherapy alone or in combination with bevacizumab: GOG 218 and ICON-7

Characteristics	GOG 218			ICON-7	
	Arm 1 CP	Arm 2 CP+Bev	Arm 3 CP+Bev →Bev maintenance	Arm 1 CP maintenance	Arm 2 CP+Bev →Bev maintenance
Median PFS					
RECIST or CA-125	10.3 mos	11.2 mos	14.1 mos	NA	NA
RECIST only	12.0 mos	NR	18.0 mos	16 mos	18.3 mos
Select adverse events					
Hypertension	7.2% ^a	16.5% ^a	22.9% ^a	2.1% ^b	18.3% ^b
GIP/fistula	1.2%	2.8%	2.6%	1.3%	2.1%
Venous thromboembolism	5.8%	5.3%	6.7%	1.7%	4.3%
Arterial thromboembolism	0.8%	0.7%	0.7%	1.3%	2.7%
Proteinuria	0.7%	0.7%	1.6%	0.1%	0.5%

^a grade ≥ 2

^b grade ≥ 3

Abbreviations: Bev, Bevacizumab; CP, carboplatin plus paclitaxel; GIP, gastrointestinal perforation; NA, not applicable; NR, not reported; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors.

Table 3. Ongoing first-line phase III trials with bevacizumab in ovarian cancer

Trial	Type	No. patients	Patients characteristics	Design	End point
GOG-262	Open label, randomized	625	Stage III or IV, suboptimal debulking	Carboplatin + paclitaxel q21 ± (optional) Bev→Bev maintenance vs carboplatin q21 + paclitaxel q7 ± (optional) Bev→Bev maintenance	PFS
GOG-252	Open label, randomized	1500	Stage II-IV, optimal or suboptimal debulking	Paclitaxel IV + carboplatin IV + Bev→Bev maintenance vs paclitaxel IV + carboplatin IP + Bev→Bev maintenance vs paclitaxel IV and IP + cisplatin IP + Bev→Bev maintenance	PFS

Abbreviations: Bev, bevacizumab; IP, intraperitoneal; IV, intravenous; PFS, progression-free survival; q7, every week; q21, every 21 days; vs, versus.

Table 4. Ongoing phase III trials with bevacizumab in relapsed ovarian cancer

Trial	Type	No. patients	Patients characteristics	Design	End point
GOG 213	Open label, randomized	660	CR to first-line platinum-taxane therapy and DFI ≥ 6 months; previous Bev allowed	Carboplatin + paclitaxel ± Bev→Bev maintenance in surgical and non-surgical candidates	OS
OCEANS	Placebo-controlled, double-blind randomized	487	Platinum-sensitive	Carboplatin + gemcitabine ± Bev→Bev maintenance	PFS
AURELIA	Open label, randomized	300	Platinum-resistant	Liposomal doxorubicin or paclitaxel or Topotecan ± Bev→Bev maintenance	PFS

Abbreviations: Bev, bevacizumab; CR, complete response; DFI, disease-free interval; OS, overall survival; PFS, progression-free survival; vs, versus.

Table 5. Published phase II trials of non-bevacizumab VEGF pathway inhibitors

Study	No. Patients	Target	Treatment	Response Rate (RECIST)	SD	Outcomes (months)	OS (months)
Tew et al. (2007) [43]	162	VEGF-Trap	Aflibercept	11%	NR	NR	NR
Hirte et al. (2008) [45]	60	VEGFR1,-2,-3, PDGFR	Cediranib	PI-s: 2 pts confir. PI-r: 1pt unconfir.	NR	TTP: 4.1	11.9
Matulonis et al. (2009) [46]	46	VEGFR1,-2,-3, PDGFR	Cediranib	PI-s: 2 pts PI-r: 6 pts	6 pts	PFS 5.2	Not reached
Matei et al. (2010) [48]	71	VEGFR,PDGFR, FLT3, c-KIT, Raf	Sorafenib	3.4%	34%	6mPFS: 24%	NR
Welch et al. (2010) [47]	43	VEGFR,PDGFR, FLT3, c-KIT	Sorafenib + gemcitabine	4.7% 27.9% (CA-125)	23.3	TTP: 5.4	13.3
Biagi et al. (2011) [50]	30	VEGFR, PDGFR, c-KIT	Sunitinib	3.3% 10% (CA-125)	53%	PFS: 4.1	NR
Friedlander et al. (2010) [51]	36	VEGFR, PDGFR, c-KIT	Pazopanib	31% (CA-125)	NR	MDR: 113 days	NR
Ledermann et al. (2009) [53]	84	VEGFR, PDGFR, FGFR	BIBF-1120	NR	NR	9mPFS: 15.6 vs 2.9*	NR

*placebo

Abbreviations: conf., confirmed; unconf, unconfirmed; FGFR, fibroblast growth factor receptor; MDR, median duration of response; NR, not reported; MDR, median duration of response; OS, overall survival; PDGFR, platelet-derived growth factor receptors; PFS, progression-free survival (6m, 6 months; 9m, 9 months); PI-r, platinum resistant; PI-s, platinum-sensitive; pt(s), patient(s); pt(s), patient(s); RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; TTP, time to progression; VEGF, vascular endothelial growth factor ; VEGFR, vascular endothelial growth factor receptor; vs, versus.

Table 6. Published phase II trials of human epidermal growth factor receptor (HER) inhibitors

Trial	No. patients	Target	Treatment	Response	SD	PFS
Schielder et al. (2009) [75]	25	EGFR	Cetuximab	1 pt	9 pts	2.1 mos
Gordon et al. (2006) [78]	61	HER-2 dimerization	Pertuzumab	4.3%	6.8%	6.6 wks
Makhija et al. (2010) [79]	130	HER-2 dimerization	Pertuzumab+ gemcitabine	13.8% vs 4.6%*	NR	NR
Bookman et al. (2003) – GOG 160 [80]	41	HER-2	Trastuzumab	7.3%	NR	NR
Posadas et al. (2007) [81]	24	EGFR	Gefitinib	0%	37%	NR
Schielder et al. (2005) – GOG 170 C [82]	27	EGFR	Gefitinib	1 pt	NR	6mPFS: 15%
Wagner et al. (2007) [83]	56	EGFR, ER	Gefitinib + tamoxifeno	0%	29%	NR
Gordon et al. (2005) [84]	34	EGFR	Erlotinib	6%	4%	NR
Nimeri et al. (2008) [85]	13	EGFR, VEGF	Erlotinib + bevacizumab	2 pt	7 pts	NR

*gemcitabine and placebo

Abbreviations: HER, human epidermal growth factor receptor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; NR, non reported; PFS, progression-free survival (6m, 6 months); pt(s), patient(s); SD, stable disease; vs, versus.

REFERENCES

- 1 Jemal A, Siegel R, Xu J et al. Cancer Statistics, 2010. *CA Cancer J Clin* 2010;60:277-300.
- 2 McGuire WP, Hoskins WJ, Brady MF et al. Cyclophosphamide and cisplatin compared with paclitaxel and cisplatin in patients with stage III and stage IV ovarian cancer. *N Engl J Med* 1996;334:1-6.
- 3 Armstrong DK. Relapsed ovarian cancer: challenges and management strategies for a chronic disease. *The Oncologist* 2002;7(suppl 5):20-28.
- 4 Markman M, Bookman MA. Second-line treatment of ovarian cancer. *The Oncologist* 2000;5:26-35.
- 5 Spriggs D. Optimal sequencing in the treatment of recurrent ovarian cancer. *Gynecol Oncol* 2003;90(suppl 1):39-44.
- 6 International Collaboration for Ovarian Neoplasia Group. Paclitaxel plus platinum based chemotherapy versus conventional platinum-based chemotherapy in women with relapsed ovarian cancer: the ICON4/AGO-OVAR-2.2 trial. *Lancet* 2003;361:2099–2106.
- 7 Pfisterer J, Plante M, Vergote I et al. Gemcitabine plus carboplatin compared with carboplatin in patients with platinum-sensitive recurrent ovarian cancer: an intergroup trial of the AGO-OVAR, the NCIC CTG, and the EORTC GCG. *J Clin Oncol* 2006;24:4699–4707.
- 8 Pujade-Lauraine E, Wagner U, Aavall-Lundqvist E et al. Pegylated liposomal doxorubicin and carboplatin compared with paclitaxel and carboplatin for patients with platinum-sensitive ovarian cancer in late relapse. *J Clin Oncol* 2010;28:3323–3329.
- 9 Monk BJ, Herzog TJ, Kaye SB et al. Trabectedin plus pegylated liposomal doxorubicin in recurrent ovarian cancer. *J Clin Oncol* 2010;28:3107-3114.
- 10 Markman M, Rothman R, Hakes T et al: Second-line platinum therapy in patients with ovarian cancer previously treated with cisplatin. *J Clin Oncol* 1991;9:389-393.
- 11 Cannistra SA. Is there a "best" choice of second-line agent in the treatment of recurrent, potentially platinum-sensitive ovarian cancer?. *J Clin Oncol*;2002;20:1158-1160.
- 12 Cannistra SA. Cancer of the ovary. *N Engl J Med* 2004;351:2519-2529.

- 13 Hollingsworth HC, Kohn EC, Steinberg SM et al. Tumor angiogenesis in advanced stage ovarian carcinoma. *Am J Pathol* 1995;147:33-41.
- 14 Angeles AA, Krigman HR, Whitaker RS et al. The prognostic significance of angiogenesis in epithelial ovarian carcinoma. *Clin Cancer Res* 1999;5:587-591.
- 15 Martin L, Schilder R. Novel approaches in advancing the treatment of epithelial ovarian cancer: the role of angiogenesis inhibition. *J Clin Oncol* 2007;25:2894-2901.
- 16 Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008;358:2039-2049.
- 17 Nagy JA, Dvorak AM, Dvorak HF. VEGF-A and the induction of pathological angiogenesis. *Annu Rev Pathol* 2007;2:251–275.
- 18 Ferrara N, Kerbel RS. Angiogenesis as a therapeutic target. *Nature* 2005;438:967-974.
- 19 Cooper BC, Ritchie JM, Broghammer CL et al. Preoperative serum vascular endothelial growth factor levels: significance in ovarian cancer. *Clin Cancer Res* 2002;8:3193–3197.
- 20 Li L, Wang L, Zhang W et al. Correlation of serum VEGF levels with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. *Anticancer Res* 2004;24:1973–1979.
- 21 Chen CA, Cheng WF, Lee CN et al. Serum vascular endothelial growth factor in epithelial ovarian neoplasms: correlation with patient survival. *Gynecol Oncol* 1999;74:235-240.
- 22 Duncan TJ, Attar AA, Rolland P et al. Vascular endothelial growth factor expression in ovarian cancer: a model for targeted use of novel therapies?. *Clin Cancer Res* 2008;14:3030-3035.
- 23 Yamamoto S, Konishil I, Mandal M et al. Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. *Br J Cancer* 1997;76:1221-1227.
- 24 Cross MJ, Claesson-Welsh L. FGF and VEGF function in angiogenesis: signalling pathways, biological responses and therapeutic inhibition. *Trends Pharmacol Sci* 2001;22:201-207.
- 25 Dempke WC, Heinemann V. Resistance to EGF-R (erbB-1) and VEGF-R modulating agents. *Eur J Cancer* 2009;45:1117–1128.

- 26 Burger RA, Sill MW, Monk BJ et al. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group study. *J Clin Oncol* 2007;25:5165-5171.
- 27 Cannistra SA, Matulonis UA, Penson RT et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. *J Clin Oncol* 2007;25:5180-5186.
- 28 Micha JP, Goldstein BH, Rettenmaier MA et al. A phase II study of outpatient first-line paclitaxel, carboplatin, and bevacizumab for advanced-stage epithelial ovarian, peritoneal, and fallopian tube cancer. *Int J Gynecol Cancer* 2007;17:771-776.
- 29 Penson RT, Dizon DS, Cannistra SA et al. Phase II study of carboplatin, paclitaxel, and bevacizumab with maintenance bevacizumab as first-line chemotherapy for advanced mullerian tumors. *J Clin Oncol* 2010;28:154-159.
- 30 Klement G, Baruchel S, Rak J, et al: Continuous low dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J Clin Invest* 2000 105:15-24.
- 31 Klement G, Huang P, Mayer B, et al: Differences in therapeutic indexes of combination metronomic chemotherapy and an anti-vegfr-2 antibody in multidrug resistant human breast cancer xenografts. *Clin Cancer Res* 2000;8:221-232.
- 32 Colleoni M, Rocca A, Sandri MT et al. Low-dose oral methotrexate and cyclophosphamide in metastatic breast cancer: antitumor activity and correlation with vascular endothelial growth factor levels. *Ann Oncol* 2002;13:73-80.
- 33 Chura JC, Iseghem KV, Downs Jr. LS et al. Bevacizumab plus cyclophosphamide in heavily pretreated patients with recurrent ovarian cancer. *Gynecol Oncol* 2007;107:326-330.
- 34 Garcia AA, Hirte H, Fleming G et al. Phase II clinical trial of bevacizumab and low-dose metronomic oral cyclophosphamide in recurrent ovarian cancer: a trial of the california, chicago, and princess margaret hospital phase II consortia. *J Clin Oncol* 2008;26:76-82.
- 35 Burger RA, Bookman MA, Walker JL et al. Phase III trial of bevacizumab in the primary treatment of advanced epithelial ovarian, primary peritoneal, or fallopian tube cancer: a Gynecologic Oncology Group (GOG) study. *J Clin Oncol*. 2010;28:18s (suppl; abstr LBA1).

- 36 Perren T, Swart AM, Pfisterer J et al. ICON7: a phase III gynaecologic cancer intergroup (GCIG) trial of adding bevacizumab to standard chemotherapy in women with newly diagnosed epithelial ovarian, primary peritoneal or fallopian tube cancer. *Ann Oncol* 2010;21:8 (suppl; abstr LBA4).
- 37 Gordon MS, Cunningham D. Managing patients treated with bevacizumab combination therapy. *Oncology* 2005;69(suppl 3):25–33.
- 38 Avastin full prescribing information warnings:
<http://www.gene.com/gene/products/information/oncology/avastin/insert.jsp#warnings>
- 39 Sfakianos GP, Numnum TM, Halverson CB et al. The risk of gastrointestinal perforation and/or fistula in patients with recurrent ovarian cancer receiving bevacizumab compared to standard chemotherapy: a retrospective cohort study. *Gynecol Oncol* 2009;114:424-426.
- 40 Diaz JP, Tew WP, Zivanovic O et al. Incidence and management of bevacizumab-associated gastrointestinal perforations in patients with recurrent ovarian carcinoma. *Gynecol Oncol* 2010;116:335-339.
- 41 Byrne AT, Ross L, Holash J et al. Vascular endothelial growth factor-trap decreases tumor burden, inhibits ascites, and causes dramatic vascular remodeling in an ovarian cancer model. *Clin Cancer Res* 2003;9:5721–5728.
- 42 Hu L, Hofmann J, Holash J et al. Vascular endothelial growth factor trap combined with paclitaxel strikingly inhibits tumor and ascites, prolonging survival in a human ovarian cancer model. *Clin Cancer Res* 2005;11:6966–6971.
- 43 Tew WP, Colombo N, Ray-Coquard I et al. VEGF-Trap for patients (pts) with recurrent platinum-resistant epithelial ovarian cancer (EOC): preliminary results of a randomized, multicenter phase II study. *J Clin Oncol* 2007;25(18 suppl):Abstract 5508.
- 44 Colombo N, Mangili G, Mammoliti S et al. Aflibercept (VEGF Trap) for advanced epithelial ovarian cancer (EOC) patients (pts) with symptomatic malignant ascites: Preliminary results of a pilot study. *J Clin Oncol* 2008;26:(20 suppl):Abstract 14598.
- 45 Hirte HW, Vidal L, Fleming GF et al. A phase II study of cediranib (AZD2171) in recurrent or persistent ovarian, peritoneal or fallopian tube cancer: Final results of a PMH, Chicago and California consortia trial. *J Clin Oncol* 2008;26(15 suppl):Abstract 5521.

- 46 Matulonis UA, Berlin S, Ivy P et al. Cediranib, an oral inhibitor of vascular endothelial growth factor receptor kinases, is an active drug in recurrent epithelial ovarian, fallopian tube, and peritoneal cancer. *J Clin Oncol* 2009;27:5601–5606.
- 47 Welch SA, Hirte HW, Elit L et al. Sorafenib in combination with gemcitabine in recurrent epithelial ovarian cancer: a study of the Princess Margaret Hospital Phase II Consortium. *Int J Gynecol Cancer* 2010;20:787-793.
- 48 Matei D, Sill MW, Lankes HA et al. Activity of sorafenib in recurrent ovarian cancer and primary peritoneal carcinomatosis: a gynecologic oncology group trial. *J Clin Oncol* 2011; 29:69-75.
- 49 Azad NS, Posadas EM, Kwitkowski VE et al. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. *J Clin Oncol* 2008; 26:3709-3714.
- 50 Biagi JJ, Oza AM, ChalChal HI et al. A phase II study of sunitinib in patients with recurrent epithelial ovarian and primary peritoneal carcinoma: an NCIC Clinical Trials Group Study. *Ann Oncol* 2011; 22: 335-340.
- 51 Friedlander M, Hancock KC, Rischin D et al. A Phase II, open-label study evaluating pazopanib in patients with recurrent ovarian cancer. *Gynecol Oncol* 2010;119:32-37.
- 52 Hilberg F, Roth GJ, Krssak M et al. BIBF 1120: Triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res* 2008;68:4774-4782.
- 53 Ledermann JA, Rustin GJ, Hackshaw A et al. A randomized phase II placebo-controlled trial using maintenance therapy to evaluate the vascular targeting agent BIBF 1120 following treatment of relapsed ovarian cancer (OC). *J Clin Oncol* 2009;27(15 suppl):Abstract 5501.
- 54 Coleman RL, Broaddus RR, Bodurka DC et al. Phase II trial of imatinib mesylate in patients with recurrent platinum and taxane resistant epithelial ovarian and primary peritoneal cancers. *Gynecol Oncol* 2006;10:126-131.
- 55 Posadas EM, Kwitkowski V, Kotz HL et al. A prospective analysis of imatinib-induced c-KIT modulation in ovarian cancer: a phase II clinical study with proteomic profiling. *Cancer* 2007;110:309–317.
- 56 Wooster R, Weber B. Breast and ovarian cancer. *N Engl J Med* 2003;348:2339-2347.

- 57 Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature* 2001;411:366-374.
- 58 Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 2002;108:171–182.
- 59 Yap TA, Sandhu SK, Carden CP et al. Poly(ADP-Ribose) Polymerase (PARP) inhibitors: exploiting a synthetic lethal strategy in the clinic. *CA Cancer J Clin* 2011;61:31–49.
- 60 Bryant HE, Schultz N, Thomas HD et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005;434:913-917.
- 61 Ashworth A. A synthetic lethal therapeutic approach: Poly(ADP)Ribose Polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J Clin Oncol* 2006;26:3785-3790.
- 62 Audeh MW, Penson RT, Friedlander M. Phase II trial of the oral PARP inhibitor olaparib (AZD2281) in BRCA-deficient advanced ovarian cancer. *J Clin Oncol* 2009;27(15 suppl).
- 63 Fong PC, Yap TA, Boss DS. Poly(ADP)-Ribose Polymerase Inhibition: frequent durable responses in *BRCA* carrier ovarian cancer correlating with platinum-free Interval. *J Clin Oncol* 2010; 28:2512-2519.
- 64 Bast Jr RC, Mills GB. Personalizing therapy for ovarian cancer: BRCAness and beyond. *Journal of Clinical Oncology* 2010;28:3545-3551.
- 65 Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. *Nat Rev Cancer*. 2004;4(10):814-819.
- 66 Baldwin RL, Nemeth E, Tran H et al. BRCA1 promoter region hypermethylation in ovarian carcinoma: a population-based study. *Cancer Res*. 2000;60:5329-5333.
- 67 Olayioye MA, Neve RM, Lane HA et al. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J* 2000;19:3159-3167.
- 68 Bianco R, Gelardi T, Damiano V et al. Rational bases for the development of EGFR inhibitors for cancer treatment. *Int J Biochem Cell Biol* 2007;39:1416–1431.
- 69 El-Rayes BF, LoRusso PM. Targeting the epidermal growth factor receptor. *Br J Cancer* 2004;91:418–424.

- 70 Sheng Q, Liu J. The therapeutic potential of targeting the EGFR family in epithelial ovarian cancer. *Br J Cancer* 2011;104: 1241–1245.
- 71 Felip E, Del Campo JM, Rubio D et al. Overexpression of c-erbB-2 in epithelial ovarian cancer. Prognostic value and relationship with response to chemotherapy. *Cancer* 1995;75:2147-2152.
- 72 Berchuck A, Kamel A, Whitaker R et al. Overexpression of Her2 is associated with poor survival in advanced epithelial ovarian cancer. *Cancer Res* 1990;50:4087–4091.
- 73 Rubin SC, Finstad CL, Wong GY et al. Prognostic significance of Her-2 expression in advanced epithelial ovarian cancer: a multivariate analysis. *Am J Obstet Gynecol* 1993;168:162–169.
- 74 Farley J, Fuchiuj S, Darcy KM et al. Associations between ERBB2 amplification and progression-free survival and overall survival in advanced stage, suboptimally-resected epithelial ovarian cancers: A Gynecologic Oncology Group study. *Gynecol Oncol* 2009;113:341–347.
- 75 Schilder RJ, Pathak HB, Lokshin AE et al. Phase II trial of single agent cetuximab in patients with persistent or recurrent epithelial ovarian or primary peritoneal carcinoma with the potential for dose escalation to rash. *Gynecol Oncol* 2009;113:21–27.
- 76 Secord AA, Blessing JA, Armstrong DK et al. Phase II trial of cetuximab and carboplatin in relapsed platinum-sensitive ovarian cancer and evaluation of epidermal growth factor receptor expression: a Gynecologic Oncology Group study. *Gynecol Oncol* 2008;108:493–499.
- 77 Konner J, Schilder RJ, DeRosa FA et al. A phase II study of cetuximab/paclitaxel/carboplatin for the initial treatment of advanced-stage ovarian, primary peritoneal, or fallopian tube cancer. *Gynecol Oncol* 2008;110: 140–145.
- 78 Gordon MS, Matei D, Aghajanian C et al. Clinical activity of pertuzumab (rhuMAb 2C4), a HER dimerization inhibitor, in advanced ovarian cancer: potential predictive relationship with tumor HER2 activation status. *J Clin Oncol* 2006;24:4324-4332.
- 79 Makhija S, Amler LC, Glenn D et al. Clinical activity of gemcitabine plus pertuzumab in platinum-resistant ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. *J Clin Oncol*. 2010;28:1215-23.

- 80 Bookman MA, Darcy KM, Clarke-Pearson D et al. Evaluation of monoclonal humanized anti-HER2 Antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: A phase II trial of the Gynecologic Oncology Group. *J Clinl Oncol* 2003;2:283-290.
- 81 Posadas EM, Liel MS, Kwitkowski V et al. A phase II and pharmacodynamic study of gefitinib in patients with refractory or recurrent epithelial ovarian cancer. *Cancer* 2007;109:1323–1330.
- 82 Schilder RJ, Sill MW, Chen X et al. Phase II study of gefitinib in patients with relapsed or persistent ovarian or primary peritoneal carcinoma and evaluation of epidermal growth factor receptor mutations and immunohistochemical expression: a Gynecologic Oncology Group study. *Clin Cancer Res* 2005;11:5539–5548.
- 83 Wagner U, du Bois A, Pfisterer J et al. Gefitinib in combination with tamoxifen in patients with ovarian cancer refractory or resistant to platinum-taxane based therapy—a phase II trial of the AGO Ovarian Cancer Study Group (AGO-OVAR 2.6). *Gynecol Oncol* 2007;105:132–137.
- 84 Gordon AN, Finkler N, Edwards RP et al. Efficacy and safety of erlotinib HCl, an epidermal growth factor receptor (HER1/EGFR) tyrosine kinase inhibitor, in patients with advanced ovarian carcinoma: Results from a phase II multicenter study. *Int J Gynecol Cancer* 2005;15:785–792.
- 85 Nimeiri HS, Oza AM, Morgan RJ et al. Efficacy and safety of bevacizumab plus erlotinib for patients with recurrent ovarian, primary peritoneal, and fallopian tube cancer: A trial of the Chicago, PMH, and California Phase II Consortia. *Gynecol Oncol* 2008;110:49 –55.
- 86 Williams C, Simera I, Bryant A. Tamoxifen for relapse of ovarian cancer (Review). *Cochrane Database Syst Rev* 2010;(3):CD001034.
- 87 Smyth JF, Gourley C, Walker G et al. Antiestrogen therapy is active in selected ovarian cancer cases: The use of letrozole in estrogen receptor positive patients. *Clin Cancer Res* 2007;13:3617–3622.
- 88 Bowman A, Gabra H, Langdon SP et al. CA125 response is associated with estrogen receptor expression in a phase II trial of letrozole in ovarian cancer: Identification of an endocrine-sensitive subgroup. *Clin Cancer Res* 2002;8:2233–2239.

- 89 Papadimitriou CA, Markaki S, Siapkarakas J et al. Hormonal therapy with letrozole for relapsed epithelial ovarian cancer. Long-term results of a phase II study. *Oncology* 2004;66:112–117.
- 90 Kavanagh JJ, Hu W, Fu S et al. Anti-tumor activity of letrozole in patients with recurrent advanced low malignant potential or low-grade serous ovarian tumors. *J Clin Oncol* 2007;25(18 suppl):Abstract 5582.
- 91 Berek J, Taylor P, McGuire W et al. Oregovomab maintenance monoimmunotherapy does not improve outcomes in advanced ovarian cancer. *J Clin Oncol* 2009;27:418-425.
- 92 Kalli KR, Oberg AL, Keeney GL et al. Folate receptor alpha as a tumor target in epithelial ovarian cancer. *Gynecol Oncol* 2008;108:619–626.
- 93 Karlan BY, Ozra AM, Hansen VL et al. Randomized, double-blind, placebo controlled phase II study of AMG 386 combined with weekly paclitaxel in patients (pts) with recurrent ovarian carcinoma. *J Clin Oncol* 2010;28:Abstract 5000.