Title: New Strategies in the treatment of ovarian cancer- current clinical perspectives and future potential

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Running Title: New Treatment in Ovarian Cancer
Abstract

The treatment of ovarian cancer is set to undergo rapid changes, as strategies incorporating molecular targeted therapies begin to take shape. These are based on a better appreciation of approaches targeting the tumour microenvironment as well as specific subtypes of the disease, with distinct molecular aberrations. Targeting the VEGF pathway through bevacizumab is clearly effective, with positive randomised trials at all disease stages; targeting defective homologous recombination repair pathways with PARP inhibitors is also proving successful in a substantial proportion of patients with high grade serous ovarian cancer. In this article we will review progress in these two leading areas and also discuss the potential for targeting other pathways and receptors which may be activated in ovarian cancer, including the RAS/RAF/MEK and PI3K/AKT/mToR pathways; the ErbB and IGF family of receptors; mitotic check points and also the folate receptor. Here as well as single agent therapy in selected cases, the way forward should include combination treatments aimed at dealing with the key problem of cytotoxic drug resistance and rational approaches to patient selection will become an essential component of future strategies.

Background

Ovarian cancer (OC) is estimated to be diagnosed in over 225,000 women per year worldwide and remains a significant cause of gynaecological cancer mortality (approximately 140,000 deaths per year) (1). Unfortunately, the majority of women continue to present at advanced stages and the overall five-year survival rate is around 40%. The current standard of care for newly-diagnosed ovarian cancer is a combination of optimal cytoreductive surgery and platinum-based chemotherapy. Key advances in radical surgery and chemotherapy strategies have led to improved, albeit modest, clinical outcomes. Despite advances, there remains a significant risk of recurrence and resistance to therapy and when this occurs, ovarian cancer is currently incurable. Hence there is an urgent need to develop smarter treatment options.
Epithelial ovarian cancer is recognized as a heterogeneous disease and is divided according to histological subtypes: high grade serous, low grade serous, clear cell, endometrioid and mucinous (2). Each histological subtype is associated with a distinct clinical behaviour (response to chemotherapy, pattern of metastases, survival) but has historically been treated as one entity. The identification of distinct molecular pathways characteristic of individual subtypes (3) has fuelled enthusiasm for the development of targeted therapies (4) directed at specific subtypes of ovarian cancer (Figure 1).

Molecularly-targeted agents hold the promise of greater selectivity with lower toxicity than conventional chemotherapy. Over the last few years, there have been several landmark reports in epithelial ovarian cancer (EOC) giving rise to the development of molecular-driven, patient-selective clinical trials and changes in clinical practice.

On the Horizon

Angiogenesis inhibitors
Vascular Endothelial Growth Factor (VEGF) is a key mediator of angiogenesis, a process that is important in ovarian cancer growth and metastasis. Phase III clinical trials of bevacizumab, a monoclonal antibody against VEGF-A, have shown significant clinical activity in EOC. Two first line studies, GOG-0218 (5) and ICON7 (6) addressed the addition of bevacizumab to the carboplatin and paclitaxel combination followed by maintenance therapy for a defined period. In both phase III trials, significant improvements in the primary end point, progression-free survival (PFS) were attained through the use of concurrent and maintenance bevacizumab despite key differences in trial design (GOG-0218 HR 0.72, P<0.001; ICON7 HR 0.81, P<0.004). Furthermore, in ICON7, an overall survival (OS) advantage of almost 8 months (28.8 months vs. 36.6 months; HR 0.64, 95 % confidence interval 0.48–0.85, P <0.002) was reported in the bevacizumab arm for the subgroup of patients with a poor prognosis (high-risk group defined as FIGO stage IV disease or FIGO stage III disease and more than 1.0 cm of residual disease after debulking surgery) (6). Although the mature OS results for
GOG-0218 and ICON7 are awaited, on the basis of the above results, the European Medicines Agency approved the use of bevacizumab in combination with carboplatin and paclitaxel as first-line therapy.

The next question was whether bevacizumab has a role in relapsed ovarian cancer. The OCEANS study (7), a phase III trial of bevacizumab in combination with chemotherapy (carboplatin with gemcitabine) followed by maintenance therapy until progression in first-line platinum-sensitive (recurrence >6 months after front-line platinum-based therapy) relapse also reported a significant improvement in the primary end point, PFS, with the addition of bevacizumab (8.4 months vs. 12.4 months; HR 0.48, P<0.0001). For patients with platinum-resistant disease, an impressive, statistically significant improvement in PFS (3.4 months vs. 6.7 months; HR 0.48, P<0.001) was demonstrated in the AURELIA study (8), a phase III trial of bevacizumab in combination with chemotherapy (pegylated liposomal doxorubicin, topotecan or weekly paclitaxel) until progression. This is the first phase III study in platinum-resistant OC to have shown benefit with a targeted therapy. Despite the lack of final OS results, the findings from both OCEANS and AURELIA strongly support a role for bevacizumab in recurrent disease and the European Medicines Agency have given a favourable opinion for approval in first-line platinum-sensitive relapse. However, in the US at this stage, approval has not yet been sought for the use of bevacizumab in ovarian cancer, and debate continues about the most appropriate use of this agent in this disease.

Other agents that directly inhibit VEGF include aflibercept, a VEGF-ligand-binding fusion protein that acts as a decoy receptor for the binding of VEGF. This approach in combination with chemotherapy appears to have substantial clinical efficacy (overall response rate 54% in combination with docetaxel) although the advantages over bevacizumab are unclear (9).

The main limitations of bevacizumab, apart from cost, are toxicities (eg. bowel perforation, hypertension) and resistance to treatment. The key challenges to address next therefore include 1) how to select which patients will derive most
benefit and at which point during the treatment pathway? (ie. upfront, platinum-sensitive or platinum-resistant setting); 2) how to overcome resistance to bevacizumab? At present, there are no validated biomarkers predicting clinical efficacy following bevacizumab in ovarian cancer although studies investigating gene expression arrays and isoform-specific plasma VEGF-A measurements are ongoing. Advances in imaging techniques eg. multiparametric MR imaging, fluorodeoxyglucose-positron emission tomography (10, 11) appear promising and prospective clinical trials including imaging based endpoints are planned. Resistance mechanisms include the upregulation of alternative pro-angiogenic signaling pathways (Fibroblast growth factor (FGF), platelet-derived growth factor (PDGFR), c-Met) and have raised the question of whether targeting additional pathways will be a successful strategy. Several tyrosine kinase inhibitors (TKIs) that target VEGF receptors also inhibit other pro-angiogenic molecules eg. FGF- nintedanib, brivanib, dovitinib; PDGFR- cediranib, pazopanib; c-Met- cabozantinib and are under investigation in various trials in ovarian cancer. These include randomized trials (first line or second line) involving nintedanib, pazopanib and cediranib, and in addition the VEGFR TKI sunitinib is under evaluation in clear cell ovarian cancer, which is often resistant to conventional therapy.

Trebananib (AMG 386) is a peptide-Fc fusion protein which prevents interactions between angiopoetin-1 and angiopoetin-2 expressed on vascular endothelial cells with the Tie2 receptor thereby inhibiting vascular maturation and reducing the impact of VEGF stimulation. In clinical trials so far, trebananib is administered weekly in combination with chemotherapy and as maintenance therapy. Efficacy seems to be dose-dependent and the toxicity profile appears to differ from bevacizumab: peripheral oedema, presumably due to disruption of the angiopoetin axis, is common, whereas hypertension and proteinuria are not seen (12). Important questions are (a) based on efficacy and a more tolerable toxicity profile, could trebananib replace bevacizumab and (b) can targeting the angiopoetin axis address resistance to bevacizumab in patients progressing on this treatment? The ongoing first line (TRINOVA-3) and recurrent (TRINOVA-1 and 2) ovarian cancer clinical trials will help answer the above.
As an increasing number of patients will have received bevacizumab, it will be increasingly important to identify rational treatment options in patients that progress on bevacizumab. In a randomized phase II discontinuation study of brivanib (targets VEGFR and FGFR), which included patients that had received antiangiogenic agents, clinical efficacy was seen in patients previously treated with VEGF inhibitors (mainly bevacizumab: 17% partial response; 30% disease stabilization and may relate to FGF inhibition (13). Data on other antiangiogenic agents in this patient population will be important to ascertain. Options to potentially overcome resistance include combination approaches- either ‘vertical’ eg. Bevacizumab plus VEGFR inhibitor sorafenib/sunitinib or ‘horizontal’ eg. bevacizumab and either a vascular disrupting agent or angiopoietin antagonist, AMG386. The increased toxicity potentially associated with combination strategies needs to be carefully considered (14) and a sequential approach with an alternative single agent antiangiogenic post bevacizumab may be an alternative strategy. In addition, the role for repeating or continuing bevacizumab at progression and substituting an alternative chemotherapy is being explored.

There are a number of other antiangiogenic targets that have emerged that are becoming more clinically relevant and include Zeste homolog 2 (EZH2) and the Notch/Delta-like ligand 4 (Dll4). EZH2 has been linked to increased angiogenesis through methylation and silencing of the antiangiogenic factor, vasohibin 1. Preclinical studies have shown that silencing of EZH2 using siRNA inhibits angiogenesis and ovarian cancer growth (15). Dll4 has been associated with poor outcome following anti-VEGF therapy and RNAi-mediated silencing of Dll4 has been shown to reduce angiogenesis and tumour growth in ovarian cancer models (16). This approach appears promising and a phase I clinical trial of REGN 421, a monoclonal antibody against Dll4 in underway.

In addition, a mechanistic link has recently been made between thrombocytosis and poor survival in ovarian cancer, which may be relevant to anti-angiogenesis. This appears to involve tumour-derived IL6 stimulation of
hepatic thrombopoietin with a consequent increase in PDGF particularly affecting pericytes (17). Mouse models have shown that antiplatelet antibody significantly reduced tumour angiogenesis and growth. This approach needs further exploration in clinical trials.

**PARP inhibitors**

PARP inhibitors exploit the concept of “synthetic lethality”- targeting one of the genes in a synthetic lethal pair, where the other is defective (eg. BRCA mutation), selectively kills tumour cells while sparing normal cells (thereby limiting toxicity), potentially creating a substantial therapeutic window (18). Patients harbouring mutations in BRCA1/2 were predicted to be highly susceptible to treatment with PARP Inhibitors (19, 20) and this proof of concept was supported in a phase II study of olaparib in patients with germline BRCA1 or BRCA2 mutations with recurrent ovarian cancer (33% RECIST response rate at 400mg bd) which included patients with platinum-resistant disease (21). A wider utility of this approach was envisaged in view of the fact that up to 50% of high-grade serous, sporadic ovarian cancers have defective homologous recombination repair pathways (including BRCA methylation and somatic BRCA mutations) which may confer sensitivity to PARP inhibition(22). Efficacy was indeed confirmed in a Phase II study of olaparib in this patient population although responders were mainly seen in those with platinum sensitive disease with a response rate of 50% (23). This was further explored in a double-blind, placebo-controlled randomized phase II study in which patients with platinum-sensitive, recurrent, high-grade serous ovarian cancer (who had achieved a response following their most recent platinum-based regimen) were randomized to either olaparib or placebo maintenance therapy (24). 22% of patients were known to have a BRCA mutation and 64% had unknown BRCA status. PFS (according to RECIST criteria), was significantly prolonged with olaparib compared to the placebo arm (median, 8.4 months vs. 4.8 months; HR 0.35, P<0.001), although an initial analysis indicated that this does not translate into an overall survival benefit. The possibility exists that although PARP inhibitors may delay disease progression, treatment could subsequently impact on response to further chemotherapy. However, an analysis of post olaparib chemotherapy in
patients with germ-line BRCA mutations indicates that sensitivity at least in this population appears to be maintained (25). The impact of PARP inhibitors may differ according to the BRCA mutation status and preliminary analysis suggests that the benefit of olaparib maintenance therapy, at least in terms of PFS, was larger in known BRCA germline mutation carriers (PFS HR 0.10).

The key issues for the development of PARP inhibitors are patient selection and single vs combination strategies. There is little doubt that PARP inhibitors should be further developed towards registration in BRCA- mutation associated ovarian cancer (26), and a maintenance treatment approach is particularly promising. For patients with BRCA associated platinum-resistant disease, a registration strategy for PARP inhibitors incorporating randomised controlled trials is less straightforward as it is becoming clear that higher response rates may be seen with certain chemotherapy agents such as liposomal doxorubicin in BRCA mutation carriers (27). Combination approaches with chemotherapy, based on the hypothesis of a chemosensitisation effect, are being tested. However, a limitation is the increased myelosuppression seen with these regimens so far and the optimal duration of PARP inhibition with chemotherapy is not yet defined. In a randomized phase II study of olaparib with carboplatin and paclitaxel, the response rate was not increased compared to chemotherapy alone (28). However, in keeping with the maintenance therapy trial previously reported (24), the PFS was significantly longer in the olaparib arm which is likely to be due to the maintenance treatment rather than the combination effect with chemotherapy.

Other combination strategies of interest are PARP inhibitors with PI3K inhibitors or antiangiogenic agents. Preclinical models of breast cancer have identified that in the context of an upregulated PI3K pathway, PI3K inhibition is associated with the loss of homologous recombination repair capability resulting in sensitisation to PARP inhibitors (29, 30). The rationale for the PARP inhibitor/anti-angiogenic combination is based on observations (a) that PARP inhibitors may lead to increased VEGFR2 phosphorylation and subsequent activation of endothelial cell survival - an effect which has been
shown to be reversed by a VEGFR2 inhibitor (31), and (b) that VEGFR2 inhibition leads to hypoxia which can lead to acquisition of HR defects and sensitivity to PARP inhibitors in hypoxic cancer cells (32). In addition to olaparib, other PARP inhibitors under investigation in this disease include rucaparib, veliparib, niraparib and BMN-673.

Ras/Raf/MEK/ERK pathway

Low grade serous ovarian carcinoma (LGSOC), a rare subtype of epithelial ovarian carcinoma, has a distinct clinical behavior characterized by younger age at presentation, more indolent growth pattern and poor responses to systemic therapy (33). Activation of the MAPK signaling pathway may be very important as BRAF and KRAS mutations were initially reported in up to 68% of cases (33% BRAF, 35% KRAS- mutually exclusive) (34). Although the incidence of BRAF mutations appears lower in recent reports (35), the Ras/MEK/ERK pathway is still an attractive therapeutic target in this notoriously difficult disease. A phase II trial of the MEK 1/2 inhibitor, Selumetinib (AZD6244) in 52 patients with recurrent LGSOC has shown promising results (36): the overall response rate was 15.4%; disease stabilization 65%; median PFS 11 months. In this study, 6% BRAF, 41% KRAS, 15% NRAS mutations were identified and response was not correlated with mutational status. Phase II trials of other MEK inhibitors such as GSK1120212 (Trametinib) are planned and combination strategies of MEK and AKT inhibitors (currently in phase I trials) are also under consideration.

In primary mucinous ovarian cancer, which is frequently resistant to conventional chemotherapy, the Ras/Raf pathway is also an appropriate therapeutic target.
Overcoming platinum and taxane resistance

**PI3K/AKT/mTOR pathway**

Activation of the PI3Kinase pathway through mutations of PIK3CA, AKT or inactivating mutations of PTEN is rare in the high grade serous subtype (<5%) although may be seen in up to 30% of clear cell and endometrioid ovarian carcinomas. However, it is unclear whether an abberation in this pathway in an individual’s tumour is the critical driver of cancer growth and therefore susceptible to targeted inhibition in ovarian cancer. Several agents are being explored in clinical trials of ovarian cancer and it will be important to correlate any evidence of clinical activity with pathway alterations. Probably of wider clinical applicability are preclinical studies which have suggested a potential for modulation of this pathway to overcome resistance to chemotherapy in ovarian cancer (37) and clinical trials of chemotherapy in combination with either AKT or TORC1/2 inhibitors are planned.

**ErbB family**

Although increased expression of EGFR is common in ovarian cancer (up to 60%) mutations are rare (<4%) (38) and clinical trial results with single agent EGFR inhibitors (erlotinib, gefitinib) are disappointing (39, 40). Similarly, HER2 targeted therapy (trastuzumab, pertuzumab) has proved to be of no benefit in unselected cases (41-43) although it has been proposed that HER2 activation as measured by phosphorylation of HER2, may be more predictive of sensitivity to HER2 targeted agents (42). HER2 overexpression or amplification has been described in up to 18% of advanced mucinous carcinomas and HER2- directed treatment approaches for this subgroup of patients should be considered (44). However, targeting ErB3 (HER 3) may be more promising for a larger group of patients. ErbB3 (HER3) forms a heterodimer with ErbB2 (HER2) and stimulates cell survival pathways through activation of MAPK and AKT pathways. HER3 has been associated with poor prognosis (45) and resistance to chemotherapy including taxanes (46). An autocrine NRG1-driven/activated ErbB3 loop promoting ovarian cancer cell proliferation has been described and disruption of this circuit with a monoclonal ErbB3-directed antibody (MM-121), significantly inhibited tumour
growth in mouse xenograft models (47). MM-121 is currently under investigation in phase II trials combined with paclitaxel in ovarian cancer.

**Other**

Multiple other signaling molecules are also implicated in overcoming resistance to chemotherapy and targeted agents in ovarian cancer including the insulin growth factor (IGF) receptor and Src. A phase II study of the Src inhibitor, dasatinib, in relapsed ovarian cancer was disappointing with no objective response reported (48). In addition, saracatinib (Src inhibitor) in combination with carboplatin and paclitaxel failed to show benefit in platinum-sensitive disease (49). However, preclinical studies suggest that Src inhibition has the potential to reverse paclitaxel resistance and merits further exploration in ovarian cancer (50). The IGF pathway has also been shown to modulate paclitaxel resistance (51). Saracatinib (Src inhibitor) and OSI-906 [linsitinib] (a small molecule dual kinase inhibitor of both insulin-like growth factor-1 receptor and insulin receptor) have entered phase II clinical trials in combination with weekly paclitaxel in platinum-resistant ovarian cancer. Furthermore, overexpression of IGF-1 and growth inhibition with OSI-906 was reported in preclinical models of low grade serous ovarian carcinoma (52) and therefore, in addition to MEK inhibition, targeting the IGF pathway may be another potential therapeutic approach in this setting.

Another potential target is Wee-1 kinase which regulates the G2/M checkpoint. Inhibition of Wee-1 kinase may lead to chemosensitisation of p53-deficient tumour cells (53) which are characteristic of high grade serous ovarian cancer. Based on the encouraging activity seen in phase I clinical trials of MK-1775 (54), a selective inhibitor of Wee-1 kinase, a randomised trial in platinum-sensitive relapsed disease is underway. Other mitotic checkpoint inhibitors include the selective aurora kinase A inhibitor, MLN8237 (alisertib) and although it has limited single agent clinical activity in platinum-resistant ovarian cancer (55), randomized trials in combination with paclitaxel are ongoing.)
The folate receptor is overexpressed in >90% of ovarian cancers and several anti-folate receptor strategies are under investigation including farletuzumab (MORab-003, Morphotek Inc.), a monoclonal antibody directed against the α-folate receptor. EC145, a conjugate of a vinblastine analogue to folate appears promising. An interim analysis of a randomised, phase II study of EC145 + liposomal doxorubicin reported a greater than 2-fold increase in median PFS with the addition of EC145 (24 weeks vs 11.7 weeks HR 0.50, p=0.014) in platinum-resistant ovarian cancer and clinical benefit was most clearly seen in the subgroup of patients with high folate receptor activity as assessed on whole body SPECT scanning using Tc-labelled folate (56).

Drug resistance has generally been considered to be characteristic of so-called ‘stem cells’, although these have proved difficult to isolate and characterize. However, a study on ascites in patients with relapsed disease identified EZH2 as playing a key role in the maintenance of a drug-resistant stem cell-like subpopulation of tumour cells (57) and this is an area of new drug development.

At present, key mutations identified in genes such as ARID1A (in clear cell and endometrioid subtype) (58) and TP53 (in high grade serous subtype) are (22) not directly ‘druggable’. However, advances in high throughput technologies are providing the opportunity for genomic-based drug discovery studies which may lead to the identification of new agents in the treatment of ovarian cancer.

**Conclusions**

Understanding more about how best to utilize our increasing knowledge of the molecular abnormalities involved in ovarian cancer will be critical in improving clinical outcome in ovarian cancer. Of the many targeted therapies currently under evaluation in phase I/II and III studies (Figure 2), the most promising strategies developed so far are the anti-angiogenic agents and PARP inhibitors. Challenges facing the success of targeted therapy include the identification of the correct population to treat, as well as a better appreciation
of mechanisms underlying drug resistance. It is generally accepted that tumour biopsies taken at the time of progression are likely to yield important information for molecular profiling in order to direct targeted agents. However, the recognition of inter and intra tumour heterogeneity poses a further challenge in terms of how best to interpret these results in the clinic. To complement biopsy data, information from other sources such as circulating tumour DNA, taken together with novel approaches to molecular imaging, should form part of a comprehensive approach to predictive biomarker validation in clinical trials of ovarian cancer.

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Conflicts of Interest
SBK Advisory boards: Roche, Astrazeneca, Clovis, Array, Sanofi-Aventis, Astellas
SB Advisory boards: Tesaro, Array
Figure 1. Histological subtypes of epithelial ovarian carcinoma and associated mutations/molecular aberrations.

*CHK2, BARD1, BRIP1, PALB2, RAD50, RAD51C, ATM, ATR, EMSY, Fanconi anaemia genes. MMR- mismatch repair. References (22, 34, 44, 58-65)

Figure 2. Targeted therapies in ovarian cancer.
References


Figure 1:

Ovarian cancer

Epithelial

High grade serous
Low grade serous
Mucinous
Clear cell
Endometrioid
Sex cord-stromal
Others including germ cell

Non-epithelial

Granulosa cell
Sertoli-Leydig cell
DICER1
MMR deficiency

Pathway alterations:
PI3K/RAS/NOTCH/FOXM1

TP53
BRCA1 and 2
NF1
RB1
CDK12
Homologous recombination repair genes

KRAS
HER2 amplification
ARID1A
PIK3CA
PTEN
CTNNB1
PPP2R1α

BRAF
KRAS
NRAS
ERBB2
ARID1A
PIK3CA
PTEN
PPP2R1α

TP53
BRCA1 and 2
NF1
RB1
CDK12
Homologous recombination repair genes

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