The role of targeted therapy in ovarian cancer

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Abstract

Ovarian cancer is the second most common gynaecological malignancy and the leading cause of death from gynaecological cancer. Although in some cases treatment is initially effective, there is a considerable risk of disease recurrence and resistance to therapy. Therapies targeting molecular alterations in tumours offer the promise of significantly improved treatment. So far, the most promising targeted agents are angiogenesis inhibitors and PARP inhibitors. Here, we review the various targeted therapeutic approaches under clinical investigation in phase I and II trials of ovarian cancer and the challenges facing their future success in the clinic.

Introduction

There are over 6600 diagnoses of ovarian cancer per year in the UK and more than 4400 deaths from the disease [1]. In the USA, the relative incidence and proportional mortality are similar, with 21,650 cases and over 15,500 deaths attributed to ovarian cancer in 2008 [2]. Many women are diagnosed with advanced disease with little prospect of cure; the five-year survival rate for advanced ovarian cancer is only 30–40%, largely due to frequent late presentation. The current standard of care consists of the combination of radical surgery and platinum-based chemotherapy. Some important advances have been made in both surgical and chemotherapeutic strategies, but only modest improvements in outcome have resulted.

Biologically targeted agents have shown some success in a variety of malignancies such as leukaemias and breast, colon and renal cancers. These agents are designed to specifically target tumour cells and/or the microenvironment by exploiting specific molecular abnormalities in the tumour (Fig. 1). The approach holds the promise of greater selectivity and lower toxicity than traditional modalities such as chemotherapy. It is now widely accepted that ovarian cancer is a heterogeneous disease. The diversity of histological subtypes of the disease is associated with strikingly different molecular characteristics [3]. Type I ovarian cancers (low-grade serous, borderline serous cancers, low-grade endometrioid, mucinous and clear-cell cancers) retain functional p53 and harbour activating mutations of either *BRAF* or *KRAS* and either *PTEN* or *PIK3CA*. In contrast, type II cancers (high-grade serous) usually have inactivation of p53, genomic instability and BRCA dysfunction [3]. The need for improved treatment options linked to a greater understanding of tumour genetics and biology has driven the development of new therapeutic approaches for ovarian cancer [4].

Targeting tumour vasculature and VEGF

Angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is a critical component of cancer growth and metastasis [5]. Vascular Endothelial Growth Factor (VEGF) and its receptors play a pivotal role in this process. The VEGF protein family consists of at least seven structurally related glycoproteins of which VEGFA (also known as VEGF) is the best understood. VEGF actions are mediated by binding to VEGF tyrosine kinase receptors (VEGFR-1 [FLT-1], VEGFR-2 [KDR/FLK-1] and VEGFR-3 [FLT-4]) [6]. This leads to receptor dimerisation, phosphorylation and downstream activation of signalling cascades, including the MAPK and PI3/AKT pathways, which eventually culminates in endothelial cell survival, proliferation, invasion and migration. It is likely that VEGFR-2 mediates the majority of the pro-angiogenic effects of VEGF. VEGF also increases vascular permeability and vasodilation resulting in interstitial hypertension and a leaky neovasculature, leading to impaired delivery of oxygen and therapeutic agents from the bloodstream to tumour cells. Furthermore, ovarian cancer cells have been reported to express VEGFR-2 and this raises the possibility that VEGF could potentially act in an autocrine fashion influencing the tumour directly as well as via angiogenesis [7,8].

Angiogenesis induced by VEGF can be blocked by either targeting the VEGF ligand itself or via

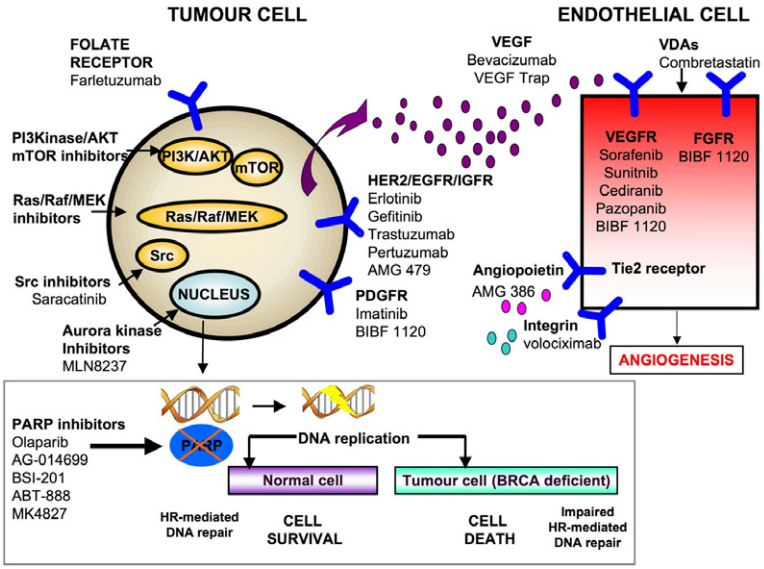

Fig. 1. Targeted therapies in ovarian cancer.

the VEGF receptors [9,10]. Bevacizumab (Avastin, Genentech, San Francisco, CA, USA) is an intravenously administered humanised monoclonal antibody directed against VEGFA that acts by binding and neutralising VEGFA. In contrast, small molecule tyrosine kinase inhibitors (TKIs) act by inhibiting the activity of VEGF receptors and therefore block downstream signalling pathways. Kinase inhibitors commonly have additional activity against other tyrosine kinases and such "multi-targeting" activity may contribute to the anti-tumour effect. For example, sorafenib inhibits Raf-1 as well as VEGFR-2, VEGFR-3 and PDGFR-β and therefore potentially targets proliferation via the Ras-ERK pathway in addition to angiogenesis. Other VEGFR TKIs include vandetanib (AstraZeneca), sunitinib (Sutent®, Pfizer, Inc., New York), BIBF 1120 (Boehringer Ingelheim) and pazopanib (GlaxoSmithKline). Additional approaches to VEGF inhibition include the utilisation of soluble receptors such as VEGF Trap (AVE0005, Aflibercept, Sanofi-Aventis). This is a recombinant fusion protein comprising the extracellular ligand-binding domains of the VEGF receptors 1 and 2 fused to the constant region of the immunoglobulin IgG and acts as a soluble decoy receptor modulating the availability of VEGF ligand. Other angiogenic targets include angiopoietins (Ang-1,2 and 4) and integrins. AMG 386 is a peptide-Fc fusion protein that prevents the interaction of Ang-1 and -2 with the Tie2 receptor, thereby inhibiting angiogenesis. Integrin receptors are involved in endothelial cell adhesion, migration and proliferation. The integrin inhibitor, volociximab, is a monoclonal antibody against α5β1 integrin that blocks binding to fibronectin and hence angiogenesis. In contrast to angiogenic agents that inhibit new blood vessels, vascular disrupting agents (VDAs) such as combretastatin, target mature, established endothelial cells and pericytes. The precise mechanisms by which angiogenesis inhibition might improve clinical outcome remain controversial. One suggestion is that antiangiogenic agents might act by suppressing tumour vasculature formation and therefore depriving the tumour of nutrients and limiting tumour growth [5,9]. Another proposed mechanism is that these agents transiently normalise the tumour vasculature, allowing chemotherapy to be delivered more efficiently [11].

Targeting the VEGF pathway has proved successful in a number of advanced cancers, most notably colorectal and renal [12–16]. This has fuelled testing of the anti-VEGF approach in other cancers, including that of the ovary (Table 1).

Clinical studies (early phase) of angiogenesis inhibition

Bevacizumab

Two prospective, phase II clinical trials of bevacizumab have provided evidence that single-agent anti-VEGF therapy is a promising strategy in recurrent ovarian cancer [17,18]. Both studies involved women with relapsed ovarian cancer and used a regimen of 15 mg/kg every 21 days. The Gynecology Oncology Group [GOG] study 170D, reported by Burger and

Table 1
Reported phase II trials of anti-angiogenic agents in ovarian cancer

Reference	Treatment	Number of patients treated	Prior regimens	OR	SD	PFS (months)
Bevacizumab monotherapy and combination treatments						
Burger (2007) [17]	Bevacizumab[a]	62	⩽2 platinum-sensitive platinum-resistant	21%	52%	4.7
Cannistra (2007) [18]	Bevacizumab[a]	44	2–3 platinum-resistant	16%	25%	4.4
Micha (2007) [19]	Bevacizumab[a] carboplatin paclitaxel	20	0	80%	5%	NR
Chura (2007) [20]	Bevacizumab[b] cyclophosphamide	15	⩾2 platinum-sensitive platinum-resistant	53%	20%	3.9
Garcia (2008) [21]	Bevacizumab[b] cyclophosphamide	70	⩽3 platinum-sensitive platinum-resistant	24%	63%	7.2 (TTP)
Nimeiri (2008) [22]	Bevacizumab[a] Erlotinib	13	⩽2 platinum-resistant	15%	54%	4.1
TKIs						
Matulonis (2009) [23]	Cediranib	47	⩽2 platinum-sensitive platinum-resistant	17%	13%	5.2
Biagi (2011) [24]	Sunitinib	30	1–2 platinum-sensitive platinum-resistant	3%	53%	4.1
Matei (2011) [25]	Sorafenib	73	1–2 platinum-sensitive platinum-resistant	3%	34%	2.1
Friedlander (2010) [26]	Pazopanib	36	platinum-sensitive platinum-resistant	31% (GCIG CA125)	56% (GCIG CA125)	Response duration 113 days 17% at 6 months

BV: bevacizumab; CR: complete response; CT: computed tomography; NR: not reported; OR: objective response; PFS: progression-free survival; SD: stable disease; TTP: time to progression.
[a] 15 mg/kg every three weeks.
[b] 10 mg/kg every two weeks.

colleagues, recruited 62 patients with either platinum-sensitive disease (>6-month platinum-free interval) or platinum-resistant disease (<6-month platinum-free interval) and was limited to patients who had received no more than two prior chemotherapy regimens [17]. Burger and colleagues reported an objective response rate of 21% and stable disease in a further 52%. Forty percent were progression-free at 6 months and the median progression-free survival and overall survival were 4.7 months and 16.9 months respectively [17]. The AVF 2949 trial was restricted to patients with platinum, topotecan or liposomal doxorubicin-resistant disease and patients who had received up to three prior chemotherapy regimens [18]. In the AVF2949 trial, a response rate of 16%, median progression-free survival of 4.4 months and median survival duration of 10.7 months at study termination was reported [18]. However, this study was stopped after

accruing 44 patients because of a higher than expected incidence of bowel perforation (11%).

The major toxicities seen in the phase II trials of bevacizumab in ovarian cancer are similar to those observed in other solid tumours and include hypertension, thrombosis, proteinuria, haemorrhage and gastrointestinal (GI) perforation. In the GOG 170D trial, six patients (9.7%) developed grade 3 hypertension, two patients had venous thromboses (1.6%), one patient had grade 4 proteinuria (1.6%), which was reversible upon discontinuation of bevacizumab, no patients had greater than grade 1 haemorrhage or GI perforations [17]. More serious adverse events occurred in the AVF2949 study; they were noted in 41% of patients and were most commonly arterial thromboembolic events, GI perforation (11%) or obstruction and three deaths (myocardial infarction, GI perforation and hypertensive encephalopathy) were suspected to be bevacizumab treatment-related [18]. The reasons for the differences in toxicity profile are not clear, but it might be relevant that patients in the AVF 2949 study were more heavily pretreated and all had platinum-resistant disease may be important. The rate of GI perforation appears substantially higher in ovarian cancer (5.4%) than colorectal cancer (3.1%) [27]. Pre-existing small bowel obstruction and platinum-resistant disease are potential predisposing factors to perforation in ovarian cancer.

VEGF-Trap

The VEGF-Trap approach has been tested in a phase II trial. Fifty-four platinum-resistant ovarian cancer patients with recurrent symptomatic malignant ascites requiring 1–4 paracenteses per month, were randomised to receive intravenous aflibercept (4.0mg/kg every two weeks) or placebo. Preliminary results in recurrent, platinum-resistant ovarian cancer indicated that VEGF-Trap can prolong the time to repeat paracentesis compared with placebo (55 days vs. 23 days, $P = 0.0019$). However, fatal gastrointestinal perforations were observed in three patients (10%) in the aflibercept group and one patient (4%) in the placebo group [28]. Further evaluation to identify patients who are most likely to benefit from this treatment are needed; the efficacy of VEGF Trap in combination with docetaxel is also being evaluated in a phase I/II trial.

Tyrosine Kinase Inhibitors (TKIs)

Several VEGFR TKIs (cediranib, pazopanib, sorafenib, sunitinib and BIBF 1120) are under evaluation either as monotherapy or combination therapy in recurrent ovarian cancer. In a phase II study of 30 patients with recurrent ovarian cancer, sunitinib monotherapy was shown to have only minimal activity; this was limited to platinum-sensitive disease (partial response 3.3%; CA125 response 10% and median PFS 4.1 months) [24]. Similarly, the reported activity of single agent sorafenib in a phase II trial of sorafenib was low (PR 3.4%; stable disease 33.9%, median PFS 2.1 months) [25]. However, the VEGFR TKI, cediranib (AZD2171, AstraZeneca), has shown encouraging results in ovarian cancer. A phase II trial of cediranib demonstrated a clinical benefit rate (defined as CR or PR, SD >16 weeks, or CA-125 non-progression >16 weeks) of 30% and median PFS of 5.2 months [23]. Grade 3 toxicities were reported in more than 20% of patients and included hypertension (46%), fatigue (24%) and diarrhoea (13%), and grade 2 hypothyroidism occurred in 43% of patients. Cediranib is currently being evaluated in patients with platinum-sensitive relapsed disease in combination with chemotherapy and as maintenance therapy in the phase III ICON-6 trial. Another phase II study of cediranib in recurrent ovarian, peritoneal or fallopian tube cancer reported a clinical benefit rate of 41% in platinum-sensitive and 29% in platinum-resistant disease. The median time to progression (TTP) and median survival time for all patients was 4.1 months and 11.9 months respectively and there was no difference in TTP or survival between the platinum-sensitive and platinum-resistant groups [29]. A phase II trial of the VEGFR TKI, pazopanib (GlaxoSmithKline), in recurrent ovarian cancer reported CA125 response in 31% of patients with median time to response of 29 days and median response duration of 113 days [26]. A randomised trial in first-line treatment in which pazopanib is given as maintenance therapy is underway.

The PDGF and FGF pathways have been implicated in resistance to anti-VEGF/VEGFR agents and counteracting compensatory mechanisms by multi-targeting other pro-angiogenic pathways may improve the efficacy of anti-VEGF therapies [30]. BIBF 1120 simultaneously targets VEGFR, PDGFR and FGFR tyrosine kinases. Preliminary results of a randomised, placebo-controlled phase II study of continuous maintenance treatment with BIBF 1120 following chemotherapy in patients with relapsed ovarian cancer are encouraging, The 36-week progression-free survival rates were 15.6% for BIBF 1120 and 2.9% for placebo and suggest that maintenance BIBF 1120 could delay disease progression [31]. A randomised trial of BIBF 1120 versus placebo together with chemotherapy as a

maintenance treatment is now underway as first-line therapy.

Preliminary findings of a phase II study of XL184 (Exelixis), a MET, VEGFR2 and RET inhibitor, suggest that this agent is active in both platinum-sensitive and resistant/refractory ovarian cancer [32]. Of 31 evaluable patients, partial response and disease stabilisation rates were 32% and 48% respectively. Furthermore, subset analyses reported a partial response in 29% and disease control rate of 59% at 12 weeks in platinum-resistant/-refractory patients. Grade 3 toxicities included hand-foot syndrome (12%), diarrhoea (7%), fatigue (5%) and vomiting (5%). These encouraging observations warrant further evaluation in the phase III setting.

In summary, several VEGFR TKIs are now under evaluation in randomised trials both in first-line and recurrent ovarian cancer, either in combination with chemotherapy and/or as maintenance monotherapy. The optimal schedule and clinical scenario for these agents is currently under investigation.

Combination treatments with angiogenesis inhibitors

Several prospective phase II trials suggest that bevacizumab, in combination with chemotherapy (carboplatin/paclitaxel, topotecan or cyclophosphamide) is efficacious in patients with recurrent ovarian cancer [19–21,33]. The largest of these phase II trials (n = 70) studied the combination of bevacizumab (10 mg/kg on days 1, 8, 15 and then every two weeks) and metronomic chemotherapy with cyclophosphamide (50 mg/day) in patients with platinum-sensitive and -resistant disease who had received no more than three prior chemotherapy regimens. A response rate of 24%, stable disease in 63% and 56% 6-month progression-free survival were reported [21]. The median time to progression and median survival was 7.2 months and 16.9 months respectively. Interestingly, patients genotyped A/A or A/T for the IL-8 T-251A gene polymorphism had a statistically significant lower response rate than those who were homozygous T/T (19% vs. 50%; $P = 0.006$), which raised the possibility that IL-8 might be a potential molecular predictor of response to bevacizumab.

The efficacy of bevacizumab in combination with chemotherapy has been addressed in the phase III setting: a detailed discussion of the results of the Gynaecologic Oncology Group (GOG) trial 218 and International Collaborative Ovarian Neoplasm (ICON) 7 trial, which tested bevacizumab in combination with carboplatin/paclitaxel in front-line ovarian cancer will be presented in an article by Bookman. The results of the OCEANS trial, a phase III study evaluating the efficacy of bevacizumab in combination with carboplatin and gemcitabine in women with platinum-sensitive recurrent ovarian cancer, are scheduled for presentation at ASCO 2011.

A phase II trial of the VEGFR TKI, sorafenib in combination with gemcitabine reported an objective response of only 4.7% in recurrent disease [34]. However, disease maintenance or stabilisation for six months was observed in 23.3% of cases and 27.9% of patients had a response using GCIG CA125 criteria; the median TTP was 5.4 months. The most common non-haematological adverse events were hand-foot syndrome, fatigue, diarrhoea and hypokalemia [34]. A phase I study demonstrated that aflibercept at 6 mg/kg in combination with docetaxel (75 mg/m^2) can be safely administered in recurrent ovarian cancer and preliminary results suggest that this has some efficacy with partial responses observed in 2/9 (22%) of patients [35]. A phase II three-arm adaptive randomisation trial studied the integrin inhibitor, volociximab (15 mg/kg q2wk or qwk) in combination with liposomal doxorubicin (PLD) compared with PLD alone. Disappointingly, the addition of volociximab was not superior to PLD alone [36]. However, early results from a phase II study of weekly taxol in combination with AMG 386 (an angiopoetin-Tie2 inhibitor) at two doses (10 mg/kg and 3 mg/kg) suggest promising activity with a dose–response effect. In this randomised placebo-controlled trial, which included 161 patients with recurrent ovarian cancer, the median PFS was 7.2 months on the paclitaxel/AMG 386 10 mg/kg arm compared with 4.6 months in patients treated with paclitaxel/placebo (HR = 0.76, $P = 0.23$) [37]. The toxicity profile of AMG 386 appears to differ from that of VEGF inhibitors; peripheral oedema, hypokalemia and thromboembolism were the most common side-effects and no grade 3 hypertension was noted. Studies of AMG in combination with either PLD or topotecan are ongoing [38]. Finally, a phase II study of the vascular disrupting agent, combretastatin (CA4P), in combination with carboplatin and paclitaxel reported a response rate of 34% by GCIC CA125 criteria and 13.5% radiological response in 44 patients with platinum-resistant disease. Neutropenia (grade $\geqslant$2) was common (75%) and other toxicities include hypertension (23%), tumour pain, fatigue and neuropathy [39].

Angiogenesis inhibition with other targeted agents

The 'vertical' approach of VEGF inhibition, i.e. targeting both the ligand and receptor, is being explored in the clinic. Although partial responses were seen in 6 out of 13 patients with ovarian cancer (43%; response duration range, 4–22+ months) within a phase I study of sorafenib in combination with bevacizumab, the regimen was not well tolerated with the majority of patients requiring dose reductions: grade 1–4 hypertension was seen in 67% patients; 79% of patients experienced hand-foot syndrome and 2 out of 13 ovarian cancer patients developed enteral fistulae [40]. An alternative approach that appears promising in early clinical trials and merits further exploration in ovarian cancer is the combination of a vascular disrupting agent, e.g. combretastatin and bevacizumab [41]. This is based on results from preclinical studies that have shown significant enhancement of anti-tumour activity with the combination [42] and reversal of combretastatin-induced mobilisation of circulating endothelial progenitor cells by VEGF inhibition [43].

Dual blockade of the VEGF and EGFR pathways has been hypothesised to provide additional clinical benefit. However, in a phase II trial that evaluated bevacizumab in combination with the EGFR inhibitor erlotinib in recurrent ovarian cancer, there was no initial indication that this combination was superior to bevacizumab alone and the incidence of grade 3 diarrhoea was higher than expected. Furthermore, fatal gastrointestinal perforations in 2 of the 13 recruited patients resulted in the closure of the study [22].

Overall, the clinical efficacy of single-agent bevacizumab in ovarian cancer demonstrated by phase II trials is considerably greater than that observed in colorectal (3%) and breast cancers (6.7%) [44] and suggests that this strategy, either as a single agent or in combination, should be considered for further study as a treatment for recurrent ovarian cancer.

Poly(ADP)Ribose Polymerase (PARP) Inhibitors

Repair pathways of DNA are critical for the maintenance of genome integrity and the response to DNA-damaging chemotherapy. Drugs have been designed to target DNA repair pathways to enhance the effects of chemotherapy and to exploit intrinsic deficiencies in specific DNA pathways. Targeting the base excision repair (BER) pathway with polyadenosine diphosphate-ribose polymerase (PARP) inhibitors appears promising in ovarian cancer, with initial data indicating efficacy in the BRCA mutation-associated disease [45]. Heterozygous carriers of germ-line *BRCA1* or *BRCA2* mutations are at a highly elevated risk of developing ovarian cancer (10–40% lifetime risk). The BRCA1 and BRCA2 proteins are critical for the maintenance of genome integrity and play important roles in the repair of DNA double-strand breaks via a homologous recombination (HR) pathway [46]. As a consequence, tumour cells arising in carriers of *BRCA1* or *BRCA2* mutations are defective in HR DNA repair. PARP is a nuclear enzyme that signals the presence of DNA damage by causing the addition of ADP-ribose polymers to DNA, which facilitates the repair of single-strand breaks via the BER. Inhibition of PARP causes the generation of specific DNA lesions, most likely double-strand breaks or collapsed replication forks that require functional BRCA1 and BRCA2 for DNA repair. Cell lines lacking wild-type BRCA1 or BRCA2 are highly sensitive to potent PARP inhibitors compared with heterozygous mutant or wild-type cells [47,48]. Patients with BRCA-associated cancers lack wild type BRCA1 or BRCA2 in tumour cells, but normal cells retain a single wild type copy of the gene. This difference between tumour and normal cells means that PARP inhibitors kill tumour cells selectively compared with the effects in normal cells. This approach utilises the concept of synthetic lethality, which describes the situation whereby two pathway defects acting individually have little effect, but when combined become lethal [46]; thus, it has been suggested that this approach might create a large therapeutic window.

The profound sensitivity of BRCA mutant cells to PARP inhibitors has led to the design of clinical trials to test this approach. This concept of "synthetic lethality" was tested in a phase I clinical trial of olaparib (formerly known as KU-0059436 and AZD2281) in patients with refractory solid tumours [49]. The study design enriched for patients with a mutation in *BRCA1* or *BRCA2*. Olaparib at doses of 60 mg twice daily and above was associated with greater than 90% inhibition of PARP. Dose-limiting toxicities were observed at 400 mg and 600 mg (twice daily), and therefore the dose-expansion cohort of *BRCA1/2* mutation carriers received 200 mg twice daily. Adverse effects were minimal (predominantly gastrointestinal and fatigue) and strikingly, partial responses in 63% of patients (12/19) were documented, including 8 patients with ovarian cancer.

This trial has now reported an expanded cohort of 50 patients with advanced *BRCA1/2* mutation associated ovarian, primary peritoneal and fallopian

Table 2
Early-phase clinical trials of PARP inhibitors in ovarian cancer

Trial	Study population	n	Treatment	RR	CBR	PFS or RD
Audeh (2010) [51]	*BRCA*-associated recurrent advanced ovarian cancer failure ⩾1 prior platinum agent	57	Olaparib 400 mg and 100 mg	400 mg dose: RECIST 33% RECIST/GCIG 61%	66%	PFS 5.8 months
Fong (2010) [50]	*BRCA*-associated ovarian cancer Progressed following platinum agent	50	Olaparib	RR (RECIST and/or GCIG) 43.5%	overall 50% Platinum-sensitive:69% Platinum-resistant: 50% Platinum-refractory:27% ($P = 0.038$)	RD 31 weeks

RR: response rate; RD: response duration

tube cancers [50]. The clinical benefit rate (RECIST and/or CA125 response was 46% (RR 40%; stable disease for more than four months, 6%) and the median duration of response was 28 weeks (range 10–86 weeks). Importantly, the overall clinical benefit rate decreased significantly with platinum insensitivity (platinum-sensitive: 69%, platinum-resistant: 46%; platinum-refractory 23%). Furthermore, there was a positive association between the overall platinum-free interval and response to olaparib ($P = 0.002$). Although the clinical efficacy of olaparib was shown to diminish with decreasing platinum-free interval, it is noteworthy that the anti-tumour activity was still substantial, particularly in platinum-resistant disease compared with other known agents (Table 2).

The promising results described above led to a phase II multi-centre single-arm, open-label sequential dosing cohort study of *BRCA1/2* mutation carriers with recurrent ovarian cancer [51]. In this trial, women received 400 mg twice daily (33 patients) or 100 mg twice daily (24 patients). Efficacy was confirmed in both dosing cohorts, but was higher in those receiving the higher dose level (33% vs. 12.5%) (Table 2). Toxicities were mild (grades 1, 2) and consisted of nausea, vomiting, fatigue and anaemia. Given the promising clinical findings seen with olaparib and the indication of a dose-response relationship, a randomised phase II study addressed the comparative efficacy of olaparib at two dose levels vs. pegylated liposomal doxorubicin (PLD, caelyx) in women with BRCA mutation-associated recurrent ovarian cancer [52]. Ninety-seven patients with a platinum-free interval of ⩽12 months were randomised in a 1:1:1 ratio to olaparib 200 mg bid or 400 mg bid continuously, or PLD 50 mg/m^2 intravenously every 28 days. No statistically significant differences in PFS (olaparib 200 mg bid 6.5 months; olaparib 400 ng bid 8.8 months; PLD 7.1 months; HR 0.88, $P = 0.66$) were observed between the combined olaparib doses and PLD, although the combined RECIST and CA125 response rate was significantly higher for olaparib 400 mg bid than for PLD (59% vs. 39%, odds ratio 2.78, $P = 0.05$). It is noteworthy that the median PFS in the PLD group of 7.1 months was considerably greater than predicted. In a large phase III randomised trial of patients with a similar mix of platinum-resistant and platinum-sensitive cancers and unknown BRCA status, who received PLD (versus topotecan), the median PFS was only four months [53]. Furthermore, a retrospective study in sporadic breast cancer demonstrated that deficiency in HR (as indicated by low RAD51 formation) was strongly predictive of pathological complete response to anthracycline-based chemotherapy [54]. This raises the possibility that patients with HR-deficient ovarian cancer may also derive a better clinical outcome from anthracyclines such as PLD compared with unselected cases. The longer than expected PFS in the PLD arm is likely to have affected the power of the study to detect superiority in PFS between olaparib and PLD. Overall, both treatments were well tolerated (<10% of discontinuation owing to toxicity) and the data did suggest that the higher dose of olaparib was more effective.

In addition to olaparib, clinical trials in ovarian cancer of a number of other PARP inhibitors as monotherapy in ovarian cancer are under investigation. These include MK4827 (Merck), PF-01367338 (Pfizer, previously AG014699), CEP-9722 (Cephalon) and ABT-888 (Abbott Laboratories). Preliminary results of a phase I study of MK4827 are particularly

encouraging, with efficacy data that are comparable to those of olaparib [55]. Further studies of single-agent PARP inhibitor therapy in BRCA mutation-associated ovarian cancer are therefore clearly warranted.

As well as patients with tumours with specific genetic defects such as BRCA mutations, PARP inhibitors may also show activity in tumours with the property of "BRCAness" [56]. This describes the situation whereby an HR DNA repair defect is present, but no germline *BRCA 1* or *BRCA2* mutation is detected. This property has been proposed to be present in a substantial subset of ovarian cancers. For example, up to 15% of sporadic ovarian cancers harbour *BRCA1* gene promoter methylation and BRCA1 protein expression is undetectable in many of these tumours, which suggests silencing of the *BRCA1* gene [57]. These and other data suggest that up to 50% of high-grade serous, sporadic ovarian cancers may have defects (including somatic BRCA mutations, BRCA methylation, dysfunction of other genes involved in HR) that confer sensitivity to PARP inhibition, suggesting a much wider utility of this approach [45,58,59].

Whether single-agent PARP inhibitor is clinically active in patients with sporadic (non-*BRCA* mutant) recurrent ovarian cancer was addressed by Gelmon and colleagues [60]. This phase II study included patients with high-grade serous/undifferentiated ovarian cancer with unknown *BRCA* status or *BRCA*-negative treated with olaparib 400 mg bid. In addition, there was a reference group known to have germ-line BRCA mutations treated within the trial. In keeping with previous studies, the objective response rate in the *BRCA* mutant patients (n = 17) was 41.2%. Strikingly, among the 47 *BRCA*-negative patients, 24% achieved a radiological objective response with a median response duration of 31 weeks. Responses were noted mostly in platinum-sensitive (radiological 50%; CA125 40%) compared with -resistant/-refractory (radiological 3.8%; CA125 17.4%) disease [61]. This is the first demonstration of meaningful clinical activity of a PARP inhibitor in sporadic ovarian cancer and provides clear evidence of the role of PARP inhibitors in non-*BRCA* mutant ovarian cancer. These data are of particular interest, as the results of a randomised placebo-controlled study in which single-agent olaparib was administered as a maintenance treatment to responding patients (including those with BRCA-negative serous sporadic ovarian cancer) after they have completed chemotherapy for platinum-sensitive relapsed disease are expected at ASCO 2011 (Clinical trials identifier: NCT00753545).

Several phase II and phase III clinical trials are currently evaluating the use of PARP inhibitors in combination with chemotherapy known to induce DNA strand breaks, such as platinum compounds and temozolomide. The rationale for this approach is that following exposure to cytotoxic therapy, DNA damage occurs and DNA repair mechanisms such as base excision repair, of which PARP is a key component, are activated. This can reverse the effects of chemotherapy resulting in resistance. Therefore, PARP inhibition in combination with DNA-damaging agents may enhance the effects of chemotherapy and potentially delay resistance to treatment. This was confirmed in preclinical studies of various PARP inhibitors in various tumour models that demonstrated that PARP inhibitors potentiate the tumour-suppressive effects of different chemotherapies [62–64].

Results of studies of PARP inhibitors with chemotherapy have not yet been reported in ovarian cancer although randomised trials in first-line and recurrent disease are planned or underway. Ahead of these, results from a randomised phase II study of 123 patients with metastatic, triple-negative breast cancer (TNBC) have been provocative since TNBC is considered to share some properties with BRCA mutation-associated disease. Iniparib (BSI-201) in combination with gemcitabine and carboplatin significantly improved both progression-free survival (3.6 vs. 5.9 months, HR 0.59, $P = 0.01$) and overall survival (HR 0.57, $P = 0.01$; median OS BSI-201 and chemotherapy 12.3 months, chemotherapy alone 7.7 months) compared with chemotherapy alone [65]. The treatment schedules were particularly noteworthy; both gemcitabine and carboplatin (AUC 2) were given on days 1 and 8 of a 21-day cycle, and iniparib was given i.v. on days 1, 4, 8 and 11 of each cycle. Curiously, the addition of iniparib to chemotherapy did not appear to enhance toxicity and there was no difference in dose reduction rates between the study arms. This is contrary to experience with various other combinations of chemotherapy with other PARP inhibitors where enhanced myelosuppression is invariably seen [66,67]. The explanation for the remarkably positive results seen with iniparib is not clear. The precise mechanisms of action of iniparib are not fully known and these results may not necessarily be reproduced with a different PARP inhibitor/chemotherapy combination. Nevertheless, based on the striking results, a phase III trial of gemcitabine/carboplatin ± iniparib in TNBC patients with metastatic disease was launched and intriguingly, although the detailed results of this trial are still awaited, BiPar Sciences and Sanofi-Aventis have recently announced that this study of 519 patients

Table 3
Published phase II trials of targeted therapy in ovarian cancer (excluding anti-angiogenic agents and PARP inhibitors)

Reference	Number of patients	Treatment	Result
EGFR			
Schilder (2005) [71]	27	Gefitinib	RR 4%
Wagner (2007) [72]	56	Gefitinib + tamoxifen	RR 0%, SD 29%
Posadas (2007) [73]	24	Gefitinib	RR 0%, SD 37%
Gordon (2005) [70]	34	Erlotinib	RR 6%, SD 44%
HER2			
Bookman (2003) [69]	41	Trastuzumab	RR 7.3%
Gordon (2006) [74]	61	Pertuzumab	RR 4.3%, SD 6.8%, PFS 6.6 wks
Other			
Coleman (2006) [75]	16	Imatinib	RR 0%, SD 33%

RR: response rate; SD: stable disease; PFS: progression-free survival.

failed to show a benefit overall from the addition of iniparib in terms of the co-endpoints of PFS and OS [68].

EGFR and HER2 inhibitors

Tyrosine kinase receptors EGFR and HER2 are involved in cell proliferation and survival and there is clinical evidence to suggest that these might be potential therapeutic targets in ovarian cancer. However, results from phase II trials of erlotinib, gefitinib (EGFR TKIs), trastuzumab (monoclonal antibody targeting HER2) and pertuzumab (monoclonal antibody targeting HER2 dimerisation inhibitor) have all been relatively disappointing (Table 3) [69–73]. A phase II study (GOG 170-C) of gefitinib monotherapy in relapsed ovarian cancer (unselected on the basis of EGFR status) demonstrated low activity (1/27 [4%] objective response and 4/27 [15%] progression-free at six months). Interestingly, the only patient with an objective response was later shown to have a mutation altering the catalytic domain of *EGFR* [71]. Erlotinib was studied in combination with docetaxel and carboplatin in ovarian cancer and although responses were noted, this regimen was associated with significant grade 3 neutropenia [76]. In this study, following six cycles of treatment, patients were permitted to receive erlotinib (150 mg/day) as maintenance therapy. Forty-four percent of patients required dose interruptions or modifications because of cutaneous toxicity. The results of the phase III EORTC55041/OVO7 trial will determine whether there is a role for maintenance erlotinib following first-line platinum-based chemotherapy. The GOG 160 phase II trial evaluated trastuzumab in ovarian cancer and reported an overall response rate of 7.3% in the 41 eligible patients with HER2 overexpression. The effectiveness of anti-HER2 therapies may have been limited by low levels of HER2 "over-expression" (11%) and gene amplification [69]. HER2 activation may be a better predictor of sensitivity to HER2-targeted agents than HER2 expression. In support of this, patients with HER2 phosphorylation (pHER2+) had a better outcome following pertuzumab compared with pHER2-negative tumours (PFS 20.9 weeks vs. 5.8 weeks; $P = 0.14$) [74]. EGFR and/or HER2 inhibitors combined with chemotherapy could potentially improve the efficiency of chemotherapy. However, so far, the results of this approach are generally disappointing. A phase II trial of topotecan in combination with lapatinib showed only modest activity in recurrent ovarian cancer [77]. The combination of gemcitabine with pertuzumab was studied in a randomised, placebo-controlled phase II study of women with platinum-resistant ovarian cancer. Although the improvement in PFS observed in the combination arm did not reach statistical significance (2.6 vs. 2.9 months; HR 0.66, $P = 0.07$), exploratory analyses suggested increased treatment benefit associated with low HER3 expression (PFS HR = 0.32; $P = 0.0002$) [78]. Further randomised trials in platinum-resistant disease, focussing on this patient subgroup, are planned; preclinical data suggest that tumour cells with low HER3 expression exhibit increased activity of the AKT pathway [79], modulation of which may explain the impact of pertuzumab on response to chemotherapy.

Other targeted strategies

Multiple components of signalling cascades are aberrant in ovarian cancer, resulting in activation of critical oncogenic pathways involved in processes such as cell proliferation, survival, migration and angiogenesis.

PI3K/AKT

Interest in the PI3K/AKT pathway in ovarian cancer is high because of reports of activation of this pathway [80,81]. This probably relates to amplification rather than mutations, which are rare. Increased activity may contribute to resistance to platinum [82] or taxanes [83]; hence, AKT inhibitors may have their main application in combination with chemotherapy. So far, single-agent activity in ovarian cancer in phase I trials of PI3K/AKT inhibitors has rarely been seen [84].

IGFR

The IGFR pathway has also been implicated in ovarian cancer and in overcoming resistance to chemotherapy and other targeted agents (HER2, EGFR). Several agents are under evaluation in early-phase clinical trials (CP-751,871 (figitumumab), MK-0646, AMG479, OSI-906). AMG479 (IGF-1 receptor antibody) is under evaluation as first-line therapy (patients optimally debulked) in combination with chemotherapy and as monotherapy in recurrent ovarian cancer. A phase I/II study of OSI-906, a small molecule dual kinase inhibitor of both insulin-like growth factor-1 receptor and insulin receptor, in combination with weekly paclitaxel is ongoing.

Src

Src emerged as a potential target in platinum-resistant disease through gene expression studies [85] and saracatinib (AZ0530) is a potent, selective Src inhibitor. Even though no single-agent activity has been seen, combination studies have been pursued. So far, a negative, randomised trial in platinum-sensitive disease has been reported in which saracatinib was added to carboplatin and paclitaxel [86]. A randomised trial with weekly paclitaxel in platinum-resistant disease is underway.

Ras/Raf MEK

Studies of mutation analyses [87,88] suggest that RAS/Raf/MEK inhibitors may be of particular relevance in low-grade invasive disease (representing 10–15% serous ovarian cancer), which has limited treatment options at the advanced stage. A GOG trial of the MEK inhibitor AZD6244 recently completed recruitment and results are expected at ASCO 2011.

Cell Cycle dysregulation

Aurora kinase A is involved in microtubule formation and stabilisation at the spindle pole during chromosome segregation. Preliminary results of a phase II study of the oral, selective aurora kinase A inhibitor, MLN8237, suggest some efficacy, with durable responses in patients with platinum-resistant ovarian cancer (26% PR or durable SD; median PFS 77 days); this may merit further exploration in selected patient subgroups [89]. Another potential target is Wee-1 kinase. This kinase regulates the G2/M checkpoint and it has been proposed that inhibition of Wee-1 kinase may lead to chemosensitisation of p53-deficient tumour cells. A preliminary report of a phase I study of MK-1775, a potent and selective inhibitor of Wee-1 kinase, as monotherapy and in combination with chemotherapy shows encouraging activity [90]. This agent, in combination with carboplatin, has entered phase II trials in ovarian cancer, and randomised trials in platinum-sensitive relapsed disease are underway.

Hedgehog

Ligand-dependent activation of the hedgehog signalling pathway has been reported in ovarian cancer [91], leading to tumour growth and spread. GDC-0449 is an oral agent that inhibits this pathway and has marked activity in basal cell cancer, in which increased hedgehog-pathway activity is mutation-driven. However, a phase II, randomised, double-blind, placebo-controlled trial of GDC-0449 as maintenance therapy for patients with ovarian cancer in second or third remission failed to demonstrate a statistically significant increase in PFS (7.5 months in the GDC-0449 arm vs. 5.8 months in the placebo arm) [92]. Careful patient selection is critical for the future of agents of this type.

Folate receptor

The folate receptor is overexpressed in >90% of ovarian cancers. Farletuzumab (MORab-003, Morphotek) is a monoclonal antibody directed against the α-folate receptor. It was evaluated in a phase II multicentre study in which platinum-sensitive relapsed patients received carboplatin/paclitaxel together with farletuzumab. In 9/44 (21%) patients, the second remission was equal to, or longer, than the first

remission [93]. Farletuzumab is currently being investigated in phase III trials. EC145 is a conjugate of a vinblastine analogue to folate and is being investigated in ovarian cancer. An interim analysis of a randomised, phase II study of EC145 + PLD compared with PLD alone, in patients with platinum-resistant ovarian cancer reported a greater than doubling in median PFS with the addition of EC145 (24 weeks vs. 11.7 weeks HR 0.50, $P = 0.014$) [94].

Challenges facing the optimal use of targeted therapies

Several important issues remain to be addressed if the potential of targeted therapies in ovarian cancer is to be fully realised. Undoubtedly, the major challenge is the identification of patients who are most likely to benefit from treatment. The successes of trastuzumab in HER2-positive breast cancer [95] and imatinib in GIST [96] are examples of the importance of treatment directed by patient selection based on molecular characteristics. So far, no reliable biomarkers have been identified as prognostic or predictive following anti-VEGF therapy. Circulating angiogenic factors such as VEGF and soluble VEGF receptors are under investigation as surrogate endpoints and need further validation [97]. A report suggested that interleukin-8 and VEGF polymorphisms could be potential markers of clinical outcome following bevacizumab-based chemotherapy in refractory ovarian cancer [98]. However, It is possible that no robust, predictive biomarker of anti-VEGF therapy exists, since the treatment essentially targets the tumour–stroma interaction. An alternative approach to patient selection would be to incorporate functional imaging at early time points in the treatment cycle. Techniques such as DCE-MRI and MRI spectroscopy will more directly monitor the activity of an anti-angiogenic agent than conventional radiological techniques, and measurable changes can generally be seen in the first week.

For PARP inhibitors, the situation is rather different in that there is clear evidence that a subgroup of patients, i.e. those with HR-deficient cancers, are more likely to derive benefit. The challenge is simple: how can they be identified for treatment? One option is to measure the formation of nuclear RAD51 foci after DNA damage, although this is technically challenging. The lack of formation of RAD51 foci would indicate patients who might respond to the drug [54]. Gene expression or immunohistochemical signatures of deficiency of *BRCA1* or *BRCA2* expression or HR defects are also being explored [99].

In the context of PI3K/AKT inhibition, recent work from our group has focused on the use of tumour cells isolated from ascites in patients with relapsed disease. Initial data indicate that when this pathway is most activated, response to subsequent chemotherapy is worse [100]. This may be a way of identifying patients who may benefit most from a PI3K/AKT inhibitor as a means of enhancing the effect of chemotherapy in relapsed disease.

Although clinical response or disease stabilisation may be achieved with targeted therapy, this is inevitably followed by tumour progression. The mechanisms related to resistance to targeted therapy are complex and is an area of ongoing research. For antiangiogenic agents, alterations in the expression of angiogenic factors and changes in the tumour and endothelial cells could be responsible. In addition, tumour cells could become more dependent on alternative angiogenic factors or rely on alternative methods of vascularisation to obtain the essential blood supply [101]. Strategies such as combining multiple antiangiogenic agents, antiangiogenic agents with classical agents or other targeted agents may overcome resistance. Biomarker analyses in phase III trials of targeted therapies in ovarian cancer will be critical to help to identify these mechanisms.

Potential mechanisms of resistance to PARP inhibitors have been elucidated from preclinical models, and indirectly in the clinic by studying platinum resistance in BRCA mutation carriers. One proposed mechanism of PARP inhibitor resistance is the restoration of HR secondary to gain of function mutations in the *BRCA2* allele (elimination of the c.6174delT mutation) rather than PARP directly [102]. Interestingly, some patients who have responded to olaparib and then develop resistance have been reported to retain sensitivity to further platinum-based treatment [103]. It will be essential to understand better the mechanisms of clinical resistance to PARP inhibitors so that the optimal sequencing of PARP inhibitors and platinum can be defined. The optimal dosage and scheduling of concurrent PARP inhibitor and cytotoxic agent therefore require carefully designed clinical trials linked to preclinical studies specifically addressing the above issues.

Finally, the long-term safety of targeted agents is yet to be established. Toxicities of concern with anti-VEGF agents include hypertension, thromboembolism, perforation and proteinuria [104]. VEGF stimulates neurogenesis and protects neurons from ischaemic damage and therefore the long-term consequences of VEGF inhibitors also need to be considered. Although PARP inhibitors are generally

very well tolerated, anaemia can develop with long-term treatment with a theoretical increase in the risk of second malignancies. This possibility would be particularly relevant if PARP inhibitors were to be used as a chemoprophylactic strategy to prevent the development of *BRCA* mutation-related cancers.

Conclusions

Ovarian cancer remains a treatment challenge. The most critical problem in the treatment of advanced ovarian cancer is that the disease remains incurable for the majority of patients. The hope is that if molecularly targeted therapy is better tolerated than conventional chemotherapy, patients may be able to continue treatment and derive benefit for prolonged periods of time; continuous treatment with cytotoxic chemotherapy is usually not practical owing to toxicity. However, although targeted agents are not associated with traditional chemotherapy-related toxicities such as significant bone marrow suppression or neuropathy, they are associated with side effects. Their toxicity profiles are different from those of chemotherapy and the long-term effects of these novel agents are poorly defined. Many of these toxicities, such as hypertension, are manageable, but rarer, life-threatening complications (gastrointestinal perforation, haemorrhage) may also occur. The cost-benefit as well as the risk-benefit ratio needs to be considered if these agents are to become part of routine treatment.

Numerous targeted therapies are currently being evaluated in phase I/II and III studies, which should clarify their potential clinical use. The most promising strategies developed so far are the anti-angiogenic approach and PARP inhibitors. Further challenges facing the success of targeted therapy include the identification of the correct population to treat, biomarkers to assess response, overcoming drug resistance and managing toxicities. Key issues that are under investigation include whether combination therapy is superior to monotherapy and if there is a role for maintenance therapy. Understanding more about the molecular abnormalities involved in ovarian cancer will be critical in progressing in this field and in improving clinical outcome.

Conflict of interest statement

S. Banerjee has no conflict of interest. S. Kaye has served on the advisory boards of Astra Zeneca, Roche, Boehringer Ingelheim, OSI and Sanofi-Aventis.

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