Review Article

Novel Targeted Therapies in Ovarian Cancer

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Abstract

Background: Traditional management of women with advanced ovarian cancer with surgery and chemotherapy provides limited duration of survival. Targeting specific pathways in angiogenesis or DNA repair may provide therapeutic benefit.

Results: In this manuscript, we review the use of various compounds including antibodies that target specific growth factors receptors or ligands for receptors as well as small molecule compounds that inhibit tyrosine kinase activities or regulate the DNA repair mechanism in the management of women with ovarian, fallopian tube and primary peritoneal cancers. Randomized clinical trials have shown a PFS benefit in the use of bevacizumab in the adjuvant setting and at disease recurrence. Randomized clinical trials are currently ongoing with several PARP inhibitors in the maintenance setting post-chemotherapy and in the recurrent disease setting.

Conclusion: In particular, bevacizumab an antibody to VEGF-A and the PARP inhibitors show promise in extending overall survival from this disease. Use of these agents especially in a maintenance strategy is promising.

Keywords: Ovarian cancer; PARP inhibitors; Angiogenesis

Abbreviations: AGO: Arbeitsgemeinschaft Gynaekologische Onkologie; AUC; Area Under The Curve; Brcam: BRCA Mutation; Brcawt: BRCA Wild Type; CCC: Clear Cell Cancer; CI: Confidence Intervals; CR: Complete Response; DAVLBH: Desacetylvinblastine Hydrazide; DFI: Disease Free Interval; DLT: Dose Limiting Toxicities; EC: Endometrioid Cancer; ECOG: Eastern Cooperative Oncology Group; EGFR: Epidermal Growth Factor Receptor; EOC: Epithelial Ovarian Cancer; FACT-O: Functional Assessment Of Cancer Therapy – Ovary; FGFR: Fibroblast Growth Factor Receptor; FR: Folate Receptor; FTC: Fallopian Tube Cancer; Gbrca: Germline BRCA Mutation; GI: Gastro-Intestinal; GOG: Gynecologic Oncology Group; HGSC: High Grade Serous Cance; HR: Hazard Ratio: HRD: Homologous Recombinant Deficiency; ICON: International Cooperative Group Neoplasia; IP: Intra-Peritoneal; IV: Intravenous; LGSC: Low Grade Serous Cancer; MC: Mucinous Cancer; MTD: Maximum Tolerated Dose; Mtor: Mammalian Target Of Rapamycin; OC: Ovarian Cancer; ORR: Objective Response Rate; OS: Overall Survival; PARP: Poly (ADP-Ribose) Polymerase; Parpi: PARP Inhibitor; PCFT: Proton-Coupled Folate Transporter; PLGF: Placental Growth Factor; PDGFR: P-Growth Factor Receptor; PLD: Pegylated Liposomal Doxorubicin; PFS: Progression Free Survival; PPC: Primary Peritoneal Cancer; PR: Partial Response; QOL: Quality of Life; RCT: Randomized Controlled Trial; RECIST: Response Evaluation Criteria In Solid Tumors; RFC: Reduced Folate Carrier; ROC: Recurrent Ovarian Cancer; SD: Stable Disease; SMDC: Small Molecule Drug Conjugate; Tbrca: Tissue BRCA Mutation; TILS: Tumor-Infiltrating Lymphocytes; Tregs: Regulatory T Cells; VEGF: Vascular Endothelial Growth Factor; VEGFR: VEGF-Receptor

Introduction

In 2012, ovarian cancer affected 239,000 women globally and accounted for 4% of all new cancer cases in women. It is the 8th commonest cause of death from cancer in women [1] and the most lethal gynecologic malignancy. Of those women who present with ovarian cancer, 90% have epithelial histologic type of ovarian cancer. Most of these women have advanced disease (Stage 3,4). Treatment options include cytoreductive surgery followed by platinum-taxane based chemotherapy or interval debulking surgery with chemotherapy

preceding and following the operation. Outcomes are similar with either approach [2]. Although initial response to treatment is very good, 5 year survival rates are poor (5-yr survival 44%) [3].In other words, 70% of women will relapse with this disease. Although use of IV/IP chemotherapy or weekly dose dense paclitaxel has been studied, patients eventually acquire drug resistance. New and more effective treatments and treatment strategies are needed to optimize quality and duration of life for women with this disease.

In the past, oncologists treated all women with epithelial ovarian cancers (EOC) the same. However, the various histologic types of EOC have different responses to chemotherapy and we have since learned that they display divergent molecular characteristics [4,5]. EOC can be dividend histologically into high grade serous cancer (HGSC), clear cell cancer (CCC), endometrioid cancer (EC), mucinous cancer (MC) and low grade serous cancer (LGSC). However there is also an important use of this classification based on potential targets for management. Table 1 provides a condensed version of that found in Bookman's paper [4]. Up until this time, the evaluation of novel targeted agents has by-enlarge been applied to all EOCs, with in some situations, an attempt to understand whether molecular targets identified post hoc could inform in whom these agents would be beneficial. In the future, treatment may become more specific as biomarkers clarify which targeted agents are beneficial prior to application.

Whether or not designer agents to these potential targets will offer a significant survival advantage remains to be determined. Below we will discuss the various targeting compounds including antibodies (that target specific growth factor receptors or ligands for receptors)

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	HGSC	CCC	EC	MC	LGSC
Precursor lesions	Serous tubal intraepithelial carcinoma	Endometriosis	Endometriosis	Unknown	Serous borderline tumor
Molecular genetics	P53, BRCA1, BRCA2. HRD with genomic instability	PI3K, ARID1A, MSI	PTEN, βcatenin, ARID1A, MSI	KRAS, HER2	BRAF, KRAS, NRAS
Potential targets	PARPi, Angiogenesis	Angiogenesis PI3K	Hormone receptors, mTOR	HER2/neu	BRAF, MEK

HRD-homologous recombination deficiencies (NF1, RB1, CDK12)

Table 1: Sub classification of epithelial ovarian cancers [amended from 4].

and small molecule compounds (that inhibit tyrosine kinase activities or regulate the DNA repair machinery). The areas covered include: angiogenesis inhibitors, EGF family of inhibitors, agents targeting the folate receptor, mTOR inhibitors, targeting p53 mutated tumors, PKC-beta inhibition, MEK inhibitors, inhibition of DNA repair, and immune checkpoint inhibitors. The agents of greatest promise include anti-angiogenic agents and polyadenosine diphosphate-ribose polymerase (PARP) inhibitors (PARPi)

Angiogenesis inhibitors

Simple diffusion can nourish a tumor mass until its 3-dimensional mass is 0.125 mm³; thereafter, new vessels are required to supply nutrients and oxygen. Angiogenesis involves the process of developing new blood vessels in an area of new tissue growth like cancer. As the oxygen tension in the tissues fall below the level required for oxidative metabolism, a protein known as hypoxia inducible factor (HIF 1 alpha) is liberated. HIF 1 alpha couples with HIF-1 beta and up regulates growth factors like vascular endothelial growth factor (VEGF). The VEGF family includes 6 related molecules of which VEGF-A is the most important. VEGF and other cytokines and growth factors like placental growth factor (PGF) 1 and 2 and 3 transmembrane receptor tyrosine kinases (VEGFR 1-3) lead to loop vessel and tubule formation. Ultimately neovascularisation occurs. VEGF is also responsible for changes in vascular permeability leading to the development of ascites and pleural effusions. Anti-VEGF monoclonal antibodies can potently inhibit cell growth, lead to decreased tumor microvessel density, and inhibit tumor angiogenesis and reverse the permeability changes. Pruning the neovasculature also retards shedding of metastatic cells into circulation and improves delivery of therapeutic agents to tumor.

Antibodies, peptides (ie., bevacizumab, aflibercet, trebananib)

a. **Bevacizumab (Avastin®, Genentech):** Bevacizumab is a recombinant humanized murine antihuman VEGF-A monoclonal antibody. It neutralizes VEGF-A signaling by blocking binding to VEGFR-1 and -2, thus inhibiting endothelial and tumor cell activitation and proliferation. In other words, it reduces angiogenesis and tumor growth. Early work with bevacizumab in ovarian cancer showed that it reduced the ascites associated with gynecologic cancers [6-8]. GOG 170D was a Phase 2 study in women with persistent or recurrent EOC or primary peritoneal cancer (PPC) previously treated with 1-2 lines of chemotherapy. With bevacizumab, there was a 21% response rate and 40.3% were progression free at 6 months [9]. A different Phase 2 trial of bevacizumab was conducted in women who had 3 or less lines of chemotherapy. To be eligible, women had progressed either during or within 3 months of treatment. Response rate was 15.9% and 27.8% were progression free at 6 months [10]. More than 70 clinical trials of bevacizumab monotherapy in EOC and PPC have shown that it is effective in treating persistent or recurrent cancer.

Bevacizumab has been evaluated in combination with chemotherapy agents. For example, a Phase 2 trial of bevacizumab with oral metronomic cyclophosphamide was conducted in women with 3 or less lines of chemotherapy. Response rate was 24% and 56% were

progression-free at 6 months [11]. Median progression free survival (PFS) duration was 7.2months and median overall survival (OS) was 16.9months. It was not clear if this response is the same or better than if cyclophosphamide had been used alone.

Bevacizumab has been evaluated against standard of care in both the adjuvant and recurrent disease settings. In the adjuvant setting, bevacizumab was evaluated both concurrently with chemotherapy and as maintenance therapy following chemotherapy. GOG 218 was a 3 arm randomized controlled trial (RCT) of carboplatinum (AUC 6) and paclitaxel (175 mg/m²) every 3 weeks for 6 cycles with concurrent bevacizumab (15mg/kg) or placebo (cycle 2-6), and maintenance bevacizumab/placebo every 3 weeks for up to 16 doses [12]. Included were 1873 women with Stage 3 incompletely resected or Stage 4 EOC, PPC or FTC. PFS was superior in the bevacizumab arm compared to placebo (14.1 months bevacizumab during and after chemotherapy versus 11.2 months in bevacizumab during chemotherapy versus 10.3 months, HR 0.717, p<0.0001) (Table 2). Bevacizumab exposure during chemotherapy only gave a HR 0.908 (95% CI 0.795-1.040, p=0.16) for progression or death compared to bevacizumab through chemotherapy and maintenance (HR 0.717, 95%CI 0.625-0.824, p<0.001). There was no difference in OS 39.7 versus 39.3 months (p=0.450) respectively. Maximum curve separation was at 15 months around the completion of the bevacizumab in the maintenance arm. The survival curves converged at 24 months possibly due to the off study use of bevacizumab at recurrence. Quality of life (QOL) was assessed using the functional assessment of cancer therapy – ovary (FACT-O) just before treatment and on day 4, 7, 13, 22 and 6 months after completing the study. QOL Scores in the bevacizumab group were slight lower during chemotherapy compared to controls. Adverse events including hypertension which led to drug discontinuation in 2.4%. ICON 7 (International Cooperative Group Neoplasia) was a 2 arm RCT of carboplatin (AUC 5-6) and paclitaxel (175mg/m²) every 3 weeks versus the addition of bevacizumab (7.5 mg/kg) during chemotherapy and maintenance for 12 cycles [13]. 1528 eligible women had Stage 1 or 2A clear cell or grade 3 histology, Stage 2B-4 ECOG 0-2 ovarian cancer. At 36 months, the PFS was 20.3 months versus 21.8 months for bevacizumab arm (HR for progression or death 0.81, 95%CI 0.70-0.94, p=0.0041) in other words the progression free survival was 19 versus 17.3 months (Table 2). The interim analysis showed no survival difference between the two arms (58.6 versus 58.0 months, HR 0.99, p=0.85). A post hoc analysis showed that PFS in stage 4 and suboptimally debulked stage 3 women was 5.4months. Survival was also better in this group if they received bevacizumab (OS 39.7 months versus 30.3 months, HR 0.64, 95%CI 0.48-0.85, p=0.002). Adverse events in ICON 7 were similar to GOG 218 with rates of hypertension, proteinuria, thromboembolic events and GI perforations being higher in the bevacizumab arm. These 2 trials showed the benefits of bevacizumab use during or after chemotherapy especially on the PFS but not the OS. This benefit was most profound in the women with a higher burden of disease. Of note, these two trials differed in their eligibility criteria, the investigational arm, drug dosing, and treatment duration. ROSiA was a global single arm study assessing bevacizumab (15 mg/kg) q3weekly with 6-8 cycles of carboplatin and paclitaxel in

Agent	PFS	OS	QOL	ORR
1.Angiogenesis inhibitors 1a.Antibodies, peptides Bevacizumab Adjuvant setting Burger [12]	14.1 vs 11.2 vs 10.3 mos, HR 0.717, p<0.0001	No difference	Lower in bev arm during chemo	
Oza [13]	20.3 vs 21.8 mos HR 0.81 95%CI 0.70-0.94, p=0.0041	No difference		
Recurrent – Platinum sensitive Aghajanian [15]	12.4 vs 8.4 mos, HR 0.484 95%CI 0.388-0.605, P<0.0001	No difference		78.5% vs 57.4% p<0.0001
Recurrent – Platinum resistant Pujade-Lauraine [16]	6.7 vs 3.4 mos HR 0.48 95%CI 0,38-0.60, p<0.001	No difference		
Trebananib Monk [28]	7.2 vs 5.4 mos HR 0.66 95%CI 0.57-0.77, p<0.001	No difference		
1b.Small Molecule inhibitors Targeting VEGFR and c-kit Cediranib Recurrent -Platinum sensitive Ledermann [35]	12.6 vs 9.4 mos, HR 0.57 95%CI 0.45-0.74, p=0.00001	20.6 vs 17.6 mos HR 0.70 p=0.419		
Nintedanib Baumann [37]	17.3 vs 16.6 mos, HR 0.84, 95% CI 0.72-0.98, p=0.0239	No difference		
Targeting VEGFR, PDGFR, c-kit Pazopanib Adjuvant Du Bois [43]	17.9 vs 12.3 mos, HR 0.766, 95%CI 0.64-0.91, p=0.0021			
2. EGF Inhibitors 2a. Antibodies Pertuzumab				14% vs 5%
3. Targeting folate receptor alpha Farletuzumab Recurrent – Platinum resistant Walters [54] Recurrent – Platinum sensitive Vergote [55]	No benefit No benefit			
EC145 Recurrent – Platinum resistant Naumann [57]	Pending			
8. Inhibition of DNA repair Olaparib Adjuvant-maintenance Lheueux [76] Recurrent -Platinum sensitive (NCT01874353)	Pending Pending			
Niraparib Recurrent – Platinum sensitive (NCT01847274)	Pending			
Rucaparib Recurrent – Platinum sensitive (NCT01968213)	Pending			

Table 2: Randomized Trials of Targeted Agents in Ovarian Cancer.

women with advanced OC, FTC, or PPC. The bevacizumab was given until disease progression (i.e., up to 36 cycles) [14]. 1000 women were enrolled.

Bevacizumab was also evaluated in the recurrent setting in platinum sensitive disease (ie., disease recurrence 6 or more months after frontline chemotherapy). OCEANS (Ovarian Cancer Study Comparing Efficacy and Safety of chemotherapy and Anti-Angiogenic Therapy in Platinum-Sensitive Recurrent Disease) were an industry sponsored trial 2 RCT [15]. Eligible were those women with histologically confirmed recurrent EOC, ECOG 0-1, and measurable disease according to RECIST ver 1.0. 484 women were randomized to carboplatin/

gemcitabine and bevacizumab versus chemotherapy and placebo for 6-10 cycles. The bevacizumab or placebo was continued until disease progression. Median PFS was 12.4 versus 8.4 months respectively (HR 0.484, 95%CI 0.388-0.605, p<0.0001) (Table 2). Bevacizumab had a better objective response rate (ORR) of 78.5% versus 57.4%, p<0.0001 with most being partial responses. There was no OS difference. Adverse events included a higher rate of grade 3-4 hypertension (0.3% vs. 14.8%) and proteinuria (0.9% vs. 8.5%) with bevacizumab. There were no GI perforations if bevacizumab was not used in women with bowel obstruction or bowel infiltration with tumor. There was one death in each arm (1 MI and 1 intracranial hemorrhage).

The AURELIA study (A Study of Avastin Added to chemotherapy in patients with platinum-resistant ovarian cancer) examined the role of bevacizumab in platinum resistant settings (ie., disease growth while on platinum or within 6 months since frontline chemotherapy) [16]. This was an open label trial with investigator's choice of chemotherapy (i.e., weekly paclitaxel, weekly topotecan, liposomal doxorubicin) compared to the same chemotherapy with bevacizumab (15 mg/kg). To be eligible, patients had to have biopsy proven ROC, PPC, FTC and ≤ 2 prior anticancer regimens, no bowel obstruction or fistula, and no evidence of rectosigmoid involvement. Women were treated until disease progression or unacceptable toxicity and then crossed over. 361 women were enrolled of which 7% had prior antiagiogenic agents. 27% had disease free interval (DFI) <3 months. 2.7% developed GI perforation. Use of bevacizumab increased the PFS to 6.7 from 3.4 months (HR 0.48, 95% CI 0.38-0.60, p<0.001). There was no OS difference between the arms (16.6 versus 13.3 months, HR 0.85, 95% CI 0.66-1.08, p=0.174) [17]. Of those who were randomized upfront to placebo, 40% got bevacizumab at recurrence. Sub-group analysis showed that the addition of bevacizumab had a trend to a superior OS over weekly paclitaxel 22.4 versus 13.2 months (HR 0.65, 95%CI 0.42-1.02).

Studies either closed to recruitment or in progress include a Phase II single arm known as OCTAVIA. Here weekly paclitaxel is given with q21day carboplatin and bevacizumab (7.5 mg/kg) in women with newly diagnosed EOC. Enrolled are 189 patients. The PFS was 23.7mos with 90% of patients having completed the 6 cycles of treatment [18]. GOG 252 (NCT00951496) is a phase 3 randomized 3-arm trial in women who have been optimally cytoreduced. One arm involved IV weekly paclitaxel and day 1 carboplatin. One arm assessed IP carboplatin with weekly IV paclitaxel. The third arm involves day 1 IV paclitaxel, day 2 IP cisplatin and day 8 IP paclitaxel. Women received bevacizumab (15 mg/kg) in all 3 arms. Accrual was completed in November 2011. GOG 262 (NCT01167712) is a Phase III RCT of weekly dose dense paclitaxel with bevacizumab versus treatment of choice with bevacizumab. Currently 692 patients have been randomized and there is no difference in the PFS [19]. Subset analysis showed that the weekly paclitaxel without bevacizumab had a 4 month better PFS than the every 21day paclitaxel regimen (HR 0.596, 95% CIN 0.369-0.958, p=0.033). GOG 213 is a 4 arm study in platinum sensitive recurrent disease evaluating outcomes using carboplatin with paclitaxel versus carboplatin with gemcitabine with or without bevacizumab followed by cytoreductive surgery. GOG 241 is a Phase 3 study of adjuvant carboplatin with paclitaxel versus oxalaplatin with capecitabine with or without bevacizumab in mucinous OC or FTC.

Some of the issues that remain to be resolved concerning use of bevacizumab include optimal dose (7.5 mg/kg versus 15 mg/kg), adjuvant versus use at recurrence, maintenance versus use during chemotherapy and maintenance and if the decision is maintenance use, the duration of maintenance, and cost effectiveness.

b. Aflibercept (VEGF-trap, AVE-0005, BAY86-5321, ZALTRAP® Regeneron/Sanofi-Aventix/Bayer): Aflibercept is a unique fusion protein that acts as a high affinity soluble VEGFR decoy, inhibiting VEGF-mediated events (VEGF-A, VEGF-B) [20] and other VEGF family members like placental growth factor [21]. It has been evaluated as a single agent in ovarian cancer. A Phase 2 trial of two different doses of aflibercept (4 mg/kg and 2 mg/kg) in women with ROC showed an ORR of 7.3% and 3.8% respectively. This study did not achieve its primary endpoint. Two Phase 2 studies of Afibercept in women with EOC and symptomatic ascites were conducted. Afibercept (4.0 mg/kg

q2wks) showed the mean time to repeat paracentesis was significantly longer than with placebo (55.1 versus 23.3 days, difference 31.8days (95%CI 10.6-53.1, p=0.0019)) (NCT00327171) [22]. The other Phase 2 study (NCT00327444) using the same regimen showed prolonged PFS 59.5 (95%CI 41.0-83.0 days) and controlled refractory ascites [23]. Adverse events were in keeping with other anti VEGF agents.

Aflibercept has been evaluated in combination with chemotherapy. A single arm phase 2 study of Aflibercept with docetaxel in women with ROC showed substantial antitumor activity. Response rate was 54%. Median PFS was 6.2 months. OS was 24.3months. Adverse events were hypertension, proteinuria and bleeding [24]. This combination has promising antitumor efficacy and warrants further testing in a randomized trial [25].

c. Trebananib (AMG 386, Amgen Inc., and Takeda Bio Development Center Ltd.): Trebananib is a peptide-Fc fusion protein that inhibits angiogenesis by binding angiopoietin-1 and -2 and blocking interaction with the Tie2 receptor [26]. Trebananib was evaluated in a Phase 2 study in women with ROC who were receiving weekly paclitaxel (80 mg/m²) (NCT00479817). Women were randomized to weekly placebo or two different doses of IV trebananib (3 mg/kg or 10 mg/kg). Median PFS was 4.6, 5.7, (HR versus placebo 0.75, 95%CI, 0.56-1.00, p=0.21) and 7.2mos (HR versus placebo 0.76, 95% CI 0.57-1. 02, p=0.23) respectively [27]. Trebananib has also been evaluated against standard of care. TRINOVA-1 (NCT01204749) is a randomized Phase 3 double-blind trial in women with ROC, FTC or PPC of weekly paclitaxel (80 mg/m² weekly 3 weeks on and one off) with trebananib (15mg/kg IV) or placebo [28]. 919 women participated. They had 3 or less prior anti-cancer regimens whose progression occurred less than 12months since their last chemotherapy and performance status was 0-1. PFS was better with trebananib at 7.2 (95%CI 5.8-7.4) versus 5.4 months (95%CI 4.3-5.5) (HR 0.66, 95% CI 0.57-0.77, p<0.001) (Table 2). Interim OS was 19 compared to 17.3 months (HR 0.86, P=0.19). Adverse events included edema (64% versus 28%), ascites, and pleural effusions. Women in the trebananib arm discontinued treatment at a rate of 17% compare to 6% in the placebo arm. Trebananib was well tolerated and quality of life scores were the same between the two arms.

TRINOVA-2 is a phase III double blind placebo controlled trial with trebananib and pegylated liposomal doxorubicin (PLD) in patients who have relapsed after at least one prior chemotherapy treatment (NCT01281254). It has completed recruitment but results are pending. TRINOVA-3 is a prospective phase III randomized, double blind placebo controlled trial of first-line carboplatinum (AUC 5-6) and paclitaxel (175 mg/kg) with or without trebanananib (15 mg/kg weekly IV) women diagnosed with advanced ovarian cancer(NCT01493505). It also is currently closed to recruitment and results are pending.

Small molecule inhibitors

Targeting VEGFR: Tyrosine kinase inhibitors (TKIs) targeting the VEGF receptor signaling axis are being studied in ovarian cancer. Sorafenib (Nexavar®, BAY 43-9006, Bayer/Onyx Pharmaceutical) is an oral Raf-kinase and VEGFR-2 inhibitor. Sorafenib has been evaluated as monotherapy for example, GOG170F (Matei 2011) was a Phase 2 study of single agent sorafenib in women with ROC [29]. There were 2 women with PRs and 20 had disease stabilization. The 6 month PFS was 24%. A randomized Phase 2 trial of sorafenib (400 mg bid) versus placebo was conducted in women with ROC or PPC who had had and one prior chemotherapy regimen. This was a negative trial [41].

Sorafenib has been assessed together with other targeted agents. A Phase 1 trial of sorafenib with bevacizumab in heavily pretreated

women with ROC showed a response rate of 47% (NCT00436215) [30,31].

Sorafenib has been evaluated in combination with other chemotherapy agents. For example, a Phase 1 study of sorafenib (400 mg bid on days 1-28 each 4-weeks) was conducted with topotecan (3.5 mg/m^2 IV weekly) in platinum-resistant ROC (NCT00526799) [32]. A partial response was achieved in 16.7% and 46.7% had stable disease. Grade 3 and 4 toxicities were significant including leukocytopenia/neutropenia, thrombocytopenia, anemia, fatigue, nausea and vomiting and hand-foot syndrome. A Phase 2 trial of sorafenib (400 mg bid) was conducted with gemcitabine (1000 mg/m^2 IV) for women with ROC [33]. The partial response rate was low at 4.7%; 23.3% had an objective response or stable disease lasting longer than 6 months. The median PFS was 5.4 months and OS was 13.0 months. A randomized Phase 2 study evaluated carboplatin (AUC 6) and paclitaxel (175 mg/m^2) with or without sorafenib (400 mg orally bid) in patients with Stage 3-4 ovarian cancer following cytoreductive surgery (NCT00390611). This study is closed to recruitment and results are pending. In general, low efficacy and significant toxicity of sorafenib when combined with chemotherapy will limit its usefulness.

Targeting VEGFR and c-kit: Cediranib is an oral tyrosine kinase inhibitor (TKI) that inhibits all 3 VEGFRs (VEGFR 1,2,3) and c-kit. 2 trials in women with ovarian cancer have shown a partial response rate of 13-17% [34]. Adverse events include grade 3 hypertension (46%) and fatigue (24%). Approximately 23% of women were removed from the trial prior to cycle 2 due to toxicities.

ICON 6 was a randomized double blinded 3 arm trial in women with platinum sensitive recurrent ovarian cancer evaluating carboplatinum and paclitaxel with and with/out cediranib during chemotherapy and as maintenance [35]. 456 women were enrolled. The PFS was improved in the cediranib maintenance arm 12.6 versus 9.4mos (HR 0.57, 95%CI 0.45-0.74, p=0.00001). The PFS difference was 3.2mos with cediranib. Using the restricted mean, OS increased by 2.7 months from 17.6 to 20.3months in the cediranib concurrent plus maintenance arm (HR 0.70, log-rank test, p=0.419). There was no evidence to suggest cediranib is more active than other antiangiogenic agents; however, this was the first time a TKI had shown an OS advantage.

Targeting VEGR, PDGFR and FGFR: Nintedanib (Vargatef) (BIBF 1120) is an oral trifunctional angiokinase inhibitor that inhibits VEGFRs (1,2,3) PDGFR (alpha and beta) and FGFRs (1,2,3 –). A Phase 2 placebo controlled maintenance trial was conducted in 83 women who responded to second line chemotherapy [36]. They were randomly assigned to nintedanib (250 mg) or placebo bid. There was a 36 week PFS of 16.3% versus 5% (HR 0.65, CI 0.42-1.02, p=0.06) in favor of nintedanib. Adverse events included elevated transaminases (51.2%gr 3 and 7.5% gr 4), diarrhea (79.1% gr 3 and 37.5% gr4), nausea (76.7% gr 3 and 35% gr 4), vomiting (62.6% gr 1 and 22.5% gr 2).

AGO-OVAR12/LUME-Ovar1 was a phase 3 randomized placebo controlled trial in 1355 post operative stage 2B-4 EOC women who are receiving carboplatinum (AUC 5) and paclitaxel (175 mg/m^2) followed in a 2:1 manner with maintenance nintedanib (200 mg bid) or placebo (NCT01015118) [37]. 1366 women are enrolled and half had no macroscopic disease at the end of surgery. Nintedanib increased PFS from 16.6 to 17.3 months (HR 0.84, 95% CI 0.72-0.98, p=0.0239) (Table 2) but there was no OS difference detected. Grade 3 adverse events which led to dose reductions included diarrhea (22%), thrombocytopenia (18%), hepatic toxicity (16%), anemia (14%).

Targeting VEGFR and PDGFR: Sunitinib (SU11248, Sutent,

Pfizer) inhibits VEGFRs,PDGFRs and c-kit. AGO-OVAR-2.11 was a trial of sunitinib monotherapy and showed a response rate of 16.7% in the noncontinuous dosing arm and a response rate of 5.4% in the continuous dosing arm [37]. GOG 254 is a Phase 2 trial of sunitinib in women 2 persistent or recurrent clear cell cancer of the ovary that is still recruiting (NCT009799). Sunitinib was studied in 30 women with ROC after up to 3 prior chemotherapy regimens. One PR (3%) and 3 CA-125 PRs (10%) were seen in women with platinum-sensitive disease, with 16 (53%) having stable disease, for an overall clinical benefit of 66%. Both a 50 mg intermittent schedule given 4 weeks out of every 6, and a 37.5 mg continuous schedule were examined, with responses noted only on the intermittent dosing schedule [38].

Targeting C-Met, ALK, VEGFR: Cabozantinib (XL-184, Exelixis) is and oral TKI that inhibits c-Met, ALK, and VEGFR2 [39]. Cabozantinib in solid tumors had an overall clinical benefit with a partial response and stable disease rate of 58%. Adverse events included handfoot syndrome (10%), diarrhea (8%), and fatigue (4%). There is an study closed to recruitment of cabozantinib versus paclitaxel in persistent EOC, FTC, PPC (NCT01716715).

Targeting VEGFR and EGFR: Vandetanib is a dual VEGFR and EGFR inhibitor. A Phase 2 study of daily oral vandetanib alone in women with EOC, PPC, and FTC was negative (NCT00445549) [40]. Examining tissue signaling pathways, cytokine concentration and functional vascular imaging in the tumors of women in this study suggested that EGFR was successfully inhibited at tolerable doses. It appeared that VEGFR2 was not inhibited at the doses used. A phase 2 trial of vandetanib with PLD was negative (NCT00862836). A randomized Phase 2 in women with ROC is underway assessing docetaxol and vandetanib (NCT00872989).

SU5416 is a potent and selective inhibitor of FIk-1 (VEGFR-2). It was evaluated with carboplatin in a phase 1 study in recurrent disease (NCT00006155).

Targeting VEGFR, PDGFR and c-kit: Pazopanib (Votrient®, GlaxoSmithKline) (GW786034) is a second generation oral TKI that inhibits all 3VEGFRs (-1,-2,-3) , both PDGFRs (alpha and beta) and c-kit. Pazopanib has been studied alone in a Phase 2 trial of pazopanib (800 mg OD) in women with ovarian cancer who had 2 or more lines of chemotherapy and who have had a CA125 response to initial platinum based chemotherapy and subsequent rise in CA125 to greater than 42 [41]. With treatment, 31% (11/36) of women had a Ca125 response with a median time to response of 29 days and median duration of response of 113 days. 29% had disease stabilization. The ORR was 18% in patients with measurable disease. Adverse events included grade 2 or higher included fatigue (11%), diarrhea (8%), alanine transaminase elevation (8%), and hypertension (3%). There was one woman with grade 4 peripheral edema. Another Phase II study of pazopanib (800 mg bid) in 25 women with ROC, FTC, or PPC treated with up to 2 prior cytotoxic regimens [42]. Pazopanib was given orally at 800mg bid. Median PFS was 1.83 months (95% 1.67-2). Clinical benefit end point did not reach statistical significance at first stage so the trial was stopped.

Pazopanib has been studied in conjunction with chemotherapy such as cisplatin (NCT01165385), cyclophosphamide (NCT01238770), gemcitabine (NCT01610206), PLD (NCT01035658), paclitaxel (NCT0148909)(NCT01608009)(NCT01644825), carboplatin-paclitaxel (NCT 00561795) and topotecan (NCT01600573). Currently the EORTC 55092 (NCT01402271) is recruiting to a Phase IB-II open label multicentre ongoing feasibility study of pazopanib with weekly

paclitaxel and carboplatin in women with platinum-refractory/resistant OC, FTC, or PPC.

Pazopanib was assessed as maintenance therapy after chemotherapy for EOC. AGO-OVAR16 (POIZE) was a phase 3 randomized placebo controlled trial in 960 women who had not progressed after first line chemotherapy (with platinum and taxane) for advanced epithelial ovarian, fallopian tube or primary peritoneal cancer [43]. They received daily oral pazopanib (800 mg) or placebo for up to 2 years after primary platinum based combination therapy. Median PFS was 17.9 versus 12.3 months (HR 0.766, 95% CI 0.64-0.91, p=0.0021). Adverse events were grade 3-4, hypertension, neutropenia, hepatic toxicity and diarrhea. This study suggested a benefit with maintenance therapy.

EGF Family of Inhibitors

EGFR is a membrane tyrosine kinase expressed by most epithelial cells. EGFR is a family of 4 transmembrane receptors (EGFR 1,2,3,4). EGFR signaling is important for normal cell function but inappropriate activation or over expression can contribute to malignancy. A wide range (19-92%) of ovarian cancers has EGFR dysregulation and this is associated with worse survival [44].

Antibodies (ie., cetuximab, panitumumab, trastuzumab, petuvumab)

Cetuximab (Erbitux®, ImClone System Inc.): Cetuximab is a murine monoclonal antibody which binds to the extracellular region of the EGFR. Binding results in inhibiting cell proliferation and has an anti-apoptotic effect. A non-randomized Phase 2 trial of cetuximab (400mg/m² loading dose followed by 250 mg/m² weekly) in women with persistent or ROC or PPC showed lack of efficacy (NCT0082212). Two other trials evaluated cetuximab and matuzumab were negative [45,46]. The use of cetuximab with chemotherapy or radiation in ROC (GOG 146P) did not show significant activity. Rates of GI and metabolic toxicity were high.

Trastuzumab (Herceptin®, Genentech/ImmunoGen, Inc): Trastuzumab is a recombinant monoclonal antibody that binds the extracellular domain of EGFR2 (Her2) and prolongs survival in Her2 positive breast cancer in adjuvant and metastatic setting. There are rare cases of true mucinous ovarian cancer which expresses very high level of Her2 and have been found to respond to trastuzumab. Two other trials with trastuzumab as monotherapy (NCT01195935) or in combination with chemotherapy (NCT00189579) have closed.

Pertuzumab (Omnitarg®, Chugai Pharmaceutical): Pertuzumab binds to Her2 near the center of the domain II dimerization region. There has been a randomized Phase 2 trial assessing 2 different doses of pertuzumab in heavily pretreated women with ovarian cancer (median 5 chemo regimens). 5 of 123 participants had a partial response [47]. In another randomized trial of gemcitabine with or without pertuzumab, there was a 14% versus 5% response rate respectively. These response rates were felt to be too low to warrant further study.

Panitumumab: Panitumumab is an antibody to the EGFR. A Phase 2 study of women with ROC and treated with panitumumab and gemcitabine has terminated (NCT01296035). PROVE is another Phase 2 study of Panitumumab with carboplatin and PLD or gemcitabine recruiting patients with platinum sensitive ovarian cancer (NCT01388621).

Small Molecule Inhibitors

Studies of Erlotinib (Tarceva®, Genetech), Geftinib (Iressa®, AstraZeneca), and Lapatinib (Tykerb®, GlaxoSmithKline) as monotherapy, with chemotherapy and as maintenance therapy have shown no effect to date [44-49].

Targeting folate receptor alpha

Folate enters cells by endocytosis mediated by high affinity folate receptors (FR). Folate receptor alpha (FRα) is over expressed on various tumors including non-mucinous ovarian cancer but not in normal ovarian tissues [50]. This feature makes the receptor of great interest.

Farletuzumab (MORAb-003; Morphotek Inc, Exton, PA, USA): Farletuzumab is a humanized monoclonal antibody against FRα. A Phase 1 dose-escalation study was conducted in 25 women heavily pretreated with platinum-resistant or platinum-refractory ovarian cancer. The drug was well tolerated at doses of 12.5 to 400 mg/m². There were no Dose limiting toxicities (DLT). The stable disease rate was 36% but there were no objective responses [51]. A Phase 2 trial was conducted with 54 women who had platinum sensitive EOC in first relapse (NCT00318370). They received weekly farletuzumab as a single agent or in combination with carboplatin (AUC 5-6) and taxane q3wks. 44 (89%) women normalized their CA125 levels. 21% had a second remission that was as long or longer than their first [52,53]. These phase 1 and 2 trials showed safety and improved response rates.

This agent was assessed in combination with chemotherapy compared to standard of care. A Phase 3 randomized double blind placebo controlled study of farlatuzumab with weekly paclitaxel in platinum-resistant ovarian cancer was closed prematurely as it did not meet pre-specified criteria to continue (NCT00738699) (Table 2) [54]. Another Phase 3 randomized trial of farlatuzumab with carboplatin paclitaxel women with in platinum sensitive ROC also did not meet the primary endpoint of PFS. In a subset analysis, there was a trend toward improved PFS in some patients (NCT00849667) (Table 2) [54,55]. Unfortunately farlatuzumab did not show superiority over standard treatment in the platinum resistant or sensitive setting.

EC145 (Vintafolide; Endocyte, Inc. West LaFayette, IN, USA): EC145 is a small molecule drug conjugate (SMDC) of folic acid linked to the vinka alkaloid desacetylvinblastine hydrazide (DAVLBH), a vinca alkaloid which is a potent microtubule destabilizing agent. A Phase 2 trial was conducted in 47 women with EOC, FTC, or PPC after identification of FR expression using etarfolatide (a companion folate receptor targeted imaging agent) (NCT00507741, EC-FV-02). This study evaluated two different dosing regimens of vintafolide administered 3 times a week on week 1 and 3 of a 4 week cycle. The primary end point was percent of patients deriving a clinical benefit. Disease control rate (CR+PR+SD) at 8 weeks in patients having 3rd or 4th line vintafolide IV was 75% compared to PLD 47% second line. 3 women had a partial response. The most common toxicity was grade 3 fatigue (8.2%). There was a trend toward longer survival in those whose lesions were positive on the scan (i.e. 63.4wk versus 23.1 wk, p=0.071).

Vintafolide has been evaluated in combination with chemotherapy. PRECEDENT was a Phase 2 trial of Vintafolide in 145 women with platinum resistant ROC. Vintafolide (2.5 mg days 1,3,5 and 15,17,19 each 4 weeks) was combined with PLD (50 mg/m²) versus PLD alone. PFS was 21.7 versus 11.7 weeks respectively (HR 0.626, p=0.031) [56,57]. In those 100 women with etarfolatide positive tumors, PFS was higher at 24 versus 6.6 wk (HR 0.381, p=0.018). Some of the adverse events related to use of this agent include hematologic, nausea, mucositis, stomatitis, and less commonly peripheral neuropathy. Extravasation precautions are necessary. No significant difference in drug-related adverse events was seen between the arms [58].

PROCEED is a Phase 3 double-blind, placebo-controlled randomized trial of PLD with or without vintafolide in platinum-resistant ROC (NCT01170650). It is powered for an OS endpoint and is ongoing (Table 2). Folates can be conjugated to a radio-isotope for diagnostic purposes. In this trial, etarfolatide, a ^{99m}Tc-folate conjugate is used to identify FR-positive lesions. Those positive women are randomized to vintafolide with or without PLD (50 mg/m^2 q4wk). Placebo or vintafolide is given at 2.5 mg days 1,3,5,15,17,19 each cycle for up to 20 cycles or unacceptable toxicity). Primary objective is PFS based on RECIST criteria in the FR positive patients. Secondary objectives include OS, safety, tolerability, overall response rate and disease control rate [57].

mTOR Inhibitors

The mammalian target of rapamycin (mTOR) integrates nutrients and signals to regulate cell growth and division. It does this through molecular products of the pI3K/AKT signaling pathway. mTOR inhibitors limit tumor proliferation and progression. Tumor response to PI3K/AKT/mTOR inhibitors appears to be related to presence of PIK3CA mutations. Examples of mTOR inhibitors include Temsirolimus (CCI-779, Torisel®, Wyeth Pharmaceuticals), Everolimus (RAD001, Afinitor®, Novartis), AP23573 (ridaforolimus), Rapamycin (sirolimus).

Temsirolimus (CCI-779, torisel®, wyeth pharmaceuticals)

Temsirolimus is being evaluated as monotherapy for safety and efficacy (NCT 00926107) (NCT00429793). For example, a GOG Phase 2 trial of temsirolim (25 mg/wk IV) in women with persistent or recurrent EOC and PPC [59]. Fifty-four women were eligible for evaluation. Partial response was 9.3% and 24.1% had a PFS for 6 months or longer. These results were too low to warrant a phase III study.

Temsirolimus was evaluated in combination with chemotherapy including PLD (NCT00982631), vinorelbine (NCT01155258), and paclitaxel plus carboplatin (NCT001196429)(NCT00408655). Phase 1 and 2 trials have shown modest activity [60]. For example, temsirolimus and carboplatin/paclitaxel showed a PR of 50% and SD of 50% in 6 OC patients. Combining chemotherapy with mTOR inhibitors may show synergy and be more active than either agent is alone. Common complications with these agents included mucositis, stomatitis (75% of temsirolimus, 40% with everolimus), rash, asthenia, fatigue, myelosuppression, and pneumonitis (40% of temsirolimus).

Everolimus (RAD001, afinitor®, novartis)

Everolimus is currently being evaluated with carboplatin and PLD (NCT01281514) and with bevacizumab (NCT01031381) (NCT00886691).

Targeting p53 Mutated Tumors

Wee-1 inhibitors

Wee-1 is a tyrosine kinase that is involved in G2 checkpoint signaling. TP53 is a key regulator of G1 checkpoint so TP53-deficient tumors rely exclusively on the G2 checkpoint following DNA damage. MK-1775 is a selective small molecule inhibitor of Wee-1 kinase and leads to cell death in TP53-deficient cells when given with chemotherapy. TP53 mutations are found in 95% of high grade serous cancers. There are two phase 2 studies of MK-1775 currently recruiting. The first is in conjunction with carboplatin in women with TP53 mutated EOC and early relapse or progressing disease (NCT01164995).

The second is evaluating the MK-1775 with carboplatin and paclitaxel (NCT01357161).

PKC-beta inhibition

Enzastaurin (LY317615) is an oral serine/threonine kinase inhibitor and selective inhibitor of PKCβ inhibitor. PKCβ is a key component of VEGF signaling pathway that is critical in tumor angiogenesis. PKCβ can activate the AKT pathway. This pathway promotes tumor cell survival and proliferation, and is activated in many human cancers. Enzastaurin is anti-apoptotic and antiproliferative in cultured ovarian cancer cells and xenografts. As a single agent enzastaurin is being evaluated in persistent or ROC or PPC. (NCT00420381). Primary end point is the frequency of patients survival PF at least 6 months or objective tumor response. Another Phase 2 trial in 27 women with ROC or PPC, showed that with a 1125 mg loading dose followed by 500mg oral enzastaurin OD until disease progression there was an 11% PFS ≥ 6mos and 7% partial response. Grade 3 adverse events were constitutional (4) and GI (3). The results showed the agent was tolerable but with insufficient activity to proceed to second stage accrual [61].

In combination there are several studies. A Phase 2 study of oral enzastaurin with carboplatin and paclitaxel in 11 women with EOC or PPC showed a grade 3 clostridial infection (1), neutropenia (1), soft tissue injury and wound infection. Phase II randomized placebo-controlled study randomly assigned 142 pt FIGO stage IIB-IV OC, FTC, PPC to 6 cycles paclitaxel (175 mg/m^2), carboplatin (AUC=5) with or without enzastaurin (Cycle 1 day 1 1125 mg enzastaurin day before chemo then 500 mg oral daily and maintenance) 3weeks. Median PFS 3.7mos longer (18.9 95%CI 13.8-- versus 15.2, 95% CI 11.0-18.9) HR=0.80, 95%CI: 0.50, 1,29; p=0.37). Factors that effected outcome included optimal debulking HR=0.51 (95%CI 0.30 -0.85; p=0.009). Safety was the same between arms. Grade 3 and 4 adverse events included constipation (1.5%), diarrhea 1.5%, dyspnea 3.0%, edema of head and neck 3%. The arm with enzastaurin was NOT superior to standard of care.

There was a Phase I of 67 patients who received enzastaurin (500 mg od then 250 mg bid, 375 mg bid, 500 mg bid, 750 mg bid) with bevacizumab (5 mg/kg q2wk, 10 mg/kg q2wk, 15 mg/kg q2w3) until dose limiting toxicity in 2 of 6 patients [62]. In the cohort were 31 with EOC. The median progression time was 8.3mos, ORR 32.3% and delayed disease progression after 6 months was 50.4%. Adverse events were fatigue (4 grade 3), cerebral hemorrhage (grade 4) and elevated AST (grade 3). MDT 750 mg bid with any bevacizumab dose. Recommended enzastaurin dose was 500 mg OD up to 500 mg bid.

MEK inhibitors

MAPK are a network of signal transducing proteins that link extracellular signals to gene expression. MAPK cascade is also known as the Ras/Raf/MEK/Erk pathway which regulates cell proliferation, cell cycle progression and cell migration. MEK inhibitors are small molecules that inhibit MEK phosphorylation. Low grade serous ovarian cancers make up 10% of serous cancers. They tend to occur in younger women (median age 46 yo)[5] and these tumors tend to have an indolent clinical course. They have a poor response to chemotherapy. Mutations in BRAF and KRAS are identified in two-thirds of patient [5]. These are components of the mitogen-activated protein kinase (MAPK) signaling cascade. 44-85% of mucinous adenocarcinomas of the ovary also harbor a KRAS mutation, suggesting targeting the MAPK pathway may also be an option here [5].

Selumetinib (AZD-6244). Selumetinib is an MEK1/2 inhibitor. A

Phase 2 study of selumetinib in 52 women with LGSOC showed an ORR of 15.4% and a stable disease rate of 65% [63]. Median PFS was 11 months. Of those with tumor for analysis 6% had a BRAF mutation and 41% a KRAS mutation. Mutational status of BRAF and KRAS did not correlate with response. Adverse events included one grade 4 cardiac, one pain and one pulmonary issue. Grade 3 and 4 adverse events included GI (13), dermatologic (9) and metabolic (7). Dose reductions took place in 42% of women while 25% were removed from study due to toxicity. Response to treatment did not align with mutation analysis. There is currently a Phase 3 MILO MEK Inhibitor in Low-Grade Serous Ovarian Cancer (ENGOT-ov11/MILO) randomized women with recurrent or persistent low-grade serous ovarian, fallopian tube or peritoneal cancer to MEK162 or physician choice cytotoxic chemotherapy (liposomal doxorubicin, weekly paclitaxel, topotecan) (NCT01849874).

SAR245409 (also known as XL765) is a selective potent pan Class I PI3K inhibitor with inhibitory activity against the mammalian Target of Rapamycin inhibitor Complex 1 and 2. Pimasertib (also known as MSC1936369B and AS703026) is a selective and potent, adenosine triphophate un-competitive MEK inhibitory. PIM/SAR was a phase 2 radnomized double blind placebo controlled trial of combination pimasertib with SAR245409 or of Pimasertib with SAR245409 placebo in women with previously treated unresectable LGOC (Protocol EMR 200066 012).

There are studies addressing MEK inhibitors with chemotherapy. A Phase I trial of MEK162 with paclitaxel in EOC, FTC, PPC (NCT01649336).

Inhibition of DNA repair

Mutations in BRCA 1 or BRCA2 occur in 10-15% of women with epithelial ovarian cancer. They are most commonly seen in high grade serous or endometrioid ovarian cancer, occurring in up to 20% of these patient. Women who have a BRCA1 or 2 mutation are at a 40-63% risk of developing ovarian cancer and 87% risk of developing breast cancer [64]. PARPi have particular utility in treating patients with BRCA-mutated ovarian cancer. These drugs block the PARP complex and lead to disturbance of single stranded DNA repair. These cells then become more prone to double strand injuries that require homologous recombination to be repaired. When there is a BRCA mutation, there is impaired homologous recombination activity and so if DNA damage cannot be repaired, this leads to apoptosis.

Olaparib (KU-0059436/AZD2281, AstraZeneca): Olaparib is a potent oral PARP-1 inhibitor that preferentially kills cancer cells by exploiting DNA repair pathways.

Phase 1 studies of olaparib included women with refractory solid tumors but enriched for women with BRCA mutations. MTD was 400 mg bid with minimal side effects such as gastro-intestinal and fatigue. Of 19 patients BRCA mutation patients, 9 had a partial response (47%) and 8/9 of these had OC. 63% had stable disease for 4 or more months [65]. This study was expanded to give olaparib to 50 patients with BRCA 1 and 2 mutated ovarian, fallopian tube and primary peritoneal cancer. Clinical benefit was 46% with 40% having a RECIST radiologic or Ca125 response [66]. Median duration of response was 28 week. Those with platinum-resistant or platinum-refractory tumors had a 46% and 23% benefit respectively versus a 69% in platinum-sensitive women (p=0.038). There was an association between platinum sensitivity interval and antitumor response. Another Phase 1 study combined olaparib with cisplatin and gemcitabine in 23 patients with advanced solid tumors with treatment given at time of progression. 3/23 had OC.

There were 2 PR and 13 SD. Adverse events included 35% anemia, 61% neutropenia and 57% thrombocytopenia [67].

If hypoxia –mediated defects in DNA repair can lead to genetic instability, this may drive oncogenesis. Thus antiangiogenic agents have been investigated with olaparib. A Phase 1 trial of olaparib (200mg bid capsules) and cediranib (30mg OD) in 28 women with ROC (n=20) or Triple Negative Breast Cancer (n=8). There was 1 CR and 7 PRs for a clinical benefit of 61%. Dose limiting toxicity was neutropenia and thrombocytopenia. Adverse events were hypertension (25%), fatigue (18%), and neutropenia (11%). There was benefit in platinum-sensitive and –resistant populations and BRCA-mutated and BRCA –wild-type patients [68].

A Phase 1 dose escalation trial of olaparib added to carboplatin in BRCA1/2 mutational carriers with breast or OC [69]. Olaparib was given at 100 or 200 mg bid with carboplatin AUC 3 on day 8 every 21 days. Then olaparib was given 200 or 400mg bid on days 1-7 with carboplatin AUC 3 on day 2 and escalated to AUC 5. DLT was bone marrow suppression. PRs were seen in 8/23 women and SD in 11/23 women.

A Phase 2 study was conducted to assess two dose levels of olaparib. This was an international multicentre trial of two sequential cohorts of 57 women with confirmed gBRCA1 or 2 mutation and measurable ROC [70]. The ORR was 33% (95%CI 20-51) in 33 women who received olaparib 400 mg bid compared to 13% (95% CI 4-31) in 24 women who received 100 mg bid. The ORR was 16% in those with BRCA1 mutations and 0% in those with BRCA2 mutations. The median PFS was 5.8 versus 1.9 months respectively. The grade 3 adverse events were nausea (48% versus 6%), fatigue (33% versus 3%), and anemia (18% versus 7%). This study was a positive proof of concept for efficacy and tolerability of olaparib in advanced BRCA-mutated ovarian cancer.

Olaparib has been assessed in non-mutation carriers (or BRCA wild-type) ovarian cancer with the rationale that there is a homologous recombinant DNA repair defect but no gBRCA1 or 2 mutation in up to 50% of ovarian cancers. A Phase 2 trial of single-agent olaparib was conducted in high grade serous/undifferentiated OC with unknown BRCA status or BRCA-negative disease [71]. There was a BRCA mutation reference group. The ORR with olaparib (400mg bid) was 41% (95%CI 22-64) in 17 women with gBRCA1 or 2 and median PFS of 221 days (95%CI 106-383). This is compared to the ORR in the 46 women with BRCA mutation negative of 24% (95%CI 14-38) and PFS of 192 days (95%CI 109-267). A post hoc assessment in platinum sensitive women showed an ORR of 50% and the gBRCA-negative cohort and 60% in the gBRCA-mutated cohort. In the platinum-resistant group, it was 33% in the gBRCA-mutation positive group and 4% in the gBRCA-mutation negative group. This trial showed the benefit of PARP inhibitors in sporadic ovarian cancer and that platinum sensitivity can be used as a surrogate marker for HR deficiency [64].

A Phase 2 open-label randomized international trial of efficacy with two dose levels of olaparib (NCT0062851) were compared to that in monthly liposomal doxorubicin in 97 women with gBRCA-mutated platinum resistant ROC [72]. Response rate was 25% in olaparib 200mg bid and 33% in women with olaparib 400 mg bid compared to 18% in liposomal doxorubicin. PFS were similar - 6.5 month (95%CI 5.5-10.1), 8.8 months (95%CI 5.4-9.2) and 7.1 months (95% CI 3.7-10.7). Grade 4 adverse events were 2 fold higher in the PLD group. Adverse events were fatigue (56%), nausea (72%), vomiting (47%), abdominal pain (25%), anemia (13%), fatigue (9%). No significant difference in PFSs across the groups but PFS was higher in PLD than previously reported.

It was hypothesized that this may be related to enhanced chemotherapy sensitivity of tumors harboring gBRCA mutations.

A randomized, double-blind, placebo-controlled phase 2 study of olaparib maintenance therapy was conducted in patients with platinum-sensitive ovarian cancer [73]. 265 women had received 2 or more platinum based regimens and had a partial or complete response in their most recent regimen. Randomized to maintenance were 136 women to Olaparib (400 mg bid) and 129 to placebo. Median PFS was 8.4 versus 4.8mos respectively (HR for progression or death 0.35, 95%CI 0.25-0.49, p<0.00001). There was no survival advantage; however, it must be noted that 22.6% of placebo patients switched to PARPi. In those with BRCA mutation, PFS was 11.2 versus 4.1 months (HR 0.17 95%CI 0.09, 0.32 p<0.001) and QOL measured by Trial Outcome Index odds ratio was 4.08 (95%CI 1.11-19.85 p=0.03). Adverse events were mild (grade 1-2) nausea (66% vs. 35%), fatigue (42% vs. 34%), vomiting (29% vs. 13%) and anemia (12% vs. 4%). This study again supports the benefits of olaparib in women with ovarian cancer regardless of BRCA mutational status and highlights the need to develop biomarkers that predict HR deficiency [64]. This study also showed maintenance therapy to be an effective treatment strategy. PFS for PARPi was superior in BRCAm but there was benefit in BRCAwt. 19/265 patients remained on PARPi for more than 3 years. Significant QOL improvements occurred with olaparib.

A Phase 2 multicenter study of olaparib (200 mg bid) day 1-10 with carboplatin (AUC 4 IV) paclitaxel (175 mg/m²IV) day 1 every 21days followed by olaparib (400 mg bid) maintenance versus chemotherapy (AUC 6/175 mg/m² q21days) alone was conducted in 162 women with platinum-sensitive ROC (NCT01081951) [74]. PFS was superior in the former arm (12.2 versus 9.6 months HR 0.51, 95% CI 0.34-0.77, P=0.0012) and OS was 64 versus 58% respectively. Adverse events were similar between the arms (65% versus 57%) and as expected in the maintenance arm (29% vs. 16%). Greatest clinical benefit was in patients with g/tBRCA (PFS HR 0.21, 95% CI 0.08-0.55, p=0.0015]. This study showed that safety during chemotherapy was acceptable with dose reductions in carboplatin and olaparib. The greatest benefit was in the g/tBRCA (t-somatic) patients.

A Phase 2 study of olaparib (200mg bid versus 400 mg bid) with or without cediranib (30 mg OD) in platinum sensitive ROC was conducted [75]. Median PFS was 17.7 versus 9.0 months (HR 0.42, 95% CI 0.23-0.76, P=0.005). Single agent olaparib provided superior outcome in BRCAm (16.5mos versus 5.7mos). Cediranib with olaparib provided a similar outcome in BRCAm or BRCA unknown (19.4 versus 16.5 month).

SOLO-1 is an ongoing phase 3 randomized double blind placebo controlled trial of olaparib in gBRCA-mutated high grade serous ovarian cancer patients who have responded to platinum-based chemotherapy (Table 2) [76]. SOLO-2 is an ongoing phase 3 randomized double blind placebo controlled trial of olaparib monotherapy in platinum sensitive relapsed gBRCA mutated high grade serous ovarian cancer who responded to platinum (NCT01874353) (Table 2). Olaparib (300 mg bid tablet) or placebo will be given until disease progression. Efficacy outcome is PFS [76].

Iniparib (BSI-201, Sanofi-Aventis). Iniparib is a PAPR inhibitor: Iniparib is being evaluated as monotherapy in a Phase II (NCT00677069) in women with BRCA positive relapsed or refractory ovarian cancer or primary peritoneal cancer. Results are pending. Iniparib is being evaluated with chemotherapy in a Phase II study with carboplatin and gemcitabine in platinum-resistant ROC, FTC

and PPC. Response rate was 31.6% with a median PFS of 5.9 months (NCT01033292) [77]. The Phase 2 study in platinum sensitive disease is closed but results are pending (NCT01033123).

Veliparib (ABT-888): Valiparib as a monotherapy is being assessed in a Phase II trial of ROC, FTC, PPC (01540565). Recruitment is complete but results are pending.

Veliparib is being assessed in combination with cyclophosphamide (NCT01306032), PLD (NCT01145430), Topotecan (NCT01012817) (NCT01690598), Temozolomide versus PLD (NCT01113957) and with carboplatin, paclitaxel and bevacizumab (NCT0098965).

Niraparib (MK-4827): Niraparib is an oral PARPi shown to induce selective lethality in HRD tumors and BRCA loss or non-BRCA HR defects [64]. A Phase I dose escalation trial involved 100 patients with advanced cancer given 10 successive dose levels [78]. MTD is 300 mg/day. 40% of those with BRCA1 or 2 mutation carriers with OC had Partial response. 3/9 platinum resistant BRCA1 mutant or BRCA2 mutant OC had RECIST and CA125 responses.1 pt had stable disease >120 days. In platinum sensitive disease 50% with BRCA1 or BRCA2 mutations had RECIST and CA125 response. 1 patient with BRCA1mutation and platinum-refractory OC had SD from 130 days. Adverse events were anemia in 48%, nausea in 42%, fatigue in 42%, and thrombocytopenia in 35%.

A Phase 3 multicenter randomized double blind placebo-controlled study of maintenance niraparib in platinum-sensitive OC with gBRCA mutation with high grade serous histology who have responded to a platinum agent (ENGOT-ov-14) is ongoing (NCT01847274) (Table 2).

BMN 673 (LT-00673): BMN 673 is a highly potent PARPi that has single-agent activity in BRCA1 and BRCA2 mutant cells specific to tumors bearing DNA-repair deficiencies. A Phase 1 single-arm, open-label study of BMN 673 is being conducted to assess the safety, pharmacokinetics, pharmacodynamics and preliminary efficacy in patients with advanced tumors with DNA-repair pathway deficiencies (NCT01286987).

Rucaparib (CO-338, formerly AG 014699 and PF-01367338): Rucaparib is a PARPi with oral bioavailability. Rucaparib as monotherapy was evaluated in 17 women with ovarian cancer [79]. Clinical benefit was seen in 32% and the ORR was 5%; 26% achieved stable disease for 4 or more months. A Phase 2 dose escalation study of rucaparib (40-120 mg per day) in women with gBRCA mutation ovarian cancer is ongoing (NCT01482715). Another Phase 1 dose-escalation and pharmacokinetic study of oral rucaparib was performed in 29 patients at doses of 48 up to 500 mg OD and 240 mg bid). 2 patients (of which one had OC) at 300 mg OD had partial response at wk 6. 10 had stable disease >12wk. Disease control rate in OC was 86% (6/7). Adverse events included fatigue in 5, anorexia in 3, nausea in 3, vomiting in 3, diarrhea in 2 [80].

Rucaparib in combination with chemotherapy has been evaluated. A Phase 1 study of oral rucaparib with carboplatin was used in 23 patients with advanced solid tumors. Oral rucaparib was given on day -10 and -5 followed by IV carboplatin (AUC 3, 4,5 on day 1) and oral rucaparib (days 1-14 every 3 week). There was no DLT at doses 80, 120, 180, 240, 360 mg [81].

Rucaparib is being evaluated in platinum sensitive ROC (ARIEL 2- NCT0189134)(ARIEL 3-NCT01968213). ARIEL 2 is a phase 2 open-label study of rucaparib in patients with platinum-sensitive, relapsed, high-grade EOC, FTC, or PPC with at least one prior platinum based chemotherapy. ARIEL -3 is a multicenter, RCT of rucaparib as switch

maintenance following platinum-based chemotherapy in patients with platinum-sensitive, HGSOC or endometrioid OC, PPC, or FTC with 2 or more prior platinum-based chemotherapy regimens (Table 2).

Immune Checkpoint Inhibitors

Immune system exerts a major effort to avoid immune over activation (prevention of autoimmune disease and to prevent rejection of the developing fetus during pregnancy). Cancer takes advantage of this by exploiting a number of immune escape mechanisms that were developed to avoid autoimmunity [82]. There is evidence to suggest that the immune system plays a key role in the outcomes of HGSC of the ovary [83-87]. The presence of tumor-infiltrating lymphocytes (TILS) in ovarian cancer, has been correlated with improved outcomes [85,88-90]. Therefore, immune based treatments, with a focus on reversing the immunosuppressive environment in the tumor milieu are of marked interest.

Daclizumab (zenaprax)

Regulatory T cells (Tregs) are the subset of T cells that mediate immune suppression. They are involved in the prevention of autoimmunity and they also suppress immune activation by down-regulating co-stimulatory molecules that are necessary for T-cell activation on dendritic cells [91,92]. Regulatory T cells are CD4+ and CD25+, with CD25 being the interleukin (IL)-2 receptor α chain [93]. Malignant ascites from previously untreated ovarian cancer patients has been shown to contain significant numbers of CD4+ CD25+ CD3+ T cells, whereas as opposed to non-malignant ascites where these cells were rarely seen. In advanced ovarian cancer (FIGO stages III–IV) CD4+ CD25+ cells were found to be more abundant than in stages I–II [94]. Thus, strategies that could inhibit the function or deplete these cells in the tumor environment could prove useful in treating cancer patients.

Daclizumab (Zenapax) is a cell-depleting humanized IgG1 monoclonal antibody specific for CD25. It is currently being evaluated in clinical trials as an immunotherapy for a variety of cancers, including ovarian cancer [95].

Cytotoxic T-lymphocytes-associated antigen 4 (CTLA4) inhibition - Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a homologue of CD28 expressed on the surface of activated T cells. It serves as a key molecule in the down-regulation of T-cell activity as a means to limit auto-immunity. It inhibits the activation of T cells by effectively outcompeting CD28 for its ligands on antigen presenting cells or delivering inhibitory signals to T cells, functioning as an immune-checkpoint receptor. CTLA-4 is also constitutively expressed on regulatory T cells contributing to how they mediate immune suppression [96]. The use of therapeutic agents to block CTLA-4 function prevents immune inactivation and is a rational approach to evoke effective anti-tumor immune responses.

Ipilimumab (MDX-CTLA-4, yervoy)

Ipilimumab is a fully human IgG1 monoclonal antibody, which binds to and blocks the activity of CTLA-4. It was recently approved by the FDA for the treatment of advanced melanoma [97]. Given the results of long-term survival in subgroups of patients with metastatic melanoma treated with ipilimumab [98], there is great interest in assessing such approaches in ovarian cancer. In a phase I/II trial on 11 patients with FIGO stage IV ovarian cancers which had previously either received chemotherapy or a vaccine (GVAX comprised on autologous, irradiated tumor cells engineered to secrete the immune stimulatory

cytokine, granulocyte macrophage colony stimulating factor). Ipilimumab administration was well tolerated with the exception of some grade 3 inflammatory toxicities [99]. Anti-tumor effects were noted in two ovarian cancer patients with reduction of intraperitoneal disease and a fall in CA125 levels in one patient and reduction of pain and ascites and stabilization of CA125 in another. Ipilimumab is being evaluated in an ongoing phase II study as monotherapy in recurrent platinum sensitive ovarian cancer (NCT01611558).

Programmed cell death 1 receptor and ligands - Programmed death 1 is a type I membrane protein of 268 amino acids and is a member of the extended CD28/CTLA-4 family of T cell regulators [100]. PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family [101,102]. Monoclonal antibodies targeting PD-1 that boost the immune system are being developed for the treatment of cancer. Many tumor cells, including ovarian cancers, express PD-L1, an immunosuppressive PD-1 ligand; inhibition of the interaction between PD-1 and PD-L1 can enhance T-cell responses *in vitro* and mediate preclinical antitumor activity.

Nivolumab

Nivolumab an anti-PD-1 antibody, produced complete or partial responses in non-small-cell lung cancer, melanoma, and renal-cell cancer [103]. Although no trials have reported on the effects of targeting PD-1 or its ligands in ovarian cancer, given the emerging evidence of the activity of such agents in a variety of solid tumors, there is great interest in exploring these in this disease.

Discussion and Conclusions

Given the generally poor outcomes with the present standard of treatment for ovarian cancer, there has been great interest in applying a number of targeted approaches to the treatment of this disease.

Although targeting of angiogenesis was felt to be very promising in ovarian cancer, especially given the role of VEGF in the biology of this disease, the evidence at the present time does not support the use of any of these agents in the routine management of ovarian cancer. It has been a challenge to define and select patients most likely to respond to such approaches. At the present time, there are no predictive biomarkers to determine likelihood of response to anti-angiogenic agents. Also, understanding mechanisms of resistance to these agents may allow more effective approaches to anti-angiogenic therapy.

The PARP inhibitors represent one of the most exciting new treatment approaches in ovarian cancer. Emerging data indicates that these agents appear to be effective in patients with BRCA mutations when given as a maintenance treatment after completion of chemotherapy. Approximately 20% of patients with high grade serous ovarian cancer have BRCA mutations, but in addition to this, up to 50% of patients with high grade serous ovarian cancer may have loss of BRCA function. Tumors with this "BRCA"ness appear to behave like BRCA-1 or BRCA-2 deficient tumors, even though no germ-line mutations and have loss of BRCA function by other genetic or epigenetic events (e.g. hypermethylation). These tumors may also be candidates for treatment with PARP inhibition. The questions being address through ongoing studies with these agents are their role in first-line therapy, when given concurrent with chemotherapy and or as maintenance therapy, their efficacy when combined with chemotherapy or given as maintenance therapy in recurrent disease and their role in BRCA wild type (non-mutated) tumors.

Lastly, there is great interest in enhancing the immune response

to ovarian cancer. A number of these agents are being assessed in ongoing studies and will define how these approaches could be used in conjunction with other treatment options in these patients.

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