Review

Advanced Epithelial Ovarian Cancer: From Standard Chemotherapy to Promising Molecular Pathway Targets – Where Are we Now?

JOHN SYRIOS, SUSANA BANERJEE and STANLEY B. KAYE

Gynaecology Unit and the Drug Development Unit, The Royal Marsden NHS Foundation Trust, London, U.K.

Abstract. *Ovarian cancer is the most frequent cause of death from gynaecological malignancy in the Western countries, and differences in outcome among different histological subtypes are being increasingly recognized. It is generally considered as chemosensitive, but resistant clones evolve in the majority of cases, at varying rates. In this brief review, we describe advances in conventional chemotherapy, particularly the use of weekly paclitaxel. We then focus on new promising agents that target certain pathways which drive the genesis and evolution of ovarian cancer; these include poly(ADP-ribose) polymerase (PARP) inhibitors targeting tumor cells deficient in homologous recombination. We also discuss other targets including the folate receptor. Ovarian cancer has also proved to be one of the most sensitive types of cancer to an anti-angiogenic approach and we summarize recent experience using a range of agents.*

Ovarian cancer ranks second in incidence among gynaecological malignancies in developed countries, with the majority of patients being diagnosed with advanced disease. During the past three to four decades, the 5-year relative survival has ranged from 92% for patients with localized disease to 27% for those with distant disease (1).

Patients with advanced epithelial ovarian cancer (EOC) should be treated with optimal cytoreductive surgery, together with adjuvant carboplatin–paclitaxel chemotherapy, in view of the chemosensitivity of the disease. Patients with unresectable disease at diagnosis are treated with up-front chemotherapy. The standard first-line treatment includes carboplatin dosed at an area under the curve (AUC) of 6 mg/ml/min (according to the Calvert formula), along with paclitaxel at 175 mg/m^2 repeated every three weeks (2, 3).

Even though survival rates of patients with advanced disease have improved over the past decade, there is still an urgent need for a more thorough insight into the molecular pathways that drive cancer progression of the several diverse histological subtypes of ovarian cancer.

Updates in the Treatment of the Advanced EOC

I. Advances in Standard Chemotherapy

An impressive overall survival benefit was recently reported in patients with advanced disease treated with weekly compared to three-weekly paclitaxel along with carboplatin (4). In this study, patients with advanced disease all received carboplatin dosed using the Calvert formula at AUC 6 mg/ml/min every three weeks, and were randomly assigned to receive weekly paclitaxel (dose-dense) or the conventional three-weekly regimen. The overall survival (OS) at three years was higher in the dose-dense treatment group (72.1%) compared to the conventional treatment group (65.1%; hazard ratio (HR)=0.75, 95% confidence interval (CI)=0.57-0.98; p=0.03). In a recent survival analysis of the same study (5), the benefit was most clearly evident in the patients who underwent suboptimal debulking surgery. The median OS observed for patients with residual disease ≥1 cm was 51.2 (95% CI=40.1-58.3) months with the intensified regimen compared to 33.5 (95% CI=29.3-43.6) months with the standard 3-weekly one (HR=0.75, 95% CI=0.57-0.97; p=0.0027). In patients undergoing optimal debulking surgery, there was no statistically significant survival benefit with the intensified regimen but this may be due to lack of sufficient number of events so far with optimally-debulked patients, and further follow-up is, therefore, necessary since the median OS has not been reached in either arm.

This article is freely accessible online.

Correspondence to: John Syrios, MD, Ph.D., Medical Oncologist, 6A Samou Street, 15344 Gerakas, Attiki, Greece. Tel: +30 6976516355, Fax: +30 2106617299, e-mail: syriosi@yahoo.gr

Key Words: Conventional chemotherapy, epithelial ovarian cancer, histological subtypes, molecular pathway targets, PARP inhibitors, tumour angiogenesis, tyrosine kinase inhibitors, review.

II. Targeting Tumor Angiogenesis

Data from a number of trials has confirmed that anti-angiogenic therapy has an important role to play at various stages of ovarian cancer and this probably relates to the central role played by the key growth factors in the biology of the healthy ovary (6-20).

Anti-angiogenesis in the First-line Treatment Setting

Bevacizumab. A humanized monoclonal antibody, bevacizumab binds the vascular endothelial growth factor (VEGF) and prevents it from binding to its receptor. In the ICON7 trial (6), patients with advanced or metastatic EOC were assigned to carboplatin/paclitaxel, given every three weeks for six cycles, or to carboplatin–paclitaxel plus bevacizumab (7.5 mg/kg), given concurrently every three weeks for five or six cycles and continued for 12 additional cycles or until progression of disease as maintenance. Progression-free survival (PFS) at 42 months of follow-up has improved from 22.4 months with standard therapy to 24.1 months with the addition of bevacizumab (p=0.04). The greatest benefit from bevacizumab was seen in patients at high risk for progression, with PFS at 42 months of follow-up of 14.5 months with standard therapy and 18.1 months when bevacizumab was added, with median OS of 28.8 and 36.6 months, respectively. In a recent subgroup analysis update, patients with stage IV disease and those who had suboptimal debulking surgery had an OS benefit of 4 months when treated with bevacizumab (39 months for the high-risk patients who received bevacizumab compared to 35 months for the control arm). In ICON7, the survival curves demonstrate non-proportionality and the significant benefit of bevacizumab is, therefore, best calculated using a restricted means analysis (7).

In the initial analysis of the parallel US GOG 218 trial, the combination of carboplatin–paclitaxel with bevacizumab followed by bevacizumab maintenance therapy improved PFS but not OS compared to the same regimen without bevacizumab (8).

The differences between GOG 218 and ICON7 may relate to a higher use of bevacizumab at relapse in the USA; interestingly, however, a recent subgroup analysis of GOG 218 also shows an overall survival benefit of 7.8 months for patients with stage IV disease who received bevacizumab (HR=0.72; 95% CI=0.53-0.97). The median OS for patients treated with chemotherapy and bevacizumab was 40.6 months compared to 32.8 months for those treated without bevacizumab (9).

As a result of these data, bevacizumab has been incorporated into the standard-of-care for first-line treatment in some Centres. However, other investigators have instead preferred to use it as part of the treatment strategy for relapsed disease (see below).

Tyrosine kinase inhibitors. Two large randomized trials have recently been concluded with interesting data as follows: Pazopanib is a tyrosine kinase inhibitor which targets the vascular endothelial growth factor receptors (VEGFR) 1, -2 and -3, the platelet-derived growth factor receptor (PDGFR)-α/β, the fibroblast growth factor receptors (FGFR) -1 and -3, and the tyrosine-proteine kinase Kit (c-KIT) (10). In the recently presented AGO-OVAR16 study (11), 940 patients with advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer who showed no evidence of progression after surgery and five or more cycles of platinum-taxane chemotherapy were randomly assigned at a ratio of 1:1 to receive 800 mg pazopanib once daily or placebo for up to 24 months. The addition of pazopanib extended the PFS by 5.6 months compared to placebo (HR=0.766; p=0.0021). The median time-to-disease progression was improved to 17.9 months with pazopanib compared to 12.3 months with the placebo. In the first interim analysis for OS, there was no difference between the two arms, however, data have yet to mature. Nonetheless, there is concern about the toxicity and serious adverse events seen in patients treated with pazopanib, which notably included hypertension, diarrhea, nausea, headache and fatigue.

Nintedanib is a tyrosine kinase oral inhibitor which targets VEGFR, PDGFR, and FGFR. In a recently presented study, patients with stage IIB-IV ovarian cancer according to the International Federation of Gynecologists and Obstetricians (FIGO) treated with upfront debulking surgery were randomized to receive the conventional chemotherapy (carboplatin–paclitaxel) with either placebo or nintedanib at 200 mg *bid* (12). In addition to concurrent treatment, nintedanib or placebo was then continued as maintenance therapy for up to 120 weeks. Nintedanib toxicity was manageable, comprising mainly of diarrhea and raised transaminases. After 752 observed events, analysis of the total population showed that patients who received nintedanib had a marginally-longer median PFS (17.3 *vs.* 16.6 months; HR=0.84; 95% CI=0.72-0.98; p=0.0239).

Interestingly, and for unknown reasons, in both the pazopanib and nintedanib trials, the benefit of these molecules seemed most clear in patients with optimal rather than suboptimal surgery, in contrast to the subgroup analyses of ICON7 and GOG-218 with bevacizumab (6, 8, 11, 12). For example, 85% of the patients in the pazopanib trial had no evidence of residual disease at the time of enrollment (11), while in the nintedanib study, low-risk patients (as defined in ICON-7) treated with the investigational agent and chemotherapy had an impressive PFS of 27.1 months compared to 20.8 months for similar patients on placebo (12).

Anti-angiogenesis in Relapsed Disease

Bevacizumab. Supporting the use of bevacizumab in the treatment of patients with relapsed EOC are the data from the OCEANS trial (13). In this phase III study, patients with platinum-sensitive relapsed EOC received carboplatin with gemcitabine with the addition of bevacizumab or placebo until progression. The investigational arm experienced a 4-month prolongation of PFS compared to the arm that received the placebo (median PFS 12.4 *vs*. 8.4 months; HR=0.484; 95% CI=0.388-0.605; $p<0.0001$). Interestingly, no OS benefit was observed. Patients in the placebo did receive bevacizumab at subsequent relapse more frequently than in the other arm and this could partly explain the lack of OS benefit.

AURELIA was a phase III study which evaluated the impact of adding bevacizumab to single-agent chemotherapy based either on weekly paclitaxel, topotecan or doxorubicin in patients with platinum-resistant EOC (14). The data on OS were recently presented and, probably due partly to the use of bevacizumab at disease progression, did not reveal any OS benefit between the patients treated with chemotherapy alone and those treated with the addition of bevacizumab, at least for the whole group. However, in a subgroup analysis, patients treated with weekly paclitaxel had a median OS of 13 months, which was significantly increased to 22 months with the addition of bevacizumab (15).

Tyrosine kinase inhibitors. Cediranib is a potent oral inhibitor of VEGFR1, -2, one -3 that inhibits VEGF signaling. In the ICON6 phase III trial (16), patients with recurrent platinum-sensitive EOC were randomized to three cohorts: platinum-based chemotherapy with placebo maintenance; concurrent cediranib at 20 mg/day during chemotherapy followed by placebo for up to 18 months; or cediranib at 20 mg/day followed by maintenance cediranib. In a restricted means analysis, OS increased from 17.6 to 20.3 months in the concurrent-plus-maintenance cediranib arm over the reference arm, (HR=0.70; $p=0.0419$). Notably, it was the first time in the treatment of the EOC that a survival benefit was seen with a tyrosine kinase inhibitor. Using restricted means analysis, the PFS for the reference *versus* the concurrent-plus-maintenance arm demonstrated a 3.1 month difference favoring cediranib (9.4 months *vs*. 12.5 months) and a PFS benefit was also seen for the concurrent arm (no maintenance) compared to the reference one (with a PFS of 10.1 *vs*. 11.4 months, respectively). This indicates that cediranib has significant activity when used both during and after chemotherapy. It was, however, clear that the major benefit in OS was due to the maintenance component of cediranib. The toxicity patients derived from the investigational agent included mainly hypertension, diarrhea, hypothyroidism, hoarseness, haemorrhage, proteinuria and fatigue.

To assess whether agents of this type can also provide a benefit in patients with recurrent platinum-resistant disease, an ongoing randomized phase IIB study of weekly paclitaxel with/without pazopanib is underway (17).

Targeting the angiopoietins. AMG386 is a novel molecular agent, a peptide-Fc fusion protein which is designed to inhibit angiogenesis by sequestering the angiopoietins (ANG)-1 and -2, thereby preventing their interaction with the endothelium-specific receptor tyrosine kinase TIE2 receptor. Its activity was evaluated in a phase II study (18) in which all patients with recurrent EOC received weekly paclitaxel at 80 mg/m^2 (three weeks on, one week off) and were randomly assigned 1:1:1 to also receive AMG386 at 10 mg/kg (arm A), AMG386 at 3 mg/kg (arm B), or placebo (arm C). The median PFS was 7.2 months (95% CI=5.3-8.1 months) in arm A, 5.7 months (95% CI=4.6-8.0 months) in arm B, and 4.6 months (95% CI=1.9-6.7 months) in arm C; a dose–response trend was observed for median PFS. The HR for arms A and B combined *vs*. arm C was 0.76 (95% CI=0.52-1.12; $p=0.165$). In view of the activity of AMG386, a phase III trial, named TRINOVA-1 (19) was conducted. In that study, patients with recurrent EOC were allocated to two investigational arms. Notably, patients previously treated with bevacizumab were not excluded from the trial, although they comprised a small minority (only 5%) of treated patients. The first arm was treated with AMG386 plus paclitaxel and the second with placebo plus paclitaxel. Patients treated with the investigational drug experienced a PFS prolonged by 52%; median PFS was 7.2 months in the AMG386 arm *vs*. 5.4 months in the control arm (HR=0.66, CI=0.56-0.76; $p<0.001$). The data regarding OS are expected to mature in 2014, however, an interim analysis revealed a trend towards improved OS of median 19.0 *vs*. 17.3 months (HR=0.86; $p=0.19$) in favour of AMG386.

To further assess the potential benefit of AMG386, another study TRINOVA-2 is underway (20). Patients with recurrent partially platinum-sensitive or resistant EOC are treated with pegylated liposomal doxorubicin (PLD) combined with AMG386 at a dose of 15 mg/kg or PLD plus placebo. Outcome measures are PFS (primary) and OS (secondary).

These data with AMG386 are certainly of interest; a key issue for the future will be the assessment of its activity in patients previously treated with bevacizumab and further clinical experience should be analyzed with this in mind. Following recurrence of disease, the optimal treatment sequence targeting diverse routes of tumor angiogenesis has yet to be defined, but this will be a key question for further randomized studies.

III. Treatment of Breast Cancer Tumour Suppressor Gene (BRCA) Mutation Carriers

BRCA1 and *BRCA2* are tumour suppressor genes that control the repair of genetic alterations throughout cellular division.

Approximately 14% of patients with ovarian cancer carry a germline *BRCA* mutation; this proportion is higher for those with high-grade serous histology (22.6%). In addition, a minority of patients (7%) in whom no germline *BRCA* mutation is detected harbour a somatic *BRCA* mutation (21).

The aberrant expression of *BRCA1* and *BRCA2* is associated with an intrinsic sensitivity to poly(ADP ribose) polymerase (PARP) inhibitors which inhibit the repair of single-strand DNA breaks during normal DNA replication, leading to accumulation of DNA double-strand breaks at replication forks. Under normal circumstances, these are repaired *via* the efficient BRCA pathway-dependent homologous recombination mechanism. Tumors lacking BRCA function have to rely on double-strand repair through other means, such as non-homologous end joining; these are error prone and lead to cell death. Such carcinomas are sensitive to PARP inhibitors alone (22). These molecules exploit the 'synthetic lethality' phenomenon since they target one gene in a synthetic lethal pair in which the other gene is defective (23).

The activity of single-agent PARP inhibitor in patients with ovarian, breast or prostate cancer carrying a *BRCA* mutation was first described by Fong *et al*. in 2009 (22). In a subsequent study, Gelmon *et al*. showed that PARP inhibitors may also exert substantial antitumor activity in patients with sporadic high grade serous carcinoma, although most responses were seen in patients with platinum-sensitive disease (24). In that study, 46 patients with relapsed disease received olaparib (AZD2281) at 400 mg twice-a-day continuously. Among the 11 responders, 10 (50%) had platinum-sensitive and one (4%) platinum-resistant disease. This relates to the fact that a proportion of sporadic high-grade serous carcinomas have lost *BRCA1* or *BRCA2* due to somatic mutations or epigenetic events (25).

In a randomized maintenance trial, patients with high-grade serous, platinum-sensitive EOC, whose disease responded to the most recent platinum-based treatment were randomized to receive either olaparib at 400 mg twice daily as a maintenance therapy or placebo (26). The PFS of patients on olaparib reached 8.4 months compared to 4.8 months for those on placebo (HR=0.35; 95% CI=0.25-0.49; $p<0.001$). In an interim analysis of OS (58% maturity), patients on olaparib had a median OS of 29.8 *vs*. 27.8 months for those on placebo (HR=0.88; 95% CI=0.64-1.21) (27). Although initially over 60% of patients were of unknown *BRCA* mutation status, a subsequent analysis of stored blood (and tumor) samples revealed that 51% of cases had a germline/tumor *BRCA1/2* mutation. In this subgroup, the benefit in respect of PFS was most obvious. The OS was also improved for the *BRCA* mutation-positive subgroup, although less so than the PFS, possibly because of a degree of crossover to olaparib in patients initially receiving placebo. The time to second subsequent therapy reached a median of 23.8 months for patients with BRCA mutation on olaparib compared to 15.3 months for those on placebo (HR=0.46; 95% CI=0.30-0.70, $p<0.0003$). The toxicity related to olaparib involved mainly nausea, fatigue, vomiting and anaemia, but generally treatment was well tolerated.

A phase III trial evaluating the role of olaparib maintenance (first-line) is currently open and recruiting patients with BRCA mutatIon who are at high risk of experiencing relapse (FIGO stages III-IV). Patients are randomly assigned to receive either olaparib tablets at 300 mg orally twice daily, or placebo, for up to two years or until objective radiological disease progression (28). This trial, and a repeat of the second-line maintenance trial (29), use a new tablet formulation of olaparib, which should prove easier for patients than the capsule, since it involves taking three tablets instead of eight capsules twice a day.

Another novel PARP1 and PARP2 inhibitor, MK4827 (niraparib), was studied in *BRCA* mutation carriers and in patients with sporadic advanced tumours in a phase I dose-escalation trial (30). In this trial, eight out of 20 (40%) patients with ovarian cancer with *BRCA* mutation experienced a partial response, including five out of 10 *BRCA1/2* mutation carriers with platinum-sensitive disease. The median duration of response was an impressive 431 days. Interestingly, three out of nine patients with *BRCA* mutation, platinum-resistant disease showed a partial response, whilst one had long-lasting (>120 days) stable disease. One *BRCA1* mutation carrier with platinum-refractory disease had stable disease. The response to niraparib was not limited to patients with *BRCA* mutation but was also reported in sporadic high-grade serous ovarian cancer cases. Two out of 3 patients with platinum-sensitive, and 3 out of 19 (16%) with platinum-resistant high-grade serous ovarian cancer experienced a partial response, whilst 3 (16%) of the latter group had stable disease. Niraparib, is currently being tested in a phase III, randomized, double-blind, placebo-controlled study as maintenance in patients with platinum-sensitive ovarian cancer who have either germline *BRCA* mutation or a high-grade serous tumour and who have responded to their most recent chemotherapy containing a platinum agent (31). A key element of this trial will be the validation of a biomarker for homologous recombination deficiency, which could predict efficacy of PARP inhibition in patients with no evidence of *BRCA* mutation. A similar maintenance trial is also planned for another PARP inhibitor, rucaparib (32).

Finally, another PARP inhibitor undergoing active clinical investigation is BMN673. In a recently presented study, 22 patients with platinum-sensitive, 4 with platinum-resistant, and two with platinum-refractory relapsed EOC were treated with this agent, which was generally well-tolerated (33). Twenty patients had *BRCA1* and 8 *BRCA2* mutation. The response rate was approximately 50% for

platinum-sensitive and 30% for platinum-resistant patients. Notably, seven patients with *BRCA1* mutation experienced a partial response compared to four with *BRCA2* mutation. The median duration of response was 26.9 weeks and the median PFS was 32.3 weeks. The maximum tolerated dose of BMN673 is 1 mg/day, which indicates the high potency of this molecule, which had previously been noted in *in vitro* studies.

The combination of a PARP inhibitor with chemotherapy has so far not shown clear evidence of benefit. In a recently presented study (34), patients with platinum-sensitive recurrent EOC were treated with chemotherapy of carboplatin–paclitaxel along with olaparib and then continued with olaparib maintenance. Patients with germline *BRCA* mutations treated with olaparib did have a superior PFS (although not OS) but this was almost certainly related to the impact of single-maintenance agent olaparib which had already been established as having efficacy. When response to chemotherapy with olaparib was compared to chemotherapy alone there was no significant difference.

Other combinations based on pre-clinical data, which include PARP inhibitors with anti-angiogenic agents, or phosphoinositide 3-kinase / serine-threonine-specific protein kinase AKT (PI3K/AKT) inhibitors, may prove to be more beneficial than those with chemotherapy, and appropriate studies are now being planned (35, 36).

IV. Targeting Other Molecular Pathways

Interfering with cell-cycle checkpoints. During the progression of the cellular cycle there are certain checkpoints which control the integrity of the dividing cells and the abundance of the appropriate molecular signs that direct cells towards differentiation. Specifically, the cell cycle is regulated by several cyclin-dependent kinases (CDKs) which control checkpoints. In the case of DNA damage, the CDKs halt the cellular cycle at the G_1, S, and G_2 phases, allowing time for the cell to repair DNA damage or to initiate apoptosis. Selective inhibition of checkpoints in tumour cells may therefore enhance the efficacy of DNA-damaging agents as long as the DNA repair mechanisms of the host are impaired (37).

MK-1775 is a small molecule inhibitor of WEE1, a nuclear tyrosine kinase belonging to the serine/threonine family, particularly involved in regulation of the G_2 checkpoint. For tumors that harbour mutation of *p53* gene, which is a tumor-suppressor gene that mainly governs the G_1 checkpoint, the S and G_2 checkpoints are crucial for differentiation (38). MK-1775 inhibits WEE1 activity by inhibition of the phosphorylation of its direct substrate. Thus, cancer cells harbouring *p53* mutation should undergo apoptosis, given that the WEE1-dependent checkpoint is also by passed (39). The hypothesis suggesting that MK-1775 potentiates the activity of cytotoxic agents or radiotherapy has been tested in tumour cell lines such as in human sarcomas, melanoma, neuroblastoma, glioblastoma and pancreatic xenografts (40-44).

In the clinical setting, the ongoing MK-1775 trial is investigating the efficacy, safety, pharmacokinetics, and tolerability of MK-1775 in combination with carboplatin–paclitaxel in patients with relapsed platinum-sensitive, p53-mutant ovarian cancer (45).

Targeting the Folate Receptor (FR). The FR is commonly expressed in more than 80% of epithelial carcinomas, including ovarian cancer. One rationale for targeting the FR is that folic acid may be used as a vector to which a cytotoxic agent is conjugated in order to penetrate the tumor cell, maximizing its antitumor effect, and eventually minimizing toxicity (46).

In a phase II study, 100 out of 149 patients with recurrent platinum-resistant EOC were treated with vintafolide (EC145), which is a folate–desacetylvinblastine monohydrazide conjugate designed to directly target FR-expressing cells, along with PLD (47). Patients were randomized to receive the investigational drug along with PLD (100 patients) or PLD alone (49 patients). Importantly, a functional evaluation of the FR expression was performed with imaging with etarfolatide. Patients were categorized as FR 100% (all lesions FR-positive), FR 10% to 90% (if at least one FR-positive lesion was identified), and FR 0% (no FR-positive lesions). The median PFS was 5.0 months for the vintafolide group compared to 2.7 months for the single-agent PLD group (HR=0.63, p=0.031). The highest median PFS was observed in patients with 100% FR activity (5.5 *vs*. 1.5 months, HR=0.38, p=0.013). However, no statistically significant PFS benefit was found for patients with 10%-90% positive lesions or those with 0% positive lesions. Further evidence is expected to emerge from an ongoing larger randomized phase III trial, which is comparing EC145 and PLD in combination *vs*. PLD in patients with recurrent platinum-resistant EOC (48).

Inhibiting Insulin-like Growth Factor (IGF). Another tyrosine kinase receptor whose activation enhances tumorigenesis is the receptor for IGF (49, 50). The IGF family plays a crucial role in the development, progression, and dissemination of multiple cancer types (51). The activation and the increased expression of the receptor 1 of the IGF correlates to the pace and prognosis of the disease. Activated AKT in turn activates the mammalian target of rapamycin (mTOR) pathway, which promotes cell growth and survival, production of transcription factors, and protein synthesis, and regulates the cell cycle (52, 53).

OSI-906 is a low-molecular weight TKI of both insulin receptor and IGF1R, which in turn regulates AKT signaling. In a phase I trial of OSI-906 in patients with recurrent EOC

in combination with paclitaxel, five out of 29 patients experienced a partial response and 11 had stable disease (54). A phase I/II study evaluating OSI-906 in combination with weekly paclitaxel in patients with recurrent EOC recently closed and results are awaited (55).

IV. Treatment Tailored to Histology

Low-grade EOC. Among the diverse histological subtypes of EOC there are certain molecular imprints and distinct genetic abnormalities which may have a predictive impact. For instance, low-grade serous ovarian carcinomas (LGSC) frequently harbour mutations in the Kirsten rat sarcoma viral oncogene (*KRAS*) or the murine sarcoma viral proto-oncogene homolog B1 (*BRAF*), and express active mitogen-activated protein kinase (MAPK) (56). Singer *et al*. reported mutations in *BRAF* (codon 599) in 33% of LGSC samples and *KRAS* mutations (codons 12 and 13) in 35% of the cases, mutations which notably were mutually exclusive (57). In the COSMIC database, 39% of LGSC samples had the V600E allele *BRAF* mutation (58, 59). Further molecular insight revealed that these LGSC do not harbour mutations in *p53* (60).

In 2008, Nakayama *et al*. suggested that in patients with ovarian cancer harbouring *KRAS* or *BRAF* mutations, activated ERK1/2 pathway is critical to tumour growth and survival, and these patients may, thus,benefit from agents that inactivate ERK1/2 (61). Recently, in a phase II study (62) patients with recurrent LGSC of the ovary or peritoneum were treated with selumetinib, a potent, selective inhibitor of MEK1/2. Eight out of 52 assessable patients, experienced partial or complete response (15%; 90% CI=7.9-26.1%). Median PFS was 11.0 months (95% CI=3.6-15.9 months) with 33 patients having a PFS of longer than six months. Interestingly, response to selumetinib was not related to *KRAS* or *BRAF* mutation status. The activity of MEK1/2 inhibitor is therefore promising and warrants further clinical testing. Two large-scale randomized trials involving different single-agent MEK inhibitors are now underway (63, 64).

The combination of a MEK inhibitor with an AKT inhibitor may prove superior in enhancing apoptosis given the cross-talk between MEK and PI3K downstream of receptor tyrosine kinases; this combination is being tested at the feasibility stage and may ultimately prove more effective in LGSC.

Taking into consideration the example of patients with melanoma, vemurafenib, a BRAF kinase inhibitor, is highly active, conferring response rates as high as 57% (65). Patients with LGSC of the ovary may also be candidates for treatment with vemurafenib. The NCRN396 VE BASKET trial is an open-label, phase II study evaluating the role of vemurafenib in patients with *BRAF* V600 mutation-positive ovarian cancer (66).

Clear Cell EOC. Clear cell ovarian carcinomas constitute a minority, accounting for only 5-13% of all EOCs (67). Their aggressive behaviour and resistance to conventional chemotherapy mandates a further insight into their biology. The molecular pathways implicated lead to new options for treatment. PI3K is found to be mutated in 33% of patients with clear cell ovarian cancer (68), whilst loss of the phosphatase and tensin homolog (*PTEN*) gene expression is noted in 40% (69) and amplification of *AKT2* was noted in 5/21 samples in a separate study (70). Various inhibitors of the PI3K/AKT pathway are therefore currently in clinical studies and there is anecdotal evidence of efficacy. In a phase II trial, the addition of temsirolimus to carboplatin and paclitaxel together will be studied in patients with newly diagnosed stage III or stage IV clear cell ovarian cancer (71). An alternative approach, based on similarities in genomic profile between clear cell ovarian and renal cancer (72) involves the antiangiogenic agent sunitinib. This agent is currently being evaluated in patients with persistent or recurrent clear cell ovarian cancer (73).

Mucinous ovarian carcinoma. Mucinous ovarian carcinoma is a rare malignancy comprising less than 4% of ovarian cancer cases. It commonly presents as a stage I tumour but when more advanced, the response rate to standard chemotherapy is lower compared to other more common pathological subtypes (74). Interestingly, 20% of mucinous carcinomas were found to express the human epidermal growth factor receptor 2 (HER2) according to immuno-histochemistry (IHC) and fluorescence *in situ* hybidization (FISH) criteria (75,76). In a study of 33 cases (76), 5 of the carcinomas (3+ in 4 cases, 2+ in 1 case) had HER2 overexpression by IHC, whilst by FISH there was high-level *HER2* amplification in six cases (18.2%).

Trastuzumab, a monoclonal antibody that targets the HER2 protein, did not show efficacy in a phase II trial of unselected patients with ovarian cancer (77), but further evaluation does seem indicated, specifically in patients with $HER2^+$ mucinous pathology.

Conclusion

The development of more sophisticated molecular agents targeting key pathways of cancer signaling and differentiation is defining a new era in the treatment of advanced ovarian cancer. More phase III studies are, however, needed to further evaluate the efficacy and toxicity of these new agents. Importantly, the identification of certain predictive biomarkers may optimize treatment's efficacy distinguishing the target group that would derive the most benefit through this patient-tailored approach.

Acknowledgements

J. Syrios' fellowship is supported by a grant of the European School of Oncology and by the Hellenic Society of Medical Oncology scholarship.

The Gynaecology Unit and the Drug Development Unit of the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research acknowledge joint support from the National Institute of Health Research Biomedical Research Centre, and also from Cancer Research UK through an Experimental Cancer Medicine Center grant.

References

1 Howlader N, Noone AM, Krapcho M *et al*: SEER Cancer Statistics Review, 1975-2010, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2010/, based on November 2012 SEER data submission, posted to the SEER web site, 2013.

2 Du Bois A, Luck HJ, Meier W *et al*: A randomized clinical trial of cisplatin/paclitaxel *versus* carboplatin/paclitaxel as first-line treatment of ovarian cancer. J Natl Cancer Inst *95*: 1320-1330, 2003.

3 Thigpen T, Dubois A, McAlpine J *et al*: First-line therapy in ovarian cancer trials. Int J Gynecol Cancer *21*: 756-762, 2011.

4 Katsumata N, Yasuda M, Takahashi F *et al*: Dose-dense paclitaxel once a week in combination with carboplatin every three weeks for advanced ovarian cancer: a phase III, open-label, randomised controlled trial. Lancet *374(9698)*: 1331-1338, 2009.

5 Katsumata N, Yasuda M, Isonishi S *et al*: Long-term results of dose-dense paclitaxel and carboplatin *versus* conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (JGOG 3016): a randomised, controlled, open-label trial. Lancet Oncol *14(10)*: 1020-1026, 2013.

6 Perren TJ, Swart AM, Pfisterer J *et al*: A phase III trial of bevacizumab in ovarian cancer. N Engl J Med *365(26)*: 2484-2496, 2011.

7 Oza A, Perren T, Swart AM *et al*: ICON7 final overall survival results in the GCIG phase III randomized trial of bevacizumab in women with newly diagnosed ovarian cancer. Program and abstracts of the European Cancer Congress, 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract LBA6.

8 Burger RA, Brady MD, Bookman MA *et al*: Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med *365(26)*: 2473-2483, 2011.

9 Randall L, Burger R, Nguyen H *et al*: Outcome differences in patients with advanced epithelial ovarian, primary peritoneal, and fallopian tube cancers treated with and without Bevacizumab. Presented at the Society for Gynecologic Oncology Annual Meeting on Women's Cancer; March 9-12, 2013, Los Angeles, CA, USA.

10 Kumar R, Knick VB, Rudolph SK *et al*: Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. Mol Cancer Ther *6(7)*: 2012-2021, 2007.

11 Du Bois A, Floquet A, Kim JW *et al*: Randomized, double-blind, phase III trial of pazopanib *versus* placebo in women who have not progressed after first-line chemotherapy for advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (AEOC): Results of an international Intergroup trial (AGO-OVAR16). J Clin Oncol 31(suppl; LBA 5503), 2013.

12 Du Bois A, Kristensen G, Ray-Coquard I *et al*: AGO-OVAR 12: A randomized placebo-controlled GCIG/ENGOT-Intergroup phase III trial of standard frontline chemotherapy +/- nintedanib for advanced ovarian cancer. Int J Gynecol Cancer Vol. 23, Issue 8, Supplement 1, 2013.

13 Aghajanian C, Blank SV, Goff BA *et al*: OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or withoutbevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol *30(17)*: 2039-2045, 2012.

14 Pujade-Lauraine E, Hilpert F, Weber B *et al*: (2012) AURELIA: A randomized phase III trial evaluating bevacizumab (BEV) plus chemotherapy (CT) for platinum (PT)-resistant recurrent ovarian cancer (OC) (LBA5002). J Clin Oncol 30(Suppl. Abstracts): LBA5002, 2012.

15 Witteveen P, Lortholary A, Fehm T *et al*: Final overall survival (OS) results from AURELIA, an open-label randomised phase III trial of chemotherapy (CT) with or without bevacizumab (BEV) for platinum-resistant recurrent ovarian cancer (OC). Program and abstracts of the European Cancer Congress, September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract LBA 5, 2013.

16 Ledermann JA, Perren T, Raja FA *et al*: Randomised double-blind phase III trial of cediranib (AZD 2171) in relapsed platinum sensitive ovarian cancer: Results of the ICON6 trial. Program and abstracts of the European Cancer Congress, September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract LBA 10, 2013.

17 ClinicalTrials.gov identifier: NCT01468909.

18 Karlan BY, Oza AM, Richardson GE *et al*: Randomized, double-blind, placebo-controlled phase II study of AMG 386 combined with weekly paclitaxel in patients with recurrent ovarian cancer. J Clin Oncol *30(4)*: 362-371, 2012.

19 Monk BJ, Poveda A, Vergote I *et al*: A phase III, randomized, double-blind trial of weekly paclitaxel plus the angiopoeitin 1 and 2 inhibitor, trebananib, or placebo in women with recurrent ovarian cancer: TRINOVA-1. 17th ECCO – 38th ESMO – 32nd ESTRO European Cancer Congress. 2013,1 October during the Gynaecological Cancer Proffered Papers Session (Abstract E17-7107).

20 ClinicalTrials.gov identifier: NCT01281254.

21 Alsop K, Fereday S, Meldrum C *et al*: BRCA mutation frequency and patterns of treatment response in *BRCA* mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol *30(21)*: 2654-2663, 2012.

22 Fong PC, Boss DS, Yap TA *et al*: Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. N Engl J Med *361(2)*: 123-134, 2009.

23 Ashworth A: A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol *26*: 3785-3790, 2008.

24 Gelmon KA, Tischkowitz M, Mackay H *et al*: Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase II, multicentre, open-label, non-randomised study. Lancet Oncol *12(9)*: 852-861, 2011.
25 Press JZ, De Luca A, Boyd N *et al*: Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer *8*: 17, 2008.
26 Ledermann JA, Harter P, Gourley C *et al*: Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med *366(15)*: 1382-1392, 2012.
27 Ledermann JA, Harter P, Gourley C *et al*: Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer (SOC) and a *BRCA* mutation (BRCAm). J Clin Oncol 31, (suppl; abstr 5505), 2013.
28 ClinicalTrials.gov Identifier: NCT01844986.
29 ClinicalTrials.gov Identifier: NCT01874353.
30 Sandhu SK, Schelman WR, Wilding G *et al*: The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in *BRCA* mutation carriers and patients with sporadic cancer: a phase I dose-escalation trial. Lancet Oncol *14(9)*: 882-892, 2013.
31 ClinicalTrials.gov Identifier: NCT01847274.
32 ClinicalTrials.gov Identifier: NCT01891344.
33 Ramanathan R, Wainberg Z, Mina L *et al*: PARP inhibition with BMN 673 in ovarian and breast cancer patients with deleterious mutations of *BRCA1* and *BRCA2*. Program and abstracts of the European Cancer Congress, September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract LBA 29, 2013.
34 Oza AM, Cibula D, Benzaquen AO *et al*: Olaparib plus chemotherapy, followed by maintenance monotherapy, in women with platinum-sensitive recurrent serous ovarian cancer (PSR SOC):BRCA1/2 mutation (BRCAm) and interim overall survival analyses. Program and abstracts of the European Cancer Congress, September 27-October 1, 2013; Amstersdam, The Netherlands. Abstract 3002, 2013.
35 Sessa C. Update on PARP1 inhibitors in ovarian cancer. Ann Oncol *22(Suppl 8)*: viii72-viii76, 2011.
36 Rehman FL, Lord CJ, Ashworth A. The promise of combining inhibition of PI3K and PARP as cancer therapy. Cancer Discov *2(11)*: 982-984, 2012.
37 Sherr CJ. Cancer cell cycles. Science *274(5293)*: 1672-1677, 1996.
38 Nigro JM, Baker SJ, Preisinger AC *et al*: Mutations in the p53 gene occur in diverse human tumour types. Nature *342(6250)*: 705-708, 1989.
39 Wang Y, Li J, Booher RN *et al*: Radiosensitization of p53 Mutant Cells by PD0166285, a Novel G2 Checkpoint Abrogator. Cancer Res *61(22)*: 8211-8217, 2001.
40 Kreahling JM, Foroutan P, Reed D *et al*: WEE1 inhibition by MK-1775 leads to tumor inhibition and enhances efficacy of gemcitabine in human sarcomas. PLoS One *8(3)*: e57523, 2013.
41 Haarberg HE, Paraiso KH, Wood E *et al*: Inhibition of WEE1, AKT, and CDK4 underlies the efficacy of the HSP90 inhibitor XL888 in an *in vivo* model of *NRAS*-mutant melanoma. Mol Cancer Ther *12(6)*: 901-912, 2013.
42 Russell MR, Levin K, Rader J *et al*: Combination therapy targeting the Chk1 and Wee1 kinases shows therapeutic efficacy in neuroblastoma. Cancer Res *73(2)*: 776-784, 2013.
43 Sarcar B, Kahali S, Prabhu AH *et al*: Targeting radiation-induced G(2) checkpoint activation with the WEE-1 inhibitor MK-1775 in glioblastoma cell lines. Mol Cancer Ther *10(12)*: 2405-2414, 2011.
44 Rajeshkumar NV, De Oliveira E, Ottenhof N *et al*: MK-1775, a potent WEE1 inhibitor, synergizes with gemcitabine to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts. Clin Cancer Res *17(9)*: 2799-2806, 2011.
45 ClinicalTrials.gov Identifier: NCT01357161.
46 Kalli KR, Oberg AL, Keeney GL *et al*: Folate receptor alpha as a tumor target in epithelial ovarian cancer. Gynecol Oncol *108*: 619-626, 2008.
47 Naumann RW, Coleman RL, Burger RA *et al*: PRECEDENT: A randomized phase II trial comparing vintafolide (EC145) and Pegylated Liposomal Doxorubicin (PLD) in combination *versus* PLD alone in patients with platinum-resistant ovarian cancer. J Clin Oncol *31(35)*: 4400-4406, 2013.
48 ClinicalTrials.gov Identifier: NCT01170650.
49 Muller M, Dietel M, Turzynski A and Wiechen K: Antisense phosphorothioate oligodeoxynucleotide down-regulation of the insulin-like growth factor 1 receptor in ovarian cancer Cells. Int J Cancer *77(4)*: 567-571, 1998.
50 Kurmasheva RT and Houghton PJ: IGF-I mediated survival pathways in normal and malignant cells. Biochim Biophys Acta *1766(1)*: 1-22, 2006.
51 Pollak M. Insulin and insulin-like growth factor signaling in neoplasia. Nature Rev Cancer *8*: 915-928, 2008.
52 Lu L, Katsaros D, Wiley A *et al*: The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-alpha expression to disease progression in epithelial ovarian cancer. Clin Cancer Res *12(4)*: 1208-1214, 2006.
53 Sayer R, Lancaster JM, Pittman J *et al*: High insulin-like growth factor-2 (IGF-2) gene expression is an independent predictor of poor survival for patients with advanced-stage serous epithelial ovarian cancer. Gynecol Oncol *96(2)*: 355-361, 2005.
54 Harb WA, Sessa C, Hirte HW *et al*: A phase I study evaluating the combination of OSI-906, a dual inhibitor of insulin growth factor-1 receptor (IGF-1R) and insulin receptor (IR) with weekly paclitaxel (PAC) in patients with advanced solid tumors. J Clin Oncol (Meeting Abstracts) *29(Suppl No15)*: 3099, 2011.
55 ClinicalTrials.gov Identifier: NCT00889382.
56 Pohl G, Ho CL, Kurman RJ *et al*: Inactivation of the mitogen-activated protein kinase pathway as a potential target-based therapy in ovarian serous tumors with *KRAS* or *BRAF* mutations. Cancer Res *65*: 1994-2000, 2005.
57 Singer G, Oldt R 3rd, Cohen Y *et al*: Mutations in *BRAF* and *KRAS* characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst *95*: 484-486, 2003.
58 Forbes SA, Bhamra G, Bamford S *et al*: The Catalogue of Somatic Mutations in Cancer (COSMIC). Curr Protoc Hum Genet Chapter 10: Unit 10 1, 2008.
59 Forbes SA, Bindal N, Bamford S *et al*: COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res *39*: 945-950, 2011.
60 Sieben NL, Macropoulos P, Roemen GM *et al*: In ovarian neoplasms, *BRAF*, but not *KRAS*, mutations are restricted to low-grade serous tumours. J Pathol *202*: 336-340, 2004.
61 Nakayama N, Nakayama K, Yeasmin S *et al*: *KRAS* or *BRAF* mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancer. Br J Cancer *99*: 2020-2028, 2008.

62 Farley J, Brady WE, Vathipadiekal V *et al*: Selumetinib in women with recurrent low-grade serous carcinoma of the ovary or peritoneum: an open-label, single-arm, phase II study. Lancet Oncol *14*: 134-140, 2013.
63 ClinicalTrials.gov Identifier: NCT01849874.
64 ClinicalTrials.gov Identifier: NCT01155453.
65 Chapman PB, Hauschild A, Robert C *et al*: Updated overall survival (OS) results for BRIM-3, a phase III randomized, open-label, multicenter trial comparing BRAF inhibitor vemurafenib (vem) with dacarbazine (DTIC) in previously untreated patients with BRAFV600E-mutated melanoma. J Clin Oncol 2012; ASCO Annual Meeting Proceedings (Post-Meeting Edition).Vol 30, No 15_suppl (May 20 Supplement) 8502, 2012.
66 http://public.ukcrn.org.uk/search/StudyDetail.aspx?StudyID=12071. The UK Clinical Research Network Study Portfolio.
67 Chan JK, Teoh D, Hu JM *et al*: Do clear cell ovarian carcinomas have poorer prognosis compared to other epithelial cell types? A study of 1411 clear cell ovarian cancers. Gynecol Oncol *109(3)*: 370-376, 2008.
68 Kuo KT, Mao TL, Jones S *et al*: Frequent activating mutations of PIK3CA in ovarian clear cell carcinoma. Am J Pathol *174(5)*: 1597-1601, 2009.
69 Hashiguchi Y, Tsuda H, Inoue T *et al*: PTEN expression in clear cell adenocarcinoma of the ovary. Gynecol Oncol *101(1)*: 71-75, 2006.
70 Yamashita Y, Akatsuka S, Shinjo K *et al*: *MET* is the most frequently amplified gene in endometriosis-associated ovarian clear cell adenocarcinoma and correlates with worsened prognosis. PLoS One *8(3)*: e57724, 2013.
71 ClinicalTrials.gov Identifier: NCT01196429.
72 Stany MP, Vathipadiekal V, Ozbun L *et al*: Identification of novel therapeutic targets in microdissected clear cell ovarian cancers. PLoS One *6(7)*: e21121, 2011.
73 ClinicalTrials.gov Identifier: NCT00979992.
74 Winter WE 3rd, Maxwell GL, Tian C *et al*: Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. J Clin Oncol *25*: 3621-3627, 2007.
75 Anglesio MS, Kommoss S, Tolcher M *et al*: Molecular characterization of mucinous ovarian tumors supports a stratified treatment approach with HER2 targeting in 19% of carcinomas. J Pathol *229(1)*: 111-120, 2013.
76 McAlpine JN, Wiegand KC, Vang R *et al*: HER2 overexpression and amplification is present in a subset of ovarian mucinous carcinomas and can be targeted with trastuzumab therapy. BMC Cancer *9*: 433, 2009.
77 Bookman MA, Darcy KM, Clarke-Pearson D *et al*: Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: a phase II trial of the Gynecologic Oncology Group. J Clin Oncol *21(2)*: 283-290, 2003.

Received March 2, 2014
Revised March 15, 2014
Accepted March 17, 2014