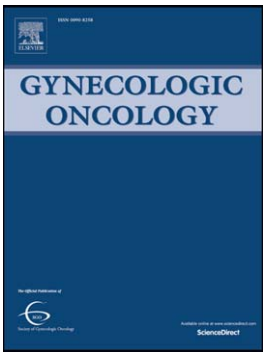
Prediction and failure of anti-angiogenesis escape



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Prediction and failure of anti-angiogenesis escape

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HIGHLIGHTS

• A better understanding of the resistance and escape mechanisms of anti-angiogenesis therapy will facilitate the choices of predictive biomarkers.

• Precision medicine has the potential to improve the prognosis of patients with gynecologic cancer treated with anti-angiogenesis agents.

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Contents

ABSTRACT

Many clinical trials have demonstrated the benefit of anti-angiogenesis therapy in the treatment of gynecologic cancer. However, these benefits have often been in terms of progression-free rather than overall survival and in some cases, the magnitude of benefit demonstrated in the pivotal phase 3 trials has been disappointing when compared with the percentage of patients who responded in earlier phase 2 trials. Two potential explanations for this are the current inability to stratify patients according to chance of benefit and the development of resistance mechanisms within the tumor. In this article, we review the prediction of response and the proposed resistance and escape mechanisms involved in anti-angiogenesis therapy, including the up-regulation of alternative proangiogenic pathways, vascular co-option, and resistance to hypoxia. These insights may offer a personalized strategy for anti-angiogenesis therapy and help us to consider the best selection of other therapies that should be combined with anti-angiogenesis therapy to improve the outcome of patients with gynecologic cancer.

1. Introduction

Oxygen supplied by the vasculature is crucial for cell function and survival, and all cells typically reside within 100 μm of a capillary blood vessel [1]. Angiogenesis, the formation of new blood vessels, is required for tumor growth beyond a millimeter and for metastasis [2]. Tumors can recruit vasculature by releasing various growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and placental growth factor. The successful inhibition of human tumor xenograft growth by a monoclonal antibody specific for VEGF [3] motivated the development of anti-angiogenesis therapy. In 2004, bevacizumab became the first anti-VEGF agent to be approved by the U.S. Food and Drug Administration (FDA) for cancer patients. In phase 3 trials, bevacizumab showed evidence of efficacy in metastatic colorectal [4], lung, [5], breast [6], and renal [7] cancers and in glioblastoma [8].

In gynecologic cancers, the phase 3 first-line GOG-218 trial enrolled 1,873 women with previously untreated macroscopic residual stage III or IV epithelial ovarian, primary peritoneal, or fallopian tube carcinoma [9]. In this three-arm study, patients received carboplatin and paclitaxel chemotherapy (all three arms) and either concomitant and maintenance placebo every 3 weeks (with up to 16 cycles of maintenance) (control group), or

concomitant bevacizumab and maintenance placebo (the bevacizumab-initiation group), or concomitant and maintenance bevacizumab (the bevacizumab-throughout group). The median progression-free survival (PFS) was 10.3 months in the control group, 11.2 months in the bevacizumab-initiation group, and 14.1 months in the bevacizumab-throughout group.

The other phase 3 first-line trial (ICON7) randomized 1,528 women with high-risk early-stage or advanced epithelial ovarian, primary peritoneal, or fallopian tube carcinoma. When PFS was assessed at 36 months, it was determined that PFS was 20.3 months with standard therapy (carboplatin and paclitaxel chemotherapy) but was 21.8 months (a statistically significant increase of 1.5 months) when bevacizumab was added to standard therapy and continued as subsequent maintenance for up to 12 additional cycles. The addition of bevacizumab, however, did not increase overall survival in the intention-to-treat population [10]. Two randomized trials in recurrent ovarian cancer have shown significant improvement in PFS as a result of adding bevacizumab to conventionally administered chemotherapy in patients with platinum-sensitive or platinum-resistant disease. In the phase 3 OCEANS trial, 484 patients with platinum-sensitive recurrent ovarian, primary peritoneal, or fallopian tube cancer and measurable disease were randomly assigned to gemcitabine and carboplatin plus either bevacizumab or placebo. The median PFS was 12.4 vs 8.4 months,

respectively [11]. In the phase 3 AURELIA trial, 361 women with measurable epithelial ovarian, primary peritoneal, or fallopian tube cancer that had progressed within 6 months after the women had completed platinum-based therapy (platinum-resistant) were randomized to one of six arms (paclitaxel, topotecan, or pegylated liposomal doxorubicin with or without bevacizumab). This trial showed that bevacizumab plus chemotherapy doubled the median PFS duration of women who received chemotherapy alone (median PFS: 6.7 vs. 3.4 months) [12]. VEGF receptor tyrosine kinase inhibitors have also demonstrated encouraging activity in patients with ovarian cancer, primary peritoneal, or fallopian tube cancer. Pazopanib is a multitargeted tyrosine kinase inhibitor whose main targets include VEGF and PDGF receptor families. Maintenance pazopanib therapy provided a median improvement of 5.6 months in PFS in patients with advanced ovarian cancer whose disease had not progressed after first-line chemotherapy [13]. Phase 2 studies of cediranib monotherapy (an inhibitor of VEGFR 1-3 and C-KIT) showed response rates of 17%–23% in heavily pretreated ovarian cancer patients [14, 15].

For patients with recurrent, persistent, or metastatic cervical cancer, the GOG-0240 clinical trial showed that the addition of bevacizumab to chemotherapy was associated with increased overall survival (17.0 vs. 13.3 months, respectively) and higher response rates

(48% vs. 36%) [16]. A subsequent randomized phase 2 study in the same patient population demonstrated that when cediranib was administered concomitantly with chemotherapy and then as maintenance thereafter, it significantly increased PFS compared with placebo [17].

However, some tumors do not respond and others eventually become unresponsive. As such, PFS or overall survival benefits in patients receiving anti-angiogenesis therapy for gynecologic malignancies are usually measured in months. Therapeutic resistance and escape have become practical limitations. In this article, we summarize clinical and translational research on predictors of response to anti-angiogenesis therapy and also review potential resistance and escape mechanisms to such therapy in gynecologic malignancies.

2. Prediction of response

There is now clear molecular evidence that ovarian cancer is a highly heterogeneous disease. The five main immunohistological subtypes (by order of incidence: high-grade serous, endometrioid, clear cell, low-grade serous, and mucinous) differ vastly in terms of their stage of presentation [18], chemosensitivity [19], overall survival, and driver genetic mutations [20]. Within high-grade serous ovarian cancer, numerous studies have demonstrated heterogeneity at the level of gene sequence, gene expression, copy number, or

methylation [21-27]. Despite these clear differences, most clinical trials of anti-angiogenesis therapy have been performed in unselected patient populations, and progress in terms of defining clinical or molecular predictors of sensitivity has been limited.

In the setting of cervical cancer, again clinical studies have largely been performed in unselected patient populations, and little progress has been made in defining subpopulations of patients according to their expected benefit from anti-angiogenesis therapy.

2.1. Clinical predictors of response to anti-angiogenesis therapy in ovarian cancer

A retrospective but predefined subgroup analysis of 502 patients with high-risk disease (defined as suboptimally debulked stage III or stage IV) from the ICON7 study demonstrated a significant overall survival benefit for women who received bevacizumab plus chemotherapy compared with those who received chemotherapy alone (restricted mean survival time was 34.5 months with standard chemotherapy compared with 39.3 months with bevacizumab) [28]. When a similar retrospective analysis was performed in the GOG218 dataset [29], no overall survival benefit was demonstrated in suboptimally debulked stage III patients (total of 751 patients in three arms). However, in patients with stage IV disease who received concomitant and maintenance bevacizumab, the overall survival duration was 40.6

months compared with 32.8 months in the control arm. These findings raise the possibility that patients with stage IV disease potentially gain more from first-line bevacizumab therapy (overall survival benefits were not seen in the intention-to-treat populations in either the ICON7 or GOG218 studies). Investigation of the clinical utility of anti-angiogenesis therapy within a histotype-specific ovarian cancer context has to date been extremely limited. Grisham et al. [30] performed a retrospective analysis of 17 patients with low-grade serous or serous borderline ovarian tumors who received bevacizumab and demonstrated a response rate of 40% (55% in the low-grade serous ovarian tumors alone). Although many of these patients also received chemotherapy, it has become clear that the response rate to chemotherapy alone in low-grade serous ovarian cancer is approximately 5% [31, 32], suggesting that bevacizumab has significant efficacy in this subtype. An attempt to establish the value of bevacizumab in mucinous ovarian cancer in the mEOC/GOG241 trial was unsuccessful; the study had to be closed because of poor patient recruitment, despite international collaboration. Likewise, the efficacy of anti-angiogenesis therapy in clear cell ovarian cancer remains poorly defined. These areas are major priorities for future research.

2.2. Molecular predictors of response to anti-angiogenesis therapy

A number of blood or tissue biomarkers have been postulated to predict sensitivity to anti-angiogenesis agents on the basis of preclinical studies, but convincing clinical validation remains elusive. Collinson et al. [33] demonstrated that a signature of four serum proteomic biomarkers (mesothelin, fms-like tyrosine kinase-4, α_1-acid glycoprotein, and CA125) was able to predict benefit from bevacizumab in a cohort of patients from the ICON7 study. The predictive ability was less strong in a second cohort from the same study, but patient numbers were small and it is likely that the validation analysis was underpowered. In another translational plasma-based study using material from patients in the ICON7 study, the combination of high plasma ANG-1 and low plasma TIE-2 was found to predict improved PFS in bevacizumab-treated patients [34]. However, in a large retrospective analysis of plasma biomarkers in prospectively collected samples from the GOG218 study, no predictive biomarkers for bevacizumab efficacy were identified [35].

Tissue biomarkers have been sought using translational research samples from both the GOG218 and ICON7 studies. Birrer at al. [36] performed an exploratory retrospective analysis of candidate predictive biomarkers of efficacy in the GOG218 trial. Of the five biomarkers explored, one (CD31, a marker of blood vessel density) was able to discriminate patients according to their degree of benefit from bevacizumab when a median cut-off level

was used (test for interaction, p=0.003 and p=0.016 for PFS and OS, respectively). When the cut-off was adjusted to the highest quartile, the test was even more discriminatory in terms of OS benefit from bevacizumab (test for interaction, p=0.008).

The translational specimens from the ICON7 study have been analyzed in two cohorts. Winterhoff et al. used the four gene expression subtypes from the Cancer Genome Atlas (TCGA) project [21] to classify 425 tumors from patients enrolled into ICON7 [37]. Patients with serous cancer in the mesenchymal subgroup had a 9.5-month PFS benefit if they received bevacizumab (25.5 vs. 16 months, p=0.053). The extent of median PFS benefit from bevacizumab in the three other molecular subtypes (differentiated, immunoreactive, and proliferative) was 5.8, 3.4, and 3.2 months, respectively.

Previous gene expression analysis and unsupervised hierarchical clustering of tumors from a conventionally treated Scottish cohort of patients with high-grade serous ovarian cancer identified three molecular subgroups: the Immune subgroup, characterized by up-regulation of genes involved in immune response; the Angio subgroup, characterized by up-regulation of genes involved in angiogenesis; and the Angioimmune subgroup, characterized by up-regulation of both immune response and angiogenesis genes [38]. Patients in the Immune subgroup were found to have improved survival compared with

patients in the other two subgroups. Since this subgroup was characterized by repression of angiogenic processes, it was hypothesized that these patients may not derive as much benefit from anti-angiogenesis therapy, and a 63-gene signature was generated to identify this subgroup. When this signature was applied to 284 translational research samples from the ICON7 study, it was found that the 63-gene signature was able to predict benefit from bevacizumab therapy (test for interaction, p=0.016) [39]. Patients in the Immune subgroup had inferior PFS (HR = 1.73 [1.12-2.68]) and OS (HR = 2.00 [1.11-3.61]) if they received bevacizumab compared with chemotherapy alone. In patients outside of the Immune subgroup, there was a nonsignificant trend to improved PFS for the addition of bevacizumab (median, 17.4 vs 12.3 months in controls). The findings from both of these translational studies in the ICON7 dataset require urgent validation in the GOG218 dataset in order to determine not only whether the degree of benefit can be predicted but also whether there is a subgroup of patients who are actually harmed if they receive first-line bevacizumab in the fashion administered in these trials.

3. Resistance and escape mechanisms

3.1. Alternatives to angiogenesis for neovascularization

It has become clear that angiogenesis is not the only mechanism by which tumors develop a vascular supply; there is increasing evidence of adaptation by invasion without angiogenesis. One of these mechanisms is vascular co-option [40, 41], also known as perivascular tumor invasion. Glioma, the most typical example, grows by co-opting existing host vessels. These tumor cells do not rely on a cytokine-driven neovascular response and are favored in an environment exposed to anti-angiogenesis therapy.

For gynecologic cancers, vessel co-option has been observed in mouse ovarian cancer models [42]. In such cases, the secondary effect of direct impairment of tumor cell viability by multitargeted anti-angiogenesis agents such as sorafenib and cabozantinib would be expected, in addition to the original anti-angiogenesis effect on the new lesion that is dependent on neovascularization. Although sorafenib (a kinase inhibitor targeting RAF, VEGF receptor [VEGFR], and platelet-derived growth factor receptor [PDGFR]) has modest antitumor activity, it caused significant toxic effects in several trials [43, 44]. Cabozantinib, a kinase inhibitor of MET, VEGFR2, FLT3, c-KIT, and TIE-2, is currently undergoing clinical trials for patients with recurrent or progressive ovarian, fallopian tube, or primary peritoneal cancer. In advanced, progressive epithelial ovarian cancer, this agent showed a promising clinical response rate (overall, 24%). Dose reductions and permanent discontinuations for

adverse effects occurred in 43% and 10% of cases, respectively, which is consistent with rates for other tyrosine kinase inhibitors [45].

Vascular mimicry refers to the formation of fluid-conducting channels by highly invasive and genetically dysregulated tumor cells [46]. Immunohistochemical analysis in an ovarian cancer model showed that matrix metalloproteinases (MMP)-1, -2, and -9 and MT1-MMP were discretely localized to these networks. Because the formation of these networks was inhibited by treatment with MMP inhibitors [47], these inhibitors could be effective in combination with anti-angiogenesis drugs.

3.2. Acute hypoxia

At the peak of the response phase of anti-angiogenesis therapy, tumors have regions of acute hypoxia [48]. Hypoxia is often implicated in the promotion of tumor progression and resistance to therapy [49]. High intratumoral hypoxia is associated with worse clinical outcomes in cancer patients [50]. A clinical investigation involving a study of glioblastoma patients being treated with the VEGFR inhibitor cediranib pointed to adaptive resistance mechanisms involving FGF-dependent revascularization [51]. Tumors subjected to hypoxic conditions also expressed higher levels of proangiogenic factors including angiopoietin [48]

and hepatocyte growth factor receptor (MET) [52, 53] than did unperturbed tumors, suggesting that these angiogenic pathways are potential targets to overcome adaptive resistance induced by hypoxia.

Drugs targeting the mechanisms described above have entered into clinical testing. BIBF-1120 is a triple-angiokinase inhibitor of VEGFR, PDGFR, and FGF receptor. In a randomized, phase 2 placebo-controlled trial, patients who had completed chemotherapy for relapsed ovarian cancer with evidence of response were treated with BIBF-1120. Three-year PFS rates were 16.3% and 5.0% in the BIBF-1120 and placebo groups, respectively [54]. A Phase 3 (NCT01015118) clinical trial is evaluating the addition of BIBF-1120 to carboplatin/paclitaxel in first-line chemotherapy in ovarian cancer. AMG386 is an angiopoietin antagonist that selectively binds angiopoietin 1 (ANG1) and angiopoietin 2 (ANG2). This binding prevents the interaction of ANG1 and ANG2 with TEK tyrosine kinase, endothelial (TIE-2) and inhibits tumor endothelial cell proliferation and tumor growth [55]. In a phase 2 study of AMG386 combined with paclitaxel, the addition of AMG386 to paclitaxel demonstrated dose-responsive improvements in PFS with a manageable safety profile distinct from that of VEGF inhibition [56]. AMG386 has entered phase 3 clinical investigation in the setting of recurrent ovarian cancer.

Certain anti-angiogenesis agents can transiently normalize the abnormal structure and function of tumor vasculature to make it more efficient for oxygen and drug delivery. In patients with glioblastoma, cediranib, a pan-VEGFR inhibitor, resulted in decreased tumor edema by dynamic magnetic resonance imaging. Although this vascular normalization is still controversial, drugs that induce vascular normalization can alleviate hypoxia and increase the efficacy of conventional therapies if carefully scheduled. Administration of metronomic topotecan was able to significantly decrease cell proliferation and angiogenesis by reducing VEGF and HIF-1α [14]; thus, its combination with anti-VEGF therapy could be beneficial.

3.3. Recruitment of bone marrow–derived cells

3.3.1. Monocytes and macrophages

Hypoxia caused by vessel regression during the course of anti-angiogenesis therapy can lead not only to up-regulation of proangiogenic factors within the tumors but also to the recruitment of various bone marrow–derived cells, including monocytes, macrophages, and endothelial progenitors, which have the capacity to fuel tumors by eliciting new blood vessels [48]. Monocytes and macrophages deliver VEGF and other angiogenic molecules in a temporal and spatial fashion to avascular areas, resulting in angiogenesis [57]. Compared

with controls, macrophages co-cultured with ovarian cancer cells showed significant up-regulation of proangiogenic genes including tumor necrosis factor α (TNF-α), CXCR4, CXCL12 (also known as SDF-1), colony-stimulating factor 1 (CSF-1), and VEGF [58], suggesting that these proangiogenic factors are potential targets for therapy under settings of adaptive resistance. Etanercept is a recombinant human soluble p75 TNF receptor that binds to TNF-α renders it biologically unavailable, and infliximab is an anti–TNF-α monoclonal antibody. These agents showed biologic activity (17% to 20% of patients experienced disease stabilization) and safety in recurrent and advanced ovarian cancer in a phase 1 trial [59, 60]. For CXCR4, the phase 1 trial of the CXCR4 receptor antagonist plerixafor is ongoing in patients with advanced pancreatic, ovarian, and colorectal cancers. Macrophage CSF-1 is widely overexpressed in ovarian cancer [58, 61], and the expression is highest at the invasive edge, a site abundantly populated by macrophages. The PLX3397 clinical trial, designed to target the receptor for CSF-1, is ongoing in ovarian cancer patients.

3.3.2. Endothelial progenitors

Proangiogenic bone marrow–derived cells also include endothelial progenitors that differentiate into endothelial cells to form the inner lining of blood vessel walls. Recruitment

of endothelial progenitors is an essential step for tumors that have undergone anti-angiogenesis therapy to acquire adaptive resistance by re-neovascularization. Cancer-associated fibroblasts promote angiogenesis by recruiting endothelial progenitor cells into carcinomas, in part through their ability to secrete CXCL12 (SDF-1), an independent predictor of tumor progression and poor survival in ovarian cancer [62, 63]. The antagonist of CXCR4 that is a receptor of CXCL12, for example plerixafor, mentioned earlier, could be active in preventing this resistance mechanism.

The origin of endothelium may be either bone marrow–derived endothelial progenitors or local endothelial progenitors rooted within organs or vascular parenchyma. The only source of endothelial progenitors was believed to be a hematopoietic stem-cell–containing CD34 population. However, a population of CD11c+ cells exhibiting simultaneous expression of both endothelial and dendritic cell markers (vascular leukocytes) was recently discovered; this population of cells is highly represented in human ovarian cancers and can assemble into functional blood vessels. Since a decrease in tumor growth was associated with a reduced number of CD11c+ infiltrating cells, vascular leukocytes could be therapeutic targets. Antibodies directed against specific antigens and conjugated to toxins may contribute to blocking tumor vascularization [64].

3.4. Pericyte coverage

One of the potential mechanisms of resistance to anti-angiogenesis therapy is the increased coverage of blood vessel with pericytes. The morphology of resistant tumor vessels is distinguishable from the typically dilated tumor vessels of untreated animals, which are, by contrast, variably covered with less closely associated pericytes. Tumor vessels lacking adequate pericyte coverage are more vulnerable to VEGF inhibition [65, 66].

The platelet-derived growth factor (PDGF) ligand/receptor system is one of the major signaling pathways for regulating pericyte coverage. In orthotopic murine models of advanced ovarian carcinoma, dual targeting of endothelial cells and pericytes has been evaluated [67]. PDGF/PDGFR-targeting agents, including pazopanib, sunitinib, BIBF-1120, and dovitinib, are being tested in clinical trials. The most promising of these is a phase 3 trial of pazopanib, VEGFR-1, -2, -3, c-kit, and PDGFR inhibitor, in advanced renal cell carcinoma. Compared with placebo, pazopanib increased PFS from 4.2 to 9.2 months. The difference in PFS was even more striking in patients who were treatment-naive (11.1 vs 2.8 months). The response rate was also improved (30% vs 3%), and the median duration of response was more than 1 year [68]. Additional testing is necessary to determine the full potential of dual

targeting of endothelial cells and pericytes in the tumor microenvironment.

4. Discussion

There is non-conclusive but accumulating evidence to suggest that certain histological subtypes of ovarian cancer (e.g., high-grade serous and low-grade serous) are more responsive to anti-angiogenesis therapy than are other subtypes (such as clear cell ovarian cancer). First-line phase 3 bevacizumab studies suggest that patients presenting with later-stage disease derive greater benefit. More work is required to clarify the value of stratification on the basis of these simple clinicopathological factors in both ovarian and cervical cancers. In terms of molecular markers, some candidate proteomic markers were suggested from ICON7, but further validation is required. In addition, molecular subgroups based on gene expression analysis from two translational ICON7 datasets suggest that stratification on this basis may have value. In GOG218, CD31 expression (as a surrogate of vascular density) appears to predict benefit from bevacizumab. The translational studies performed in ICON7 require additional validation.

In terms of precision medicine, tumor imaging or biopsy could be useful for patient selection, but such approaches require further development. Dynamic contrast-enhanced MRI

enables quantitation of blood volume, blood flow, relative vessel size, and vascular permeability in measurable tumor [51]. This kind of vascular imaging could be used to evaluate neovascularization and vascular response. Tumor biopsy either percutaneously or *via* minimally invasive surgery [69, 70] could enable assessment of potential resistance mechanisms.

Recently, clinical trials of anti-angiogenesis therapy in combination with other targeted therapies such as PARP inhibitors have produced intriguing results. In addition, the ongoing MITO16MANGO2b study is evaluating whether administering bevacizumab in combination with chemotherapy as second-line therapy to patients with recurrent ovarian cancer who have received first-line bevacizumab will be more effective than chemotherapy alone. A greater understanding of optimal stratification based on tumor/stromal characteristics and resistance mechanisms will allow us to benefit from the results of these clinical trials by personalizing anti-angiogenesis therapy for each patient.

Conflict of interest statement

Charlie Gourley's employer (University of Edinburgh) has received compensation for his

advisory board attendance (Roche, AstraZenenca, GlaxoSmithKline and Nucana) and for his lecturing (Roche and AstraZeneca). Charlie Gourley (through the University of Edinburgh) has also received research funding from AstraZeneca, GlaxoSmithKline, and Aprea.

REFERENCES

[1] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100 (1):57-70.

[2] Hillen F, Griffioen AW. Tumour vascularization: sprouting angiogenesis and beyond. Cancer Metastasis Rev 2007;26 (3-4):489-502.

[3] Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993;362 (6423):841-4.

[4] Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350 (23):2335-42.

[5] Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355 (24):2542-50.

[6] Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357 (26):2666-76.

[7] Rini BI, Halabi S, Rosenberg JE, Stadler WM, Vaena DA, Ou SS, et al. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. J Clin Oncol 2008;26 (33):5422-8.

[8] Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009;27 (28):4733-40.

[9] Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med 2011;365 (26):2473-83.

[10] Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med 2011;365 (26):2484-96.

[11] Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin

Oncol 2012;30 (17):2039-45.

[12] Pujade-Lauraine E, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. J Clin Oncol 2014;32 (13):1302-8.

[13] du Bois A, Floquet A, Kim JW, Rau J, del Campo JM, Friedlander M, et al. Incorporation of pazopanib in maintenance therapy of ovarian cancer. J Clin Oncol 2014;32 (30):3374-82.

[14] Hirte HW, Vidal L, Fleming GF, Sugimoto AK, Morgan RJ, Biagi JJ, et al. A phase II study of cediranib (AZD2171) in recurrent or persistent ovarian, peritoneal or fallopian tube cancer: Final results of a PMH, Chicago and California consortia trial. ASCO Meeting Abstracts 2008;26 (15_suppl):5521.

[15] Matulonis UA, Berlin S, Ivy P, Tyburski K, Krasner C, Zarwan C, et al. Cediranib, an oral inhibitor of vascular endothelial growth factor receptor kinases, is an active drug in recurrent epithelial ovarian, fallopian tube, and peritoneal cancer. J Clin Oncol 2009;27 (33):5601-6.

[16] Tewari KS, Sill MW, Long HJ, 3rd, Penson RT, Huang H, Ramondetta

LM, et al. Improved survival with bevacizumab in advanced cervical cancer. N Engl J Med 2014;370 (8):734-43.

[17] Symonds R, Gourley C, Davidson S, Carty K, McCartney E, Rai D, et al. Cediranib combined with carboplatin and paclitaxel in patients with metastatic or recurrent cervix cancer (CIRCCa): a randomised, double blind, placebo controlled phase 2 trial. Lancet Oncol 2015;manuscript in press

[18] Morris CR, Sands MT, Smith LH. Ovarian cancer: predictors of early-stage diagnosis. Cancer Causes Control 2010;21 (8):1203-11.

[19] Prat J. New insights into ovarian cancer pathology. Annals of Oncology 2012;23 (suppl 10):x111-x7.

[20] Vaughan S, Coward JI, Bast RC, Jr., Berchuck A, Berek JS, Brenton JD, et al. Rethinking ovarian cancer: recommendations for improving outcomes. Nat Rev Cancer 2011;11 (10):719-25.

[21] Integrated genomic analyses of ovarian carcinoma. Nature 2011;474 (7353):609-15.

[22] Bentink S, Haibe-Kains B, Risch T, Fan JB, Hirsch MS, Holton K, et al. Angiogenic mRNA and microRNA gene expression signature predicts a novel

subtype of serous ovarian cancer. PLoS One 2012;7 (2):e30269.

[23] Bonome T, Levine DA, Shih J, Randonovich M, Pise-Masison CA, Bogomolniy F, et al. A gene signature predicting for survival in suboptimally debulked patients with ovarian cancer. Cancer Res 2008;68 (13):5478-86.

[24] Sfakianos GP, Iversen ES, Whitaker R, Akushevich L, Schildkraut JM, Murphy SK, et al. Validation of ovarian cancer gene expression signatures for survival and subtype in formalin fixed paraffin embedded tissues. Gynecol Oncol 2013;129 (1):159-64.

[25] Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S, et al. Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res 2008;14 (16):5198-208.

[26] Verhaak RG, Tamayo P, Yang JY, Hubbard D, Zhang H, Creighton CJ, et al. Prognostically relevant gene signatures of high-grade serous ovarian carcinoma. J Clin Invest 2013;123 (1):517-25.

[27] McBride DJ, Etemadmoghadam D, Cooke SL, Alsop K, George J, Butler A, et al. Tandem duplication of chromosomal segments is common in ovarian and breast cancer genomes. J Pathol 2012;227 (4):446-55.

[28] Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, et al. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. The Lancet Oncology 2015;16 (8):928-36.

[29] Randall LM, Burger RA, Nguyen H, Kong G, Bookman MA, Fleming GF, et al. Outcome Differences in Patients with Advanced Epithelial Ovarian, Primary Peritoneal and Fallopian Tube Cancers Treated With and Without Bevacizumab. In: Society of Gynecologic Oncology annual meeting. Los Angeles; 2013.

[30] Grisham RN, Iyer G, Sala E, Zhou Q, Iasonos A, DeLair D, et al. Bevacizumab shows activity in patients with low-grade serous ovarian and primary peritoneal cancer. International Journal of Gynecological Cancer 2014;24 (6):1010-4.

[31] Schmeler KM, Sun CC, Bodurka DC, Deavers MT, Malpica A, Coleman RL, et al. Neoadjuvant chemotherapy for low-grade serous carcinoma of the ovary or peritoneum. Gynecol Oncol 2008;108 (3):510-4.

[32] Gershenson DM, Sun CC, Bodurka D, Coleman RL, Lu KH, Sood AK, et

al. Recurrent low-grade serous ovarian carcinoma is relatively chemoresistant. Gynecol Oncol 2009;114 (1):48-52.

[33] Collinson F, Hutchinson M, Craven RA, Cairns DA, Zougman A, Wind TC, et al. Predicting response to bevacizumab in ovarian cancer: a panel of potential biomarkers informing treatment selection. Clin Cancer Res 2013;19 (18):5227-39.

[34] Backen A, Renehan AG, Clamp AR, Berzuini C, Zhou C, Oza A, et al. The combination of circulating Ang1 and Tie2 levels predicts progression-free survival advantage in bevacizumab-treated patients with ovarian cancer. Clin Cancer Res 2014;20 (17):4549-58.

[35] Birrer MJ, Lankes H, Burger RA, Mannel R, Homesley H, Henschel V, et al. Biomarker (BM) results from GOG-0218, a phase 3 trial of front-line bevacizumab (BV) + chemotherapy (CT) for ovarian cancer (OC). In: The ESMO Congress. Vienna, Austria; 2012. p. 198.

[36] Birrer MJ, Choi Y, Brady MF, Mannel RS, Burger RA, WEI W, et al. Retrospective analysis of candidate predictive tumor biomarkers (BMs) for efficacy in the GOG-0218 trial evaluating front-line carboplatin-paclitaxel (CP)

{+/-} bevacizumab (BEV) for epithelial ovarian cancer (EOC). ASCO Meeting Abstracts 2015;33 (15_suppl):5505.

[37] Winterhoff BJN, Kommoss S, Oberg AL, Wang C, Riska SM, Konecny GE, et al. Bevacizumab and improvement of progression-free survival (PFS) for patients with the mesenchymal molecular subtype of ovarian cancer. ASCO Meeting Abstracts 2014;32 (15_suppl):5509.

[38] Gourley C, Michie CO, Keating KE, Deharo S, O'Brien EJ, Winter A, et al. Establishing a molecular taxonomy for epithelial ovarian cancer (EOC) from 363 formalin-fixed paraffin embedded (FFPE) specimens. ASCO Meeting Abstracts 2011;29 (15_suppl):5000.

[39] Gourley C, McCavigan A, Perren T, Paul J, Michie CO, Churchman M, et al. Molecular subgroup of high-grade serous ovarian cancer (HGSOC) as a predictor of outcome following bevacizumab. ASCO Meeting Abstracts 2014;32 (15_suppl):5502.

[40] Rubenstein JL, Kim J, Ozawa T, Zhang M, Westphal M, Deen DF, et al. Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. Neoplasia 2000;2 (4):306-14.

[41] Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 1999;284 (5422):1994-8.

[42] Yokoyama Y, Sedgewick G, Ramakrishnan S. Endostatin binding to ovarian cancer cells inhibits peritoneal attachment and dissemination. Cancer Res 2007;67 (22):10813-22.

[43] Matei D, Sill MW, Lankes HA, DeGeest K, Bristow RE, Mutch D, et al. Activity of sorafenib in recurrent ovarian cancer and primary peritoneal carcinomatosis: a gynecologic oncology group trial. J Clin Oncol 2011;29 (1):69-75.

[44] Ramasubbaiah R, Perkins SM, Schilder J, Whalen C, Johnson CS, Callahan M, et al. Sorafenib in combination with weekly topotecan in recurrent ovarian cancer, a phase I/II study of the Hoosier Oncology Group. Gynecol Oncol 2011;123 (3):499-504.

[45] Buckanovich RJ, Berger R, Sella A, Sikic BI, Shen X, Ramies DA, et al. Activity of cabozantinib (XL184) in advanced ovarian cancer patients (pts): Results from a phase II randomized discontinuation trial (RDT). ASCO Meeting Abstracts 2011;29 (15_suppl):5008.

[46] Folberg R, Maniotis AJ. Vasculogenic mimicry. APMIS 2004;112 (7-8):508-25.

[47] Sood AK, Seftor EA, Fletcher MS, Gardner LM, Heidger PM, Buller RE, et al. Molecular determinants of ovarian cancer plasticity. Am J Pathol 2001;158 (4):1279-88.

[48] Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8 (8):592-603.

[49] Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2 (1):38-47.

[50] Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev 2007;26 (2):225-39.

[51] Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 2007;11 (1):83-95.

[52] Knudsen BS, Vande Woude G. Showering c-MET-dependent cancers with drugs. Curr Opin Genet Dev 2008;18 (1):87-96.

[53] Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3 (4):347-61.

[54] Ledermann JA, Hackshaw A, Kaye S, Jayson G, Gabra H, McNeish I, et al. Randomized phase II placebo-controlled trial of maintenance therapy using the oral triple angiokinase inhibitor BIBF 1120 after chemotherapy for relapsed ovarian cancer. J Clin Oncol 2011;29 (28):3798-804.

[55] Herbst RS, Hong D, Chap L, Kurzrock R, Jackson E, Silverman JM, et al. Safety, pharmacokinetics, and antitumor activity of AMG 386, a selective angiopoietin inhibitor, in adult patients with advanced solid tumors. J Clin Oncol 2009;27 (21):3557-65.

[56] Karlan BY, Oza AM, Richardson GE, Provencher DM, Hansen VL, Buck M, 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 2012;30 (4):362-71.

[57] Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. Cell 2010;141 (1):39-51.

[58] Hagemann T, Wilson J, Burke F, Kulbe H, Li NF, Pluddemann A, et al. Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. J Immunol 2006;176 (8):5023-32.

[59] Madhusudan S, Muthuramalingam SR, Braybrooke JP, Wilner S, Kaur K, Han C, et al. Study of etanercept, a tumor necrosis factor-alpha inhibitor, in recurrent ovarian cancer. J Clin Oncol 2005;23 (25):5950-9.

[60] Brown ER, Charles KA, Hoare SA, Rye RL, Jodrell DI, Aird RE, et al. A clinical study assessing the tolerability and biological effects of infliximab, a TNF-alpha inhibitor, in patients with advanced cancer. Ann Oncol 2008;19 (7):1340-6.

[61] Kacinski BM. CSF-1 and its receptor in ovarian, endometrial and breast cancer. Ann Med 1995;27 (1):79-85.

[62] Jiang YP, Wu XH, Shi B, Wu WX, Yin GR. Expression of chemokine CXCL12 and its receptor CXCR4 in human epithelial ovarian cancer: an independent prognostic factor for tumor progression. Gynecol Oncol 2006;103 (1):226-33.

[63] Popple A, Durrant LG, Spendlove I, Rolland P, Scott IV, Deen S, et al.

The chemokine, CXCL12, is an independent predictor of poor survival in ovarian cancer. Br J Cancer 2012;106 (7):1306-13.

[64] Coukos G, Benencia F, Buckanovich RJ, Conejo-Garcia JR. The role of dendritic cell precursors in tumour vasculogenesis. Br J Cancer 2005;92 (7):1182-7.

[65] Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003;111 (9):1287-95.

[66] Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development 1998;125 (9):1591-8.

[67] Lu C, Kamat AA, Lin YG, Merritt WM, Landen CN, Kim TJ, et al. Dual targeting of endothelial cells and pericytes in antivascular therapy for ovarian carcinoma. Clin Cancer Res 2007;13 (14):4209-17.

[68] Sternberg CN, Davis ID, Mardiak J, Szczylik C, Lee E, Wagstaff J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. J Clin Oncol 2010;28 (6):1061-8.

[69] Magrina JF, Cetta RL, Chang YH, Guevara G, Magtibay PM. Analysis of secondary cytoreduction for recurrent ovarian cancer by robotics, laparoscopy and laparotomy. Gynecol Oncol 2013;129 (2):336-40.

[70] Nezhat FR, Pejovic T, Finger TN, Khalil SS. Role of Minimally Invasive Surgery in Ovarian Cancer. Journal of Minimally Invasive Gynecology 2013;20 (6):754-65.