trials testing new molecules, new indications and new combinations are in progress, including in gynecological cancers, including ovarian cancer. Compared to the benefits expected based on preclinical models, patient benefits in term of long-term survival, however, remained modest. Recent experimental results have demonstrated that tumors treated with anti-angiogenic therapies, contrary to initial assumptions, can develop evasive resistance and rapidly progress to become invasive and metastatic. Thus, in spite of the undisputed success of this new therapeutic approach some old questions on tumor angiogenesis have remained unanswered and new ones have emerged. They include the understanding about how anti-angiogenic therapy and chemotherapy synergize, the characterization of the biological consequences of sustained suppression of angiogenesis on tumor biology and normal tissue homeostasis, and the mechanisms of tumor escape from anti-angiogenesis. Bone marrow-derived and tumor-mobilized cells recruited at tumor sites are emerging as critical determinant of resistance to anti-angiogenic therapy and may represent novel therapeutic targets. Furthermore, although it has been suggested that biomarkers of angiogenesis would greatly facilitate the clinical development of anti-angiogenic therapies, so far there are no validated biomarkers of angiogenesis and surrogate biomarkers of anti-angiogenesis. In order to improve the clinical use of available anti-angiogenic drugs and the development of new ones it will be important to challenge some of the basic concepts of tumor angiogenesis biology and the relationship between tumor vessels and tumor cells. In this lecture I will review some of the emerging critical issues in tumor angiogenesis and discuss their impact on the development of anti-angiogenic therapies.

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Clinical experience with antiangiogenic targeting in ovarian cancer

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The biology of Vascular Permeability Factor (VPF) was first described in 1983, followed by the demonstration of constitutive expression in ovarian cancer in 1994. This led to efforts at antibody targeting in tumor model systems, with promising control of ascites and tumor growth. VPF is now recognized as Vascular Endothelial Growth Factor (VEGF), and our knowledge of VEGF expression, receptor biology, and signal transduction has expanded considerably over the last 10 years, culminating in successful targeting strategies through ligand sequestration, inhibition of receptor activation, interference with internal signaling pathways, and gene expression. In addition, a variety of other factors have been identified that contribute to a regulatory network of tumor-associated angiogenesis, introducing an array of potential targets and combinations.

The most well-studied agent has been bevacizumab, a monoclonal antibody that sequesters VEGF, as well as aflibercept, an antibody-like protein constructed of VEGF binding domains. Although single-agent activity with bevacizumab in lung, colorectal, and breast cancer was limited, phase III trials in combination with chemotherapy have demonstrated modest improvements in long-term clinical outcomes. Interest in ovarian cancer was accelerated based on phase II trials demonstrating a 20% RECIST response rate in patients with recurrent disease, together with control of ascites. While generally well-tolerated with a predictable toxicity profile, there was initial concern regarding the risk of bowel perforation that appears largely related to patient selection criteria. As a result, single-agent phase II trials were rapidly followed by 2 front-line phase III trials (in combination with chemotherapy) coordinated by GOG-US and MRC-UK. Accrual has been completed on both studies, and results are pending.

Inhibitors of VEGF-associated tryosine kinase (TKI) have also been evaluated in phase II trials, including sorafinib, cediranib, and pazopanib, and phase III studies of maintenance or consolidation have been initiated, with additional plans for front-line trials. Combinations of bevacizumab with TKI appear to have a high response rate, but at the expense of increased serious toxicity, and more studies with newer agents are needed. There are also limited data emerging with regard to other network components involved in tumor angiogenesis, such as angiopoietin-2, protein kinase-cβ, AKT, mTOR, or regulation of HIF1α activity.

Many questions remain with regard to the optimal clinical strategy for incorporation of these agents, including timing (front-line, maintenance, or recurrence), as well as combinations with cytotoxic chemotherapy or other molecular-targeted agents. In addition, there are not yet any comparative data to guide selection of the best agents, or class of agents, for future study.

Finally, the mechanism of action in ovarian cancer remains to be elucidated. Are these agents acting on tumor-associated vessels to normalize blood flow and reduce capillary permeability (as originally proposed), or are they acting directly on tumor cells, or perhaps accelerating the immune response through maturation of dendritic cells? Clearly, more randomized phase II trials with comparative and translational endpoints are needed to guide future investigations.

196 INVITED

Emerging new targets in ovarian cancer

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In the era of molecular targeted therapy, ovarian cancer provides a particularly exciting opportunity for clinical new drug testing, in which pathway-specific agents are being linked to predictive biomarkers aimed at identifying patients most likely to benefit. The paradigm is the remarkable activity of single agent PARP inhibitor therapy for patients with BRCA 1/2 mutation positive ovarian cancer. This is based on the exquisite sensitivity of cancer cells which are deficient in the ability to repair DNA damage through homologous recombination (HR); the challenge for the future is to assess this new form of treatment in that larger group of ovarian cancer patients, with sporadic disease, who are also likely to have HR deficiency. We already have preliminary evidence to indicate that efficacy in these patients is possible.

Another pathway likely to provide a rich seam of novel agents is the PI3 kinase/AKT/mTOR pathway, since amplifications and mutations are well recognised in ovarian cancer. A number of agents are already in the clinic; here it is likely that a combination strategy will ultimately be employed, aimed at dealing with cytotoxic drug resistance through modulation of this pathway. Similarly, potent inhibitors of other relevant targets such as the SRC oncogene hold particular promise, based on molecular analysis of clinical material indicating the likely relevance of this target in ovarian cancer.

A key aim for the future is the identification of novel targets in so-called 'stem cells', which are increasingly being identified in ovarian cancer patients. These may include novel pathways such as the sonic hedgehog pathway, as well as well-recognised transport proteins (from the ABC transporter family) which may play a particular role in stem cell biology.

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Molecular determinants of acquired resistance

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Resistance to platinum-based chemotherapy is a major problem in the treatment of ovarian cancer. The reduced tendency of ovarian cancer cells to undergo apoptosis contributes to drug resistance. In order to gain more insight in the molecular mechanisms underlying platinum resistance, we profiled 9 paired stage III/IV serous ovarian cancer specimens obtained before and after platinum-based chemotherapy using oligonucleotide microarrays. The prognostic value of differentially expressed genes and deregulated biological pathways was assessed in an independent set of 157 previously profiled late stage serous ovarian cancers. Immunohistochemical staining of MB1 as representative for proteasome pathways confirmed the prognostic value of these pathways at the protein level. Our analyses reveal both well-known as well as novel genes and pathways tentatively involved in platinum resistance, including the insulin-like growth factor (IGF)-axis. High IGF-1 receptor (IGF-1R) and insulin receptor (IR) expression were observed in 51.1% and 19.9% of ovarian cancers, respectively. In univariate analysis for stage III/IV ovarian cancers, high IGF-1R expression was related to improved prognosis. In contrast, high IR expression was independently associated with poor disease specific survival (HR 2.0, 95%CI 1.30−3.09). Almost all cancers expressed IGF-I (100%), IGF-II (100%), IGF-1R (73.3%) and both IR-A and IR-B isoforms (94.4%) but none insulin mRNA. IGF-II levels in cyst fluid were elevated compared to cystadenomas suggesting a possible autocrine/paracrine activation of the IGF-axis. We investigated whether the IR inhibitor hydroxy-2-naphthalenylmethylphosphonic acid (HNMPA) treatment could sensitize the cisplatin-sensitive ovarian cancer cell line A2780 and its cisplatin-resistant subline C30 to cisplatin-induced apoptosis. A2780 and C30 showed membrane expression of IGF-1R and IR. Addition of IGF-I, IGF-II or insulin resulted in activation of the IGF-1R/IR signaling in A2780 and C30. A combination of HNMPA and cisplatin strongly enhanced apoptosis and decreased survival in both cell lines, indicating that inhibition of pro-survival signaling enhances cisplatin-induced apoptosis. Another strategy for targeting ovarian cancer involves shifting cellular balance in favor of cell death via activation of the intrinsic (mitochondrial) and extrinsic apoptotic pathway. In cisplatin resistant ovarian cancer cells we found reduced activation of p53 and reduced apoptosis-induction by recombinant human form of the death ligand TNF related apoptosis inducing ligand (rhTRAIL). Combination of cisplatin and rhTRAIL enhanced apoptosis in A2780 and a cisplatin resistant subline. Both rhTRAIL and rhTRAIL-DR5, a rhTRAIL variant that specifically binds to pro-apoptotic DR5 receptor induced high levels of apoptosis in combination with cisplatin with rhTRAIL-DR5 being most potent. Anti-tumor efficacy of rhTRAIL-DR5 or rhTRAIL in combination with cisplatin was determined in an intraperitoneal growing bioluminescent A2780 xenograft model. Intraperitoneal administration of rhTRAIL or rhTRAIL-DR5 plus cisplatin resulted in 85% ($p = 0.003$) and