

# Antiangiogenesis and Drug Delivery to Tumors: Bench to Bedside and Back

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## Abstract

**After over 30 years of preclinical and clinical development, antiangiogenic agents have recently entered the clinic as attractive targeted therapeutics for the treatment of cancer. Fueled by exciting new developments in the field, the AACR Special Conference was designed to broadly survey critical scientific advances in the antiangiogenic therapy of cancer. Because these advances have come primarily with the use of combinations of antiangiogenic agents with chemotherapy, or with antiangiogenic agents that also directly target the cancer cells, the central theme included the issue of drug delivery to tumors. These two major issues were addressed in concert, from basic mechanisms of action of antiangiogenic agents to new combination approaches to cancer treatment. Nearly 300 participants from 20 countries registered for the conference, drawn both from academia and industry, with a wide range in experience and background. Dr. Rakesh Jain, along with conference co-chairs, Drs. Lee Ellis and Luisa Iruela-Arispe, assembled an outstanding lineup of speakers for this conference that included many of the pioneers in the fields of angiogenesis and drug delivery from the U.S. and abroad. This resulted in an excellent overview of the advances in our understanding of cellular and molecular aspects of tumor angiogenesis and antiangiogenic therapy of cancer in combination with conventional therapy. (Cancer Res 2006; 66(8): 3967-70)**

## Introduction

In his opening remarks to the attendees, conference chair Dr. Rakesh Jain summarized the milestones in angiogenesis and drug delivery research, the importance of addressing them in the same meeting, and the relevance of having Boston as a most fitting place to organize it. According to Dr. Jain, "Boston is where Dr. Folkman put forward his visionary hypothesis that antiangiogenic therapy can control tumor growth, and where Drs. Dvorak, Teicher, and Langer performed pioneering work in the areas of tumor angiogenesis, combination therapies, and drug delivery systems—all of which are now in use in patients." And Boston is where Dr. Jain put forward the hypothesis of vascular normalization by antiangiogenic agents.

An important lesson learned from successful phase III trials is that the antiangiogenic therapies increased survival in patients in the context of direct and simultaneous cytotoxic therapy directed

at cancer cells. Therefore, a better understanding of how each of these therapies affects the delivery and efficacy of the other is needed to improve outcome. The goal of this AACR special conference was to form a bridge between these two fields and to understand their interrelation. This integrative approach is expected to allow translation of findings from the bench-to-bedside, and from the bedside back to the bench, with the ultimate goal of optimizing antiangiogenic treatment. Realizing such an advance may permit the use of the vascular endothelial growth factor (VEGF)-specific antibody bevacizumab (approved by the Food and Drug Administration in 2004 for combination with standard chemotherapy for patients with metastatic colorectal cancer) and of other antiangiogenic agents—recently approved or in the process of being approved—to increase the overall and/or progression-free survival beyond 2 to 5 months achieved to date in four phase III clinical trials of bevacizumab with chemotherapy in patients with colorectal, lung, and breast cancers.

## “Tumor Vascular Normalization” and “Wiring Nerves and Blood Vessels”

The opening session featured keynote lectures by Dr. Rakesh Jain and by Dr. Marc Tessier-Lavigne. Dr. Jain described the work in his laboratory which was aimed at understanding the vascular, interstitial, and lymphatic biology of tumors with the ultimate goal of improving delivery of therapeutic agents to tumors. He presented preclinical and clinical evidence consistent with the concept of vascular “normalization” by anti-VEGF agents for improved drug delivery and efficacy (1, 2). Dr. Tessier-Lavigne presented his work on the functional characterization of four families of molecules (ephrins, netrins, semaphorins, and slits)—originally associated with the development of the nervous system—on the development of the vascular tree and angiogenesis. Dr. Tessier-Lavigne’s description of parallels between axonal guidance molecules and angiogenesis-related molecules raised exciting implications for both therapeutic angiogenesis and antiangiogenic therapies (3, 4). Each of the keynote lecturers, and the speakers in the following days, tailored their presentations very well to the more general scientific audience present, describing the conceptual bases for key basic and clinical concepts in the antiangiogenic therapy of cancer.

## Status of the Clinical Development in Antiangiogenic Therapy of Cancer

The first session was dedicated to discussions related to the use of antiangiogenic agents in the clinical setting. The session chair, Dr. Lee Ellis, presented a comprehensive overview of the completed phase III clinical trials involving anti-VEGF agents (bevacizumab, semaxinib, vatalanib, sorafenib, and sunitinib), and underscored the challenges ahead in improving preclinical models and clinical research to achieve further increases in survival. Dr. Ellis also addressed the fact that VEGF receptors (VEGFR) are functional in

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certain cancer cells, and therefore hypothesized that the beneficial effects of anti-VEGF therapy may, in part, be due to the direct effects of these agents on tumor cells (5). Next, Dr. Christopher Willett presented studies done in a phase I trial of bevacizumab as a component of neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer (6). He described the changes in the molecular, cellular, and functional variables measured in patients with cancer during the initial cycle of therapy in which bevacizumab was delivered as a single agent: decreases in microvascular density, blood flow, circulating endothelial cells (CEC) and progenitor cells, and increases in tumor cell apoptosis and proliferation, pericyte coverage, and plasma VEGF and placental-derived growth factor (PlGF) content. Finally, Dr. Lee Rosen presented a comprehensive summary of the toxicity profile of antiangiogenic agents seen in clinical trials, and pointed out, in concert with other speakers, that this type of therapy may not be appropriately evaluated with the criteria generally used for cytotoxic agents (7). He emphasized that agents targeting the tumor vasculature have generally mild toxicities, and that many grade 3 and grade 4 adverse effects have been manageable with medical treatment with ongoing therapy.

### The Role of Sequencing in Antiangiogenic Therapy

The second session was devoted to the identification of optimal schedules for the delivery of antiangiogenic agents. The first speaker and the session chair, Dr. Robert Kerbel, described a variety of approaches tested in his laboratory aimed at maximizing the efficacy of antiangiogenic and cytotoxic agents and preventing tumor escape from antiangiogenic therapy. These approaches included the combination of antiangiogenic low-dose "metronomic" chemotherapy with bolus administration of the chemotherapeutic and combination of antivascular and antiangiogenic agents, as well as the use of circulating endothelial progenitor cells to identify the optimal biological dose of antiangiogenic agents (8). Dr. Gordon Jayson presented an overview of the imaging techniques used in the clinic to evaluate the response to antiangiogenic agents in patients with cancer (9). Special focus was dedicated to dynamic contrast-enhanced magnetic resonance imaging, which produced data that correlated with histologic, biochemical, and clinical outcome in patients with cancer. This session ended with two short talks selected from the abstracts. Dr. Dan Duda presented a study of cell phenotype and circulation kinetics of subpopulations of CECs—emerging biomarkers of antiangiogenic therapy—in patients with cancer treated with bevacizumab (10). Dr. Kyoko Hida reported the cytogenetic abnormalities of endothelial cells in tumors and raised questions regarding the relevance of these findings on tumor response to antiangiogenic therapies (11).

### Vascular Hyperpermeability and Drug Delivery

Dr. Donald McDonald chaired the third session, which addressed the implications of antiangiogenic therapy on the structure and function of the vessel wall in normal tissues and tumors, as well as on delivery of drugs. The session's chair described the effects of VEGF pathway inhibition by agents under clinical development on tumor and normal vessel architecture at microscopic and ultrastructural levels. Although the VEGF inhibition pruned away up to 75% of the tumor vessels, it also affected the vasculature of 11 of 17 organs analyzed by inducing extensive but reversible capillary regression

(up to 68% in the thyroid; ref. 12). Next, Dr. William Sessa described the role of endothelial nitric oxide synthase and hyperpermeability in the progression of tumors. Using genetic models and pharmacologic inhibition of endothelial nitric oxide synthase, Dr. Sessa offered evidence for the direct effects exerted by nitric oxide on permeability (via protein kinase G) and angiogenesis (13). Finally, Dr. David Cheresh presented his work on dissecting the role of VEGF on increased vascular permeability in tumors (14). Dr. Cheresh identified Src as a critical component downstream of VEGFR activation, and proposed Semaphorin 3A as a new vascular permeability factor.

### Lymphangiogenesis and Lymphatic Function

The first presentation of this session was given by the session chair, Dr. Harold Dvorak, who first presented work aimed at the identification of targets using laser-capture microdissection of endothelium and multigene transcriptional profiling (15). In the second part, Dr. Dvorak described the blood and lymphatic vessels' abnormalities associated with a transient overexpression of VEGF. Dr. Peter Carmeliet gave an overview of the pleiotropic roles of PlGF in pathologic angiogenesis, which is explored in gene knockout models and by using antibody blockade: PlGF blockade delayed tumor growth and plaque formation, blocked autoimmune arthritis by inhibiting angiogenesis and immune cell infiltration, and was not associated with some of the unwanted effects of VEGF withdrawal on fetal growth or on normal vasculature (e.g., trachea) in adult mice. Next, he described the use of *Xenopus* tadpoles to study the development and molecular regulation of the lymphatic system (16). The session included a short talk by Dr. Timothy Padera, who described the mechanism by which the compressive force generated by proliferating cancer cells collapses blood and lymphatic vessels in tumors (17).

### The Extracellular Matrix and Drug Delivery

The session dedicated to the interstitial matrix and transport was chaired by Dr. Luisa Iruela-Arispe. Dr. Yves Boucher described two major barriers to drug delivery to tumors: interstitial hypertension and interstitial matrix. Dr. Boucher presented evidence for increase penetration of macromolecules upon alleviation of interstitial hypertension using anti-VEGF blockade (18) or modification of the collagen fibers in tumors. Dr. Luisa Iruela-Arispe described some of the molecular underpinnings of the release of VEGF bound to matrices: the cleavage mediated by matrix metalloproteinase (e.g., MMP-3, -7, -9, -12, and -19) activity, and the inhibition by thrombospondin 1. Matrix-bound VEGF and nontethered VEGF provided different signaling outcomes, and generated blood vessels with different branching patterns, tortuosity and diameter (19). In the last talk of the session, Dr. Zena Werb, offered insights into the biology of hematopoietic cells (macrophages, neutrophils, and T cells) in tumors (20) by real-time analyses using a spinning disc confocal microscope system. In tumors, MMP-9 regulated pericyte recruitment and was expressed at the tumor edge by inflammatory cells; the motility of the inflammatory cells was related to their phagocytic capacity and degree of hypoxia.

### Targeting of Pericytes

This session was devoted to the targeting of pericytes for the treatment of cancer and was chaired by Dr. Douglas Hanahan. Dr. Gabriele Bergers presented evidence for the bone marrow origin

and the function of platelet-derived growth factor receptor  $\beta$  (PDGFR- $\beta$ )-positive pericyte precursors recruited by insulinoma, glioma, or mammary carcinoma in mice (21). Combined targeting of pericytes by anti-PDGFR- $\beta$  antibody and endothelial cells by anti-VEGFR2 antibody provided an additive antitumor effect. Along the same lines, Dr. Hanahan presented data in support of the concept that concomitant targeting of VEGF and PDGF signaling will increase endothelial sensitivity to antiangiogenic and cytotoxic treatments. The speaker also offered insight into the mechanisms of advanced insulinoma escape from anti-VEGF therapy, which was found to depend on basic fibroblast growth factor and EphA pathways (22). The session ended with two short talks. Dr. Carsten Ley proposed that angiopoietin 4 could decrease interstitial fluid pressure in tumors and inhibit angiogenesis by interfering with the VEGF pathway. Dr. Lingge Lu reported that mammary carcinoma growth is delayed in transgenic mice overexpressing canstatin (a fragment of collagen type IV, the main component of the vascular basement membrane).

### The Role of Endothelial Progenitor Cells in Angiogenesis and Delivery of Therapeutics

This session was dedicated to discussions on the role of bone marrow-derived progenitors in tumor progression, a subject that has been recently under intense investigation and debate. The session was chaired by Dr. Shahin Rafii, who summarized the discoveries in the field and described the findings of his lab on the role of endothelial progenitor cells and "proangiogenic" hematopoietic precursors in pathologic neovascularization and their potential role in therapeutic vessel formation (23). Dr. Rafii also described the current understanding of the molecular pathways (e.g., VEGF, stromal-derived factor 1, and angiopoietin 1) involved in bone marrow-derived cells' contribution to new vessel formation. Dr. David Lyden proposed the concept that bone marrow-derived hematopoietic progenitor cells create "metastatic niches" in organs such as the lung or liver (24). According to this new paradigm, metastatic cancer cells home preferentially in these sites where neovascularization is promoted by the hematopoietic precursor cells. The lecture of Dr. Luigi Naldini was dedicated to the discovery by his group of a new population of hematopoietic (myeloid cells) that express the endothelial marker Tie2 (Tie2-expressing mononuclear cells). The speaker described the expression of this marker on local tissue-derived mesenchymal cells in tumors, and underscored the importance of these two stromal cell populations in tumor neovascularization (25).

### The Delivery of Therapeutic Loads to the Tumor Vascular "Zip Codes"

The second morning session of the last day of the conference was dedicated to the delivery of therapeutics specifically to the tumor vasculature. Dr. Erkki Ruoslahti, the chair of the session, presented seminal work done in his laboratory on the discovery of peptides that specifically recognize tumor blood or lymphatic vessels, and discussed their potential therapeutic applications. Several notable examples were LyP-1 (26), a peptide specific to tumor lymphatics, F3, a peptide that binds certain bone marrow cells and angiogenic vessels in tumors, and clt-1 and clt-2, which both bind fibrin networks in blood clots. Dr. Philip Thorpe presented a new approach of targeting tumor endothelium using an antibody-3G4, currently in phase I clinical trials, that recognizes anionic phospholipids on tumor endothelial cell plasma mem-

brane. In mice, the efficacy of this approach was enhanced by chemotherapy or radiotherapy, which increase the expression of phosphatidylserine on tumor endothelium, thus expanding the target for the antibody therapy (27). The last talk of the session was given by Dr. Beverly Teicher, who presented the work of her laboratory on the discovery of genes specifically up-regulated in tumor endothelial cells (28). The process involved the creation of gene expression libraries from endothelial cells of human tumor specimens using serial analysis of gene expression, followed by target validation in preclinical *in vivo* models.

### Key Initiatives Supported by Funding from the National Cancer Institute for Research on Angiogenesis

The topic portfolio sampled all of the key initiatives of the National Cancer Institute for research on tumor angiogenesis, presented and discussed in a special session by Drs. Suresh Mohla and Mary Wolpert. Drs. Mohla and Wolpert described the Trans-Institute Angiogenesis Research Program initiative, created in response to the increasing need of centralizing the research efforts on a variety of pathologies that involve vascular abnormalities, and presented the concept of exploratory (or phase 0) clinical studies.<sup>1</sup>

### "Broad Spectrum Angiogenesis Inhibitors" and "Drug Delivery Systems for Angiogenesis Inhibitors and Other Biomolecules"

The closing session featured two other outstanding keynote lecturers, Drs. Judah Folkman and Robert Langer. Dr. Folkman described the preclinical and clinical development of two broad-spectrum antiangiogenic agents: endostatin (which has recently received State Food and Drug Administration approval in China for lung cancer) and TNP-470 (a synthetic analogue of fumagillin tested in clinical trials; refs. 29, 30). Dr. Folkman proposed that the continued success of the antiangiogenic therapy would depend on the ability to effectively block multiple angiogenic pathways. Dr. Langer offered a historical perspective of the fields of tissue engineering and controlled release of therapeutics, including inhibitors of angiogenesis in tumors (31, 32). These new delivery systems are expected to greatly enhance the ability to deliver drugs locally and in therapeutic concentrations. This session provided an exceptional overview of the development of antiangiogenic therapies and novel drug delivery systems from pioneers in these two fields.

The poster sessions were well attended, with a critical mass of presenters and visitors. The 80 poster presentations sampled a wide range of topics similar to the oral presentations. Fifteen young researchers were recognized with Awards in Cancer Research by the AACR.

### Conclusion

This AACR special conference featured some of the most important advances in the research on angiogenesis and drug delivery. In this way, the leading oncologists and researchers in the world invited and inspired new research efforts from the conference attendees.

<sup>1</sup> See [http://www.cancer.gov/ncicancerbulletin/NCI\\_Cancer\\_Bulletin\\_051804/page3](http://www.cancer.gov/ncicancerbulletin/NCI_Cancer_Bulletin_051804/page3) and [http://www.cancer.gov/ncicancerbulletin/NCI\\_Cancer\\_Bulletin\\_011706/page2](http://www.cancer.gov/ncicancerbulletin/NCI_Cancer_Bulletin_011706/page2) (accessed on January 18, 2006).

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