Evolution of Antiangiogenic Therapies

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The earliest *in vivo* images of tumor angiogenesis

“One is almost forced to the conclusion that there is, associated with the viable growing tumor, some blood vessel growth stimulating factor”.

Tumor Angiogenesis

“Anti-angiogenesis may provide a form of cancer therapy worthy of serious exploration”.

Angiogenic factors

Direct
- Acidic fibroblast growth factor (aFGF)
- Basic fibroblast growth factor (bFGF)
- Epidermal growth factor (EGF)
- Transforming growth factor (TGF)-α

Indirect
- Angiogenin
- TGF-β
- Tumor necrosis factor (TNF)-α
- Lipids
- Prostaglandin E2 (PGE-2)

"... it is now obvious that there are a number of angiogenic factors. Since the distribution and action of these factors is not yet known, it is difficult to speculate on their relative contribution to angiogenesis”

Isolation and cloning of an angiogenic factor secreted by follicular cells

Vascular Endothelial Growth Factor Is a Secreted Angiogenic Mitogen

DAVID W. LEUNG, GEORGE CACHIANES, WUN-JING KUANG,
DAVID V. GOEDEDEL, NAPOLEONE FERRARA*

Fig. 1. Nucleotide sequence and deduced amino acid sequence of bovine (A) and a human (B) VEGF cDNA clone. The amino acid sequence derived from NH2-terminal sequence analysis is underlined. The protein sequence is numbered starting with 1 at the mature NH2-terminal alanine. The putative glycosylation site is boxed. ATG and stop codons found in the 5' non-coding region and the poly(A) signal AAATAAA are also underlined. DNA sequence analysis was performed by the dideoxy chain termination method with cDNA fragments subcloned into plasmid vectors (25).

Abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
Vascular endothelial growth factor (VEGF) promotes angiogenesis

- Mitogen and survival factor for endothelial cells
- Permeability factor
- Chemotactic
- Homodimeric glycoprotein
- Molecular weight: 45,000Da
VEGF-A is spliced into different isoforms that show distinct heparin affinity and diffusibility in tissues.
Plasmin cleaves the heparin-binding domain from VEGF-A and generates bioactive and more diffusible fragments.
The three VEGF tyrosine kinase receptors show distinct functions and binding to VEGF family members.
Neuropilins as Receptors for Semaphorins and the Heparin-Binding VEGF Isoforms

Sema 3A, C, E

Sema 3A

VEGF189

VEGF165

VEGF-C

VEGF-D

VEGF165

Sema 3F

Plexin D1

Plexin As

Nrp-1

Nrp-2

VEGFRs
Loss of a single VEGF-A allele results in embryonic lethality

Angiogenic sprouting and balance between DLL4 and VEGF

- Tip cells
- DLL4/Notch1

VEGF blockade

- Sprouting
- EC proliferation
- EC survival
- Vascular organization
- Tumor vascular density
- Tumor vessel perfusion
- Tumor growth

DLL4 blockade

- Sprouting
- EC proliferation
- Vessel lumen size
- Vascular organization
- Tumor vascular density
- Tumor vessel perfusion
- Tumor growth
A wide variety of tumors show increased expression of VEGF mRNA
Tumor characteristics and microenvironment promote VEGF expression

PDGF = platelet-derived growth factor; IGF-1 = insulin-like growth factor 1
IL-8 = insulin-like growth factor 8
Inhibition of VEGF-mediated angiogenesis suppresses tumor growth *in vivo*

**Diagram:**
- Graph showing tumor size (mm³) over weeks for different treatment groups.
- Bar graph showing tumor weight (g) for different tumor cell lines.

**Legend:**
- Vehicle
- A4.6.1 10μg
- A4.6.1 50μg
- A4.6.1 100μg
- A4.6.1 200μg
- A4.6.1 400μg
- Control Mab 400μg

**Tumor cell lines:**
- A673
- G55
- SK-LMS-1

**Source:**
Anti-human VEGF-A Mab (A.4.6.1) inhibits growth of liver metastases of HM-7 colorectal cancer cells

A4.6.1 Mab  Control Mab

Tumour volume (mm$^3$)

Time (days)

MAb = monoclonal antibody

Anti-VEGF Mab A.4.6.1 inhibits tumor growth in the SKOV-3 ovarian cancer model as long as the treatment is continued. After cessation of anti-VEGF treatment, growth and revascularization of s.c. or i.p. implanted tumors resumed and the animals had to be killed within a few weeks.

Schema depicting tumor vascular normalization as proposed by Jain


Multiple angiogenesis inhibitors fail to show efficacy in clinical trials despite strong activity in animal models

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batimastat</td>
<td>MMP Inhibitor</td>
</tr>
<tr>
<td>Marimastat</td>
<td>MMP Inhibitor</td>
</tr>
<tr>
<td>Neovastat</td>
<td>MMP Inhibitor</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Collagen XVIII fragment</td>
</tr>
<tr>
<td>TNP470</td>
<td>Fumagillin analog</td>
</tr>
<tr>
<td>SU-5416</td>
<td>VEGFR/PDGFR TKI</td>
</tr>
<tr>
<td>PTK787</td>
<td>VEGFR/PDGFR TKI</td>
</tr>
<tr>
<td>Vitaxin</td>
<td>Antibody targeting Av integrins</td>
</tr>
</tbody>
</table>
A murine anti-VEGF monoclonal antibody was humanized to generate Bevacizumab (Avastin)

Recombinant humanized monoclonal antibody (MAb) targeting VEGF-A

- 93% human, 7% murine
- does not induce immune response in humans
- binds to primate VEGF and rabbit VEGF but not to rat or mouse VEGF
- binds to all isoforms of VEGF-A
Phase III trial AVF2107g: bevacizumab increases survival in previously untreated metastatic CRC

Median survival (months)
IFL + placebo: 15.6 (95% CI: 14.3–17.0) vs IFL + Avastin: 20.3 (95% CI: 18.5–24.2)
HR=0.66 (95% CI: 0.54–0.81)
p<0.001

Previously untreated epithelial ovarian, primary peritoneal, or fallopian tube cancer

- Stage III optimal (macroscopic)
- Stage III suboptimal
- Stage IV

n=1800 (planned)

Stratification variables:
- GOG performance status
- Stage/debulking status

GOG-0218 study schema

1:1:1

Arm

I
(CP + PLA → PLA)

Carboplatin AUC 6
Paclitaxel 175 mg/m²
Placebo

II
(CP + BEV → PLA)

Carboplatin AUC 6
Paclitaxel 175 mg/m²
Bevacizumab 15 mg/kg
Placebo

III
(CP + BEV → BEV)

Carboplatin AUC 6
Paclitaxel 175 mg/m²
Bevacizumab 15 mg/kg

Cytotoxic (6 cycles)
Maintenance (16 cycles)
15 months

Progression-free survival in ovarian cancer patients is dependent on the duration of bevacizumab treatment.

NCI Press Release

**Bevacizumab significantly improves survival for patients with recurrent and metastatic cervical cancer**

Patients with advanced, recurrent, or persistent cervical cancer that was not curable with standard treatment who received the drug bevacizumab (Avastin) lived 3.7 months longer than patients who did not receive the drug, according to an interim analysis of a large, randomized clinical trial.

The clinical trial, known as GOG240, was sponsored by the National Cancer Institute (NCI), part of the National Institutes of Health, and conducted by a network of researchers led by the Gynecologic Oncology Group (GOG). Genentech, South San Francisco, Calif., the drug manufacturer, provided support for the trial under the Cooperative Research and Development Agreement (CRADA) with NCI for the clinical development of bevacizumab.

The data safety monitoring committee overseeing the trial recommended that the results of a recent interim analysis be made public because the study had met its primary endpoint of demonstrating improved overall survival in patients who received bevacizumab, which also means that it delayed the chance of dying from the disease.
VEGF signaling pathways and potential therapeutic targets

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Type</th>
<th>Mechanism of action</th>
<th>Clinical stage</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>Humanized mAb</td>
<td>Blocks VEGF-A binding to receptors</td>
<td>Approved for metastatic CRC, NSCLC, recurrent CRC</td>
<td>Genentech/Roche (Basel, Switzerland)</td>
</tr>
<tr>
<td>Sunitinib (Sutent)</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs, PDGFRs, FLT3, CSF1R</td>
<td>Approved for metastatic RCC, imatinib-resistant GIST</td>
<td>Pfizer (New York, NY)</td>
</tr>
<tr>
<td>Sorafenib (Nexavar)</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs Raf, PDGFRs, KIT</td>
<td>Approved for metastatic RCC, HPCC</td>
<td>Bayer/Onyx (South San Francisco, CA)</td>
</tr>
<tr>
<td>Pazopanib (Votrient)</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, KIT</td>
<td>Approved for metastatic RCC</td>
<td>GlaxoSmithKline (London, UK)</td>
</tr>
<tr>
<td>Vandetanib (Caprelsa)</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, EGFR</td>
<td>Approved for metastatic medullary thyroid cancer</td>
<td>AstraZeneca (London, UK)</td>
</tr>
<tr>
<td>Axitinib (Inlyta)</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, KIT</td>
<td>Approved for RCC that failed first-line therapy</td>
<td>Pfizer (New York, NY)</td>
</tr>
<tr>
<td>Aflibercept (Zaltrap)</td>
<td>Chimeric soluble receptor</td>
<td>Binds VEGF-A, VEGF-B and PIGF</td>
<td>Phase 3 multiple tumor types</td>
<td>Regeneron/Sanofi Aventis (Paris)</td>
</tr>
<tr>
<td>AGM386</td>
<td>Peptidobody</td>
<td>Binds Angiopoietin-1 and -2</td>
<td>Phase 3 multiple tumor types</td>
<td>Amgen (Thousand Oaks, CA)</td>
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<tr>
<td>Motesanib</td>
<td>Small-molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, KIT</td>
<td>Phase 3 multiple tumor types</td>
<td>Amgen</td>
</tr>
<tr>
<td>Cediranib (Recentin)</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, KIT</td>
<td>Phase 3 multiple tumor types</td>
<td>AstraZeneca</td>
</tr>
<tr>
<td>Cabozantinib</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, MET, RET, KIT</td>
<td>Phase 3 multiple tumor types</td>
<td>Exelixis (South San Francisco, CA)</td>
</tr>
<tr>
<td>Tivozanib</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs PDGFRs, KIT</td>
<td>Phase 3 metastatic RCC</td>
<td>Aveo (Cambridge, MA)</td>
</tr>
<tr>
<td>Regorafenib</td>
<td>Small molecule RTK inhibitor</td>
<td>Inhibits signaling of VEGFRs Raf, PDGFRs, KIT</td>
<td>Phase 3 relapsed CRC and other tumors</td>
<td>Bayer/Onyx</td>
</tr>
<tr>
<td>Ramucirumab</td>
<td>Human mAb</td>
<td>Blocks VEGFR-2 signaling</td>
<td>Phase 3 multiple tumor types</td>
<td>ImClone/Lilly (Indianapolis, IN)</td>
</tr>
<tr>
<td>Cilengitide</td>
<td>Cyclic peptide</td>
<td>Blocks αv integrins</td>
<td>Phase 3 GBM</td>
<td>Merck KGaA (Darmstadt, Germany)</td>
</tr>
<tr>
<td>Volociximab</td>
<td>Chimeric mAb</td>
<td>Blocks α5β1 integrin</td>
<td>Phase 2 multiple tumor types</td>
<td>PDL/Biogen Idec (Cambridge, MA)</td>
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<tr>
<td>IMC-18F1</td>
<td>Human mAb</td>
<td>Blocks VEGFR-1 signaling</td>
<td>Phase 2 multiple tumor types</td>
<td>ImClone/Lilly</td>
</tr>
<tr>
<td>TB-403</td>
<td>Humanized mAb</td>
<td>Blocks PIGF binding to VEGFR-1</td>
<td>Phase 2 multiple tumor types</td>
<td>Thrombogenix/Roche</td>
</tr>
<tr>
<td>Anti-EGF7</td>
<td>Humanized mAb</td>
<td>Blocks EGFL7, a protein implicated in vascular maturation</td>
<td>Phase 2 multiple tumor types</td>
<td>Genentech/Roche</td>
</tr>
</tbody>
</table>

TKI, tyrosine kinase inhibitor; CRC, colorectal cancer; NSCLC, non–small cell lung carcinoma; RCC, renal-cell carcinoma; GBM, glioblastoma multiforme; GIST, gastrointestinal stromal tumor; HPCC, hepatocellular carcinoma.
Biomarker Challenges by complexity of cancer biology

**Most biomarkers**

- **Tumour cell**
  - Cell surface: overexpressed or overactive receptors
  - Intracellular: mutated or overactive signaling proteins

**Biomarkers difficult to identify**

- **Tumour microenvironment**
  - Stromal cells
  - Infiltrating immune cells
  - Endothelial cells
  - Pericytes

Microenvironment: complex milieu – difficult to classify. Heterogeneous environment due to multiple cell types and signaling molecules among them.
pVEGF-A: potentially predictive in mBC, mGC and mPaC

GS Jayson et al.
Identification of Genes Expressed in The VDV Compartment

VDV signature: VEGF/VEGFR-2 signaling inhibition + subsequent VDV ablation
NO16966: Overall Survival in XELOX treated arms, VDV analysis

NO16966/CONSORT: Ph III 1st Line; Metastatic CRC N=2034

XELOX (n=317)

XELOX + Placebo (n=350)

XELOX + bevacizumab (n=350)

Pre-treatment tumor RNA analyzable from 103 patients

Effect of Bevacizumab on OS

Effect of bevacizumab and VDV gene set expression on OS

26-gene subset stratifies OS response to bevacizumab+chemotherapy vs chemo
Before Therapy  
After 15 Weeks on PLX4032  
Relapse after 23 Weeks of Therapy

Patient with BRAF mutant melanoma

Identified two mouse tumor cell lines (LLC and EL4) that are resistant to anti-VEGF treatment.
Anti-VEGF resistant tumors recruit large numbers of CD11b+Gr1+ myeloid cells compared to sensitive tumors.
The key hematopoietic growth factor G-CSF up-regulates expression of *Bv8/PK2* in Cd11b+Gr1+ myeloid cells by a Stat3-dependent mechanism.

Anti-Bv8 antibodies suppress tumor angiogenesis and growth

HM7 tumor

Control    Anti-VEGF    Anti-Bv8

HP-30120 Anti-BV8 “++” signal in colon adenocarcinoma
G-CSF and Bv8 Contribute to anti-VEGF Resistance

**Sensitive**

Angiogenesis is largely driven by tumor- and stroma-derived VEGF-A

**Refractory**

Tumor and/or stromal cells secrete VEGF and myeloid growth factors such as G-CSF. Myeloid cells can produce Bv8 and other angiogenic factors.
Multiple cell types and pro-angiogenic molecules are likely implicated in resistance to VEGF inhibitors.
Neovascular age-related macular degeneration
Ranibizumab results in increased visual acuity in patients with wet AMD

- PDT (n=143)
- Ranibizumab 0.3 mg (n=140)
- Ranibizumab 0.5 mg (n=139)

**Month**

ETDRS letters

- +11.3
- +10.7
- +8.1
- 20.5 letter benefit *
- 17.9 letter benefit *
- -9.6
- -9.8

* P < 0.0001

**Diagram:**
- Erythrocyte
- Pericyte
- Microvascular endothelium
- CNV-associated vessel

**Labels:**
- VEGF
- Short term
- Long term
- Ranibizumab
- No treatment
- Exudation Hemorrhage
Neovascular AMD no longer may be the leading cause of blindness in people over the age of 50 in the U.S. and throughout the world where access to monthly ranibizumab (and potentially other VEGF inhibitors) is available.
- **Impact of VEGF Inhibitors on Disease**
  - Benefit in several tumor types. VEGF inhibitors now represent standard of therapy for multiple malignancies.
  - Dramatic benefit in patients with intraocular neovascular diseases such as wet AMD and retinal vein occlusion following treatment with ranibizumab, bevacizumab or aflibercept

- **Present Challenges**
  - Identification of predictive biomarkers
  - Establishing optimal treatment duration/combinations
  - Elucidating mechanisms of inherent refractoriness/resistance
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