Anti-angiogenic tumor therapy - novel insights and translational approaches

Targeting the vasculature of visceral tumors: current therapeutic strategies and translational approaches

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01.04.2014, Heidelberg
Early history of angiogenesis

„Tumor growth is angiogenesis-dependent; each increment of tumor growth requires an increment of capillary growth.“

„Vascular endothelial growth factor is a secreted angiogenic mitogen."
Napolone Ferrara and colleagues, Science 1989

2004 First approved anti-angiogenic therapy - Avastin™ (Bevacizumab = VEGF-antibody)
48,720 papers published on angiogenesis from 2005-2014.
1. “... solid tumors are dependent upon new capillary sprouts...”

2. “… without neovascularization solid tumors might become completely dormant…”

3. “the term “anti-angiogenesis” is proposed to mean the prevention of new vessel sprouts from penetrating into an early tumor implant.”

4. “the necrotic center of a large tumor was at one time well vascularized, however, the high pressures which build up in a large tumor could diminish blood flow to the center.”
Cancer detection not possible using current methods

 Clinically detectable cancer

Angiogenic switch

Time:

Up to 10 years

1 to 5 years

Cell number

Size: Microscopic Milligrams

1 mm³

10^3

10^9

10^12


Angiogenic factors and receptors

**EC “specific” factors/receptors:**
- VEGF-A, PLGF
- VEGF-A
- VEGF-C
- ANG1,2,3,4
- Ephrins

**VEGFR**
- VEGFR1
- VEGFR2
- VEGFR3
- TIE1, TIE2
- Eph-Receptors

**Non EC-specific factors:**
- bFGF
- PDGF
- TGF-b

**EFGR**
- FGFR-1-4
- PDGF-R
- TGF-bR-1-3

Etc.
Tumor angiogenesis is facilitated by developmental pathways

Sonic Hedgehog
Notch
Bone Morphogenic Proteins

Relationship between angiogenesis and CSC niche maintenance

(A) CSCs generate pro-angiogenic factors to stimulate angiogenesis while the tumor vasculature aids in maintaining CSC self-renewal.

(B) Anti-angiogenic agents disrupt angiogenesis and may also interrupt vascular derived CSC maintenance cues.
Impact of angiogenesis within tumor progression ~ 1990s

In situ tumor: (Dormant, microscopic)

Angiogenesis

Expansion of the tumor population

Enter circulation

Exit

Limited growth in target organ

Angiogenesis

Expansion into vascularized metastasis

Dormant
In situ tumor: (Dormant, microscopic)

Angiogenesis

Expansion of the tumor population

Survival of exiting tumor cells in endothelial-dependent.

Dormancy due to blocked angiogenesis.

Limited growth in target organ

Angiogenesis

Expansion into vascularized metastasis

Paracrine interactions between endothelial cells and tumor cells.

Antiangiogenic therapy does not induce drug resistance.

P53 control of angiogenic switching

Ras-dependent angiogenic switching.
Arbiser et al, PNAS 1997; 94:861.

Angiogenic switching.

Metastasis is angiogenesis-dependent.

Tumor growth is angiogenesis-dependent.

Primary tumor can suppress secondary tumors.

Human Melanoma: dormancy in the absence of angiogenesis.

Angiogenic progression.
Reif et al, Cancer Res 1997; 57:963.

Leukemia is angiogenic.
Perz-Atayde Amer J Pathol 1997; 150:815.
Clinical translation
2000 - -

_In situ_ tumor:
(Dormant, microscopic)

**Angiogenesis**

- Expansion of the tumor population
  - **Current angiogenesis inhibitors**

- Enter circulation

- Exit
  - Limited growth in target organ

**Angiogenesis**

- Expansion into vascularized metastasis
  - **Current angiogenesis inhibitors**
Expansion of the tumor population

Tumor cells

Prevention of the angiogenic switch?

Expansion into vascularized metastasis

Limited growth in target organ

Exit

Dormant

Enter circulation

Angiogenesis

Prevention of the angiogenic switch?

Expansion of the tumor population

Future: prevention of the angiogenic switch?
**Strategies for angiogenesis-based cancer therapy**

**Growth Factor Inhibitors**
- Antibodies, Traps
- Decoy Receptors
- RNAi

**Inhibitors of Invasion**
- MMP Inhibitors

**Radioligands**
- $^{125}$I-anti-endoglin

**EndoTAG-1**
- Cationic liposomal-encapsulated paclitaxel

**Tumor**
- VEGF

**Receptor Antagonists**
- Peptides
- Small Molecules
- RNAi

**Targeting miRs**
- polymer-based nanoparticles
- antagonirs

**Signal Transduction Inhibitors**
- Tyrosine Kinase Inhibitors

**EPC, MSC, etc.**
- as gene therapy vehicles
# Targeting the vasculature of visceral tumors

Leading anti-angiogenic and vascular-targeting agents being approved or investigated in clinical trials

<table>
<thead>
<tr>
<th>Name</th>
<th>Target</th>
<th>Cancer type</th>
<th>Phase of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>VEGF-A</td>
<td>CRC, RCC, HCC</td>
<td>Approved</td>
</tr>
<tr>
<td>Bavituximab</td>
<td>Phospholipid membrane of endothelial cells</td>
<td>NSCLC, CRC, PDAC</td>
<td>Phase II</td>
</tr>
<tr>
<td>Ramucirumab</td>
<td>VEGFR-2</td>
<td>CRC, HCC</td>
<td>Phase II</td>
</tr>
<tr>
<td>Everolimus</td>
<td>mTOR</td>
<td>RCC</td>
<td>Approved</td>
</tr>
<tr>
<td>Tensirolimus</td>
<td>mTOR</td>
<td>RCC</td>
<td>Approved</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>VEGFR, PDGFR, c-kit</td>
<td>RCC</td>
<td>Approved</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>VEGFR-1/-2, PGDFR, Flt-3, c-Kit</td>
<td>RCC, HCC, GIST</td>
<td>Approved</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>VEGFR-1,-2,-3, PDGFR</td>
<td>HCC, RCC</td>
<td>Approved</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>EGFR</td>
<td>HCC; PDAC</td>
<td>Approved; phase III</td>
</tr>
<tr>
<td>Aflibercept</td>
<td>Decoy receptor for VEGF</td>
<td>CRC</td>
<td>Phase III</td>
</tr>
<tr>
<td>Axitinib</td>
<td>VEGFR</td>
<td>RCC; PDAC</td>
<td>Phase III; phase I</td>
</tr>
<tr>
<td>EndoTAG-1</td>
<td>Tumor endothelium</td>
<td>PDAC, breast cancer</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

*L. V. Klotz. et al, Langenbecks Arch Surg, 2012*
Tumor vasculature targeting-Updates

TPZ-tirapazamine:
- competitive NOS inhibitor
- Hypoxic cytotoxin

-poorly oxygenated tumour vessels
-abnormally organized
-inadequate smooth muscle cells can be successfully targeted by inhibition of NOS and hypoxia-activated prodrug toxicity.

-a novel use of hypoxia-activated cytotoxic prodrugs as vascular targeting agents, and also represents a novel mechanism for targeting tumour vessels.

Endothelial Tie1 deficiency inhibits tumor growth

Endothelial Tie1 deficiency inhibits tumor angiogenesis

Gabriela D’Amico, et al. JCI, 2014
Tumor vasculature targeting - Updates

Research article

Tumor endothelial marker 1–specific DNA vaccination targets tumor vasculature

John G. Facciponte,1 Stefano Ugel,1 Francesco De Sanctis,1,2 Chunsheng Li,1 Liping Wang,3 Gautham Nair,4 Sandy Sehgal,5 Arjun Raj,4 Ethymia Matthaiou,1 George Coukos,1 and Andrea Facciabene1

Tem1-TT vaccination inhibits CT26 tumor vascularization

J. G Facciponte, et al. JCI, 2014
Glycosylation-Dependent Binding of Gal1 to ECs Mimics VEGF-A Function

Discovery of a glycosylation-dependent pathway that compensates for the absence of cognate ligand and preserves angiogenesis in response to VEGF blockade.

Diego O. Croci et al. Cell, 2014
Role of mTOR in tumor growth and angiogenesis

mTOR
PI3-K
Akt/PKB
AMPK
LKB1
PTEN
PI3-K
Akt/PKB
mTOR
mTOR inhibitor
FKBP-12
FKBP-12
mTOR inhibitor
Rapamycin CCI-779 RAD001

growth factors
growth factors

integrins

nutrients
amino acids

Energie

Energie

transcription

transcription

cell growth

cell growth

proliferation

proliferation

protein production

protein production

Tumor cell

Endothelial cell

VEGF production

VEGF production

cell growth and proliferation

cell growth and proliferation

4E-BP1

4E-BP1

p70S6K

p70S6K

Role of mTOR in tumor growth and angiogenesis
mTOR inhibition in pancreatic cancer

Effect of rapamycin on tumor growth of mice with orthotopically implanted L3.6pl pancreatic tumors

Rapamycin treatment induces the selective shutdown of initially functional tumor vessels

Proposed pathways for rapamycin-induced TF production in endothelial cells.

Combination of anti-angiogenic therapy using the mTOR-inhibitor everolimus and low-dose chemotherapy for locally advanced and/or metastatic pancreatic cancer – a dose finding study


MTD:
400 mg/m²/week gemcitabine and 5 mg/d everolimus

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Patients</th>
<th>Everolimus</th>
<th>Gemcitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N = 3</td>
<td>5 mg/2nd day</td>
<td>400 mg/m²/week</td>
</tr>
<tr>
<td>2</td>
<td>N = 4</td>
<td>5 mg/2nd day</td>
<td>500 mg/m²/week</td>
</tr>
<tr>
<td>3</td>
<td>N = 6</td>
<td>5 mg/2nd day</td>
<td>600 mg/m²/week</td>
</tr>
<tr>
<td>4</td>
<td>N = 7</td>
<td>5 mg/day</td>
<td>400 mg/m²/week</td>
</tr>
<tr>
<td>5</td>
<td>N = 7</td>
<td>5 mg/day</td>
<td>500 mg/m²/week</td>
</tr>
</tbody>
</table>

Response

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>23 (100)</td>
</tr>
<tr>
<td>Overall response rate (CR + PR)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Clinical benefit rate (CR + PR + SD)</td>
<td>18 (78)</td>
</tr>
<tr>
<td>Complete response (CR)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>15 (65)</td>
</tr>
<tr>
<td><strong>Progressive disease (PD)</strong></td>
<td>3 (13)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Evaluation missing</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

In review: Anti-cancer drugs
Engineered mesenchymal stem cells (eMSC) based anti-angiogenic therapy

Targeting of tumor angiogenesis by linking suicide gene expression (HSV-Tk) to angiopoietin-1–induced differentiation of engineered mesenchymal stem cells.

*In vivo* efficacy of Tie2/TK eMSC treatment in an murine orthotopic pancreatic carcinoma model. *In vivo* treatment of spontaneous Balb/c-neuT murine breast cancer model with Tie2/TK eMSC. Integration of exogenously added Tie2/RFP eMSC into the vasculature of growing primary mammary tumors.

eMSC based suicide gene therapy: Targeting tumor endothelium and tumor stroma

(SPP „tumor-vessel-interface“)

Hepatocellular carcinoma

Huh7

Balb/c nu/nu

CCL5-HSV-TK
Tie2-HSV-TK

Engineered MSC

MSC

Kontrolle

+ Ganciclovir

TREAT-ME I
Phase 1/2 clinical trial
Metastatic/locally advanced GI Tumors

01/2014
2 patients included

Lessons learnt from VEGF-targeted therapies and open questions

- Evidence for activity of VEGF pathway targeted drugs (bevacizumab, sunitinib, aflibercept) in some tumors (CRC, kidney cancer, breast cancer, ovarian cancer)
- Transfer in other tumor entities: HCC, sarcomas, etc.
- VEGF-targeted therapies in different disease stages (palliative, adjuvant, neoadjuvant)
- Interaction with chemotherapy, duration, scheduling
- **Surrogate markers, predictive markers**
- Mechanisms of resistance
- Paradoxical mechanisms (enhanced tumor aggressiveness)
- Personalized anti-angiogenic therapies

Vasudev et al. Angiogenesis 2014
Targeting the vasculature of visceral tumors

Profile of Soluble and Cellular Biomarkers and of Functional Imaging During Antiangiogenic Therapies in Cancer Patients
NCT01507740

• CRC, RCC, HCC, NSCLC
• Anti-angiogenic drugs: Bevacizumab, Sunitinib, Sorafinib
• CEC, CEP, soluble plasma markers: VEGF, bFGF, ICAM, sVGFR-2 IL-8, SDF1, DKK-3
• Measurable lesion, MRI scan, RECIST criteria
• Control group = 20 patients, investigational group = 40 patients
• Estimated completion date 11/2014
Lessons learnt from VEGF-targeted therapies and open questions  
Vasudev et al. Angiogenesis 2014

- Evidence for activity of VEGF pathway targeted drugs (bevacizumab, sunitinib, aflibercept) in some tumors (CRC, kidney cancer, breast cancer, ovarian cancer)
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- **Interaction with chemotherapy, duration, scheduling**
  - Predictive markers
  - Mechanisms of resistance
  - Paradoxical mechanisms (enhanced tumor aggressiveness)
  - Personalized anti-angiogenic therapies

Vasudev et al. Angiogenesis 2014
Relevance of therapy scheduling

**PEAK**

R 1:1

mFOLFOX6 + Bevacizumab

<table>
<thead>
<tr>
<th>OS Monate</th>
<th>PFS Monate</th>
<th>ORR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>28,9</td>
<td>10,1</td>
<td>60</td>
</tr>
</tbody>
</table>

HR = 0,63  
p = 0,06  
OR: n.a.  
p = n.a.

mFOLFOX6 + Panitumumab

<table>
<thead>
<tr>
<th>OS Monate</th>
<th>PFS Monate</th>
<th>ORR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>41,3</td>
<td>13,0</td>
<td>64</td>
</tr>
</tbody>
</table>

HR = 0,66  
p = 0,03  
OR: n.a.  
p = n.a.

**FIRE-3**

R 1:1

FOLFIRI + Bevacizumab

<table>
<thead>
<tr>
<th>OS Monate</th>
<th>PFS Monate</th>
<th>ORR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>25,6</td>
<td>10,2</td>
<td>59,6</td>
</tr>
</tbody>
</table>

HR = 0,70  
p = 0,011  
OR = 1,28  
p = 0,32

FOLFIRI + Cetuximab

<table>
<thead>
<tr>
<th>OS Monate</th>
<th>PFS Monate</th>
<th>ORR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>33,1</td>
<td>10,4</td>
<td>65,5</td>
</tr>
</tbody>
</table>

HR = 0,93  
p = 0,54  
OR = 1,28  
p = 0,32

Antiproliferatives Agens: S-FU, Oxaliplatin, anti-EGFR-AK;  
Angiogenesehemmer;  
HR: Hazard Ratio, OR: Odds Ratio, n.a.: nicht angegeben

Karthaus M et al. EJC 2013: 49 (suppl 3): abstract 2262+Poster  
Keine formale Hypothesentestung; prospektive geplante, retrospektive RAS-Analyse; Phase 2, 1st-Line
PEAK: Relevance of therapy scheduling Anti-EGFR therapy first line

RAS-WT mCRC

**Strategy:**
- Panitumumab first
  - 100% Patients

**Strategy:**
- Bevacizumab first
  - 100% Patients

**First line therapy**
- Panitumumab
- Bevacizumab
- VEGF
- andere

**Second line therapy**
- VEGF
- andere
- EGFR
- andere

<table>
<thead>
<tr>
<th>Median OS (months)</th>
<th>% Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>41.3</td>
<td>100%</td>
</tr>
<tr>
<td>28.9</td>
<td>100%</td>
</tr>
</tbody>
</table>

Δ 12.4 months

Karthaus M. et al. EJC 2013; 49 (suppl 3): abstract 2262 (und Poster); *Stratifiziertes Cox-Proportional-Hazards-Modell; Keine formale Hypothesentestung; Phase-2 Studie*
FIRE-3: Relevance of therapy scheduling Anti-EGFR therapy first line

**RAS-WT mCRC**

**Strategy:**
- Cetuximab first: 100% (100% Cetuximab, 46% VEGF, 44% andere)
- Bevacizumab first: 100% (100% Bevacizumab, 44% EGFR, 44% andere)

**First line therapy**
- **Strategy:** Cetuximab
  - Median OS (months): 33.1
  - HR* = 0.70, p = 0.011

**Second line therapy**
- **Strategy:** Bevacizumab
  - Median OS (months): 25.6
  - Δ 7.5 months

Heinemann V, et al. EJC 2013; 49 (suppl 3): LBA 17 (Vortrag)
*pimarer Endpunkt (ORR) nicht erreicht*
Deepness of response correlates with post-progression survival

- Data from the CRYSTAL trial indicate that tumor size reduction is more predictive for OS than PFS

<table>
<thead>
<tr>
<th></th>
<th>Cetuximab + FOLFIRI (n=315)</th>
<th>FOLFIRI (n=348)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median DpR (95% CI)</td>
<td>50.9 (18.4 - 78.6)</td>
<td>33.3 (8.0 - 58.0)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Median OS (95% CI)</td>
<td>23.5 (21.2 - 26.3)</td>
<td>20.0 (17.4 - 21.7)</td>
<td>p&lt;0.0093</td>
</tr>
</tbody>
</table>

Mansmann et al, ASCO 2013
Abstract #3630
Targeting the vasculature of visceral tumors: future directions

- Intelligent combination of anti-angiogenic therapy with conventional therapy (CTx, RTx, targeted therapy)
- Identification of surrogate markers (molecular imaging)
- Identification of subgroups with significant efficacy (predictive markers)
- Identification of the timing and schedule
- Prevention of the angiogenic switch
- CSC-targeted therapy
Rapamycin leads to massive tumor necrosis

CT-26 colon cancer in BALB/c mice

- Control
- Rapamycin 0.15 mg/kg/d
- Rapamycin 1.5 mg/kg/d
- Rapamycin 15 mg/kg/d

Tumor volume (mm$^3$) vs. Time (days)

Start of therapy

n = 6-8
**In vivo efficacy of Matuzumab +/- Gemcitabine in an orthotopic human pancreatic cancer model (L3.6pl)**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Mean body weight (g) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Pl)</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>Matuzumab (40 + 10)</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Matuzumab + Gemcitabine</td>
<td>21 ± 2</td>
</tr>
</tbody>
</table>

*P < 0.01
†P < 0.01
‡P < 0.05
§P < 0.05
¶P < 0.05
Interaction between angiogenesis and metastasis

1. Pro-angiogenic factors (VEGF, etc.)
2. Proteolytic destruction of the extracellular matrix
3. Endothelial cell proliferation and migration
4. “tumor vessel system”
5. Metastasis
6. Cancer stem cells (CSC):
   - Angiogenesis?
   - Metastasis?
   - Therapy resistance?
Tumor vasculature

Angiogenesis
- Sprouting of cells from mature endothelial cells of the vessel wall
- Crucial prerequisite in tumor growth, disease progression, and metastasis


Immature, Chaotic, Hypoxia

Gillian M. Tozer. et.al. Nature reviews cancer. 2005
**mTOR inhibition in pancreatic cancer**

*In vitro* proliferation of HUVECs and tumor cells of RAD001

Phosphorylation of s6rp as the downstream target of mTOR was absent in starved L3.6pl cells

mTOR inhibition can interrupt the radiation-induced stress response of tumor cells that should protect tumor microvasculature against radiation damage.

---

Philipp C. Manegold, C. J. Bruns. CCR. 2007
Representative color-coded maps for plasma flow (Fp) pre (A) and post therapy (B). Microvascular density, apoptosis, and proliferation in the therapy and control group (bottom).

Linear correlation coefficients (Pearson’s r) for correlation between tumor microcirculation and immunohistochemical parameters (IHC) in the therapy group.

<table>
<thead>
<tr>
<th>Perfusion/IHC</th>
<th>R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_p/Ki67</td>
<td>0.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>F_p/TUNEL</td>
<td>-0.66</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>F_p/CD-31</td>
<td>0.84</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>V_p/Ki67</td>
<td>0.25</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>V_p/TUNEL</td>
<td>-0.71</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>V_p/CD-31</td>
<td>0.66</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PS/Ki67</td>
<td>-0.04</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PS/TUNEL</td>
<td>-0.11</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>PS/CD31</td>
<td>0.71</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Regorafenib significantly suppressed tumor vascularity (plasma volume) quantified by dynamic contrast-enhanced computed tomography in experimental colon carcinomas in rats with good-to-moderate correlations to an immunohistochemical gold standard.

Conventional chemotherapy vs. anti-angiogenic chemotherapy (metronomic).

Time

Plasma Concentration

Conventional chemotherapy

3 weeks

Therapeutic window for endothelium (Metronomic)
Assessment of angiogenesis in human tumors

Noninvasive imaging
MRI, molecular imaging

Clinical chemistry
Biomarkers, CEC

Pathology
MVD

Augustin H et al.