The Biology of Angiogenesis and the Challenge of Identifying Biomarkers
ESMO Asia 2015, Singapore.

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‘Angiogenic switch’ - crucial in all stages of tumor development

Small tumor (1-2 mm)
Avascular
Dormant

Angiogenic switch by expressing multiple angiogenic factors, such as VEGF

Large tumor
Vascular
Metastatic potential
Targeted Therapy by Inhibition of Angiogenesis

The tumour angiogenesis process & therapeutic role of angiogenesis inhibitors
[Kindly provided by A. Griffoen]
### Tumor site | Name of the Study | Endpoint | Effect on PFS months (P-value) | Effect on OS Months (P-value)
--- | --- | --- | --- | ---
Prostate | CALGB 90401 | OS | 2.4 (0.0001) | 1.1 (0.18)
Pancreas | AVITA | OS | 1 (0.0002) | 1.1 (0.21)
Lung | ECOG E4599 | OS | 1.7 (<0.001) | 2 (0.003)
Lung | AVAIL | PFS, OS | 0.6 (0.0003) | 0.5 (0.42)
Kidney | CALGB 90206 | OS | 3.3 (<0.001) | 0.9 (0.097)
Breast | ECOG E2100 | PFS | 5.9 (0.001) | 1.5 (0.16)
Breast | AVADO | PFS | 1.9 (0.006) | -1.7 (0.85)
Breast | RIBBON-1 | PFS | 2.9 (0.0002) | 7.8 (0.27)
Gastric | AVAGAST | OS | 1.4 (0.0037) | 2 (0.1)
Colorectal | Hurwitz et al. (2008) | OS | 4.4 (<0.001) | 4.7 (<0.001)
Colorectal | VELOUR | OS | 2.23 (<0.001) | 1.4 (0.0032)
Colorectal | RAISE | OS | 1.2 (<0.0005) | 1.6 (0.0219)
Colorectal | CORRECT | OS | 1.4 (0.0052) | ---
Some tumors are initially resistant to VEGF inhibition, others eventually develop resistance.

Intrinsic Non-responsiveness

Response

Relapse
Intrinsic Resistance to Angiogenesis Inhibitors

- Pre-existing multiplicity of redundant pro-angiogenic signals
- Pre-existing inflammatory cell-mediated vascular protection
- Characteristic hypovascularity and indifference toward angiogenesis inhibitors

Acquired Resistance to Angiogenesis Inhibitors

- Activation &/or up-regulation of alternative signaling pathways within tumor
- Recruitment of bone marrow-derived pro-angiogenic cells
- Increased pericyte coverage of tumor vessels
- Activation and enhancement of invasion/metastasis to provide access to normal tissue vasculature

(Bergers and Hanahan Nat Rev Cancer. 8(8): 592–603. 2008)
Hallmarks of Resistance to Angiostatic Therapy

1. Angiogenesis is a complex biological process with numerous compensatory pathways that can be activated.

Biomarkers for other cancer therapies
- Tumour cell
  - Cell surface: overexpressed or overactive receptors
  - Intracellular: mutated or overactive signaling proteins

Biomarkers for anti-angiogenesis
- Tumour microenvironment
  - Stromal cells,
  - Infiltrating immune cells
  - Endothelial cells & Pericytes

Microenvironment: complex milieu – difficult to classify

Lambrechts D. et al., JCO, 2013
1. Angiogenesis is a complex biological process with numerous compensatory pathways that can be activated.

2. Bevacizumab has various mechanisms of action, which may differ between cancer types and chemotherapy.

- **Regression** of existing tumour vasculature
- **Inhibition** of new vessel growth
- **Anti-permeability** of surviving vasculature

**Vessel normalization, vessel cooption, etc**

*Lambrechts D. et al., JCO, 2013*
3. Which end-point? Poor correlation between response and survival. Effects limited to PFS. Cross-over events at progression. Perhaps imaging?

4. Bevacizumab is combined with standard chemotherapy. Response to chemo or Bevacizumab?
Multiple biomarkers from various locations within the tumor may play a role

**Potential Biomarkers:**
- Circulating angiogenic factors
- Genetic variants in germline DNA
- Tumor/stromal markers
- Molecular subtypes
- Other
Multiple biomarkers from various locations within the tumor may play a role

Potential biomarkers:

- Circulating angiogenic factors
- Genetic variants in germline DNA
- Tumor/stromal markers
- Molecular subtypes
- Other
# A meta-analysis of plasma VEGF at baseline in various cancer indications

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median (months)</th>
<th>Bevacizumab + chemo better</th>
<th>Chemo alone better</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chemo + placebo</td>
<td>Chemo + Bev</td>
<td></td>
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<tr>
<td><strong>AVF2107 in CRC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low VEGF (n=192)</td>
<td>6.93</td>
<td>11.24</td>
<td></td>
</tr>
<tr>
<td>High VEGF (n=192)</td>
<td>5.52</td>
<td>9.10</td>
<td></td>
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<tr>
<td><strong>E4599 in mBC</strong></td>
<td></td>
<td></td>
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<tr>
<td>Low VEGF (n=83)</td>
<td>5.59</td>
<td>6.57</td>
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</tr>
<tr>
<td>High VEGF (n=83)</td>
<td>3.91</td>
<td>6.44</td>
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<tr>
<td><strong>AVAiL (low dose) in NSCLC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low VEGF (n=301)</td>
<td>6.44</td>
<td>7.03</td>
<td></td>
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<tr>
<td>High VEGF (n=291)</td>
<td>5.95</td>
<td>6.67</td>
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<tr>
<td><strong>AVAiL (high dose) in NSCLC</strong></td>
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<td></td>
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<tr>
<td>Low VEGF (n=283)</td>
<td>6.44</td>
<td>7.03</td>
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</tr>
<tr>
<td>High VEGF (n=303)</td>
<td>5.95</td>
<td>6.47</td>
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<tr>
<td><strong>AVOREN in RCC</strong></td>
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</tr>
<tr>
<td>Low VEGF (n=192)</td>
<td>7.43</td>
<td>12.94</td>
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</tr>
<tr>
<td>High VEGF (n=192)</td>
<td>3.81</td>
<td>7.72</td>
<td></td>
</tr>
</tbody>
</table>

High plasma VEGF is associated with worse outcome regardless of bevacizumab therapy. Plasma VEGF is a prognostic rather than a predictive marker.

Hegde et al., Clin Can Res, 2013
Diffusion of short VEGF isoforms

Short VEGF isoforms diffuse over long ranges and could provide a better read-out of the tumor-derived VEGF
Short VEGF: potentially predictive in metastatic breast, gastric and pancreatic cancer
Short VEGF: prognostic, but not predictive in mCRC, mNSCLC and mRCC
## Summary of short VEGF data

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Trial</th>
<th>Prognostic</th>
<th>Potentially Predictive</th>
<th>Sample Buffer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PFS</td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>AVADO</td>
<td>✔</td>
<td>✔</td>
<td>✔ ✗ *</td>
</tr>
<tr>
<td>Gastric</td>
<td>AVAGAST</td>
<td>✔</td>
<td>✔</td>
<td>✔ ✔</td>
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<tr>
<td>Pancreatic</td>
<td>AViTA</td>
<td>✔</td>
<td>✔</td>
<td>✔ ✔</td>
</tr>
<tr>
<td>Colorectal</td>
<td>AVF2107</td>
<td>✗</td>
<td>✔</td>
<td>✗ ✗</td>
</tr>
<tr>
<td>Lung (NSCLC)</td>
<td>AVAiL</td>
<td>✔</td>
<td>✔</td>
<td>✗ ✗ *</td>
</tr>
<tr>
<td>Renal (RCC)</td>
<td>AVOREN</td>
<td>✔</td>
<td>✔</td>
<td>✗ ✗ *</td>
</tr>
</tbody>
</table>

Jayson et al. EMCC 2011; Gianni et al. SABCS 2011
MERiDiAN GO25632 for metastatic breast cancer

Stratification:
- VEGF-A level
- Adjuvant therapy (y/n)
- Hormonal status (ER +/−)
- Region

First biomarker-driven trial for anti-angiogenesis
Compensatory Upregulation of other Angiogenic Markers after VEGF inhibition

Adaptive Angiogenesis

Hypoxia

PIGF

Angiopoietins

FGFs
Dynamic Plasma Marker Analyses in the CRC phase 2 trial testing Afibercept in the first-line (AFFIRM):

Measure 27 circulating angiogenic factors in plasma from mCRC patients receiving Afibercept

Factors measured:
Ang-1, Ang-2, CSF2, SDF1a, Endostatin, FGF acidic, FGF basic, FIGF, HGF, IFNG, IL-10, IL-12, IL-1B, IL-2, IL-4, IL-5, IL-6, IL-8, PDGF-A, PDGF-B, PIGF, sFLT4, sKDR, TSP-2, TNF, VEGF, VEGF-C
Baseline IL8 and changes in IL8 expression correlate with PFS in the Aflibercept arm

Lambrechts D. et al., Brit J Cancer 2015
Multiple biomarkers from various locations within the tumor may play a role

Potential biomarkers:

- Circulating angiogenic factors
- Genetic variants in germline DNA
- Tumor/stromal markers
- Molecular subtypes
- Other
Pancreatic cancer patients (AviTA) with rs7993418 A allele have improved OS in the bevacizumab arm

Overall survival

Time on study (months)

D Lambrechts, ... E Van Cutsem, Lancet Oncology 2012
Pancreatic cancer patients with rs7993418 A allele have no improved OS in the placebo arm.

Overall survival

Time on study (months)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>1.6</td>
<td>0.063</td>
</tr>
<tr>
<td>CC</td>
<td>1.5</td>
<td>0.24</td>
</tr>
</tbody>
</table>

D Lambrechts, ... E Van Cutsem, Lancet Oncology 2012
Is the VEGFR1 locus also predictive for tyrosine kinase inhibitors?

VEGFR-1 rs7993418 C allele correlates with reduced OS in 91 sunitinib-treated renal cell carcinoma

Current biomarker evidence for high VEGFR-1 levels as a negative predictive marker

sVEGFR-1 in plasma (before treatment):

- High sVEGFR-1 ~ bevacizumab poor outcome in:
  - Rectal Cancer (Willett et al., JCO, 2009; Duda et al., Oncologist, 2010)
  - NSCLC (Heist et al., PNAS, 2015)
  - CRC (Aoyagi et al., Oncol Lett, 2010)
  - Breast (Tolaney et al., JCO, 2009)

- High sVEGFR-1 ~ cediranib poor outcome in:
  - HCC (Zhu et al., Clin Canc Res, 2013)
  - GBM (Batchelor et al., PNAS 2013)

VEGFR-1 on tumor (IHC, before treatment):

- High VEGFR-1 expression ~ bevacizumab poor outcome in CRC (NO16966) and gastric cancer (AVAGAST):
  (Weickhardt, ASCO 2011; Van Cutsem, JCO 2012)
Similar data for soluble neuropilin-1 in mCRC treated with either BEV or tivozanib

sNRP-1 Low

Tivozanib (n=52)
PFS=17.9 months

Bevacizumab (n=28)
PFS=11.2 months

sNRP-1 High

Tivozanib (n=56)
PFS=7.3 months

Bevacizumab (n=28)
PFS=7.5 months

sNRP-1 levels predict response to Bevacizumab and the VEGFR inhibitor Tivozanib in mCRC

Jie Lin et al. 2015, and Al. B Benson III et al., poster presentations by AVEO Oncology, 2015
Endogenous levels of soluble anti-angiogenic VEGF receptors are predictive of treatment outcome.

**High sVEGFR-1 or sNRP-1**

- VEGF
- VEGFR-2
- VEGFR-1
- No response to anti-VEGF

**Low sVEGFR-1 or sNRP-1**

- VEGF
- VEGFR-2
- VEGFR-1
- Response to anti-VEGF
Multiple biomarkers from various locations within the tumor may play a role

Potential biomarkers:

• Circulating angiogenic factors
• Genetic variants in germline DNA
• **Tumor/stromal markers**
• Molecular subtypes
• Other
Data suggest that low neuropilin expression is associated with improved PFS, similar findings for sNRP1 in various studies.

Foernzler D. et al. ASCO GI 2010
Data in AVAGAST (gastric cancer) confirm that low tumor NRP1 expression is associated with improved OS. Similar association seen for PFS.

Van Cutsem E. et al., JCO 2012
Biomarkers in gastric carcinoma, involving ramucirumab monotherapy: immunohistochemistry for VEGFR2

HR = 0.35 (0.20-0.59) in high VEGFR2 group versus HR = 0.73 (0.42-1.27) in the low VEGFR2 group. Interaction P-value of 0.05

>>> Promising

Fuchs et al, ASCO Annual Meeting 2015
Multiple biomarkers from various locations within the tumor may play a role

<table>
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<tr>
<th>Potential biomarkers:</th>
<th>Circulating angiogenic factors</th>
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<td></td>
<td><strong>Molecular subtypes</strong></td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
</tbody>
</table>
Four molecular subtypes in *IDH1* wild-type glioblastoma based on gene expression data

Sandmann et al., JCO 2015
The mesenchymal and proneural subtypes exhibited a PFS benefit from bevacizumab

**Mesenchymal**

- Placebo (n = 69)
- Bevacizumab (n = 70)
- HR, 0.57 (95% CI, 0.40 to 0.82),
- \( P = .0019 (.0076), n = 139 \)

**Proliferative**

- Placebo (n = 24)
- Bevacizumab (n = 34)
- HR, 0.74 (95% CI, 0.42 to 1.32),
- \( P = .31 (.46), n = 58 \)

**Proneural**

- Placebo (n = 58)
- Bevacizumab (n = 46)
- HR, 0.57 (95% CI, 0.37 to 0.89),
- \( P = .012 (.036), n = 103 \)

**Unclassified**

- Placebo (n = 22)
- Bevacizumab (n = 17)
- HR, 0.78 (95% CI, 0.39 to 1.53),
- \( P = .46 (.46), n = 39 \)

*Sandmann et al., JCO 2015*
A significant OS advantage for patients with proneural IDH1 wild-type tumors

Sandmann et al., JCO 2015
Several molecular subtypes in CRC

A greater benefit for Regorafenib was seen in the high-risk **mesenchymal** subgroup (C4+C6; n=26; HR=0.10; 95%CI=0.02–0.35; P=0.0009) than the low-risk subgroup (C1+C2+C3+C5; n=255; HR=0.58; 95%CI=0.44–0.77; P=0.002). *Teufel et al J Clin Oncol 33, 2015. ASCO 2015 (suppl; abstr 3558)*
The consensus molecular subtypes of colorectal cancer


Figure 2  Identification of the consensus subtypes of colorectal cancer and application of classification framework in non-consensus samples.

Guinney et al Nat Med. 2015 Nov;21(11):1350-6
Next Generation Integrative Biomarker Discovery Strategy

PATIENTS TREATED WITH CHEMOTHERAPY +/- AVASTIN

GENOMIC INTERROGATION OF WHOLE TUMOUR TEC, TC & TAM

BEVACIZUMAB (AVASTIN)

Linking genetic abnormalities with response to targeted therapy

Germline genetic variants
Somatic mutations
Copy number variations
Epigenetic changes
Transcriptomics

RESPONSE PREDICTION

Benefit
No benefit

BIOMARKER VALIDATION IN INDEPENDENT PROSPECTIVE COHORT (AC-ANGIOPREDICT PHASE 2 TRIAL)

COMPANION DIAGNOSTIC ASSAY OPTIMIZATION

RT-PCR Expression Based Companion Diagnostic
Methylation-based diagnostic assay

ANGIOPREDICT
Integrative Systems Medicine Approaches

Analytical Statistics Model

- List of important genes/loci for Avastin response
- Adding nodes and their relations from collaborators data

Network Model

- Thematic pathways related to Avastin response
- Trimming network and adding information from collaborators

Edited from Kling et al., Nucleic Acids Research, 2015
Edited partly from Shen et al., 2009

Omnics characterizations

Edited from Kling et al., Nucleic Acids Research, 2015

List of important genes/loci for Avastin response

Adding nodes and their relations from collaborators data

Thematic pathways related to Avastin response

Trimming network and adding information from collaborators

Edited from Kling et al., Nucleic Acids Research, 2015
Multi-scale Cellular Automata Models

Model inputs:
- Cell proliferation and death,
- Vessel phenotype
- Genomic data/CRC Subtype data

Predefined rules – proliferation, cell death

Tumour cells

Spatio-temporal distribution of Oxygen, VEGF, Drug

Angiogenesis

Signalling Pathway – MAPK, ERK

Predictive Tumour behaviour and Biomarker for clinical validation
Do we have a light for predictive biomarkers with anti-angiogenic therapies, or are we still lost in translation?
We are still somewhat lost in translation ...

- There is not a single biomarker that is consistent across all trials tested.
- Response to anti-angiogenesis is a complex phenotype.
- A single biomarker might not be sufficient to capture the true heterogeneity of the response.
We are still *somewhat* lost in translation …

- Response to anti-angiogenesis is a continuum.
- A panel of multiple markers may be necessary to cover the full spectrum.
- Application is clinically challenging
We are still somewhat lost in translation ...

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We are still *somewhat* lost in translation ...

- Response to anti-angiogenesis is a continuum.
- A panel of multiple markers may be necessary to cover the full spectrum.
- Application is clinically challenging.
Biomarkers provide important information on how to overcome resistance to anti-angiogenic therapies:

- **Inhibition of multiple angiogenic factors**
  - Aflibercept inhibits both VEGF and PlGF
  - Bevacizumab with other anti-angiogenic therapies

- **Destabilization of resistant tumor vessels**
  - Inhibition of VEGF and MET receptors

- **Inhibition of immune cell recruitment. Synergy with immunotherapeutics?**
Increasing clinical benefit and overcoming resistance to anti-angiogenesis therapies

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Vesalius Research Institute Belgium- Prof Diether Lambrechts

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