Predicting Benefit from Anti-Angiogenic Therapies

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Aims

- Focus on Avastin / Bevacizumab (Anti-VEGF mAb)

- Are biomarkers required?

- What data is currently available and what does(n’t) it tell us?

- Our published and unpublished data on Avastin biomarker development in clinical trials.
Anti-VEGF Therapy

Avastin/bevacizumab
Clinical Uses for Bevacizumab

Current FDA Approval for:

- **Metastatic Colorectal Cancer** (+ 1\textsuperscript{st} or 2\textsuperscript{nd} Line 5-FU)
- **Non-Squamous NSCLC** (+ 1\textsuperscript{st} Line Carboplatin + Paclitaxel)
- **Metastatic Her2 -ve Breast Cancer** (+ 1\textsuperscript{st} Line Paclitaxel)

  Under Review
- **Glioblastoma** (single agent at progression following prior therapy)
- **Metastatic Renal Cell Carcinoma** (+ IFN alpha)

Avastin Prescribing Information, 6 May 2011
Are Biomarkers Required?
Bevacizumab in Unselected Patients

AVF2107g trial of IFL +/- Bevacizumab in first-line colorectal cancer.

E3200 trial of FOLFOX4 +/- Bevacizumab in second-line colorectal cancer.

*NEJM* 2004;**350**:2335

*J Clin Oncol* 2007;**25**:1539
1. Lack of Overall Survival Benefit

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Setting</th>
<th>Control group</th>
<th>Investigational group</th>
<th>Primary endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVF2107g³ Metastatic colorectal adenocarcinoma</td>
<td>First line</td>
<td>Irinotecan, 5-fluorouracil, and leucovorin</td>
<td>Irinotecan, 5-fluorouracil, leucovorin, bevacizumab</td>
<td>OS (met)</td>
</tr>
<tr>
<td>AVF2119g¹⁴ Metastatic breast carcinoma</td>
<td>First to Fifth line</td>
<td>Capecitabine</td>
<td>Capecitabine, bevacizumab</td>
<td>PFS (Not met)</td>
</tr>
<tr>
<td>AVADO¹¹ HER2 negative, advanced/metastatic breast carcinoma</td>
<td>First line</td>
<td>Docetaxel</td>
<td>Docetaxel, bevacizumab</td>
<td>PFS (met)</td>
</tr>
<tr>
<td>AVAil¹⁵ Advanced, non-squamous, non-small-cell-lung carcinoma</td>
<td>First line</td>
<td>Cisplatin and gemcitabine</td>
<td>Cisplatin, gemcitabine, bevacizumab</td>
<td>PFS (met)</td>
</tr>
<tr>
<td>AVOREN¹⁶ Metastatic renal-cell carcinoma</td>
<td>First line</td>
<td>Interferon-α2a</td>
<td>Interferon-α 2a + bevacizumab</td>
<td>Originally OS (not Met), Revised to PFS (Met)</td>
</tr>
<tr>
<td>CALGB 90206¹⁷ Metastatic renal-cell carcinoma</td>
<td>First line</td>
<td>Interferon-α</td>
<td>Interferon-α, bevacizumab</td>
<td>OS (not met)</td>
</tr>
<tr>
<td>E2100⁹ Metastatic breast carcinoma</td>
<td>First line</td>
<td>Paclitaxel</td>
<td>Paclitaxel, bevacizumab</td>
<td>PFS (met)</td>
</tr>
<tr>
<td>E4599¹⁴ Non-squamous, non-small-cell lung carcinoma</td>
<td>First line</td>
<td>Paclitaxel and carboplatin</td>
<td>Paclitaxel, carboplatin, and bevacizumab</td>
<td>OS (met)</td>
</tr>
<tr>
<td>GOG0218¹⁸ Advanced ovarian cancer</td>
<td>First line</td>
<td>Paclitaxel and carboplatin</td>
<td>Paclitaxel, carboplatin, bevacizumab, ± follow-on bevacizumab monotherapy</td>
<td>PFS (met)</td>
</tr>
<tr>
<td>N016966³ Metastatic colorectal adenocarcinoma</td>
<td>First line</td>
<td>FOLFOX ± bevacizumab</td>
<td>XELOX ± bevacizumab</td>
<td>PFS (met)</td>
</tr>
<tr>
<td>RIBBON¹² HER2 negative, recurrent/metastatic, breast carcinoma</td>
<td>First line</td>
<td>Capecitabine or taxane or anthracycline</td>
<td>Chemotherapy, bevacizumab</td>
<td>PFS (met)</td>
</tr>
<tr>
<td>RIBBON²¹¹ HER2 negative, recurrent/metastatic, breast carcinoma</td>
<td>Second line</td>
<td>Capecitabine or gemcitabine or vinorelbine or taxane</td>
<td>Chemotherapy, bevacizumab</td>
<td>PFS (met)</td>
</tr>
</tbody>
</table>

FOLFOX=leucovorin, 5-fluorouracil, oxaliplatin. XELOX=capecitabine and oxaliplatin. OS=overall survival. PFS=progression-free survival.

Lancet Oncol 2010;11:1172
2. Lack of Sustained Benefit

- Overall Survival (%)
  - Months
  - No. at Risk
    - IFL+bevacizumab: 402, 362, 320, 178, 73, 20, 1, 0
    - IFL+placebo: 411, 363, 292, 139, 51, 12, 0, 0

3. RECIST Does not Predict Survival

### AVF2107g

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Hazard ratio (95% CI)</th>
<th>Forest plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>813</td>
<td>0.54 (0.45–0.66)</td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>323</td>
<td>0.53 (0.38–0.74)</td>
<td></td>
</tr>
<tr>
<td>Non-responders</td>
<td>490</td>
<td>0.63 (0.49–0.80)</td>
<td></td>
</tr>
</tbody>
</table>

### BRiTE

Survival Beyond First Progression (months)

*Postprogression therapy:
- No treatment
- No IIBP
- IIBP*
3. RECIST Does not Predict Survival

NO16996

AVF2119g

Response Rate (%)

Chemo+BV  Chemo+Placebo
47         49

Response Rate (%)

Chemo+BV  Chemo+Placebo
19.8      9.1

Median progression-free survival
CAP alone = 4.17 months
CAP + BV = 4.86 months

J ClinOncol 2005;23(4):792

Published Biomarker Data
VEGF as a Biomarker

Å Bevacizumab has a clear biological effect that depends exclusively on binding VEGF and blocking VEGF signalling.

Å Therefore, shouldn’t VEGF expression predict benefit from bevacizumab?
VEGF in AVF2107g

MVD also non-predictive (assessed by manual and computer-aided Chalkley Counting).

*JCO* 2006;24:217
VEGF as a Biomarker

Bevacizumab has a clear biological effect that depends exclusively on binding VEGF and blocking VEGF signalling. Therefore, shouldn’t VEGF expression predict benefit from bevacizumab?

No

1. VEGF expression within a single tumour is VERY heterogeneous (tumour cells, necrosis, inflammation)
2. VEGF expression is dynamic and regulated by multiple pathways, not dependent on a fixed mutation or amplification event
3. All colorectal cancers express some level of VEGF
4. VEGF is important but only part of the story in advanced tumours
Heterogeneity of VEGF

Tumour -, Stroma +

Perinecrotic Tumour +,
Distant Tumour -, Stroma -

Tumour +, Stroma +

Inflammation

JCO 2006;24:217
Vascular Endothelial Growth Factor Messenger RNA Expression Level Is Preserved in Liver Metastases Compared with Corresponding Primary Colorectal Cancer

Hidekazu Kuramochi, Kazuhiko Hayashi, Kazumi Uchida, Satoru Miyakura, Daisuke Shimizu, Daniel Vallböhmer, Seongjin Park, Kathleen D. Danenberg, Ken Takasaki, and Peter V. Danenberg

Clin Cancer Res 2006;12:29
Primary vs Secondary

Safety of Bevacizumab in Patients With Non–Small-Cell Lung Cancer and Brain Metastases

Mark A. Socinski, Corey J. Langer, Jane E. Huang, Margaret M. Kolb, Peter Compton, Lisa Wang, and Wallace Akerley

CD31-Endothelium
SMA-Pericyte

J ClinOncol 2009;27:5255
Br J Cancer 2011(in press)
Circulating VEGF Levels

â• Total VEGF or Free VEGF?
â• Plasma or Serum?
â• Baseline or changes with treatment?
Baseline Plasma VEGF

Genentech Plasma VEGF ELISA

No predictive effect in:
Å AVF2107g – metastatic CRC
Å E4599 - NSCLC
Å AVAiL - NSCLC
Å AVOREN – metastatic RCC

Roche Plasma VEGF ELISA
AVADO Trial (Metastatic Breast Cancer, 1\textsuperscript{st} Line Docetaxel +/- Bevacizumab)

<table>
<thead>
<tr>
<th></th>
<th>Bv7.5mg/kg</th>
<th>Bv15mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR(95%CI)</td>
<td>Interaction p-value*</td>
</tr>
<tr>
<td>VEGF low</td>
<td>0.96(0.62-1.48)</td>
<td>P=0.0136</td>
</tr>
<tr>
<td>VEGF high</td>
<td>0.52(0.33-0.81)</td>
<td></td>
</tr>
</tbody>
</table>

But not for mCRC or NSCLC

Bernaards et al. \textit{ASCO 2010}
Miles et al. \textit{SABCS 2010}
Biomarkers of Activity

Å Tells us bevacizumab has biological activity
Å Does not tell us bevacizumab has clinical efficacy
Å Need to see survival data, ideally from a clinical trial with prognostic (control arm) and predictive (bevacizumab arm) effects.

Biomarkers of Activity may not = Efficacy

Bevacizumab reduces VEGF expression in patients with relapsed and refractory acute myeloid leukemia without clinical antileukemic activity.
**p53**

![Graph showing tumor volume over days post injection for different genotypes and treatments.](Image)

*Science 2002;295(5559):1526*
**p53 in AVF2107g**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Total n</th>
<th>Placebo plus IFL</th>
<th>Bevacizumab plus IFL</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All subjects</strong></td>
<td>267</td>
<td>120 17.45</td>
<td>147 26.35</td>
<td>0.57 (0.39 - 0.85)</td>
</tr>
<tr>
<td><strong>p53 mutation status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>139</td>
<td>63 21.72</td>
<td>76 27.7</td>
<td>0.54 (0.30 - 0.95)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>66</td>
<td>31 16.36</td>
<td>35 NR</td>
<td>0.67 (0.32 - 1.42)</td>
</tr>
<tr>
<td><strong>P53 overexpression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>191</td>
<td>92 17.45</td>
<td>99 26.35</td>
<td>0.70 (0.45 - 1.10)</td>
</tr>
<tr>
<td>Negative</td>
<td>75</td>
<td>28 16.26</td>
<td>47 25.07</td>
<td>0.32 (0.15 - 0.70)</td>
</tr>
</tbody>
</table>

*J Natl Cancer Inst* 2005;97:981
K-ras and B-raf

A

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Total n</th>
<th>Placebo plus IFL Median (mo)</th>
<th>Bevacizumab plus IFL Median (mo)</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>267</td>
<td>120 17.45</td>
<td>147 26.35</td>
<td>0.57 (0.39 - 0.85)</td>
</tr>
<tr>
<td>k-ras mutation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>78</td>
<td>34 13.6</td>
<td>44 19.91</td>
<td>0.69 (0.37 - 1.31)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>152</td>
<td>67 17.64</td>
<td>85 27.7</td>
<td>0.58 (0.34 - 0.99)</td>
</tr>
<tr>
<td>b-raf mutation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>10</td>
<td>3 7.95</td>
<td>7 15.93</td>
<td>0.11 (0.01 - 1.06)</td>
</tr>
<tr>
<td>Wild-type</td>
<td>217</td>
<td>97 17.45</td>
<td>120 26.35</td>
<td>0.53 (0.34 - 0.82)</td>
</tr>
<tr>
<td>k-ras and b-raf mutation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Either mutant</td>
<td>88</td>
<td>37 13.6</td>
<td>51 19.91</td>
<td>0.67 (0.37 - 1.20)</td>
</tr>
<tr>
<td>Both wild-type</td>
<td>125</td>
<td>57 21.72</td>
<td>68 27.7</td>
<td>0.57 (0.31 - 1.06)</td>
</tr>
</tbody>
</table>

*J Natl Cancer Inst* 2005;97:981
K-ras and B-raf: Negative Data?
Hypertension

- **E2100 Breast Cancer**
  - Median survival, months: 25.3
  - No hypertension
  - Hypertension

- **Renal Cell Carcinoma**
  - Stratified log-rank $P < .001$

- **E4599 Lung Cancer**
  - Medians: 10.1, 10.3, 11.5, 15.9
  - PC, high BP (92 events/95 patients)
  - PC, no high BP (263 events/276 patients)
  - PCB, high BP (93 events/107 patients)
  - PCB, no high BP (242 events/263 patients)

References:

- *J Clin Oncol* 2010;28(13):2137
Hypertension

<table>
<thead>
<tr>
<th>SBP/DBP</th>
<th>AVF2107g</th>
<th>NO16966</th>
<th>AVADO&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RIBBON-1</th>
<th>AVAiL&lt;sup&gt;b&lt;/sup&gt;</th>
<th>AVOREN</th>
</tr>
</thead>
<tbody>
<tr>
<td>20/10 mmHg</td>
<td>0.6 (&lt;0.001)</td>
<td>0.8 (0.18)</td>
<td>0.9 (0.72)</td>
<td>0.8 (0.26)</td>
<td>1.0 (0.83)</td>
<td>0.8 (0.29)</td>
</tr>
<tr>
<td></td>
<td>0.4 (&lt;.0001)</td>
<td>0.8 (0.36)</td>
<td>0.9 (0.85)</td>
<td>0.6 (0.05)</td>
<td>1.3 (0.21)</td>
<td>1.0 (0.95)</td>
</tr>
<tr>
<td>10/5 mmHg</td>
<td>0.7 (0.07)</td>
<td>0.8 (0.10)</td>
<td>0.8 (0.46)</td>
<td>1.0 (0.95)</td>
<td>1.0 (0.78)</td>
<td>1.2 (0.46)</td>
</tr>
<tr>
<td></td>
<td>0.6 (0.04)</td>
<td>0.8 (0.36)</td>
<td>1.0 (0.92)</td>
<td>0.6 (0.03)</td>
<td>1.0 (0.90)</td>
<td>1.4 (0.24)</td>
</tr>
<tr>
<td>30/15 mmHg</td>
<td>0.5 (&lt;0.001)</td>
<td>0.8 (0.26)</td>
<td>1.0 (0.96)</td>
<td>0.9 (0.50)</td>
<td>0.9 (0.78)</td>
<td>0.8 (0.35)</td>
</tr>
<tr>
<td></td>
<td>0.4 (&lt;0.0001)</td>
<td>0.6 (0.08)</td>
<td>1.7 (0.45)</td>
<td>0.7 (0.29)</td>
<td>1.0 (0.89)</td>
<td>1.0 (0.90)</td>
</tr>
<tr>
<td>40/20 mmHg</td>
<td>0.5 (0.01)</td>
<td>1.4 (0.32)</td>
<td>0.4 (0.03)</td>
<td>0.9 (0.73)</td>
<td>0.8 (0.55)</td>
<td>0.9 (0.75)</td>
</tr>
<tr>
<td></td>
<td>0.3 (0.0001)</td>
<td>1.2 (0.74)</td>
<td>0.5 (0.44)</td>
<td>0.5 (0.11)</td>
<td>1.6 (0.31)</td>
<td>0.7 (0.46)</td>
</tr>
</tbody>
</table>

<sup>a</sup> HR from Cox model; p-values from Wald test.

<sup>b</sup> 5mg/kg/week.
Hypertension

- Early vs Late
- Definitions of hypertension
- Many trials excluded patients with hypertension
- Several trials did not fully report hypertension
- Effects of anti-hypertensive drug treatment
- Dosage reduction vs omission of therapy for hypertension
- Problems using a toxic effect as a biomarker, especially as baseline hypertension increases the likelihood of thromboembolic events in patients receiving bevacizumab (HR 1.89, p=0.03)

*J Natl Cancer Inst* 2007;99:1232
VEGF Promoter Polymorphisms

First Line Paclitaxel +/- Bevacizumab in Metastatic/Advanced Breast Cancer

### Table 3. Comparison of Combined VEGF Genotypes With Overall Survival in Experimental Arm

<table>
<thead>
<tr>
<th>VEGF -2578/-1154</th>
<th>Median Overall Survival (months)</th>
<th>% of Patients</th>
<th>P (comparison with other genotypes combined)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/AA</td>
<td>49.7</td>
<td>7.6</td>
<td>.041</td>
</tr>
<tr>
<td>AA/GA</td>
<td>30.2</td>
<td>11.4</td>
<td>.44</td>
</tr>
<tr>
<td>CA/GA</td>
<td>27.1</td>
<td>20.9</td>
<td>.40</td>
</tr>
<tr>
<td>CA/GG</td>
<td>22.5</td>
<td>21.5</td>
<td>.038</td>
</tr>
<tr>
<td>CC/GG</td>
<td>21.7</td>
<td>32.9</td>
<td>.30</td>
</tr>
<tr>
<td>Others</td>
<td>—</td>
<td>5.7</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviation: VEGF, vascular endothelial growth factor.

Validation
- Colorectal Cancer (ASCO 2010): Agreement
- Ovarian Cancer (GynecolOncol 2010;117:109: No Agreement

J Clin Oncol 2009;26(28):4672
Metastatic Breast Cancer

First Line Paclitaxel +/- Bevacizumab

Second/Third Line Capecitabine +/- Bevacizumab

(10.7% improvement in response)

J Clin Oncol 2005;23(4):792
Summary

• VEGF Expression
• Primary vs Secondary Tumours
• Biomarkers of Activity: e.g. DCE-MRI
• P53 mutations
• K-ras/B-raf mutations
• Hypertension
• VEGF Polymorphisms
Novel Biomarkers
Endothelial: Dll4

HUVEC Migration

HUVEC Proliferation

HUVEC VEGFR2/NRP1 Expression

Anti-Dll4 is Active in Anti-VEGF Resistant Xenografts

Blood 2006;107(3):931
Cancer Res 2007;67(23):11244
Stromal/Tumour: PDGF-C

*Plos One 2009;4(4):e5123
*Cancer Cell 2009;15(1):21*
Inflammatory: Myeloid Cells & Bv8

Legend | Cell line | From EL4 | Treatment
--- | --- | --- | ---
| | B16F1 | - | Control
| | B16F1 | + | Anti-VEGF
| | B16F1 | - | Anti-VEGF
| | B16F1 | + | Control

Mean tumor volume (mm$^3$)

DAYS

Control

Anti-Bv8

Anti-Vegf

Combination

Mean tumor volume (mm$^3$)

DAYS

Nat Biotech 2007;25(8):911
Nature 2007;450(7171):825
Proc Natl Acad Sci U S A 2009;106(16):6742
Neutropenia

Prognostic factors in 645 mRCC patients treated with bevacizumab, sunitinib or sorafenib from 3 centres (single arm).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Docetaxel + Placebo</th>
<th>Docetaxel + BV 7.5 mg/kg</th>
<th>Docetaxel + BV 15 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-CSF</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>N</td>
<td>28</td>
<td>213</td>
<td>33</td>
</tr>
<tr>
<td>Median PFS (mo)</td>
<td>7.4</td>
<td>8.0</td>
<td>8.4</td>
</tr>
<tr>
<td>HR</td>
<td>1.09</td>
<td>0.92</td>
<td>1.14</td>
</tr>
<tr>
<td>P value</td>
<td>0.67</td>
<td>0.63</td>
<td>0.47</td>
</tr>
</tbody>
</table>

NOTE. Total number of patients = 564.
Abbreviations: SE, standard error; KPS, Karnofsky performance status; LLN, lower limit of normal; ULN, upper limit of normal.

J Clin Oncol 2009;27(34):5794
**AVF2119g**

- 1-5th Line Metastatic/Advanced Breast Cancer including Pts progressing on therapy.
- N = 462 Randomized to Cap or Cap+BV.
- 10.7% improvement in RR, No difference in PFS or OS in unselected pts.
- Tissue from N = 223, TMAs and Whole Sections
- Examine: VEGF-A, VEGF-B, VEGF-C, PIGF, VEGF-R1, VEGF-R2, VEGF-R3, Her2, HIF1a, CA9, p53, TP, Thrombospondin-2, Dll4, NRP1, PDGF-C and Bv8
- IHC (if validated reagents) and/or ISH

*J Clin Oncol* 2005;23(4):792
Immunohistochemistry

Figure 2

A. VEGF-C
B. PDGF-C
C. Dll4
D. Dll4

Clin Cancer Res 2011;17:372
Immunohistochemistry

E
NRP1

F
NRP1

G
Bv8

H
TP

Clin Cancer Res 2011;17:372
**Frequency Data**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Cut-off Low/High</th>
<th>Score 0</th>
<th>Score 1</th>
<th>Score 2</th>
<th>Score 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A</td>
<td>0,1 vs 2,3</td>
<td>37 (20%)</td>
<td>79 (43%)</td>
<td>38 (21%)</td>
<td>29 (16%)</td>
<td>183</td>
</tr>
<tr>
<td>VEGF-B</td>
<td>0 vs 1,2</td>
<td>60 (59%)</td>
<td>41 (40%)</td>
<td>1 (1%)</td>
<td>0</td>
<td>102</td>
</tr>
<tr>
<td>Flt4</td>
<td>0 vs 1,2</td>
<td>76 (74%)</td>
<td>24 (23%)</td>
<td>3 (3%)</td>
<td>0</td>
<td>103</td>
</tr>
<tr>
<td>TSP2</td>
<td>0,1 vs 2,3</td>
<td>43 (27%)</td>
<td>55 (35%)</td>
<td>47 (30%)</td>
<td>12 (8%)</td>
<td>157</td>
</tr>
<tr>
<td>VEGF-C</td>
<td>0 vs 1-3</td>
<td>73 (45%)</td>
<td>36 (22%)</td>
<td>35 (22%)</td>
<td>17 (11%)</td>
<td>161</td>
</tr>
<tr>
<td>PDGF-C</td>
<td>0 vs 1,2</td>
<td>67 (43%)</td>
<td>71 (45%)</td>
<td>19 (12%)</td>
<td>0</td>
<td>157</td>
</tr>
<tr>
<td>Neuropilin-1</td>
<td>0,1 vs 2,3</td>
<td>18 (11%)</td>
<td>78 (48%)</td>
<td>65 (40%)</td>
<td>1 (1%)</td>
<td>162</td>
</tr>
<tr>
<td>Dll4</td>
<td>0 vs 1-3</td>
<td>33 (21%)</td>
<td>90 (56%)</td>
<td>35 (22%)</td>
<td>2 (1%)</td>
<td>160</td>
</tr>
<tr>
<td>Bv8</td>
<td>0 vs 1-3</td>
<td>136 (85%)</td>
<td>15 (9%)</td>
<td>5 (3%)</td>
<td>5 (3%)</td>
<td>161</td>
</tr>
<tr>
<td>p53</td>
<td>0 vs 1-3</td>
<td>92 (53%)</td>
<td>44 (25%)</td>
<td>10 (6%)</td>
<td>28 (16%)</td>
<td>174</td>
</tr>
<tr>
<td>TP</td>
<td>0 vs 1</td>
<td>42 (27%)</td>
<td>127 (73%)</td>
<td>0</td>
<td>0</td>
<td>174</td>
</tr>
</tbody>
</table>

Abbreviations: VEGF, vascular endothelial growth factor; Flt4, fms-like tyrosine kinase 4; TSP2, thrombospondin 2; Bv8, Bombina variagata peptide 8; PDGF-C, platelet-derived growth factor C; Dll4, delta-like ligand 4; TP, thymidine phosphorylase; vs, versus.
## Results

<table>
<thead>
<tr>
<th>Marker</th>
<th>Subset</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-C</td>
<td>High</td>
<td>1.11</td>
<td>0.64-1.91</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.59</td>
<td>0.35-1.01</td>
<td>0.05</td>
</tr>
<tr>
<td>PDGF-C</td>
<td>High</td>
<td>1.10</td>
<td>0.66-1.85</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.70</td>
<td>0.40-1.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Bv8</td>
<td>High</td>
<td>1.78</td>
<td>0.65-4.86</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.77</td>
<td>0.51-1.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Dll4</td>
<td>High</td>
<td>1.05</td>
<td>0.69-1.60</td>
<td>0.82</td>
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<tr>
<td></td>
<td>Low</td>
<td>0.31</td>
<td>0.13-0.76</td>
<td>0.01</td>
</tr>
<tr>
<td>Neuropilin-1</td>
<td>High</td>
<td>1.34</td>
<td>0.75-2.40</td>
<td>0.32</td>
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<tr>
<td></td>
<td>Low</td>
<td>0.61</td>
<td>0.36-1.03</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Progression Free Survival Curves for Dll4 Subsets

Figure 3

Dll4 Score = 0

Dll4 Score = 1-3

Cumulative Events

<table>
<thead>
<tr>
<th></th>
<th>Capecitabine + Bevacizumab</th>
<th>Capecitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>0</td>
<td>5</td>
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</tbody>
</table>
| Patients at Risk

<table>
<thead>
<tr>
<th></th>
<th>Capecitabine + Bevacizumab</th>
<th>Capecitabine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>21</td>
<td>12</td>
</tr>
</tbody>
</table>

Clin Cancer Res 2011;17:372
Summary

Å Subsets too small to provide meaningful data
Å Validated assays that could be employed to GLP
Å Frequency data to statistically power prospective biomarker trials
Å Endothelial Phenotypes e.g. Dll4/NRP1 worth investigating further as biomarkers
Å Frequency data suggest that multiple markers may be involved in resistance to bevacizumab
What’s Next?
Phase II Neoadjuvant Adv. BC

Recruitment
n=48

1 Week

Avastin
15 mg/kg
IV bolus

1 Week

FEC 3x 3wk cycles then:
Docetaxel +/- Trastuzumab
3x 3wk cycles

2 Weeks

- Routine diagnostic MRI scan
- Research DCE MRI and BOLD MRI
- Research breast core biopsies taken for in situ analyses and gene array analysis
- Blood samples for pharmacodynamic analysis

- Physical examination (incl BP)
- Any adverse event(s)
- Haematology (incl Neutrophil count)
- Biochemistry
- Coagulation
- Urine dipstick for proteinuria.
- Research DCE MRI and BOLD MRI
- Research breast core biopsies taken for in situ analyses and gene array analysis.
- Blood samples for pharmacodynamics.

Harris, Mehta
DCE-MRI

Harris, Mehta, Hughes  
*J Natl Cancer Inst* 2010:in press
Endothelial Metagene

Å Affymetrix Microarray Data

Å Normal and Malignant: Breast, Colon, Lung

Å Seed Genes: vWF, VE-Cadherin and CD31

Å Endothelial Expression “Signature”
Mount tissues on membrane slides

Quick IF stain for 2’ with anti-CD146: AlexaFluor488 at 4 degrees

Dissect vasculature with MMI UV laser 10’

Extract RNA from 20 pooled dissections per sample

2-round T7-based amplification

Agilent WHG arrays

Jubb, Tarlow, Koeppen
Endothelial Laser Capture vs Metagene

Top 100 probes in Laser Capture Data

100 randomly selected probes

Metagene Vascular correlation rank

Tarlow, Koeppen
Summary

• Phase II Single Arm Diagnostic Study
• Neoadjuvant Breast Cancer
• Numerous biomarker analyses before/after Tx
• Define DCE-MRI “Response”

• Endothelial Metagene

• Pathology on FFPE Tissue
  • VEGF, KDR, Dll4, NRP1, CA9, HIF1a, Ki67 and CD31
  • Validation of Metagene Data in situ

• Take data forward to a phase 3 study
Conclude

• We need a biomarker, especially for breast cancer
• After 10 years, we have lots of markers of activity but no biomarkers of efficacy
• Hypertension shows promise but its use as a biomarker is problematic and very premature
• Retrospective subset analyses are important but their impact is limited
• Prospectively designed trials are essential
Charles Spearman, FRS
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