USE OF PROTEIN MULTIPLEX ARRAYS TO IDENTIFY BLOOD-BASED BIOMARKERS FOR ANTI-ANGIOGENIC THERAPIES

Andrew Nixon, PhD, MBA
Department of Medicine/Medical Oncology
Duke University Medical Center


**Phase I Biomarker Laboratory**

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**Laboratory Themes**

- Develop & optimize study-specific blood-based biomarker assays
- Implement operational standardization across studies
- Execute correlative biomarker analyses in a time- and cost-effective manner
- Correlate biomarkers at baseline and on-treatment with measures of clinical benefit and toxicity
- Assess novel assay technologies and platforms

Currently serve as a Molecular Reference Laboratory for the Alliance Oncology Cooperative Group
What is cancer – the old paradigm

Breast Cancer

Lung Cancer

Colon Cancer
Personalized Medicine

“Knowledge of the molecular profile of the tumor is necessary to guide selection of therapy for patient.”

**Current Model**

All patients receive standard treatment

**Personalized Medicine Model**

Choice of treatment dependent upon molecular profile of tumor
Biomarkers

- Types of Biomarkers
  - Prognostic
  - Predictive
  - Pharmacodynamic
  - Pharmacogenomic
  - Other

- Uses
  - Regulatory
  - Optimize drug use
  - Explore mechanisms of action, toxicity, sensitivity, resistance
  - Explore other aspects of biology
  - Should be fit for purpose
    - Assay: Sample handling, Analytic methods
    - Context: Drug, Target, Pathway, Disease and setting
    - Goals: Immediate needs vs Long term uses
Tumor Angiogenesis

Multiple targets in angiogenesis

Hypoxia
Inflammation
Platelets
HIF1-alpha
COX-2
ephrinB2
VEGF
PIGF
PDGF
Others
Angiogenic Factors
Notch / Dll4
Proteases
Invasion
Integrins
VEGFRs
TEMs
Receptor Binding
Signal Transduction
mTOR
Tyrosine kinases
Akt, PI3K
Proliferation
Migration
Ang-2
Apelin
Tube Formation
Rasip-1
Homing
Endothelial Progenitor Cells (EPC)
SDF-1, CXCR4, PSGL1

Source: The Angiogenesis Foundation.
VEGF family and Receptors

VEGF family and Receptors

1971  J. Folkman: Publishes Angiogenic Hypothesis in NEJM

1983  H. Dvorak: Tumors Secrete a Vascular Permeability Factor (VPF)

1989  N. Ferrara: Cloned Vascular Endothelial Growth Factor (VEGF)

1989  D. Connolly: Cloned VPF - Identical to VEGF

1996  L. Presta: Humanized mAb to VEGF

1997  Clinical Trials of rhuMAb VEGF begin

2003  Phase III Colorectal Trial Results for Bevacizumab/ Avastin™ Reported
Resistance Mechanisms to Anti-VEGF Therapy
Blood-based Biomarkers

**Advantages**

- easy to obtain, multi-institutional studies possible
- large amounts of sample can be acquired
- defined assays allowing direct hypothesis testing
- represents a composite from several microenvironments
- can evaluate many targets at one time (multiplexing)
Comprehensive list of biomarkers

<table>
<thead>
<tr>
<th>Soluble Angiogenic Factors</th>
<th>Matrix-Derived Angiogenic Factors</th>
<th>Markers of Coagulation</th>
<th>Markers of Vascular Activation and Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG-2</td>
<td>BMP9</td>
<td>CRP</td>
<td>E-cadherin</td>
</tr>
<tr>
<td>bFGF</td>
<td>MMP2</td>
<td>D-Dimer</td>
<td>E-selectin</td>
</tr>
<tr>
<td>HGF</td>
<td>MMP9</td>
<td>PAI-1 Active</td>
<td>Gro-α</td>
</tr>
<tr>
<td>IGFBP1</td>
<td>osteopontin</td>
<td>PAI-1 Total</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>IGFBP2</td>
<td>PEDF</td>
<td>PAI-1 Active</td>
<td>IL-6</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>sEndoglin</td>
<td>Von Willebrand Factor</td>
<td>IL-8</td>
</tr>
<tr>
<td>PDGF-AA</td>
<td>TGFβRIII</td>
<td></td>
<td>MCP-1</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>TGFβ1</td>
<td></td>
<td>P-selectin</td>
</tr>
<tr>
<td>PIGF</td>
<td>TGFβ2</td>
<td></td>
<td>SDF-1</td>
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<tr>
<td>sVEGFR1</td>
<td>TSP1</td>
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<td>VCAM-1</td>
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<tr>
<td>sVEGFR2</td>
<td>TSP2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVEGFR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF-A</td>
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<td></td>
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<tr>
<td>VEGF-C</td>
<td></td>
<td></td>
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<tr>
<td>VEGF-D</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Standard ELISA assays were also included to evaluate soluble TGFβRIII and IGF1 as additional blood markers.*
# Impact on Cooperative Group Research

**All Studies Large Randomized, Phase III**

<table>
<thead>
<tr>
<th>CALGB Study</th>
<th>Title</th>
<th>Drug</th>
<th>Approach</th>
<th>Target Evaluation</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>80303</td>
<td>A Randomized Phase III Trial of Gemcitabine plus Bevacizumab Versus Gemcitabine plus Placebo in Patients with Advanced Pancreatic Cancer</td>
<td>Bevacizumab</td>
<td>Aushon (SearchLight) ELISA</td>
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<td>Nixon, et al. CCR (2013)</td>
</tr>
<tr>
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<tr>
<td>80405</td>
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<td>90401</td>
<td>Docetaxel and Prednisone With or Without Bevacizumab in Treating Patients With Prostate Cancer That Did Not Respond to Hormone Therapy</td>
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<td>A Randomized Phase III Study of Carboplatin and Paclitaxel or Carboplatin, Paclitaxel and Bevacizumab or Carboplatin, Paclitaxel and Bevacizumab plus Bevacizumab maintenance in Patients with Advanced Ovarian Cancer</td>
<td>Bevacizumab</td>
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CALGB 80303 was a randomized Phase III Trial of gemcitabine plus bevacizumab versus gemcitabine plus placebo in patients with advanced pancreatic cancer.

Pancreatic cancer is the 4th leading cause of cancer-related death in the United States\(^1\).

Bevacizumab (Genentech/Roche) is a humanized monoclonal antibody against vascular endothelial growth factor A (VEGF A) and has been shown to provide clinical benefit in a number of tumor types including colon, glioblastoma, renal, and non-small cell lung cancer\(^2\).

No clinical benefit was observed from the addition of bevacizumab in this study\(^3\).

Blood-based biomarker profiling to identify potential prognostic/predictive factors has been supported by several recent studies\(^4-7\).

2. Bevacizumab (Avastin) resource center: http://www.avastin.com
7. Tran, HT, et al. JCO. 2011; 29 (suppl 7; abstr 334).
General Objectives

- To correlate baseline values of multiple plasma-based angiogenic factors with the primary clinical outcome (Overall Survival)
  - Identify univariate and multivariate prognostic markers
    - Predict outcome independent of treatment group
  - Identify univariate and multivariate predictive markers
    - Predict outcome that is dependent upon treatment
  - Identify patterns of correlation among analytes
Predictive Markers in CALGB80303

<table>
<thead>
<tr>
<th>Predictive Marker</th>
<th>Favors Bev</th>
<th>Favors Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td>n  HR   95% CI</td>
</tr>
<tr>
<td>VEGF-D</td>
<td></td>
<td>328 1.11 (0.89,1.38)</td>
</tr>
<tr>
<td>&lt;Q1</td>
<td></td>
<td>82 0.60 (0.39,0.96)</td>
</tr>
<tr>
<td>&gt;Q1</td>
<td></td>
<td>246 1.30 (1.04,1.74)</td>
</tr>
<tr>
<td>SDF-1</td>
<td></td>
<td>164 1.40 (1.04,1.94)</td>
</tr>
<tr>
<td>&lt;med</td>
<td></td>
<td>164 0.87 (0.64,1.18)</td>
</tr>
<tr>
<td>&gt;med</td>
<td></td>
<td>164 0.82 (0.60,1.12)</td>
</tr>
<tr>
<td>Ang2</td>
<td></td>
<td>164 1.40 (1.02,1.92)</td>
</tr>
</tbody>
</table>

Hazard Ratio
Vascular endothelial growth factors (VEGF) and VEGF receptor expression as predictive biomarkers for benefit with bevacizumab in metastatic colorectal cancer (mCRC): Analysis of the phase III MAX study.

• Using Immunohistochemical (IHC) approaches, Weickhardt et al. identified VEGF-D as a potential predictor for bevacizumab response in mCRC.

• AGITG - MAX Trial (Mitomycin, Avastin, Xeloda) – 268 biomarker/471 total patients
  • capecitabine
  • capecitabine and bevacizumab
  • capecitabine, bevacizumab and mitomycin

• TMAs were stained for VEGF-D and scored as 0, 1+, 2+, 3+
  • Benefit from bevacizumab observed in patients (n=32) with low VEGF-D (0,1+)
  • Significant for OS & PFS; however, OS results not significant after multiple parameter testing (for biomarkers)

• Observation in agreement with our findings
  • Low blood levels (<Q1) of VEGF-D predicted for benefit from bevacizumab
MAX Trial: IHC Evaluation of VEGF-D

**FOREST PLOT: PROGRESSION FREE SURVIVAL AND VEGF-D**

<table>
<thead>
<tr>
<th>EXPRESSION</th>
<th>N</th>
<th>HR (95% CI)</th>
<th>p-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-D 0, 1+</td>
<td>32</td>
<td>0.21 (0.08 to 0.55)</td>
<td></td>
</tr>
<tr>
<td>VEGF-D 2+</td>
<td>117</td>
<td>0.67 (0.45 to 1.00)</td>
<td>0.02</td>
</tr>
<tr>
<td>VEGF-D 3+</td>
<td>110</td>
<td>0.77 (0.50 to 1.17)</td>
<td></td>
</tr>
<tr>
<td>C vs CB + CBM</td>
<td>471</td>
<td>0.61 (0.50 to 0.74)</td>
<td></td>
</tr>
</tbody>
</table>

**FOREST PLOT: OVERALL SURVIVAL AND VEGF-D**

<table>
<thead>
<tr>
<th>EXPRESSION</th>
<th>N</th>
<th>HR (95% CI)</th>
<th>p-interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-D 0, 1+</td>
<td>32</td>
<td>0.35 (0.13 to 0.90)</td>
<td></td>
</tr>
<tr>
<td>VEGF-D 2+</td>
<td>117</td>
<td>0.82 (0.52 to 1.30)</td>
<td>0.01</td>
</tr>
<tr>
<td>VEGF-D 3+</td>
<td>110</td>
<td>1.28 (0.79 to 2.09)</td>
<td></td>
</tr>
<tr>
<td>C vs CB + CBM</td>
<td>471</td>
<td>0.93 (0.75 to 1.16)</td>
<td></td>
</tr>
</tbody>
</table>
CALGB 90206: A Randomized Phase III Trial of IFN $\alpha \pm$ Bevacizumab

- Renal cell carcinoma (RCC) accounts for 4-5% of all new cancers diagnosed and is the most common type of kidney cancer in the US

- Although bevacizumab significantly increased PFS, no clinical benefit in OS was observed
  - IFN$\alpha$ monotherapy – 17.4 months (95% CI: 14.4, 20.0)
  - IFN$\alpha$ + bevacizumab – 18.3 months (95% CI: 16.5, 22.5)
  - Unstratified log-rank $P = 0.097$
Patient population

- Baseline plasma were available from 431 of the 732 patients treated on the parent protocol (Table 1).
- Baseline characteristics between the entire study population and the biomarker evaluable cohort were similar.

Approach

- Primary endpoint: OS defined as time from randomization to time of death of any cause.
- The data were randomly split at a 2:1 ratio into Training (n=286) and Testing (n=138) sets.
- The proportional hazards (PH) model was used to test for marker-treatment arm interaction in predicting OS.
CALGB 90206 Predictive markers in mRCC

**HGF**

- **Number at risk**:
  - High HGF, Bev+IFN: 78
  - High HGF, IFN: 65
  - Low HGF, Bev+IFN: 72
  - Low HGF, IFN: 71

- **Overall Survival (Probability)**: P Value = 0.022

**IL-6**

- **Number at risk**:
  - High IL6, Bev+IFN: 78
  - High IL6, IFN: 64
  - Low IL6, Bev+IFN: 72
  - Low IL6, IFN: 72

- **Overall Survival (Probability)**: P Value = 0.0016
Results

- Multivariable Analysis
  - A 3-way interaction between IL-6, HGF and treatment arm was noted in the Training set.
  - Evaluation of HGF and treatment arm stratified by IL-6 revealed:
    - Patients with high IL-6 levels and either high or low HGF levels benefitted from the addition of bevacizumab.
    - Patients with low IL-6 and low HGF levels benefitted from the addition of bevacizumab.
    - Patients with low IL-6 and high HGF levels did not benefit from the addition of bevacizumab (shorter OS).
## HGF and Treatment Arm, Stratified by IL-6

### Training Set

| High IL-6 Levels*  
<table>
<thead>
<tr>
<th>(n=144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Arm</td>
</tr>
<tr>
<td>Low HGF Levels</td>
</tr>
<tr>
<td>(n=50)</td>
</tr>
<tr>
<td>High HGF Levels</td>
</tr>
<tr>
<td>(n=94)</td>
</tr>
<tr>
<td>IFNα</td>
</tr>
<tr>
<td>Bev + IFNα</td>
</tr>
</tbody>
</table>

| Low IL-6 Levels   |
| (n=142)          |
| Treatment        |
| Low HGF Levels    |
| (n=93)           |
| High HGF Levels   |
| (n=49)           |
| IFNα              | 33.5 (27.7-54.6) | 27.6 (18.4-42.3) |
| Bev + IFNα        | 43.0 (31.2-58.3) | 18.7 (12.1-37.7) |

* Based on median in the Training Set
Median OS (months) and 95% CI

### Testing Set

| High IL-6 Levels*  
<table>
<thead>
<tr>
<th>(n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Arm</td>
</tr>
<tr>
<td>Low HGF Levels</td>
</tr>
<tr>
<td>(n=15)</td>
</tr>
<tr>
<td>High HGF Levels</td>
</tr>
<tr>
<td>(n=46)</td>
</tr>
<tr>
<td>IFNα</td>
</tr>
<tr>
<td>Bev + IFNα</td>
</tr>
</tbody>
</table>

| Low IL-6 Levels   |
| (n=77)          |
| Treatment        |
| Low HGF Levels    |
| (n=57)           |
| High HGF Levels   |
| (n=20)           |
| IFNα              | 26.8 (19.3-40.4) | 18.3 (11.1-NR) |
| Bev + IFNα        | 55.2 (42.0-NR) | 15.6 (5.9-NR) |

* Based on the observed median in the Training set
Median OS (months) and 95% CI
NR=not reached,
ASCO 2013 Poster Discussion
Dr. Primo Lara – UC-Davis

Bladder cancer
#4518: Hussain, et al. Gene expression profiling
#4523: Gonzalez-Ribbon, et al. IGF1R expression

Kidney cancer
#4519: Xu, et al. Germline IL8 polymorphisms
#4520: Nixon, et al. Plasma IL6 and HGF
#4521: Bailey, et al. Pdl-1/pdl-3 expression
#4522: Rathmell, et al. 34-gene signature

**BIOMARKER REPORT CARD**

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td>Biologically relevant?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Prognostic?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Predictive?</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Immediate clinical application?</td>
<td>My comments</td>
</tr>
</tbody>
</table>
**BIOMARKER REPORT CARD**

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Biologically relevant?</td>
<td>Yes</td>
<td>Results are consistent with previously reported data* on circulating IL6 as a biomarker of benefit from VEGFR-TKI</td>
</tr>
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<td>Prognostic?</td>
<td>Yes</td>
<td>Study demonstrates value of NCI-supported US Cooperative Groups in pursuing translational studies</td>
</tr>
<tr>
<td>Predictive?</td>
<td>Yes</td>
<td>These results provide additional justification for a phase III intergroup trial that will <strong>prospectively validate IL6 and LDH as predictors of benefit</strong> from VEGF and mTOR inhibitors, respectively</td>
</tr>
<tr>
<td>Immediate clinical application?</td>
<td>Almost</td>
<td></td>
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</table>

*Tran, et al Lancet Oncology 2012*
IL-6: Biomarker for anti-VEGF treatment in Renal Cancer

Overview

- Pazopanib vs best supportive care in mRCC
- Used Aushon SearchLight multiplex technology
- Identified IL-6 predicted for benefit/lack of benefit from pazopanib
- Data confirms observations made in CALGB90206 and implies that IL-6 may predict for benefit across the class of VEGF inhibitors in mRCC

IL-6: Predicts Benefit in mRCC

Pazopanib

IL-6: Predicts Benefit in mRCC

Pazopanib

Bevacizumab

**Median OS (months) Hazard ratio 95% CI**

<table>
<thead>
<tr>
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<th>Placebo</th>
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<tbody>
<tr>
<td>Low IL-6, Bev+IFN</td>
<td>29.0</td>
<td>28.0</td>
</tr>
<tr>
<td>High IL-6, Bev+IFN</td>
<td>19.0</td>
<td>8.4</td>
</tr>
<tr>
<td>High IL-6, IFN</td>
<td>27.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Low IL-6, IFN</td>
<td>23.0</td>
<td>16.0</td>
</tr>
<tr>
<td><em>p</em> value</td>
<td>&lt;0.0001</td>
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</tr>
<tr>
<td>Interaction</td>
<td>0.053</td>
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**Overall Survival (Probability)**

- **P<sub>interaction</sub> = 0.0016**

**Number at risk**

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<td>59</td>
<td>43</td>
<td>27</td>
<td>68</td>
</tr>
<tr>
<td>Placebo (low)</td>
<td>42</td>
<td>27</td>
<td>60</td>
<td>57</td>
</tr>
<tr>
<td>Placebo (high)</td>
<td>33</td>
<td>17</td>
<td>51</td>
<td>52</td>
</tr>
<tr>
<td>Overall</td>
<td>27</td>
<td>13</td>
<td>47</td>
<td>45</td>
</tr>
<tr>
<td>Number at risk</td>
<td>12</td>
<td>3</td>
<td>47</td>
<td>38</td>
</tr>
<tr>
<td>Median survival</td>
<td>23</td>
<td>8</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>Overall survival</td>
<td>16</td>
<td>3</td>
<td>14</td>
<td>32</td>
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<tr>
<td>Median survival</td>
<td>11</td>
<td>2</td>
<td>7</td>
<td>27</td>
</tr>
<tr>
<td>Overall survival</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Median survival</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Overall survival</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Median survival</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>Overall survival</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>23</td>
</tr>
</tbody>
</table>


## Impact on Cooperative Group Research
### All Studies Large Randomized, Phase III

<table>
<thead>
<tr>
<th>CALGB Study</th>
<th>Title</th>
<th>Drug</th>
<th>Approach</th>
<th>Target Evaluation</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>80303</td>
<td>A Randomized Phase III Trial of Gemcitabine plus Bevacizumab Versus Gemcitabine plus Placebo in Patients with Advanced Pancreatic Cancer</td>
<td>Bevacizumab</td>
<td>Aushon (SearchLight) ELISA</td>
<td>Angiogenic factors, VEGF-related factors, Inflammatory markers, Coagulation factors</td>
<td>Nixon, et al. CCR (2013)</td>
</tr>
<tr>
<td>90206</td>
<td>A Randomized Phase III Trial of Interferon Alfa-2B or Interferon Alfa-2B Plus Bevacizumab in Patients with Advanced Renal Carcinoma</td>
<td>Bevacizumab</td>
<td>Aushon (SearchLight) ELISA</td>
<td>Angiogenic factors (VEGF and non-VEGF related)</td>
<td>Work completed, Presented at ASCO 2013</td>
</tr>
<tr>
<td>80405</td>
<td>A Phase III Trial of Irinotecan/5-FU/Leucovorin or Oxaliplatin/5-FU/Leucovorin with Bevacizumab or Cetuximab or with the Combination of Bevacizumab and Cetuximab for Patients with Untreated Metastatic Adenocarcinoma of the Colon or Rectum</td>
<td>Bevacizumab &amp; Cetuximab</td>
<td>Aushon (SearchLight) ELISA</td>
<td>Angiogenic factors, VEGF-related factors, EGF-related factors, Inflammatory markers, Coagulation factors</td>
<td>Currently being analyzed in our Lab</td>
</tr>
<tr>
<td>90401</td>
<td>Docetaxel and Prednisone With or Without Bevacizumab in Treating Patients With Prostate Cancer That Did Not Respond to Hormone Therapy</td>
<td>Bevacizumab</td>
<td>Aushon (SearchLight) ELISA</td>
<td>Angiogenic factors (VEGF and non-VEGF related)</td>
<td>Project to be initiated Q3 of 2015</td>
</tr>
<tr>
<td>90601</td>
<td>A Randomized Phase III Study of Gemcitabine and Cisplatin or Gemcitabine, Cisplatin, and Bevacizumab in Patients with Advanced Bladder Cancer</td>
<td>Bevacizumab</td>
<td>Aushon (SearchLight) ELISA</td>
<td>Angiogenic factors, VEGF-related factors, Inflammatory markers, Coagulation factors</td>
<td>Project to be initiated Q2 of 2016</td>
</tr>
<tr>
<td>GOG218</td>
<td>A Randomized Phase III Study of Carboplatin and Paclitaxel or Carboplatin, Paclitaxel and Bevacizumab or Carboplatin, Paclitaxel and Bevacizumab plus Bevacizumab maintenance in Patients with Advanced Ovarian Cancer</td>
<td>Bevacizumab</td>
<td>Aushon (SearchLight) ELISA</td>
<td>Angiogenic factors (VEGF and non-VEGF related)</td>
<td>Analysis complete, waiting on Stats</td>
</tr>
</tbody>
</table>
CALGB 80405

CALGB/SWOG 80405: FINAL DESIGN

mCRC 1st-line
KRAS wild type (codons 12,13)
STRATA: FOLFOX/FOLFIRI
Prior adjuvant
Prior XRT

FOLFIRI or FOLFOX
MD choice

Chemo + Cetuximab

Chemo + Bevacizumab

N = 1140
1° Endpoint: Overall Survival

CALGB 80405

<table>
<thead>
<tr>
<th>Arm</th>
<th>N (Events)</th>
<th>OS (m) Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo + Cetux</td>
<td>578 (375)</td>
<td>29.9</td>
<td>27.0-32.9</td>
</tr>
<tr>
<td>Chemo + Bev</td>
<td>559 (371)</td>
<td>29.0</td>
<td>25.7-31.2</td>
</tr>
</tbody>
</table>

P=0.34
HR 0.925 (0.78-1.09)

CALGB 80405

CALGB/SWOG 80405: Progression-Free Survival
(Investigator Determined)

<table>
<thead>
<tr>
<th>Arm</th>
<th>N (Events)</th>
<th>PFS (m) Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemo + Bev</td>
<td>559 (498)</td>
<td>10.8</td>
<td>9.7-11.4</td>
</tr>
<tr>
<td>Chemo + Cetux</td>
<td>578 (499)</td>
<td>10.4</td>
<td>9.6-11.3</td>
</tr>
</tbody>
</table>

P=0.55
HR 1.04 (0.91-1.17)

1st line Metastatic Colorectal Cancer n = 238

Key inclusion criteria:
- EGFR positive
- Ras status not required

Accrual: 01/21/2004-12/03/2004
Cetuximab Efficacy Predicted by Ras Mutation

Progression-free Survival

Overall Survival

C Karapetis, NEJM 2008
Tumor Gene Expression and Response to Cetuximab in Ras WT mCRC

JB Baker et al. BJC(2011) 104:488-495
Predictive Marker – HER3

OS in KRAS WT

Cushman, SM. CCR 2014; 21(5):1078-86.
Predictive Marker – CD73

Cushman, SM. CCR 2014; 21(5):1078-86.
Biology of CD73 / 5NTE

Markers Evaluated in Plasma

- EGF
- HBEGF
- sEGFR
- sHER2
- sHER3
- sCD73

Analyte selection was driven by ELISA-compatible reagents and assay performance.
EGF Predicts Cetuximab Benefit

OS KRAS WT

$p=0.045$

sHER3 Predicts Cetuximab Benefit

Hatch, AJ. ASCO 2014.
sCD73 Predicts Cetuximab Benefit

Hatch, AJ. ASCO 2014.
Comparing mRNA and Protein

The same markers have been examined in two different contexts (tumor mRNA and plasma protein). How do they line up?
HER3: mRNA and Protein

**mRNA**

Probability

Time since study entry (months)

p = 0.029

**Plasma Protein**

Probability

Overall Survival Time

p = 0.046
CD73: mRNA and Protein

**mRNA**

- CD73 high w/o cetux
- CD73 high w/ cetux
- CD73 low w/o cetux
- CD73 low w/ cetux

\[ p = 0.026 \]

**Plasma Protein**

- CD73: high, w/o cetux
- CD73: high, w/ cetux
- CD73: low, w/o cetux
- CD73: low, w/ cetux

\[ p = 0.018 \]
Conclusions

- The purpose of biomarkers is to inform clinical decision making by matching therapies to patients to improve efficacy and reduce toxicity.
- Blood-based multiplex analyses were technically robust
- Multiple factors with strong prognostic importance were identified
- Several markers predicted for potential benefit or lack of benefit from bevacizumab
- Several markers predicted for potential benefit or lack of benefit from cetuximab
- Confirmation of results from different laboratories/approaches
- Results need validation before being applied to clinical practice
- Inclusion of multi-analyte angiome analyses in other trials is warranted
Acknowledgments

**Biomarker Lab**

*Present:*
- Mark Starr
- Chris Brady
- Ace Hatch, PhD
- Yingmiao Liu, PhD
- Jeff Clarke, MD
- Dan Bowers, MD

*Past:*
- Haiyan Li
- Susan Liu
- Stephanie Cushman, PhD
- Jingquan Jia, MD, PhD

All the clinical fellows and undergrads who have contributed to the lab

**Statistical Team**

*GI and Phase I*
- Herbert Pang, PhD
- Anu Bulusu
- Andrew Dellinger
- Jennifer Marcello
- Christel Rushing
- Donna Niedzwiecki, PhD

*GU*
- Susan Halabi, PhD
- Ivo Shterev, PhD

*Pharmacogenomic/Other*
- Kouros Owzar, PhD
- Jiang Chen, PhD
- Alex Sibley

**GI and Phase I Team**
- Herb Hurwitz, MD
- Leigh Howard
- Shawna Savage
- Wanda Honeycutt
- Neal Kaplan
- Kellen Meadows

**GU Team**
- Dan George, MD
- Kelly Mundy

**The Alliance for Clinical Trials in Oncology**