Pharmacogenomics of Angiogenesis

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This is a brief overview on pharmacogenomics of angiogenesis — a simple but clear illustration of how pharmacogenomics can be used to approach angiogenesis biomarker discovery. Today we study cancer and lump patients together based on the site of disease, whether it be breast cancer or lung cancer.
Overview: Pharmacogenetic Approach to Angiogenesis Biomarker Discovery

Source: With permission from Walgren et al. JCO 2005; 23:7342-7349
Our goal in using pharmacogenomics is to be able to identify subgroups of patients who will or will not benefit from a given drug or find a subgroup susceptible to the associated toxicity. Each color represents a different subgroup. The green group (everyone wants to fall in this subgroup) represents the group that benefits but without the toxicity and then there is the purple group that will obtain no benefit and no toxicity. Many of our recent studies have also suggested that the subgroup enriched to experience both better efficacy and more toxicity (blue group) is quite common. It is for this subgroup that we must adequately understand this benefit to toxicity ratio so we can jointly decide with the patient whether proceeding is worth it.
Hallmarks of Malignancy: A Biomarker Rich Environment?

This is a great place to start when thinking about biomarkers, in that these are the fundamental hallmarks that define cancer. And many are really specific to a given tumor cell, including the ability of a cell to evade apoptosis, having cell sufficiency and growth signaling, insensitivity to antigrowth signaling, the ability of a cancer cell to invade and metastasize, and of course, limitless replication potential.

But the hallmark of sustained angiogenesis is really quite different. Although the tumor elicits this response, it is the host that has to comply. And so this allows for a higher yield of host-imprinted genetic variability in terms of thinking about biomarkers.
Angiogenesis is Critical for Tumor Proliferation

Source: With permission from Hicklin et al, JCO, 2005: 23, 1011
Angiogenesis is important for tumor proliferation, including a variety of cytokines, growth factors and receptors that are fundamental to this process. One of the most central is vascular endothelial growth factor, or VEGF, which we know to be secreted, in part, by the tumor. This then is bound to a variety of VEGF receptors. One of the critical receptors is VEGF receptor 2, which helps recruit bone marrow-derived endothelial precursor cells to help form the vasculature to the tumor itself.
Evidence for Angiogenic Role in Tumor Pathogenesis

- Increased MVD associated with advanced stage & poor outcomes in multiple malignancies
- High pro-angiogenic factor tumor expression correlated with poor clinical outcome in malignancy
- Inhibition of angiogenesis successful in MULTIPLE tumor types
Evidence that angiogenesis is important in tumor pathogenesis is as follows: Increased microvessel density, which is the histologic surrogate for angiogenesis, has been found to be associated with advanced stage and poor outcomes in a variety of cancers. Data also exist to support high expression of proangiogenic factors and can be correlated with poor clinical outcome in a variety of malignancies. Most importantly, if you block tumor angiogenesis, it disrupts growth in a variety of tumor types, which is the strongest indication that tumor angiogenesis is important in tumor pathogenesis.
Multiple Targets to Inhibit Angiogenesis

Antiangiogenesis agents that target the VEGF receptor and its signaling pathway.

- **Bevacizumab** (monoclonal antibody)
- **VEGF Trap** (ligand sequestrant)
- **IMC-1121B** (monoclonal antibody)

**Extracellular receptor**
- VEGFR1 / VEGFR1
- VEGFR2 / VEGFR2
- VEGFR3 / VEGFR3

**Intracellular tyrosine kinase**

**Nucleus**

**Angiozyme** (ribozyme; decreases mRNA / VEGFR1 production)

There are several ways to think about targeting angiogenesis. One can disrupt vascular endothelial growth factor itself. Probably the most mature drug in this class is bevacizumab, which is a humanized monoclonal antibody against VEGF, or by use of a ligand sequestrant, such as VEGF Trap. The VEGF receptors have also become targets. Monoclonal antibodies target the extracellular membrane portion, and a variety of intracellular tyrosine kinase inhibitors such as sunitinib and sorafenib, both of which are FDA-approved in several malignancies, target the intracellular portion.
# VEGF SNPs Associated with Outcome

<table>
<thead>
<tr>
<th>VEGF Allele</th>
<th>Trial</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2578A</td>
<td>E2100 (phase III breast)</td>
<td>Improved OS</td>
</tr>
<tr>
<td>-1498C</td>
<td>E2100</td>
<td>More HTN</td>
</tr>
<tr>
<td>-1154A (tagSNP)</td>
<td>E2100</td>
<td>Improved OS</td>
</tr>
<tr>
<td>-634G</td>
<td>E2100</td>
<td>More HTN</td>
</tr>
<tr>
<td></td>
<td>E4599 (phase III lung)</td>
<td>Improved OS</td>
</tr>
<tr>
<td></td>
<td>RCCA (axitinib)</td>
<td>More HTN</td>
</tr>
</tbody>
</table>

VEGF -2578A, -1498C, -1154A, and -634G A alleles are in L.D.

**Sources:** Schneider, JCO 2008; Zhang, ASCO:abstr# 8032, 2009; Kim ASCO:abstr# 5005, 2009
Unfortunately, although these are truly targeted therapies — whether they target VEGF or the VEGF receptors — to date we really don’t have a good idea of what subpopulations to target these agents to. Data are now emerging looking for biomarkers to indicate which patients would be best suited for each drug treatment. This table demonstrates some data to date looking at VEGF single nucleotide polymorphisms.

The VEGF minus 2578 allele was associated with improved overall survival in the E2100 trial, a Phase III trial implementing bevacizumab in breast cancer. The minus 1498C allele was associated with more hypertension in E2100. The minus 1154A allele, again, was associated with improved overall survival in E2100. And the minus 634G allele was associated with more hypertension in E2100, improved overall survival in E4599, a Phase III lung cancer trial, again implementing bevacizumab, and was associated with more hypertension in a meta-analysis implementing axitinib in renal cell cancer. It’s also important to note that all of these alleles are in tight linkage disequilibrium.
Pharmacogenetic Approach to Angiogenesis Biomarker Discovery

Essential Ingredients:

- Genetic variability must have potential for biologic impact
- Genetic variability must exist in drug disposition or destination
  - Metabolizing enzymes/transporters/targets
- Drug evaluated must be heterogeneous in outcome
  - Mix of success and toxicity
- Variability must be frequent
  - Generalizability of results

Source: Walgren et al. JCO 2005; 23:7342-7349
When thinking about a pharmacogenetic approach to angiogenesis biomarker discovery, there are four essential ingredients. The first, genetic variability, must have the ability to impact the biological system that is being tested.
Genetic Variability Impacts Angiogenesis: Brief Summary

• Epidemiologic Data:
  – Variable risk & prognosis in multiple conditions where angiogenesis is important: risk/prognosis in multiple malignancies, retinopathy, nephropathy, pre-eclampsia, recurrent pregnancy loss, vasculopathy
  – Mostly VEGF, HIF1, & NOS
  – Limitations: Conflicting data, single gene/SNP approach, & clinical variables often ignored
Plenty of epidemiologic data suggest that genetic variability impacts angiogenesis. This includes variable risk and prognosis in a variety of conditions where we know angiogenesis is an important player such as, in a variety of malignancies, retinopathy, nephropathy, preeclampsia, recurrent pregnancy loss, as well as vasculopathy. The majority of these studies look at VEGR, HIF1 alpha or nitric oxide synthase; however, there are newer data looking at VEGF receptors in other critical genes.

One major limitation to these studies is conflicting outcomes. Also, many of these studies only examine a single gene or a single SNP. Finally, clinical variables, which we know to be very important in terms of altered risk, are often completely ignored in these studies.
Genetic Variability Impacts Angiogenesis: Brief Summary

- Variability may associate with site of metastasis
  - VEGF-1498 CC more common in visceral (vs. bone) mets
- VEGFR-1 promoter SNP associated with differential induction by p53: (Menendez, PNAS 2006)
- Variability in complement factor H may affect treatment outcome in macular degeneration (?biomarker): (Brantley, Ophthalmology 2007)
  - CC genotype had inferior outcome in visual acuity with intravitreal bevacizumab
Data suggests that variability may be associated with the site of metastases. One data set shows the VEGF minus 1498CC genotype was more common in visceral metastases, as opposed to bony metastases. A provocative paper by Menendez, published in the *PNAS* in 2006, demonstrated that a VEGF receptor-1 promoter polymorphism was associated with differential induction by p53. As a result, for this first time, we have been able to intimately tie the cancer pathway with the angiogenesis pathway together.

And finally, a small study showed that variability and complement factor H may actually affect treatment outcome in macular degeneration for patients receiving intravitreal bevacizumab.
Genetic Variability Impacts Angiogenesis: Brief Summary

- NOT level 1 evidence: body of data strongly suggests variability is biologically important
- Breast cancer angiogenesis as a model
This is not level one evidence, but when reviewing the body of data as a whole, it strongly suggests that variability is biologically important. I’m going to use breast cancer angiogenesis as a model, briefly.
Pharmacogenetic Approach to Angiogenesis Biomarker Discovery

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  – Mix of success and toxicity
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  – Generalizability of results

Source: Walgren et al. JCO 2005; 23:7342-7349
A second fundamental property is that genetic variability must exist in the drug’s disposition or destination, and this can include variability in the metabolizing enzymes for the drug, drug transporters or ultimately the drug target.
Excellent Genetic Variability in Angiogenesis Drug Targets

Source: Reprinted with permission from Schneider et al, CCR 15, 5297; 2009;15:5297 fig 1
Angiogenesis is a great place to look at genetic variability, as there is robust variability within VEGF itself. The majority of variability in VEGF is within the promoter and regulatory regions. Here you can see five polymorphisms. These are five SNPs that tag for the most common haplotypes and actually account for about three quarters of the variability seen in a mixed population of Caucasians and African-Americans. These polymorphisms do appear to affect the functionality of and expression of VEGF. Likewise, within VEGF receptor 2, there is a promoter polymorphism, which appears to affect the function, and two common nonsynonymous SNPs, as well.
Pharmacogenetic Approach to Angiogenesis Biomarker Discovery

Essential Ingredients:
• Genetic variability must have potential for biologic impact
• Genetic variability must exist in drug disposition or destination
  – Metabolizing enzymes/transporters/targets
• Drug evaluated must be heterogeneous in outcome
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  – Generalizability of results

Source: Walgren et al. JCO 2005; 23:7342-7349
A third important quality for a biomarker is that the drug evaluated must be heterogeneous in outcome, obviously, so you need to see a mix of success and toxicity to have a good biomarker.
Bevacizumab in Breast Cancer (E2100)

Stratify:
• DFI ≤ 24 mos. vs. > 24 mos.
• <3 vs. ≥3 metastatic sites
• Adjuvant chemotherapy: yes vs. no
• ER+ vs. ER- vs. ER unknown

Randomize
Paclitaxel
Paclitaxel + Bevacizumab

722 randomized
666 evaluated for efficacy
672 evaluated for toxicity
A good example of heterogeneous outcome is with the use of bevacizumab in breast cancer. E2100, a Phase III trial, randomized 722 patients to either paclitaxel or paclitaxel with bevacizumab as first-line therapy for metastatic breast cancer.
Bevacizumab Significantly Improved PFS

As you know from the parent trial, there was a significant prolongation in progression-free survival from about six months to 12 months with the addition of bevacizumab, and this was statistically significant with a $p$-value of less than 0.001. Likewise, there was an improvement in objective response rate from about 25 percent to 49.2 percent when evaluating measurable disease. And, again, this was statistically significant.
Bevacizumab Increased Grade 3/4 Toxicity

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>P (%)</th>
<th>P + B (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>2.9</td>
<td>9.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4.9</td>
<td>9.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>17.7</td>
<td>23.5</td>
<td>0.05</td>
</tr>
<tr>
<td>CNS ischemia</td>
<td>0</td>
<td>1.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>2.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>0</td>
<td>3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>14.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Source: Miller et al. NEJM 357:2666; 2007
Bevacizumab also increased Grade III/IV toxicities. The toxicities listed in this table were statistically significantly different. And I think these can really be broken down into three major categories, the first of which includes infection, fatigue and neuropathy. And I think this category could be explained, at least in part, due to an increased duration of taxane exposure in the experimental arm.

The second category includes CNS ischemia, headache and proteinuria. And this category represents serious bevacizumab-induced toxicities — fortunately, they were quite rare.

The final category is hypertension. This is a serious toxicity, which is frequent and clearly bevacizumab-induced. And because of its frequency, this becomes a very nice toxicity to study from a biomarker standpoint.
VEGF-2578 AA and -1154 AA Genotypes in Combination Arm Outperformed Control in E2100

Source: With permission from Schneider et al; JCO; 26, 2008: 4672-4678

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These are the Kaplan-Meier curves for the overall survival from E2100 in our pharmacogenomics substudy. This was a retrospective evaluation of E2100 where we had DNA available for 363 patients, divided approximately equally between the experimental arm and the control arm. Now, if you look at the Kaplan-Meier curve on the left, what you can see in purple is the paclitaxel median overall survival, which is 25.2 months, compared to the orange line, which is paclitaxel with the bevacizumab, which was improved only to 26.7 months, and these were not statistically different. However, if one looks at the experimental arm broken down by genotype, one can see a dramatic improvement in median overall survival to 37 months.

Equally important, if you were to break down the paclitaxel-alone arm by genotype, there is no difference. Therefore, this serves as a predictive marker, not a prognostic marker.

On the right Kaplan-Meier curve, one can see the VEGF minus 1154AA genotype has almost a two-year incremental benefit over the paclitaxel arm alone.
Genetic Variability of VEGF Predicts Clinically Significant Hypertension in E2100

<table>
<thead>
<tr>
<th>SNP</th>
<th>Percent Grade 3/4 Hypertension (no./%) by Genotype</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-634</td>
<td>CC=0% (n=27, 15.3%) vs. GC=22% (n=82, 46.3%) vs. GG=19% (n=68, 38.4%)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td><strong>CC vs. GC+GG</strong></td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>VEGF-1498</td>
<td>TT=8% (n=60, 33.9%) vs. CT=22% (n=82, 46.3%) vs. CC=23% (n=35, 19.8%)</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td><strong>TT vs. CC+CT</strong></td>
<td><strong>0.022</strong></td>
</tr>
</tbody>
</table>

Source: Schneider et al; JCO; 26, 2008: 4672-4678
Genetic variability of VEGF also predicted clinically significant hypertension in E2100. The VEGF minus 634CC genotype had essentially no Grade III/IV hypertension. And this was compared to about 20 percent for the alternate genotypes. Those with the VEGF minus 1498TT genotype had less than 10 percent Grade III/IV hypertension, and this was compared to about 20 to 25 percent for the alternate genotypes.
Grade 3/4 Hypertension Associated with Improved Median OS in E2100

**Source:** With permission from Schneider et al; *JCO*; 26, 2008: 4672-4678
We also compared this toxicity of hypertension with overall survival, retrospectively, in E2100. And what we found is that, indeed, those patients who had experienced Grade III/IV hypertension had a significant prolongation of median overall survival — 25 months for those who did not experience hypertension compared to about 38.7 months for those who did. And this was statistically significant.
## Hypertension Association with Survival

<table>
<thead>
<tr>
<th>Trial</th>
<th>Anti-VEGF</th>
<th>Definition of HTN</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E2100</strong> (breast: phase III)</td>
<td>Bevacizumab</td>
<td>CTC grade 3/4</td>
<td>Improved OS</td>
</tr>
<tr>
<td><strong>E4599</strong> (lung: phase III)</td>
<td>Bevacizumab</td>
<td>CTC any grade and &gt; 150/100</td>
<td>Improved OS and PFS</td>
</tr>
<tr>
<td><strong>NCIC BR24</strong> (lung: randomized phase II)</td>
<td>Cediranib</td>
<td>New HTN or worsening grade HTN</td>
<td>Improved RR and PFS</td>
</tr>
<tr>
<td>Axitinib Meta-analysis</td>
<td>Axitinib</td>
<td>dBP &gt; 90 mm Hg</td>
<td>Improved OS</td>
</tr>
</tbody>
</table>

**Sources:** Schneider JCO 2008; Dahlberg ASCO: abstr #8042, 2009, Goodwin ASCO: abstr #3527, 2009; Rini ASCO: abstr #3543, 2008
The association of hypertension with survival is a theme seen across several trials now. As mentioned, E2100 was associated with an improved overall survival. E4599, which was the Phase III lung cancer trial implementing bevacizumab, also was associated with improved overall survival and progression-free survival. In this study, hypertension was defined as any grade hypertension and that greater than 150/100. The NCI Canada’s BR-24 study, a Phase II trial implementing the anti-VEGF drug cediranib was associated with improved response rate and progression-free survival. They defined hypertension as new hypertension or any worsening grade of hypertension. Finally, a meta-analysis of several axitinib trials demonstrated an association with improved overall survival. They defined hypertension in that study as diastolic blood pressure greater than 90.
**E4599 Lung Cancer Trial**

**Advanced NSCLC**
- 878 patients

- Carboplatin / Paclitaxel
- Carboplatin / Paclitaxel + Bevacizumab

**Pharmacogenetics sub-study**

- N=133 eligible (67 PC arm / 66 PCB arm)

**Candidate SNPs:** VEGF, EGF, EGFR, IL-8, KDR, ICAM1, FGFR4, ERCC1, XPD, XRCC1, GSTP1, and WNK1
We’ll now look at the E4599 lung cancer trial. This trial randomized 878 patients with advanced non-small cell lung cancer to either the standard carboplatin/paclitaxel or the experimental arm of carboplatin/paclitaxel/bevacizumab. They also performed a pharmacogenetic substudy of 133 patients, again split fairly evenly between the control and experimental arm, which evaluated several candidate polymorphisms, including those from VEGF, interleukin-8 and ICAM-1.
E4599 Results

• Median OS:
  – PC arm=10.3 months (95% CI: 8.2-15.6)
  – BPC arm=13.0 months (95% CI: 10.2-16.6)

• Median PFS:
  – PC arm=4.6 months (95% CI: 3.6-5.6)
  – BPC arm=6.5 months (95% CI: 5.4-8.3)

• Treatment by genotype interactions tested for in a multivariable model:
  – Gender
  – PS (0 or 1)
  – Stage (IIIB/IV vs. recurrent)
  – Adrenal mets, liver mets, and bone mets
In the parent trial, there was a significant improvement in survival with the addition of bevacizumab from 10 to 13 months, and also an improvement in progression-free survival from 4.6 to 6.5 months. They looked at treatment by genotype interactions tested for in a multivariate model, analyzing for gender, performance status, stage and also, site of metastases.
PFS Classifying Patients by the SNPs that Selected Patients for Superior PFS
(VEGF634 GG and IL8-251 TT ≠ TT, VEGF634 GG and IL8-251 TT and ICAM469 TT)

Source: With permission from Zhang W et al. *Proc ASCO* 2009 Abstract 8032
They evaluated progression-free survival, classifying patients by their SNPs. These are a combination of polymorphisms, including the VEGF 634, the interleukin-8, as well as ICAM-1. Then they created a subgroup of patients that were categorized as a “good SNP cluster” and a “bad SNP cluster.” As you can see from the Kaplan-Meier curve, those patients that had the “good SNP profile” and received paclitaxel and bevacizumab had a long median progression-free survival of 9.2 months, as demonstrated in the blue line.

However, the experimental arm of paclitaxel/bevacizumab in the unselected, or “bad SNP arm,” had a progression-free survival of only 5.4 months. What’s also interesting is if you look in the control arm, paclitaxel alone, there was no major difference in terms of median progression-free survival, whether they had the good or bad SNP profile. And when looking at treatment by marker group interaction, this was statistically significant, with a p-value of 0.003.
E4599: OS Classifying Patients by the SNPs that Selected Patients for Superior OS

(ICAM469 TT and VEGF634 GG, ICAM469 ≠ TT and IL8-251 ≠ TT)

- Hypothesis: SNPs involved in angiogenesis pathway (VEGF, EGF, EGFR, IL-8, KDR, ICAM1, FGFR4), DNA repair pathway (ERCC1, XPD, XRCC1, GSTP1) and WNK1 will predict clinical outcome in a subset of patients enrolled on E4599.
Patients were classified by SNP category and evaluated based on overall survival. The bottom table shows that in the patients that received chemotherapy alone, those with the “bad SNP profile” had a median survival of 8.5 months compared to 10.2 months for those with the “good SNP profile.” Patients that received chemotherapy with bevacizumab and had the “bad SNP profile” had a median survival of 10.7 months and extended to 16.8 months for the “good SNP profile.”
E4599: OS Classifying Patients by the SNPs that Selected Patients for Superior OS (ICAM469 TT and VEGF634 GG, ICAM469 ≠ TT and IL8-251 ≠ TT)

Source: With permission from Zhang W et al. Proc ASCO 2009 Abstract 8032

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Demonstrated in Kaplan-Meier form, it is visually easy to see the differences here. Again, for the paclitaxel/bevacizumab with “good SNP profile” (blue line), you see a huge separation from the green line, where the median survival drops from 16.8 to 10.7 months, whereas in the paclitaxel-alone arm, you see a fair overlap of the curves. Again, when looking at treatment by marker group interaction, this was statistically significant.
### Pairing Drugs and Genes

**The New York Times**

**Pairing Drugs and Genes**

Genetic screening can help determine which patients are best suited to certain drugs. Here is a sampling of drugs for which genetic markers can help identify the suitable patients. Screening tests range in price from a few hundred dollars for tests for drugs like the breast cancer medicine Tamoxifen or the painkiller Celebrex to $3,000 for a test to guide breast cancer chemotherapy. Because experts do not always agree on whether the genetic links are conclusive or whether the screening tests are reliable, the Food and Drug Administration does not always require that drug labels mention such testing.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>USE</th>
<th>GENETIC MARKER</th>
<th>SUITABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Herceptin</strong> (trastuzumab)</td>
<td>Breast cancer</td>
<td>HER2 in tumor</td>
<td>Patients whose tumors have overabundance of Her2 protein. (1)</td>
</tr>
<tr>
<td><strong>Erbilux</strong> (etuximab) and <strong>Vextax</strong> (panitumumab)</td>
<td>Colon cancer</td>
<td>KRAS</td>
<td>Ineffective for patients whose tumors have a mutation in KRAS gene. (4)</td>
</tr>
<tr>
<td><strong>Iressa</strong> (gefitinib) and <strong>Tarceva</strong> (erlotinib)</td>
<td>Lung cancer</td>
<td>EGFR in tumor</td>
<td>Tumors with mutation in this gene may respond better to these drugs than its standard chemotherapies. (4)</td>
</tr>
<tr>
<td><strong>Tamoxifen</strong></td>
<td>Breast cancer</td>
<td></td>
<td>Women with certain variants of CYP2D6 gene may not benefit from drug. (4)</td>
</tr>
</tbody>
</table>

**Avoiding side effects**

<table>
<thead>
<tr>
<th>DRUG</th>
<th>USE</th>
<th>GENETIC MARKER</th>
<th>SUITABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Comptosar</strong> (triclocan)</td>
<td>Colon cancer</td>
<td>UGT1A1*28</td>
<td>This gene variant poses higher risk of white blood cell deficiency from drug. (2)</td>
</tr>
<tr>
<td><strong>Avastin</strong> (bevacizumab)</td>
<td>Various cancers</td>
<td>VEGF</td>
<td>Breast cancer research suggests variants of this gene might help predict which patients benefit from drug and which might be most at risk of side effects. (4)</td>
</tr>
</tbody>
</table>

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**Key:** What drug’s label says about testing:

1. Testing required
2. Testing recommended
3. Genetic relationship only mentioned
4. Test not mentioned

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**Mutational variability**

**Metabolizing enzyme SNPs**

**Host Target SNPs**
More recently, the pairing of drugs and genes has hit the lay press. This is from last year’s *New York Times*, where you can see all three major categories of variability being demonstrated to associate well with the drug of choice, the first of which is mutational variability, where HER2 gene amplification has been used successfully as a marker for the drug trastuzumab. Mutations in K-ras have successfully predicted benefit for those receiving cetuximab. Likewise, EGFR mutations have been associated with outcome for the drugs gefitinib and erlotinib. Several metabolizing enzyme polymorphisms have also been able to predict outcome, including CYP2D6 with tamoxifen and UGT1A1 for irinotecan. Most recently, variability in VEGF has become recognized as a potential biomarker for bevacizumab.
Comprehensive Analysis of Genetic Variability in E5103

1) Validate VEGF SNPs from E2100
2) GWAS
3) QoL: Patient perception of angiogenesis biomarkers
Our hope now is to prospectively validate some of the prior findings from these VEGF polymorphisms in E5103. E5103 is an adjuvant trial for lymph node-positive and high-risk lymph node-negative breast cancer. All patients have HER2-negative disease. And the randomization goal is for about 5,000 patients to be randomized to 1) a control arm of AC followed by paclitaxel, 2) an experimental arm of AC followed by paclitaxel with concurrent bevacizumab, and 3) a second experimental arm of AC followed by paclitaxel with concurrent bevacizumab followed by a maintenance phase of bevacizumab.

Here we plan to either validate or refute the VEGF polymorphisms that we looked at from E2100 and to perform a more comprehensive analysis by genome-wide association. We have also implemented a quality-of-life assessment to more thoroughly understand the risk to benefit ratio.
Conclusions

- Pharmacogenetics (biomarkers)
  - Improves therapeutic index
  - Leads to drug discovery
  - Benefits patients
In conclusion, using pharmacogenetics as biomarkers allows for improvement of the therapeutic index of existing drugs, has the potential to lead to new drug discovery, and clearly can be beneficial to our patients.
Conclusions

- Angiogenesis
  - Hallmark of malignancy
  - Inhibition effective in multiple tumor types
  - Therapeutic heterogeneity – biomarkers needed
  - Early work suggests germline genetic variability might be important
  - Validation and further understanding of molecular biology essential
Angiogenesis is a hallmark of malignancy. We know that its inhibition is effective in multiple tumor types, but there’s real therapeutic heterogeneity. Because of this, biomarkers are clearly needed to best select drugs for a given patient.

Some of the early work suggests that germline genetic variability might be important; however, validation of these findings and further understanding of molecular biology is essential before these can be used in clinical practice.