Pharmacogenomics and Tailored Treatments
SWOG Spring Meeting, 2007

Christine B. Ambrosone, PhD
Roswell Park Cancer Institute
Co-Chair, SWOG Molecular Epidemiology Committee

Treatment Outcomes

- Differential treatment outcomes for patients receiving similar therapies
  - Toxicities experienced
  - Disease-free survival
- Not all accounted for by disease characteristics, patient age, co-morbidities
- What makes some people experience toxicity, cancer recurrence, while others have few adverse outcomes?

Biochemical Individuality\(^1\)

"... every individual has his own inborn metabolic characteristics."

"...every application of biochemistry to human beings must take these differences into account."

"...all diseases, such as cancer, arthritis, heart disease, etc., are related to biochemical individuality."

Pythagoras (510 BC) ‘dangers of some, but not other, individuals who eat the fava bean’ (hemolytic anemia - glucose-6-phosphate dehydrogenase deficient)

Clinical Reports (1940s) - interindividual variations in response to drugs
- isoniazid - peripheral neuropathy in patients treated for tuberculosis; later attributed to ‘slow metabolizer phenotype’ (NAT2).
- hypotensive response in clinical trial with debrisoquine (attributed to impaired oxidative metabolism); associated with recessive alleles for CYP2D6.

Pharmacogenetics - ‘the study of variability in drug response due to heredity’
- Early roots in clinical drug metabolism observations (pharmacogenetics)
- Application to epidemiology and exposure/disease relationships (ecogenetics)
- Recent studies in relation to therapeutic response (pharmacogenomics)

Pharmacogenetics
most variability in drug response the result of ‘polymorphisms’ – commonly occurring variations in DNA of genes that metabolize drugs, participate in other pathways
DNA

- DNA from all organisms is made up of the same chemical and physical components.
- This order spells out the exact instructions required to create a particular organism with its own unique traits.

Proteins

- Although genes get a lot of attention, it's the proteins that perform most life functions and even make up the majority of cellular structures.
- Proteins are large, complex molecules made up of smaller subunits called amino acids.
Genes, DNA Structure, DNA Duplication

- Genetic information flows from DNA to RNA to protein
- Changes in DNA structure lead to altered gene expression or proteins
- Rare ‘changes’ – mutations
- Common variants (> 1%) – single nucleotide polymorphisms (SNPs)

Phenotypic and Genetic Polymorphisms

- Changes occur over thousands of years, become common variants in populations
- Effects of genetic polymorphisms depend upon where in the gene they occur, and what the gene encodes for
- If polymorphism results in decreased activity, and if protein clears drug metabolites, may result in less drug to cancer cells (lower cell kill, poor survival), or less drug to normal cells (fewer toxicities).
Phenotypic and Genetic Polymorphisms

- Drug ‘allergies’ actually differential abilities to clear drug
- Some genetic differences (SNPs) may have little effect on protein or expression
- Some variants may be very common
  - NAT2 slow acetylation phenotype/genotype (~ 60% Caucasians)

Applying Pharmacogenetics to Treatment Outcomes

- Determine pathways for metabolism of chemotherapy drugs – what enzymes activate/detoxify them
- Assess variability (identify SNPs) in the genes that encode those proteins
- Divide patients by genotypes - do toxicities or recurrence differ by genotypes?

TPMT and Thiopurines

- Treatment of childhood leukemia with mercaptopurine
- Small proportion of children experience life-threatening myelosuppression
- Thiopurine methyltransferase (TPMT) prevents formation of thioguanine nucleotides, that are incorporated into DNA, causing cell death
TPMT and Thiopurines

- TPMT polymorphism – homozygotes do not express functional enzyme
- Linked to severe myelosuppression
- St. Jude Hospital for Children (Evans, Relling) – genotyping performed, dose reduced to 1/10th normal dose in carriers
- Heterozygotes – intermediate levels, modest dose reduction

5-FU, DPD and TS

- Treatment of colorectal cancer with 5-Fluorouracil (5-FU)
- 5-FU inactivated in liver by dihydropyrimidine dehydrogenase (DPD); low DPD activity associated with numerous toxicities; polymorphisms associated with activity
- Thymidylate synthase (TS) involved in metabolism; low activity associated with better response (also target for anti-folate drugs) (Lenz)

Irinotecan and UGT1A1

- Irinotecan used for treatment of colorectal cancer, lung cancer
- UDP-glucuronosyltransferase 1A1 (UGT1A1) inactivates metabolite
- Variability reduces enzyme expression – associated with increased risk of toxicities (Ratain, Innocenti)
Pharmacogenetics and Cancer Therapy

- FDA package inserts for specific drug use note effects by genotype (TPMT, UGT1A1, CYP2D6 [codeine derivatives])
- Real application to clinical settings

Metabolic Variability and Response to Breast Cancer Treatment

- Breast cancer often treated with cyclophosphamide, adriamycin, taxanes
- Radiation therapy in addition to chemotherapy or alone
- Tamoxifen used for adjuvant therapy in ER positive women

Metabolic Variability and Response to Breast Cancer Treatment

- Potential role of metabolic variability in treatment efficacy
- Impact of genetic polymorphisms on:
  - metabolism of cyclophosphamide
  - ROS generated by adriamycin, radiation therapy
  - DNA repair
  - Tamoxifen
Chemotherapeutic agent → Detoxification

Lipid Peroxidation → Reactive Intermediates

Chemotherapeutic agent → Activation (P450s)

Excretion

Detoxification (GSTs, UGTs, SULTs)

Damage to DNA, lipids, proteins

Cell Death

DNA Repair

Cyclophosphamide

CYP3A4/5, 2C19, 2C9, 2B6, 3A4

4-OH

ALDH

Phosphoramide Mustard

Acrolein

Carboxy

GSTs

GSTP1 polymorphism, amino acid substitution (Ile105Val), with the Val variant having 2-fold lower activity towards thiopeta (6% Val/Val, 43% Val/Ile)

GSTA1, predominant hepatic GST, single nucleotide substitution in promoter region

GSTA1*B allele - lower GSTA1 levels than cytosols with *A alleles (14%*B/*B, 48% *B/*A)
Cyclophosphamide Metabolism

- Cyclophosphamide activated by CYPs, metabolites can be detoxified through conjugation with GSH catalyzed by GSTP1 and GSTA1.
- Women with high activity CYPs and low activity GST alleles will have greater levels of reactive intermediates; thus, better tumor cell kill, better survival.

Pilot Study of Genetics and Breast Cancer Treatment Outcomes

Conducted at University of Arkansas Medical Sciences
Tumor Registry review for women with incident, primary, invasive breast cancer, diagnosed 1984-1996
Therapy included chemo and/or radiation, matched with pathology – archived normal tissue (lymph node or skin)
Extracted DNA, genotyped, evaluated with survival
n=251, 71 deaths, median follow-up=58 mo.

Overall Breast Cancer Survival (n=240) by GSTP1 Ile105Val
Cancer Res, 2000
Overall Breast Cancer Survival (n=245) among women treated with chemotherapy by GSTA1

**Int J Cancer, 2003**

**SURVIVAL BY GSTM1 & GSTT1 COMBINED**

<table>
<thead>
<tr>
<th>M1 &amp; T1</th>
<th>%</th>
<th>%</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>94 (38)</td>
<td>36 (49)</td>
<td>1.0</td>
</tr>
<tr>
<td>M1 or T1 Present</td>
<td>124 (49)</td>
<td>36 (43)</td>
<td>0.6 (0.3-0.9)</td>
</tr>
<tr>
<td>M1 &amp; T1</td>
<td>33 (8)</td>
<td>6 (8)</td>
<td>0.3 (0.1-0.7)</td>
</tr>
</tbody>
</table>

Hazard Ratio adjusted for age, race, stage at diagnosis, and ER/PR status.
Test for trend: p<0.05

**Cancer Res, 2001**

Chemotherapy, Radiation and ROS

- Proximate cause of cancer cell death by radiation and numerous chemotherapeutic agents (including A, C) - generation of ROS
- Oxidative stress results in damage to DNA, lipid peroxidation, protein modification, membrane disruption, mitochondrial damage, resulting in apoptotic cascade
- Inherited variability in generation of ROS, and repair of oxidative damage, could result in differential treatment outcomes
Endogenous Oxidant and Antioxidant Capabilities

Could variability in endogenous oxidant and antioxidant capabilities modify relationships between diet and breast cancer?

Polymorphisms in Genes Related to Oxidative Stress (MPO, MnSOD, CAT) and Survival After Treatment for Breast Cancer

Previously investigated in convenience sample derived from Tumor Registry at ACRC, DNA from normal lymph node tissue

251 women, 71 deaths

MnSOD CC – HR=0.66 (95% CI 0.34-1.29)

MPO GG – HR=0.60 (CI 0.38-0.95)

Pharmacogenomics and Prognosis

- Study limitations
  - heterogeneous population in disease and treatments; no ‘control’ group; limited sample size to evaluate gene/gene interactions

- With K. Albain, initiated study in SWOG 8897 (Hutchins, PI), clinical trial of CAF and CMF (R01 CA095222)

- Identified patients with normal lymph node tissue available (‘uncertain’ risk group), slides pulled from SWOG tissue bank (thanks to I-Tien Yeh) from both treated and untreated patients
Results

Cyclophosphamide metabolism

No associations between SNPs in 2B6 and 3A4 and DFS

No associations between GSTP1, GSTA1 SNPs and DFS

MPO -463 G/A and Breast Cancer Disease-Free Survival

Presented at San Antonio Breast Cancer Symposium, 2006
**Associations between MPO Genotype and DFS, Treated and Untreated Arms**

<table>
<thead>
<tr>
<th>genotype</th>
<th>Treated failures</th>
<th>HR (95% CI)</th>
<th>Treated censor</th>
<th>HR (95% CI)</th>
<th>Untreated failures</th>
<th>HR (95% CI)</th>
<th>Untreated censor</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>11</td>
<td>1.0 (ref)</td>
<td>19</td>
<td>1.0 (ref)</td>
<td>7</td>
<td>1.0 (ref)</td>
<td>11</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>AG</td>
<td>84</td>
<td>0.51 (0.26-0.99)</td>
<td>169</td>
<td>1.27 (0.59-2.76)</td>
<td>77</td>
<td>0.41 (0.21-0.77)</td>
<td>350</td>
<td>1.06 (0.50-2.31)</td>
</tr>
<tr>
<td>GG</td>
<td>194</td>
<td>0.41 (0.21-0.77)</td>
<td>350</td>
<td>1.06 (0.50-2.31)</td>
<td>77</td>
<td>0.51 (0.26-0.99)</td>
<td>169</td>
<td>1.27 (0.59-2.76)</td>
</tr>
</tbody>
</table>

HR adjusted for menopausal status, and time between surgery and chemotherapy.

**Skin Toxicity with Radiation Therapy**

- Patients undergoing radiation therapy following lumpectomy for breast cancer (Germany)
- Calculation of biologically effective dose (BED) to account for differences in fractionation and overall treatment time
- Side effects of grade 2c and above (at least 1 moist desquamation or interruption of RT due to toxicity)
- Modeled using Cox regression models (SAS)

**Toxicity by Patient Characteristics**

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>n</th>
<th>toxicity</th>
<th>HR¹ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 25</td>
<td>229</td>
<td>23</td>
<td>1.0</td>
</tr>
<tr>
<td>26-30</td>
<td>150</td>
<td>36</td>
<td>2.5 (1.5-4.3)</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>68</td>
<td>19</td>
<td>3.3 (1.8-6.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Current Smoking</th>
<th>n</th>
<th>toxicity</th>
<th>HR¹ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>399</td>
<td>67</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>48</td>
<td>11</td>
<td>1.5 (0.8-3.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol consumption</th>
<th>n</th>
<th>toxicity</th>
<th>HR¹ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>110</td>
<td>12</td>
<td>1.0</td>
</tr>
<tr>
<td>Yes</td>
<td>321</td>
<td>61</td>
<td>1.9 (1.0-3.5)</td>
</tr>
</tbody>
</table>

¹Hazard Ratio adjusted for clinic, photon field, beam energy, boost method
Toxicity by Glutathione S-Transferase Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>toxicity</th>
<th>HR¹ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTA1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>149</td>
<td>29</td>
<td>1.0</td>
</tr>
<tr>
<td>GA</td>
<td>194</td>
<td>35</td>
<td>1.2 (0.7-2.1)</td>
</tr>
<tr>
<td>AA</td>
<td>87</td>
<td>12</td>
<td>0.9 (0.4-1.8)</td>
</tr>
<tr>
<td>GSTP1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>176</td>
<td>27</td>
<td>1.0</td>
</tr>
<tr>
<td>AG</td>
<td>213</td>
<td>39</td>
<td>1.3 (0.8-2.2)</td>
</tr>
<tr>
<td>GG</td>
<td>38</td>
<td>10</td>
<td>2.5 (1.2-5.6)</td>
</tr>
</tbody>
</table>

¹Hazard Ratio adjusted for clinic, photon field, beam energy, boost method, BMI, smoking, alcohol, hormonal therapy

Toxicity by Combined DNA Repair Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal wt</th>
<th>Overweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1</td>
<td>APE1</td>
<td></td>
</tr>
<tr>
<td>Arg399Gln</td>
<td>Asp148Glu</td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Asp/Asp</td>
<td>1.0</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>Glu</td>
<td>0.20 (0.06-0.71)</td>
</tr>
<tr>
<td>Gln</td>
<td>Asp/Asp</td>
<td>0.19 (0.05-0.73)</td>
</tr>
<tr>
<td>Gln</td>
<td>Gin</td>
<td>0.19 (0.06-0.56)</td>
</tr>
</tbody>
</table>

¹Hazard Ratio adjusted for clinic, photon field, beam energy, boost method, BMI, smoking, alcohol, hormonal therapy

DNA Repair Pathways

DNA Repair Genes (N = 130) Hoeijmakers (2001), Nature
DNA Repair and AML Therapeutic Outcomes

- 200 older (> 56 y) patients with de novo or secondary (23%) AML from Southwest Oncology Group clinical trials SWOG-9031 and SWOG-9333
- Received standard induction regimens: Cytarabine, 200mg/m² per day x 7 and Daunorubicin, 45 mg/m² per day x 3 (plus either Placebo or G-CSF on SWOG-9031)
- Genotypes of patients’ bone marrow samples determined by MALDI-TOF-MS

Logistic Regression Analyses of Toxicities

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype variant</th>
<th>Metabolic Toxicity</th>
<th>Lung Toxicity</th>
<th>Liver Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>CI</td>
<td>OR</td>
<td>CI</td>
</tr>
<tr>
<td>ERCC1</td>
<td>CC</td>
<td>1.00</td>
<td>----</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>0.41</td>
<td>0.19-0.90</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>0.23</td>
<td>0.04-1.81</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>CA OR AA</td>
<td>0.39</td>
<td>0.19-0.82</td>
<td>1.37</td>
</tr>
<tr>
<td>XRCC3</td>
<td>CC</td>
<td>1.00</td>
<td>----</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>1.18</td>
<td>0.56-2.57</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>1.84</td>
<td>0.46-12.30</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>CT OR TT</td>
<td>1.27</td>
<td>0.61-2.65</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Activation of Tamoxifen

Tamoxifen metabolized to endoxifen by CYP2D6; Extensive and poor 2D6 metabolizers

Better survival for extensive metabolizers
Also more hot flashes

Goetz, M.P. et al.
Tamoxifen and Sulfotransferase 1A1

• 4-hydroxytamoxifen, a tamoxifen metabolite, is a potent antiestrogen
• SULT1A1 sulfates 4-OH-TAM, is also upregulated 10-fold by tamoxifen
• G to A transition (Arg to His) results in enzyme with much lower catalytic activity and decreased thermostability.
• Hypothesized that low activity would result in better survival

SULT1A1 Genotype and Breast Cancer Survival among Tamoxifen Treated Patients

Nowell, JNCI 2002

SULT1A1 Genotype and Breast Cancer Survival among Patients Not Treated with Tamoxifen

Nowell, JNCI 2002
**Limitations**

- Very limited quantities of DNA – only single SNPs assessed
- Limited number of genes in pathways examined
- Limited sample size
- Need for high quality DNA (WBCs) from large clinical trials, comprehensive assessment of variability across genes in pathways

**Identifying Genes that Modify Treatment Outcomes**

I. Identify genes in candidate pathways
   - Drug metabolism, DNA repair, oxidative stress
   - Genotype for known functional SNPs
   - Haplotypes to determine variability across gene

II. Assess variability across entire genome (Genome Wide Association Study)

**S0221: Phase III Trial of Continuous Schedule AC+G vs Q2 Week Schedule AC, Followed by Paclitaxel Given Either Every 2 Weeks or Weekly for 12 Weeks as Post-Operative Adjuvant Therapy in Node-Positive or High-Risk Node Negative Breast Cancer (Budd, PI)**

*Accrual goal – 3,250*
Collection of 2 tubes of blood from consenting patients (1 red-top banked for banked serum, 1 purple top for DNA extraction)

As of 3/27/08, samples received from 903 patients

Also collection of questionnaire data

Application to TBCI for use of DNA for Genome Wide Study in relation to toxicity and DFS

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Pharmacogenetics in SWOG

- Goal to collect blood or saliva from all patients on clinical trials
- Samples useful for future pharmacogenetic studies
- For drugs with clear metabolic pathways, pharmacogenetics can lead to individualized therapy – decrease toxicities, increase likelihood of DFS