“A surgeon who uses the wrong side of the scalpel cuts her own fingers and not the patient;

if the same applied to drugs they would have been investigated very carefully a long time ago”

Rudolph Bucheim  
Beitrage zur Arzneimittellehre, 1849

The clinical problem
• Multiple active regimens for the treatment of most diseases
• Variation in response to therapy
• Unpredictable toxicity

$\ddots$

With choice comes decision
What is your intent?

**Drug Safety**

- Clinical trial
- n/exclusion

**Clinical practice**

Pharmacogenomic examples-2012

- **bcr/abl** or 9:22 translocation—imatinib mesylate*
- **HER2-neu**—trastuzumab**
- C-kit mutations—imatinib mesylate**
- Epidermal growth factor receptor mutations—gefitinib
- Thiopurine S-methyltransferase—mercaptopurine and azathioprine*
- UGT1A1—irinotecan**
- CYP2C9/VKORC1—warfarin*
- HLA-B*5701—abacavir .
- HLA-B*1502—carbamazepine .
- CYP2C19—clopidogrel
- IL28B-interferon
- Cytochrome P-450 (CYP) 2D6—5-HT3 receptor antagonists, antidepressants, ADHD drugs, and codeine derivatives, tamoxifen*
Applications of pharmacogenetics

- Explanation for untoward event (DPYD, CYP2D6)
- Required for insurance coverage (KRAS, EGFR, ABL)
- Identify low utility (KRAS)
- Dose selection (CYP2C9, CYP2C19)
- Therapy selection (CYP2C19)
- Preemptive prediction (HLA-B*5701)

What needs to be done to determine hope vs hype?

- Find the 'right' biomarkers
- Validate in robust datasets
- Apply them!
We do not know very much about Irinotecan cell drugs.
We are only beginning to try!

As of 3/10/12
Drug-related phenotypes represented
50/1196 GWA studies (4.1%)

10/50 had ≥ 500 ‘cases’

15/50 (30%) found no significant ‘hits’
29/50 PGx studies had a replication cohort

8 contributed to changes in FDA ‘package insert’

Centre d’Etude du Polymorphisme
Human (CEPH) Cell lines

• Large, multigeneration pedigrees widely studied
• Immortalized lymphoblastoid cell lines
**Methodology**

- Cells counted, plated at $1 \times 10^4$ / well
- Cells incubated with increasing concentrations of drug
- Alamar blue vital dye indicator added

Viability relative to untreated control calculated by spectrophotometry

**Significant Variation in Cellular Sensitivity to Docetaxel**
'CE-PH/F-DA' project

- 126 CEPH cell lines from 14 nuclear families
- All FDA approved cytotoxic drugs + new kinase inhibitors/MTOR/demethylation
- No antiestrogen or vitamin A analogues
- Evaluate degree of heritability, presence of QTL(s), and evidence for correlations between drug sensitivity patterns.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>$h^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temo</td>
<td>4</td>
<td>63.51</td>
</tr>
<tr>
<td>Epi</td>
<td>1</td>
<td>59.48</td>
</tr>
<tr>
<td>Oxal</td>
<td>1</td>
<td>50.03</td>
</tr>
<tr>
<td>Mitox</td>
<td>1</td>
<td>46.46</td>
</tr>
<tr>
<td>Topi</td>
<td>1</td>
<td>46.12</td>
</tr>
<tr>
<td>Pac</td>
<td>1</td>
<td>45.91</td>
</tr>
<tr>
<td>Ida</td>
<td>1</td>
<td>45.83</td>
</tr>
<tr>
<td>Cytos</td>
<td>2</td>
<td>43.8</td>
</tr>
<tr>
<td>Hydrox</td>
<td>2</td>
<td>43.22</td>
</tr>
<tr>
<td>Cytar</td>
<td>1</td>
<td>41.74</td>
</tr>
<tr>
<td>Etop</td>
<td>1</td>
<td>41.26</td>
</tr>
<tr>
<td>Daun</td>
<td>1</td>
<td>37.14</td>
</tr>
<tr>
<td>Acet</td>
<td>1</td>
<td>36.98</td>
</tr>
<tr>
<td>Tax</td>
<td>3</td>
<td>36.37</td>
</tr>
<tr>
<td>Dox</td>
<td>1</td>
<td>35.3</td>
</tr>
<tr>
<td>Vino</td>
<td>1</td>
<td>34.13</td>
</tr>
<tr>
<td>Vino</td>
<td>4</td>
<td>31.17</td>
</tr>
<tr>
<td>Eto</td>
<td>3</td>
<td>30.11</td>
</tr>
</tbody>
</table>

Maximum heritability over all doses

Peters et al 2011
Brown et al submitted cells from 563 unrelated individuals treated with Temozolomide.

In vitro GWAS as filter for candidate genes

(A) Box plots for the estimated percent viability at 0.25 mmol by genotype for rs531572.

(B) Boxplots of MGMT transcript levels differ by rs531572 genotype.

MGMT SNP is associated with chemosensitivity and mRNA expression.
What needs to be done to determine hope vs hype?

• Find the 'right' biomarkers
• Validate in robust datasets
• Apply them!

*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.

Source: American Cancer Society 2006.
Docetaxel vs. Paclitaxel
(Clinical data: SCOTROC1)

With:
- RT
- Docetaxel 75 mg/m² 1-hr IV, followed by Carboplatin AUC 5 * IV
  Repeat q 3 wk for up to 6 cycles
- Paclitaxel 175 mg/m² 3-hr IV, followed by Carboplatin AUC 5 * IV
  Repeat q 3 wk for up to 6 cycles

Study End Points
Primary: progression-free survival
Secondary: response rate, overall survival, toxicity, QOL

Sarah Glass, Alison Motsinger-Reif, Sharon Marsh, Bob Brown, Jim Paul

![Progression-free survival](Vasey et al JNCI 2004)

![Overall survival](Vasey et al JNCI 2004)
## Chemotherapy

### Biology:
- Nerve function
- Gene
- Inheritance

### SNPs

**Table 5. NCI-CTC neurotoxicity in the Scottish Randomised Trial in Ovarian Cancer**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Docetaxel–carboplatin arm (n = 537)</th>
<th>Paclitaxel–carboplatin arm (n = 532)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>22</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

*NCI-CTC = National Cancer Institute–Common Toxicity Criteria.
†Not available for two patients who died after one cycle.
‡Not available for one patient who died after one cycle.
§All statistical tests were two-sided. P value from Mann–Whitney U test.
||| Grades 1–4.
||| Total.

- Vasey et al JNCI 2004

---

### Docetaxel vs. Paclitaxel

**Clinical data: SCOTROC1**

### Pharmacology:
- Drug Action

### Genes

### Neurotoxicity:
- Candidate Gene Approach

- Illumina GoldenGate™ SNP Array

**Docetaxel vs. Paclitaxel**

**Genes**

**g + function**

**Neurotoxicity:**

- Candidate Gene Approach
The filtering of Neuro-risk genotypes

Quality Assessment
1261 SNPs

SNPs Significantly Associated in Test cohort
69 SNPs

SNPs Significantly Associated in Validation cohort
5 SNPs

Consistent direction of genetic risk
4 SNPs

Table 1: SNPs significantly associated with severe neurotoxicity in the validation cohort

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Base Change</th>
<th>Corrected P-value</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>Risk Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs139087</td>
<td>SOX10</td>
<td>C-&gt;G</td>
<td>0.001</td>
<td>2.87</td>
<td>(1.4361, 5.7530)</td>
<td>CG</td>
</tr>
<tr>
<td>rs2849380</td>
<td>BCL2</td>
<td>A-&gt;G</td>
<td>0.013</td>
<td>4.08</td>
<td>(1.5254, 10.8975)</td>
<td>AA</td>
</tr>
<tr>
<td>rs544093</td>
<td>OPRM1</td>
<td>A-&gt;C</td>
<td>0.015</td>
<td>2.25</td>
<td>(1.2365, 4.0841)</td>
<td>AA</td>
</tr>
<tr>
<td>rs879207</td>
<td>TRPV1</td>
<td>A-&gt;G</td>
<td>0.002</td>
<td>2.31</td>
<td>(1.4467, 3.6767)</td>
<td>AG</td>
</tr>
</tbody>
</table>

Table 2: Percent PAR for each SNP and joint PAR

<table>
<thead>
<tr>
<th>SNP</th>
<th>PAR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs139087</td>
<td>45.8</td>
</tr>
<tr>
<td>rs2849380</td>
<td>9.1</td>
</tr>
<tr>
<td>rs544093</td>
<td>50.2</td>
</tr>
<tr>
<td>rs879207</td>
<td>38.4</td>
</tr>
<tr>
<td>All SNPs</td>
<td>84.9</td>
</tr>
</tbody>
</table>
Cumulative impact of Neuro-risk genotypes

Figure 2: Number of Risk Genotypes by Predicted and Observed Odds Ratio

Neuro-risk genotypes not associated with outcome

Figure 3: Relationship between genotype risk score (0-2 vs 3-4) and (A), progression free survival (p=0.75) or (B) overall survival (p=0.54)
What needs to be done to determine hope vs hype?

• Find the 'right' biomarkers

• Validate in robust datasets

• Apply them!
Tamoxifen Metabolism

Endoxifen

CYP2D6

CYP3A4

CYP2C9

CYP2C19

CYP3A4

Endoxifen

Tamoxifen

partial-antagonist

1% rel. to E2

N-didesmethyl

partial-antagonist

<1% rel. to E2

Metabolite E:

full agonist

?% rel. to E2

N-desmethyl:

partial-antagonist

1% rel. to E2

3,4-hydroxy:

potent - antagonist

100% rel. to E2

4-hydroxy, N-desmethyl:

potent - antagonist

100% rel. to E2

Metabolite Y:

partial-agonist

1% rel. to E2

CYP3A4

CYP2D6

CYP2C9

CYP2C19

CYP3A4

CYP2D6

CYP3A4

CYP2C9

CYP2C19

CYP3A4

Endoxifen

Tamoxifen

partial-antagonist

1% rel. to E2

N-didesmethyl

partial-antagonist

<1% rel. to E2

Metabolite E:

full agonist

?% rel. to E2

N-desmethyl:

partial-antagonist

1% rel. to E2

3,4-hydroxy:

potent - antagonist

100% rel. to E2

4-hydroxy, N-desmethyl:

potent - antagonist

100% rel. to E2

Endoxifen

Relapse-free Survival

100

80

60

40

20

0

0 2 4 6 8 10 12

Log Rank

EM

EM-extensive metabolizer

IM-extreme metabolizer

PM-poor

2-year RFS

EM 98%

IM 92%

PM 68%

EM

IM

PM

Years after randomization


EM

IM

PM

Log Rank

P=0.009

EM-extensive metabolizer

IM-intermediate

PM-poor

Years after randomization

Log Rank

P=0.009

EM-extensive metabolizer

IM-intermediate

PM-poor

2-year RFS

EM 98%

IM 92%

PM 68%

EM

IM

PM

Log Rank

P=0.009
Adjuvant Tamoxifen and CYP2D6

- **CYP2D6 associated with recurrence**
  - Goetz et al. 2005, 2007 (USA)
  - Schroth et al. 2007 (Germany)
  - Kiyotani et al. 2008 (Japan)
  - Newman et al. 2008 (UK)
  - Xu et al. 2008 (China)
  - Okishiro et al. 2009 (Japan)
  - Ramon et al. 2009 (Spain)
  - Bijn et al. 2009 (Netherlands)
  - Schroth et al. 2009, 2010 (Germany, USA)
  - Fugisata et al. 2010 (Japan)
  - Lammers et al. 2010 (Netherlands)
  - Kiyotani et al. 2010 (Japan)
  - Thompson et al. 2010 (UK)
  - Kiyotani et al. 2012 (Japan)

- **CYP2D6 not associated with recurrence**
  - Wegman et al. 2005, 2007 (Sweden)
  - Nowell et al. 2005 (USA)
  - Abraham et al. 2010 (UK)
  - Goetz et al. 2011 (USA)
  - Rae et al. 2012 (UK)
  - Regan et al. 2012 (USA/Europe)

**Relapse-free Survival**

CYP2D6-guided tamoxifen dosing normalizes endoxifen levels in IM patients

All patients on tamoxifen 20 mg/day for 4 months then
EM-20 mg
IM-change to 40 mg

N=119

Start of study 4 months on study

P=0.84

Irvin et al J Clin Oncol 2011

Study of 500 patients across NC is nearly completed, with oversampling of African American and Hispanic patient

Implementation Science can be conducted where most patients are treated
Comprehensive optimization of patient care

Does pharmacogenetics have relevance for public health?

Pharmacogenetics for Every Nation Initiative  pgeni.org

Disease Genotypes
Infection Defense Genotypes
Supportive Care Genotypes
Toxicity-risk Genotypes
Modern medical therapy is a key component of improved health

Selection of medications for each indication is a combination of clinical consensus, access/cost of drugs, and familiarity

Medicine prioritization is a high stakes undertaking

We need to use all available data
Background: Source of data for patient therapy selection

Best option: individual

Good: relevant geographic/ethnic/racial population

Worst: inferred world population

Voltaire

• "The best is the enemy of good."
Continents are more similar than different
-but context is everything

1,936 functional mutations in 225 genes
Optimal dose for each patient differs by TPMT genotype

Relling et al. JNCI, 1999

Legend
Reference Freq. = x, Country Freq. = y

© PGENI 2012
CYP2C19 allele frequency
Type of output

**Surveillance** - identifying population subgroups at higher risk of toxicity or treatment failure

**Prioritization** - assisting the treatment selection from among WHO recommended therapies

Example:

Population Genotypes > “risk threshold” for TYMS, NAT2, MTHFR, but not TPMT
**Pharmacogenomics**

- **First Line Therapy**
  - Methotrexate (MTX)
  - RA patient
  - TYMS
  - MTX + corticosteroids
  - Add post treatment folic acid

- **Second Line Therapy**
  - Azathioprine (AZA)
  - Sulfasalazine (SSZ)
  - Therapeutic options
    - NAT2 variant
    - TPMT*1
    - Risk of SSZ induced neutropenia

**RA patient**

- *TYMS* 3'3
- *MTHFR* 677T

**Increased risk of treatment failure**

**Increased risk of toxicity**

**Risk of SSZ induced neutropenia**

**Therapeutic options**

**PGEni Recommendation for China**

- Using US Caucasian population frequency data as a reference, based on genetic variant frequency information, the following therapy strategy is suggested for China:
  - **First Line** Methotrexate (MTX) with supplemental corticosteroid to improve efficacy
  - **Second Line** Enfuaenziopine (AZA), or sulfasalazine (SSZ), as needed

**NOTE**: Pharmacogenetic information is one of many factors influencing the choice of therapy and shouldn't be used as the sole basis for drug selection.
Pharmacogenomic examples-2012

- **bcr/abl** or 9:22 translocation—imatinib mesylate*
- HER2-*neu*—trastuzumab**
- C-kit mutations—imatinib mesylate**
- Epidermal growth factor receptor mutations—gefitinib
- Thiopurine S-methyltransferase—mercaptopurine and azathioprine*
- UGT1A1-irinotecan**
- CYP2C9/VKORC1-warfarin*
- HLA-B*5701-abacavir
- HLA-B*1502-carbamazepine*
- CYP2C19-clopidogrel
- IL28B-interferon
- Cytochrome P-450 (CYP) 2D6—5-HT3 receptor antagonists, antidepressants, ADHD drugs, and codeine derivatives, tamoxifen*

*Not a rare issue!
Applications of pharmacogenetics

- Explanation for untoward event (DPYD, CYP2D6)
- Required for insurance coverage (KRAS, EGFR, ABL)
- Identify low utility (KRAS)
- Dose selection (CYP2C9, CYP2C19)
- Therapy selection (CYP2C19)
- Preemptive prediction (HLA-B*5701)

Bundled care
Patient safety
‘bounce back’ avoidance
Pharmacy & Therapeutics committee
National formulary
Others…….

Boring!
Marker Discovery \rightarrow Marker Validation

Health Economics

- $$$
- $$

Health system integration

Medical informatics

IF

THEN

Research assay to Clinical assay

Routine Clinical Use

changing old habits
Table 1: Range of Expected Therapeutic Warfarin Doses Based on CYP2C9 and VKORC1 Genotypes

<table>
<thead>
<tr>
<th>VKORC1</th>
<th>CYP2C9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1/*1</td>
</tr>
<tr>
<td>GG</td>
<td>5-7 mg</td>
</tr>
<tr>
<td>AG</td>
<td>5-7 mg</td>
</tr>
<tr>
<td>AA</td>
<td>3-4 mg</td>
</tr>
</tbody>
</table>

Ranges are derived from multiple published clinical studies. Other clinical factors (e.g., age, race, body weight, sex, concomitant medications, and comorbidities) are generally accounted for along with genotype in the ranges expressed in the Table. VKORC1 −1639 G>A (rs9923231) variant is used in this table. Other co-inherited VKORC1 variants may also be important determinants of warfarin dose. Patients with CYP2C9 *1/*3, *2/*2, *2/*3 and *3/*3 may require more prolonged time (>2 to 4 weeks) to achieve maximum INR effect for a given dosage regimen.

Factors that Correlate w/ Warfarin Dose

- Age
- Body surface area (BSA) or weight
- Amiodarone dose
- Other drugs (e.g., HMG CoA Reductase inhibitors)
- Target INR
- Race
- Sex
- Plasma vitamin K level
- Decompensated CHF or post-operative state
- CYP2C9 and VKORC1 genotype
Tools to Implement Warfarin PGx

Translational science: The steps to success

Step I: Discovery

Step II: Validation

Step III: Integration into practice

Step IV: Integration into policy

Boring!
We now have new audiences

Past
- Ourselves
- Editors/reviewers
- Study section

Now
- Clinic administrators
- Payers
- Patients

We now have new (additional) endpoints

Past
- Survival
- Stent thrombosis
- Severe neutropenia

Now
- Selection from amongst ‘equal’ therapies
- Return on investment for medical home
- Quality measures
- Patient satisfaction
I have ears, but cannot hear

- 44 year old white male (CSO at local biotech)
- AV block 2° congenital heart disease
- Presents for placement of epicardial pacemaker
- Tells cardiologist, CT surgeon, anesthesiologist, and admitting team (cardiology fellow, resident, intern) that an executive physical revealed genetic data relevant to pain control and anticoagulation
- Adequate pain control (4/10) in recovery room on MS moved to CCU and switch to oxycodone during the night, waking up in severe pain (10/10), ignored x 24 hours
- Student and PharmD recognized CYP2D6 PM and patient was switched to hydromorphone (5/10)

Thank you to the PGENIUSES!
Because everybody's therapy is not your body's therapy.