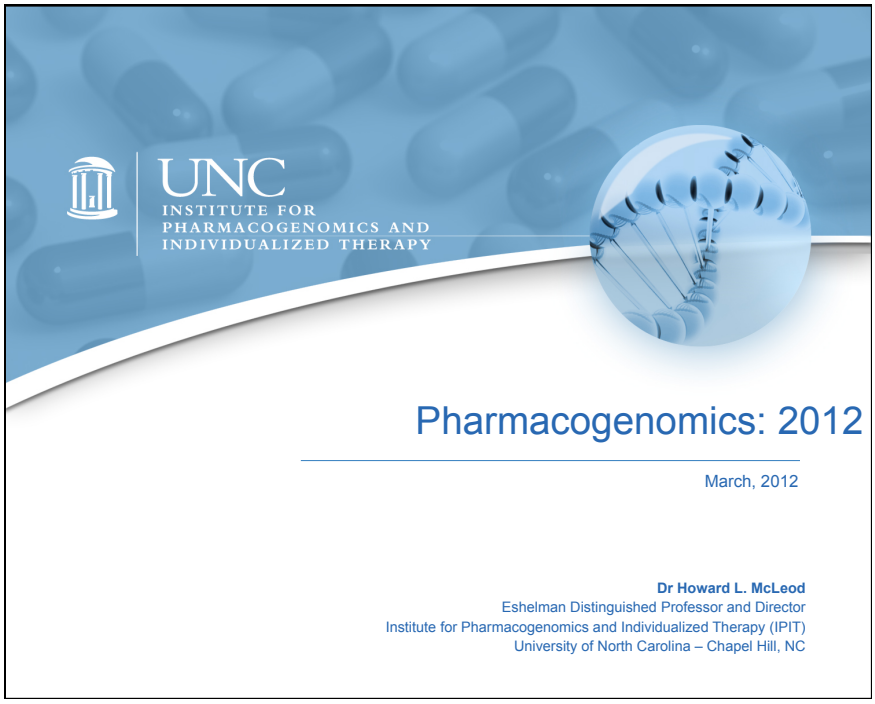
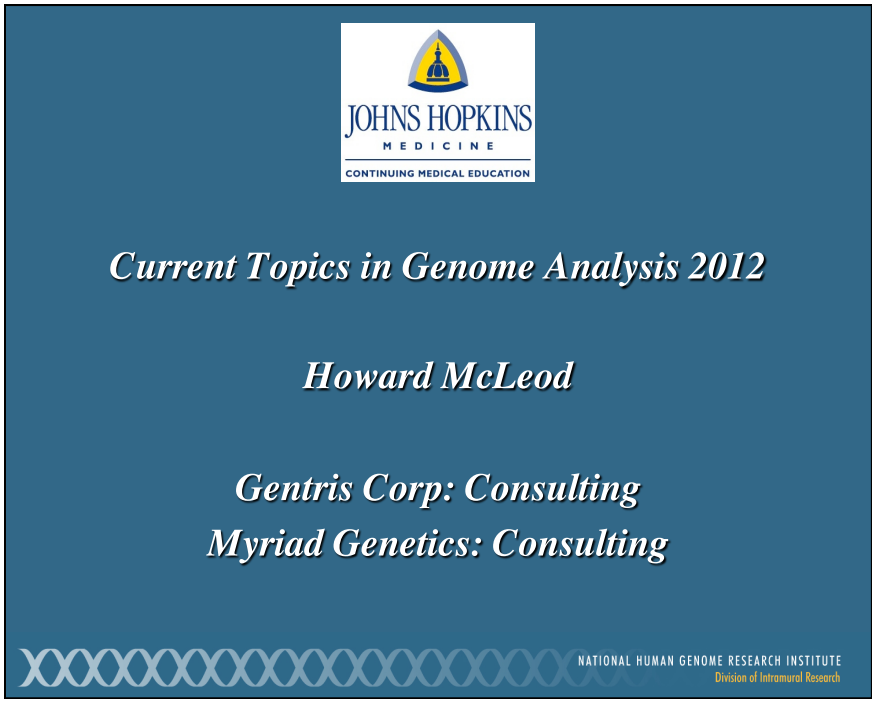
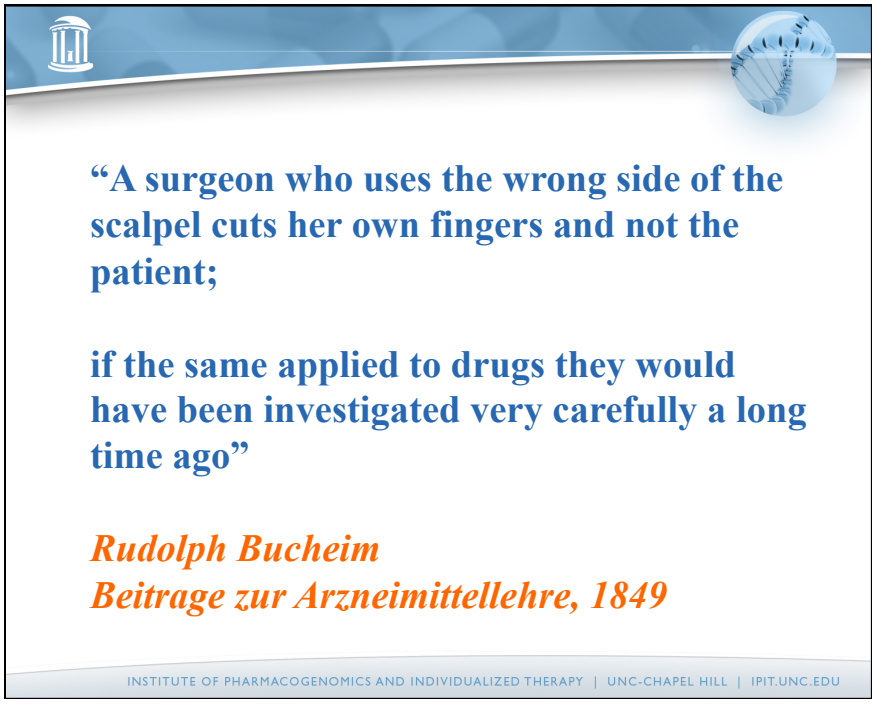

UNC
INSTITUTE FOR
PHARMACOGENOMICS AND
INDIVIDUALIZED THERAPY
Pharmacogenomics: 2012
March, 2012
Dr Howard L. McLeod
Eshelman Distinguished Professor and Director
Institute for Pharmacogenomics and Individualized Therapy (IPIT)
University of North Carolina – Chapel Hill, NC


JOHNS HOPKINS
MEDICINE
CONTINUING MEDICAL EDUCATION
Current Topics in Genome Analysis 2012
Howard McLeod
Gentris Corp: Consulting
Myriad Genetics: Consulting
NATIONAL HUMAN GENOME RESEARCH INSTITUTE
Division of Intramural Research


"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
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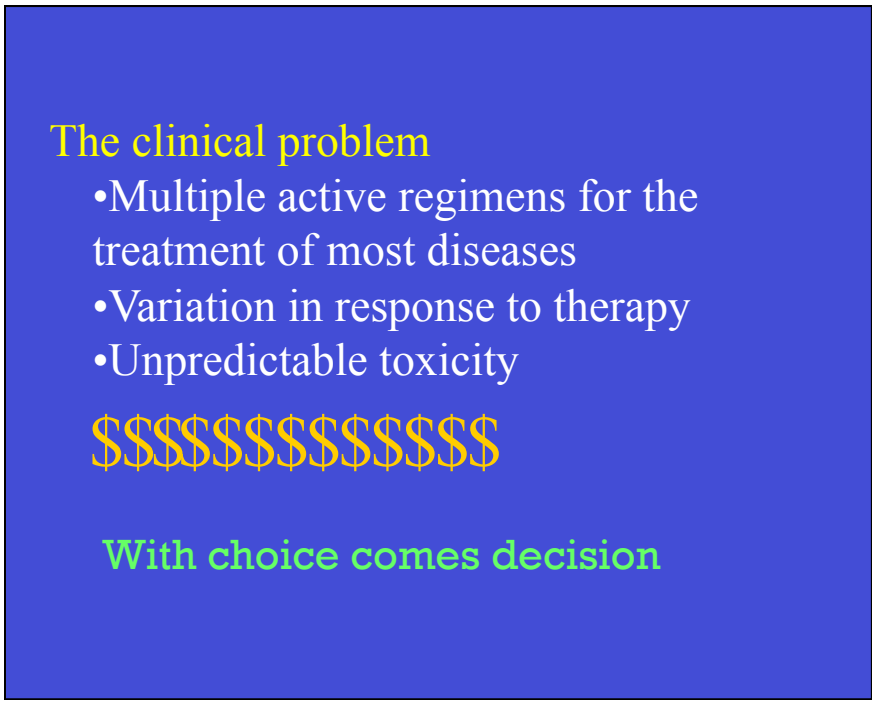
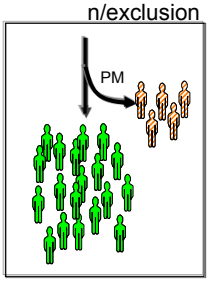

The clinical problem
•Multiple active regimens for the treatment of most diseases
•Variation in response to therapy
•Unpredictable toxicity
$$$$$$$$$$$$
With choice comes decision

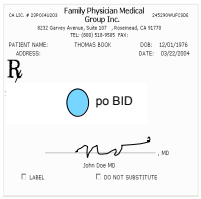
what is your intent?

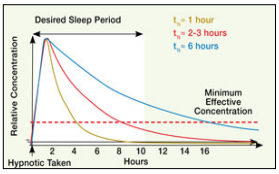
Drug Safety

cal 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*

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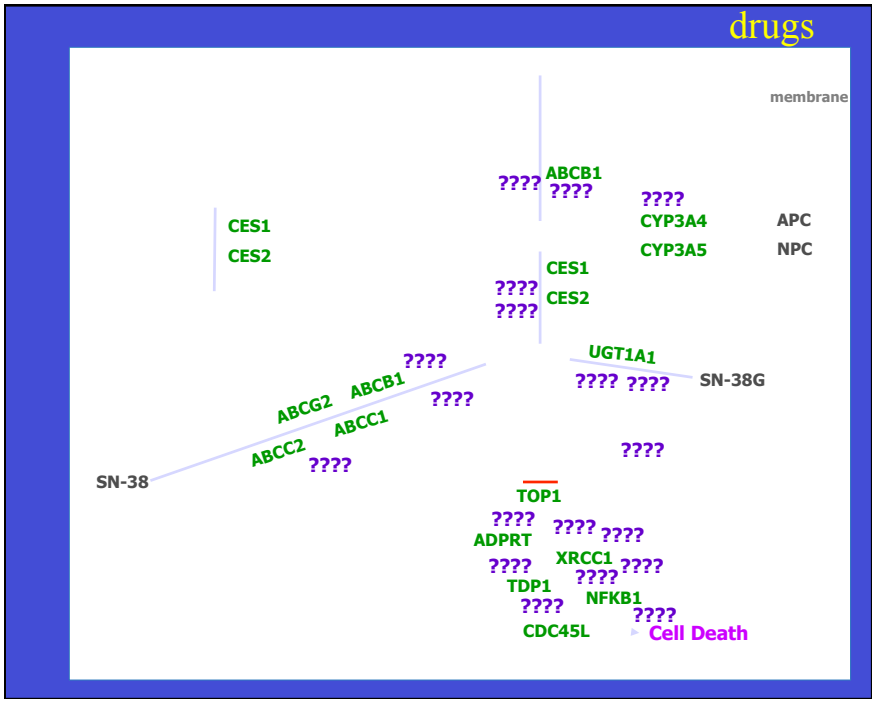
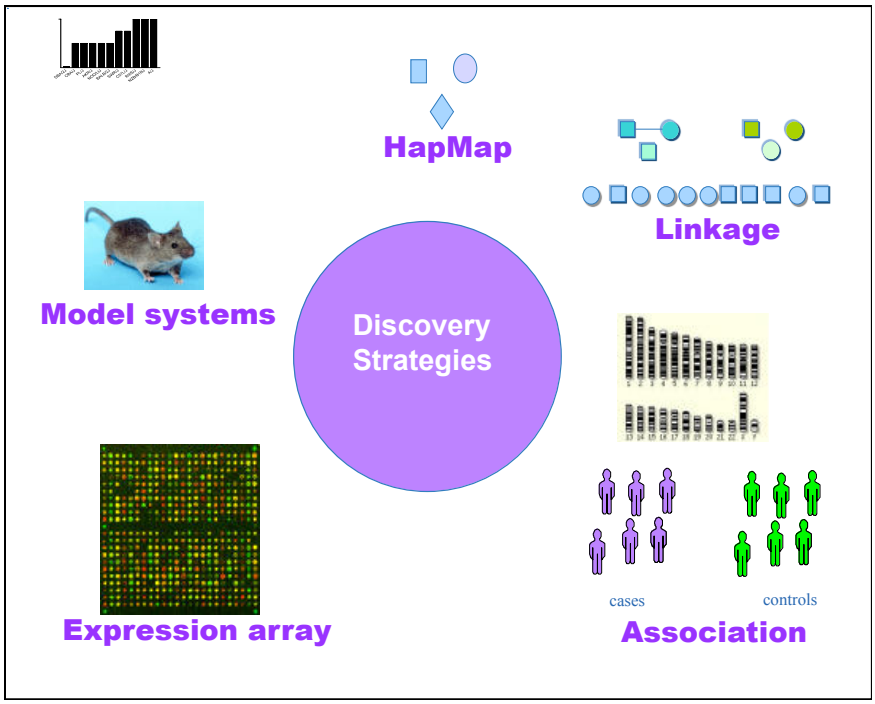
Applications of pharmacogenetics

- 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!


drugs
membrane
????
ABCB1
????
????
CYP3A4
CYP3A5
APC
NPC
CES1
CES2
CES1
????
????
CES2
UGT1A1
????
????
SN-38G
????
ABCG2
ABCB1
????
ABCC1
ABCC2
????
SN-38
????
TOP1
????
????
????
ADPRT
????
XRCC1
????
????
TDP1
NFKB1
????
????
CDC45L
Cell Death


HapMap
Linkage
Model systems
Discovery Strategies
Expression array
cases
controls
Association

We are only beginning to try!

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

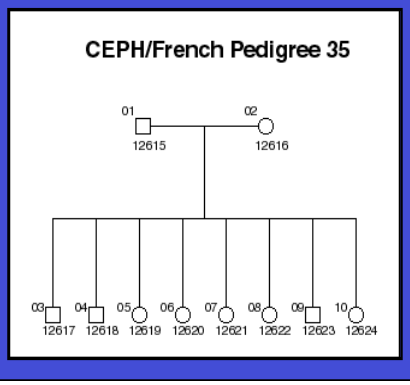
  10/50 had ≥ 500 'cases'

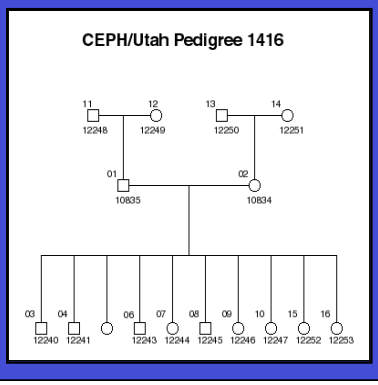
  15/50 (30%) found no significant 'hits'
  29/50 PGx studies had a replication cohort

  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



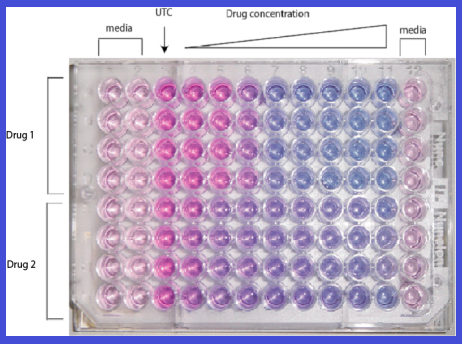


Methodology

Cells counted, plated at 1 x 10^4 / well

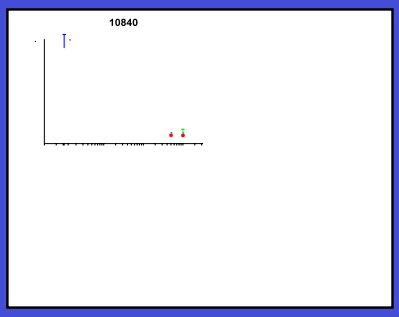
Cells incubated with increasing concentrations of drug

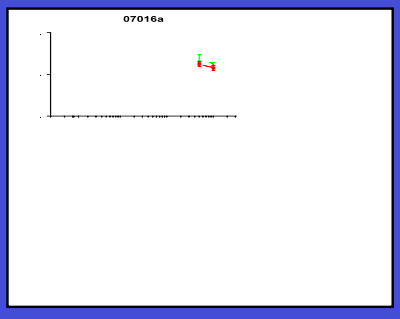
Alamar blue vital dye indicator added



Viability relative to untreated control calculated by spectrophotometry

Significant Variation in Cellular Sensitivity to Docetaxel



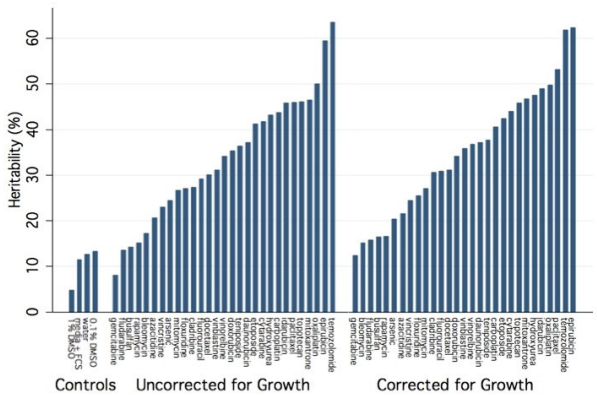


'CE-PH/F-DA' project

- 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.

Maximum heritability over all doses

Drug	Dose	h^2
Temo	4	63.51
Epi	1	59.48
Oxal	1	50.03
Mitox	1	46.46
Topo	1	46.12
Pac	1	45.91
Ida	1	45.83
Carbo	2	43.8
Hydrox	2	43.22
Cytar	1	41.74
Etop	1	41.26
Daun	1	37.14
Ace	1	36.98
Teni	3	36.37
Dox	1	35.3
Vino	1	34.13
Vinb	4	31.17
Doc	3	30.11



Peters et al 2011

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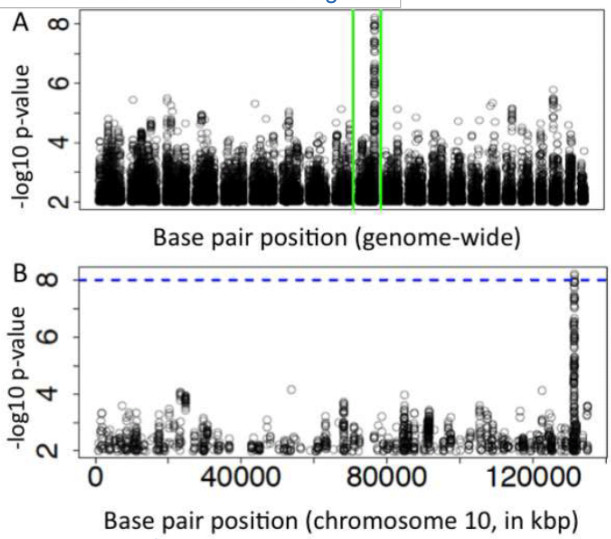
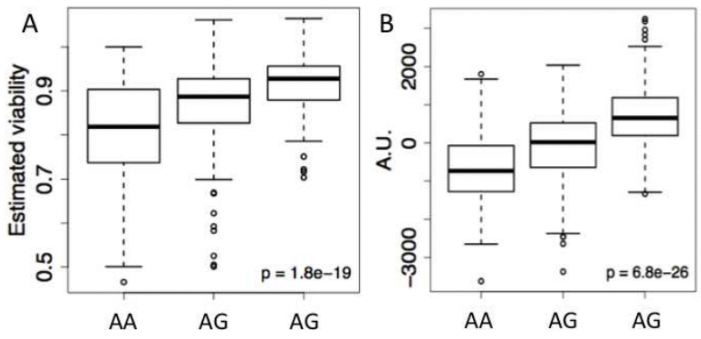
In vitro GWAS as filter for candidate genes



Brown et al submitted cells from 563 unrelated individuals Treated with Temozolomide

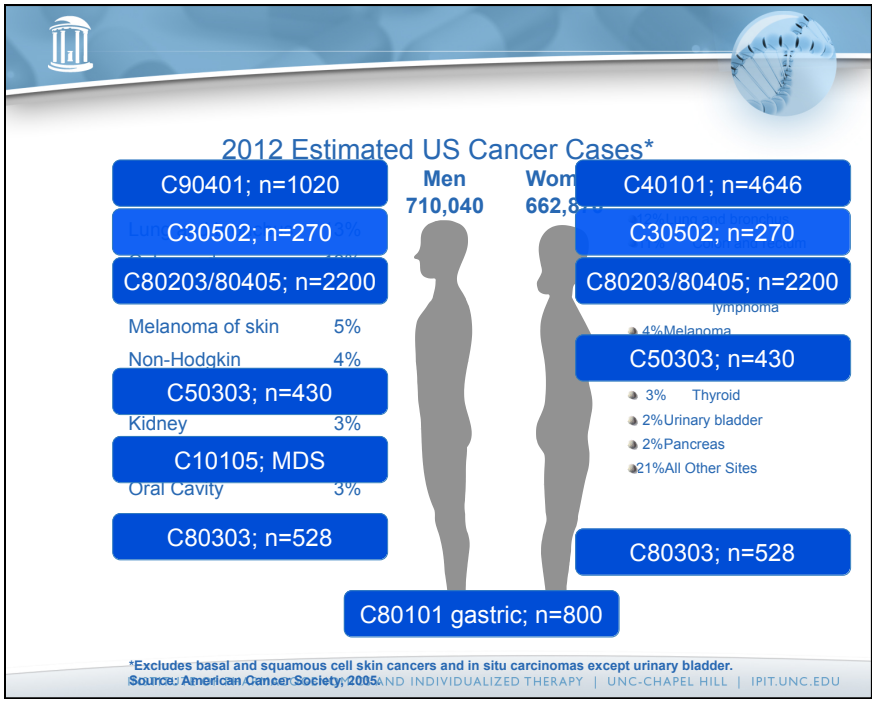
MGMT SNP is associated with chemosensitivity and mRNA expression



(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

What needs to be done to determine hope vs hype?

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



Docetaxel vs. Paclitaxel (Clinical data: SCOTROC1)

With:
rian cancer

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

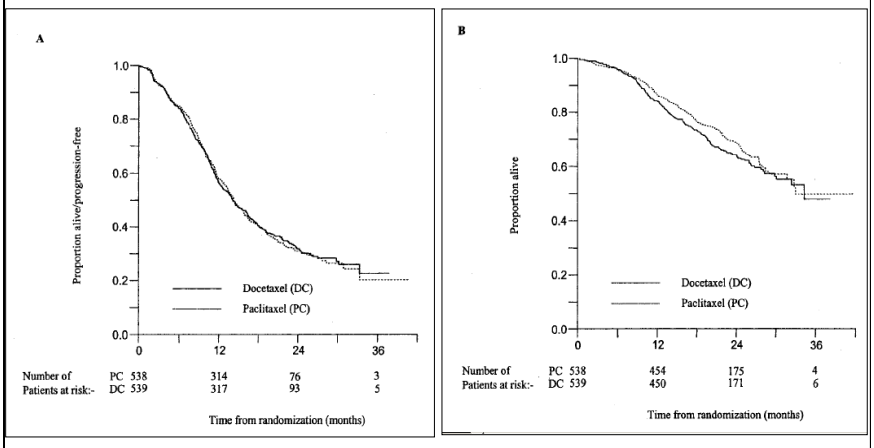
Study End Points
Primary: progression-free survival
Secondary: response rate, overall survival, toxicity, QOL

CANCER RESEARCH UK

scottish gynaecological cancer trials group

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

Docetaxel vs. Paclitaxel (Clinical data: SCOTROC1)



Progression-free survival

Overall survival

Vasey et al JNCI 2004

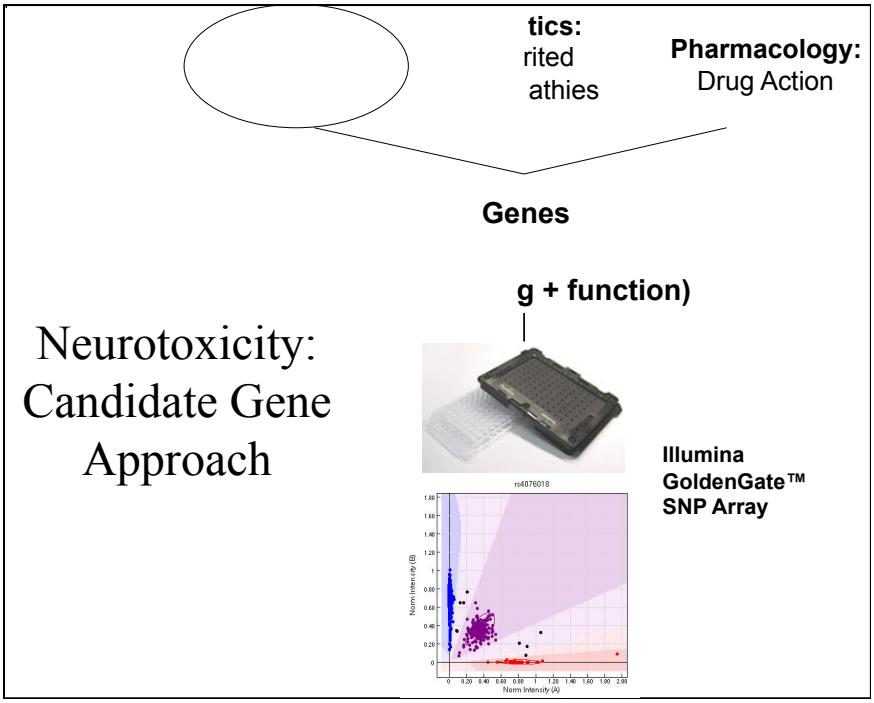
Docetaxel vs. Paclitaxel (Clinical data: SCOTROC1)

Table 5. NCI–CTC neurotoxicity in the Scottish Randomised Trial in Ovarian Cancer 1*

Grade	% of patients: Docetaxel–carboplatin arm (n = 537)†	% of patients: Paclitaxel–carboplatin arm (n = 532)‡	*P*
Sensory‖			
1	35	48	
2	9	22	
3	2	8	<.001‖
4	0	0	
Total	45	78	<.001¶
Motor¶			
1	6	9	
2	2	5	
3	1	2	.005‖
4	0	0	
Total	9	16	.001¶

*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



The filtering of Neuro-risk genotypes

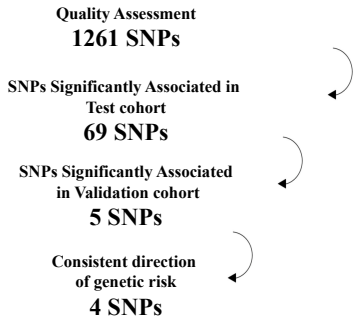


Figure 1: The workflow of the data analysis, represented by the narrowing number of SNPs at each stage of the analysis.

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

SNP	Gene	Base Change	Corrected P-value	Odds Ratio	95% CI	Risk Genotype
rs139887	SOX10	C->G	0.001	2.87	(1.4361, 5.7530)	CG
rs2849380	BCL2	A->G	0.013	4.08	(1.5254, 10.8975)	AA
rs544093	OPRM1	A->C	0.015	2.25	(1.2365, 4.0841)	AA
rs879207	TRPV1	A->G	0.002	2.31	(1.4467, 3.6767)	AG

Table 2: Percent PAR for each SNP and joint PAR

	rs139887	rs2849380	rs544093	rs879207	All SNPs
PAR (%)	**45.8**	**9.1**	**50.2**	**38.4**	**84.9**

Cumulative impact of Neuro-risk genotypes

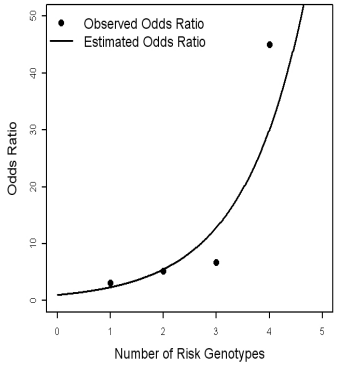


Figure 2: Number of Risk Genotypes by Predicted and Observed Odds Ratio

Neuro-risk genotypes not associated with outcome

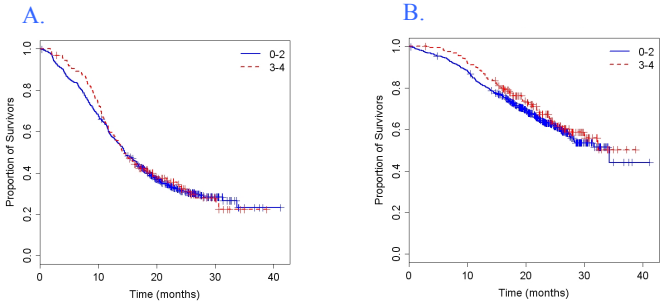
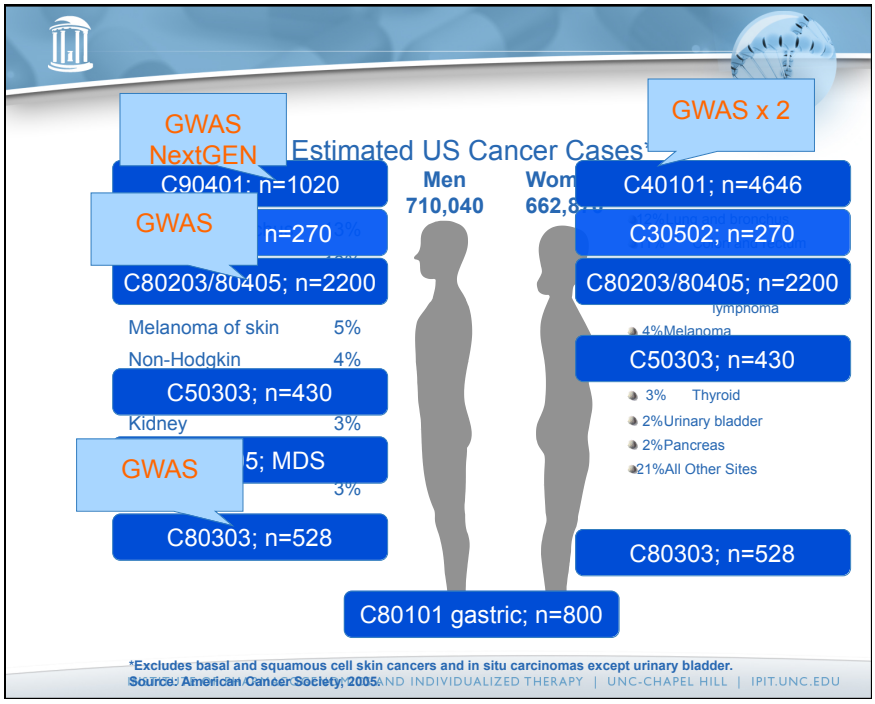


Figure 3: Relationship between genotype risk score (0-2 vs 3-4) and (A), progression free survival (p=0.75) or (B) overall survival (p=0.54)


GWAS NextGEN
GWAS x 2
Estimated US Cancer Cases*
Men
710,040
C90401; n=1020
C40101; n=4646
GWAS
n=270
C30502; n=270
C80203/80405; n=2200
C80203/80405; n=2200
Melanoma of skin 5%
Non-Hodgkin 4%
C50303; n=430
Kidney 3%
C50303; n=430
4% Melanoma
3% Thyroid
2% Urinary bladder
2% Pancreas
21% All Other Sites
GWAS
5; MDS
3%
C80303; n=528
C80303; n=528
C80101 gastric; n=800
*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.
Source: American Cancer Society, 2005.
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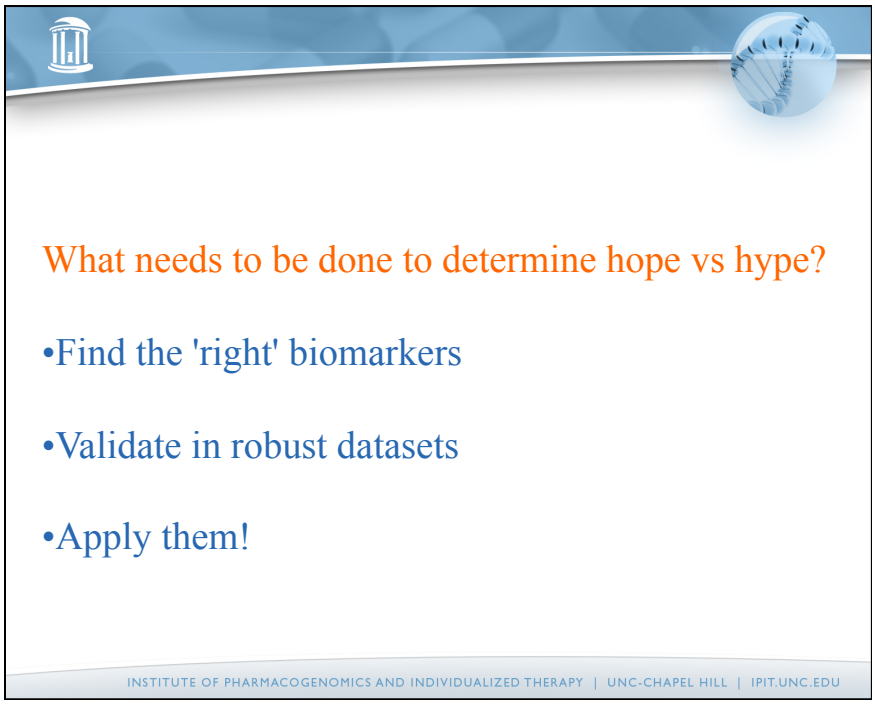
What needs to be done to determine hope vs hype?
•Find the 'right' biomarkers
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•Apply them!
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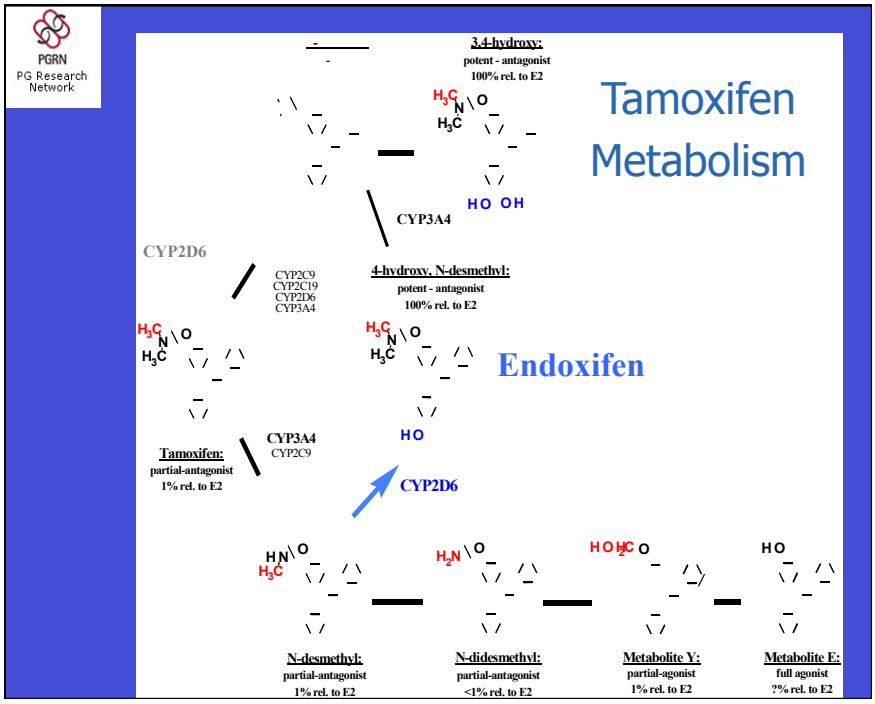
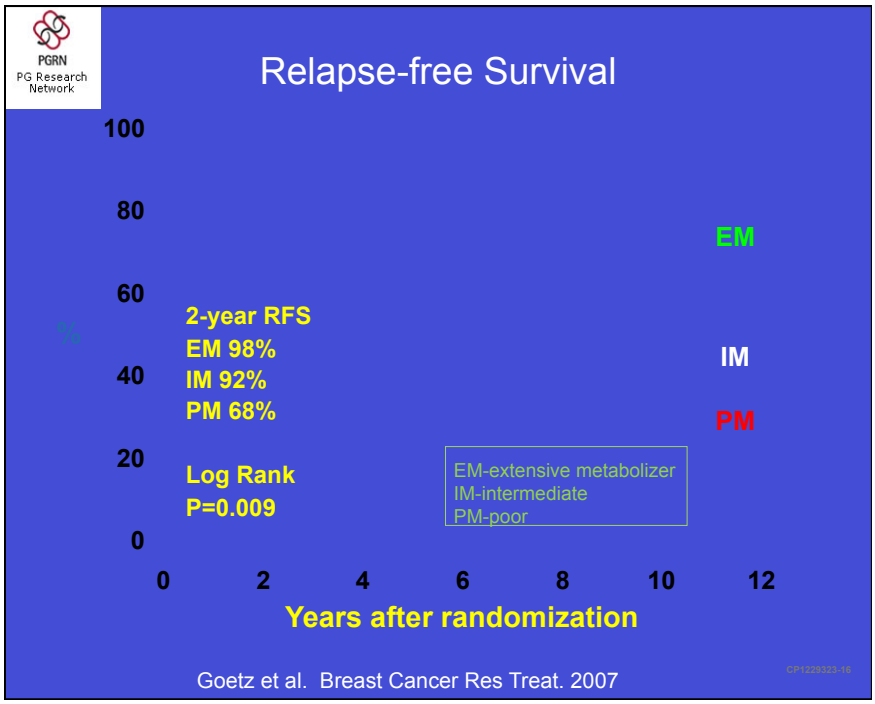

PGRN
PG Research Network
Tamoxifen Metabolism
3,4-hydroxy:
potent - antagonist
100% rel. to E2
CYP3A4
CYP2D6
CYP2C9
CYP2C19
CYP2D6
CYP3A4
4-hydroxy, N-desmethyl:
potent - antagonist
100% rel. to E2
Endoxifen
Tamoxifen:
partial-antagonist
1% rel. to E2
CYP3A4
CYP2C9
CYP2D6
N-desmethyl:
partial-antagonist
1% rel. to E2
N-didesmethyl:
partial-antagonist
<1% rel. to E2
Metabolite Y:
partial-agonist
1% rel. to E2
Metabolite E:
full agonist
?% rel. to E2


PGRN
PG Research Network
Relapse-free Survival
100
80
60
40
20
0
%
2-year RFS
EM 98%
IM 92%
PM 68%
Log Rank
P=0.009
EM
IM
PM
EM-extensive metabolizer
IM-intermediate
PM-poor
0
2
4
6
8
10
12
Years after randomization
Goetz et al. Breast Cancer Res Treat. 2007

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)
  - Bijl 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)

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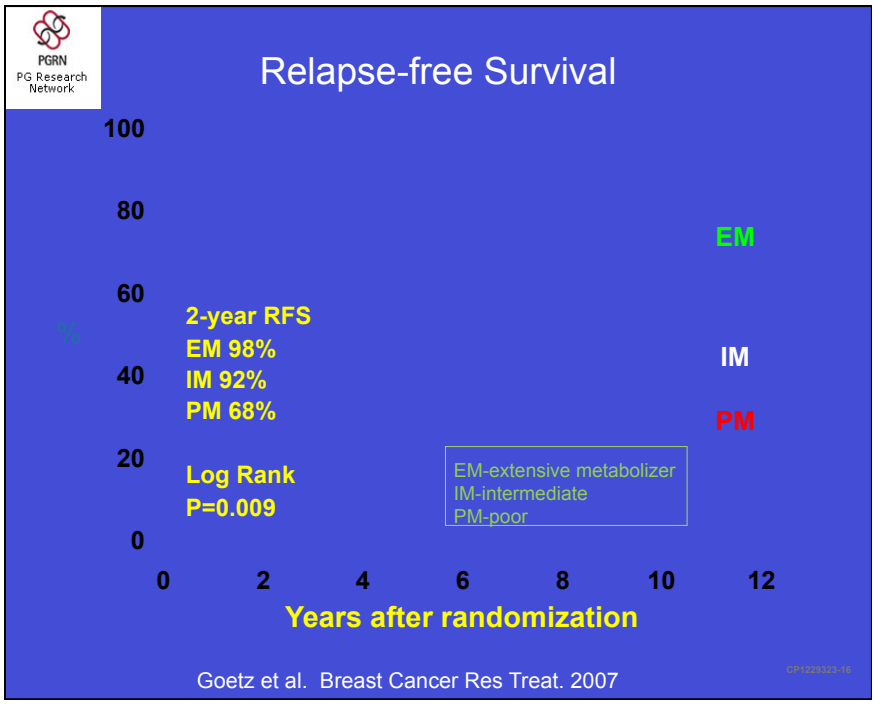
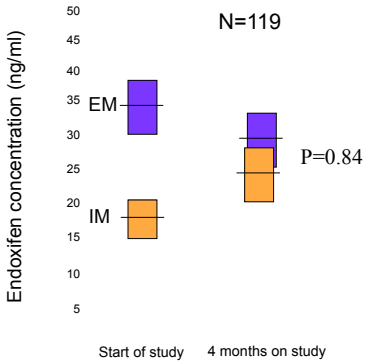


CYP2D6-guided tamoxifen dosing normalizes endoxifen levels in IM patients

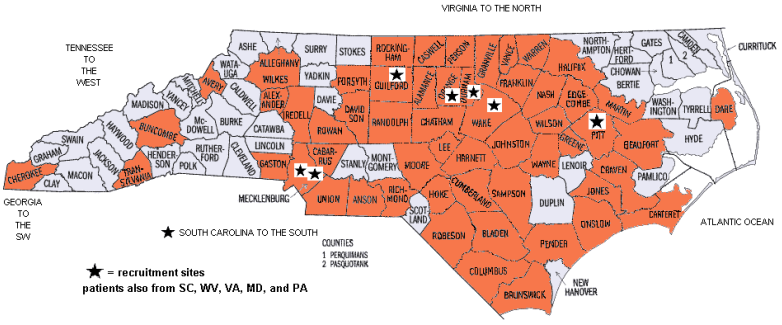
All patients on tamoxifen 20 mg/day for 4 months then
EM-20 mg
IM-change to 40 mg



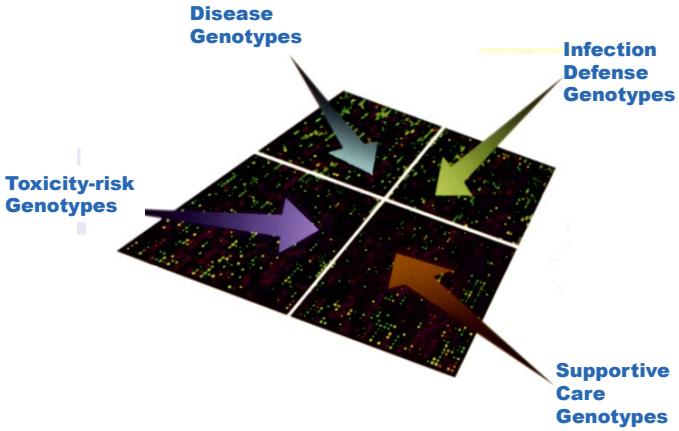
Irvin et al J Clin Oncol 2011

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?

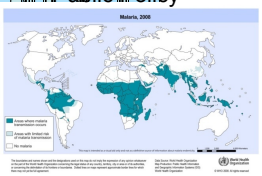
Pharmacogenetics for Every Nation Initiative pgeni.org

PGENI Treating the Population. Impacting the World.

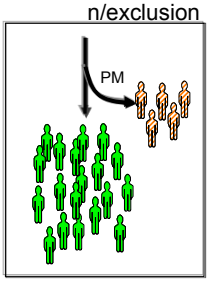
what is your intent?

Drug Safety

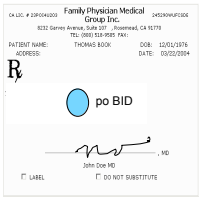
Public Policy

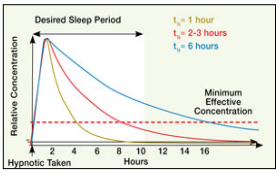


cal trial
n/exclusion



Clinical practice





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- 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

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Background: Source of data for patient therapy selection

Best option: individual

Good: relevant geographic/
ethnic/racial population

Worst: inferred world population

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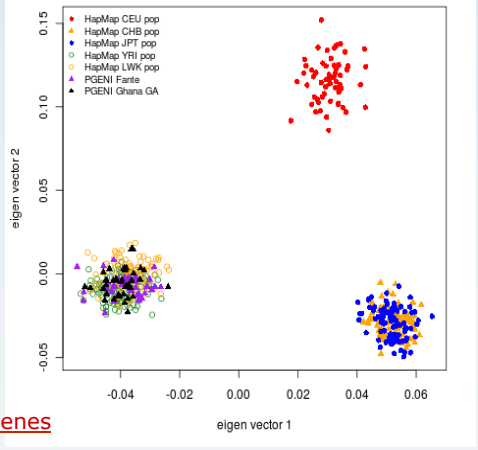
Voltaire

- **"The best is the enemy of good."**,

Continents are more similar than different

-but context is everything



1,936 functional mutations in 225 genes

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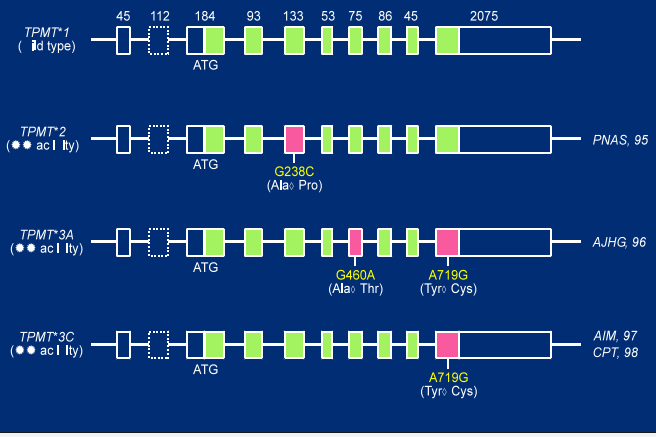
Human TPMT Gene and Mutant Alleles



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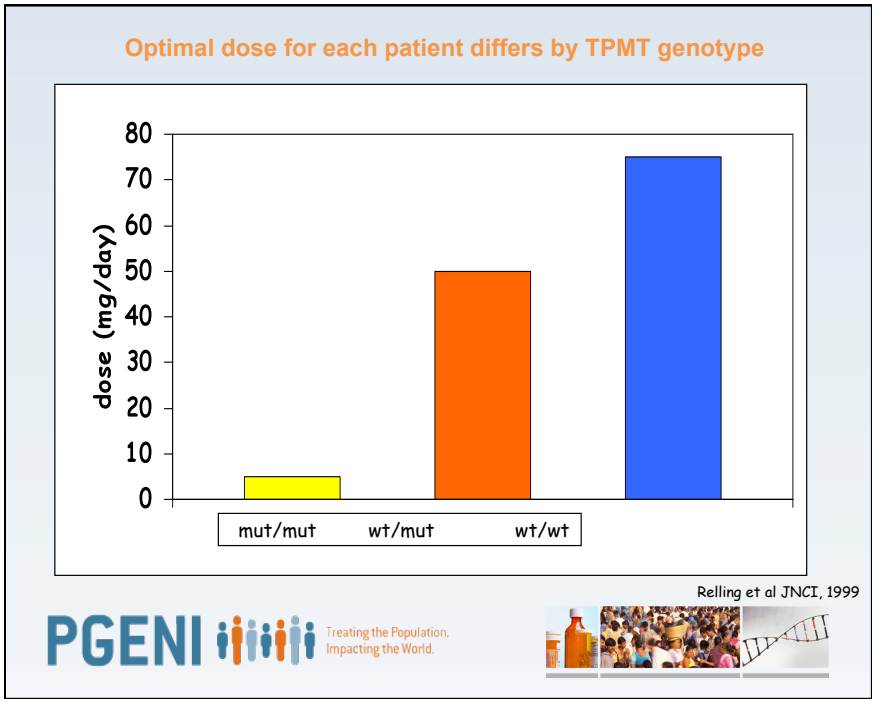
Optimal dose for each patient differs by TPMT genotype
dose (mg/day)
80
70
60
50
40
30
20
10
0
mut/mut
wt/mut
wt/wt
Relling et al JNCI, 1999
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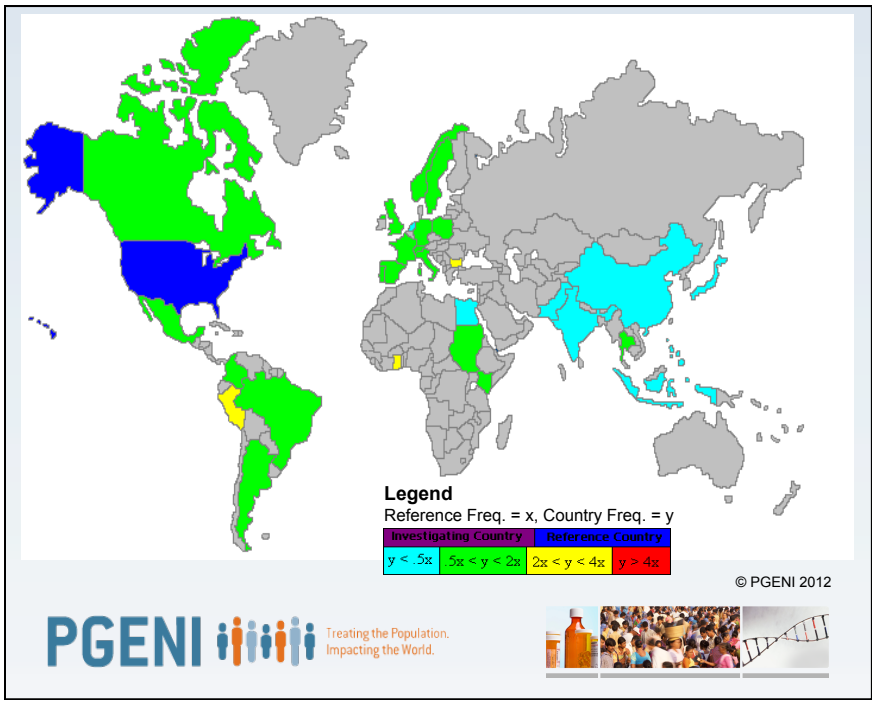
Legend
Reference Freq. = x, Country Freq. = y
Investigating Country
Reference Country
y < .5x
.5x < y < 2x
2x < y < 4x
y > 4x
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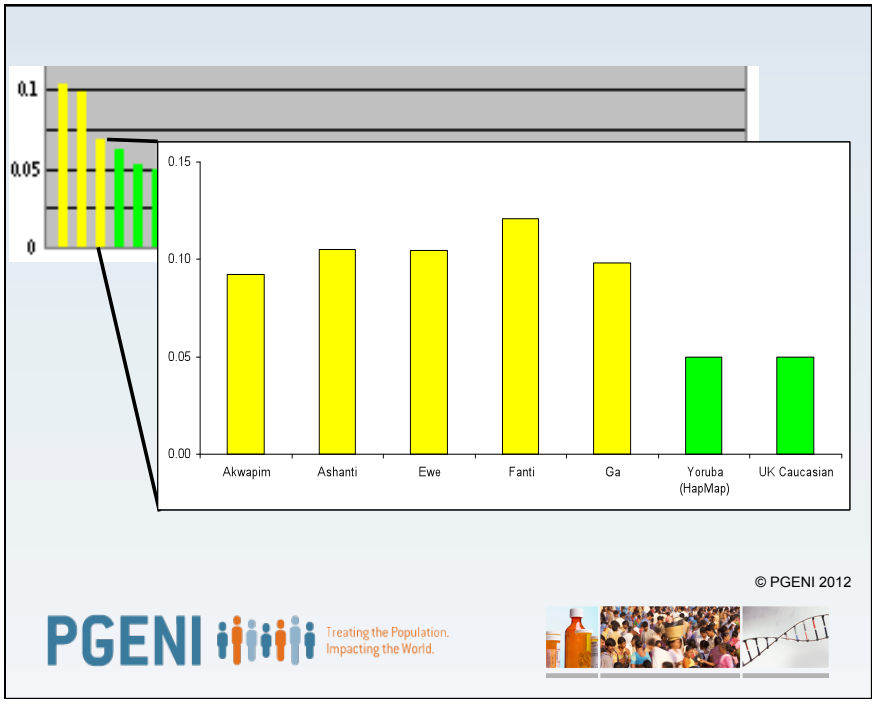
0.1
0.05
0
0.15
0.10
0.05
0.00
Akwapim
Ashanti
Ewe
Fanti
Ga
Yoruba (HapMap)
UK Caucasian
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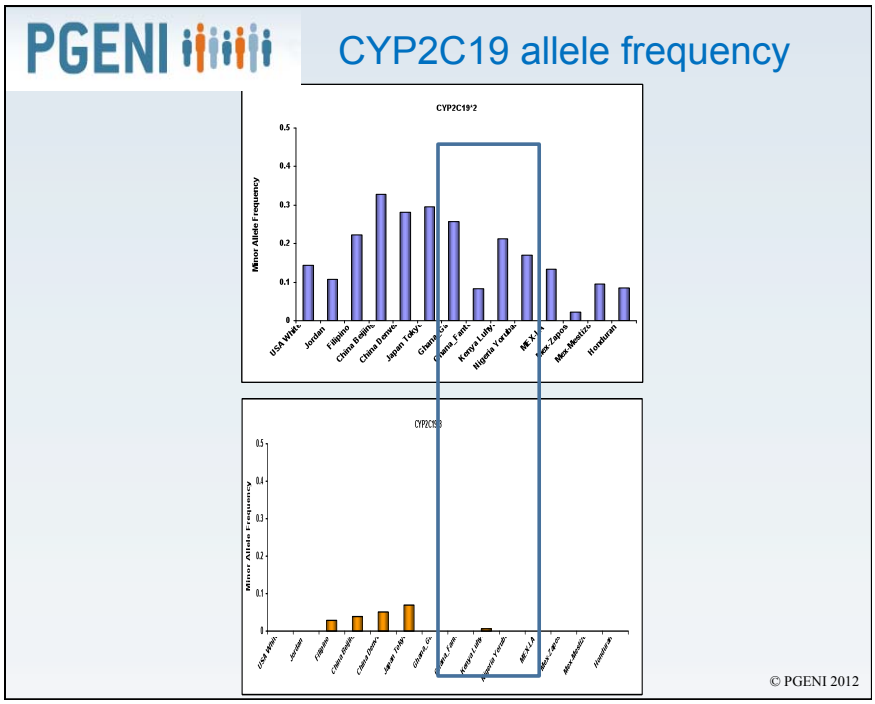
PGENI
CYP2C19 allele frequency
CYP2C19*2
Minor Allele Frequency
0.5
0.4
0.3
0.2
0.1
0
USA White
Jordan
Filipino
China Beijing
China Denver
Japan Tokyo
Ghana_Ga
Ghana_Fanti
Kenya Luhya
Nigeria Yoruba
MEX LA
Nic Zapos
Mex Mestizo
Honduran
CYP2C19
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Type of output

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

Prioritization - assisting the treatment selection from among WHO recommended therapies

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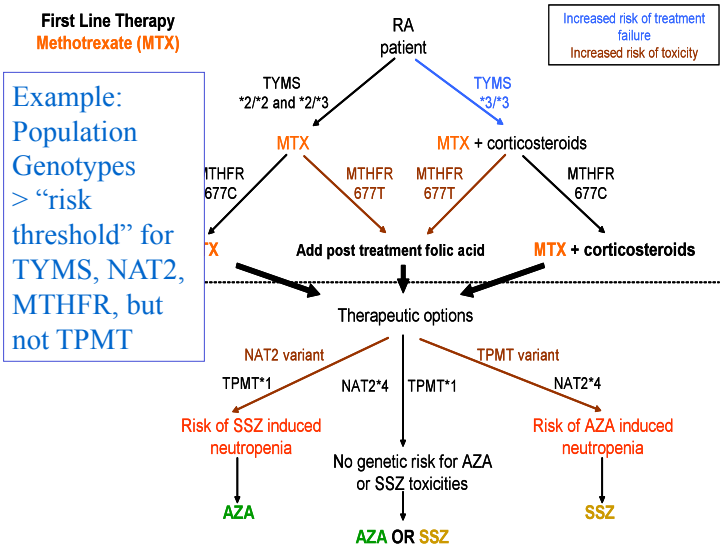

Example: Population Genotypes > "risk threshold" for TYMS, NAT2, MTHFR, but not TPMT

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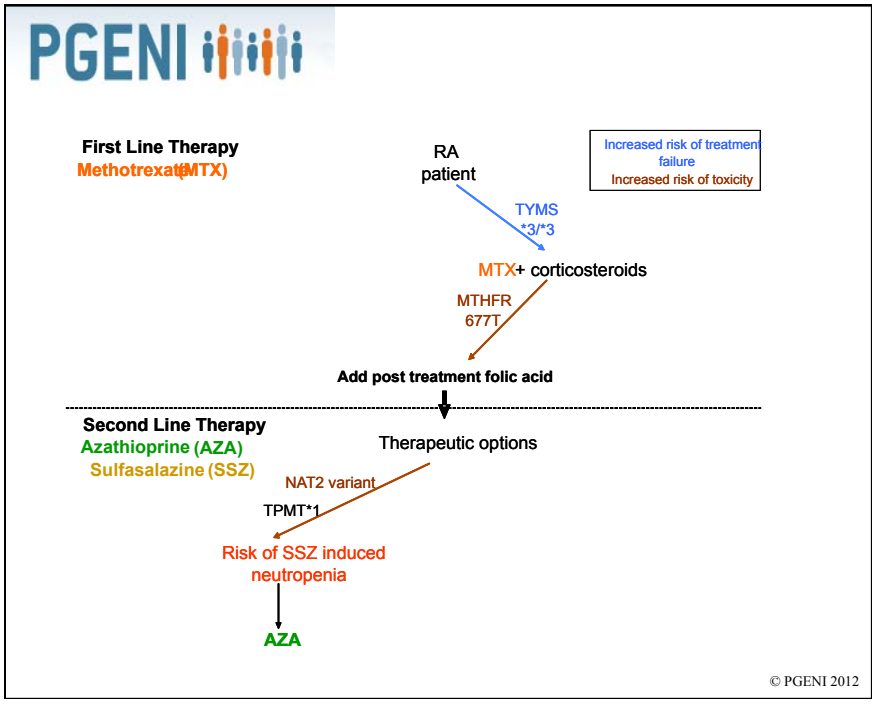
PGENI
First Line Therapy
Methotrexate (MTX)
RA patient
Increased risk of treatment failure
Increased risk of toxicity
TYMS *3/*3
MTX + corticosteroids
MTHFR 677T
Add post treatment folic acid
Second Line Therapy
Azathioprine (AZA)
Sulfasalazine (SSZ)
Therapeutic options
NAT2 variant
TPMT*1
Risk of SSZ induced neutropenia
AZA
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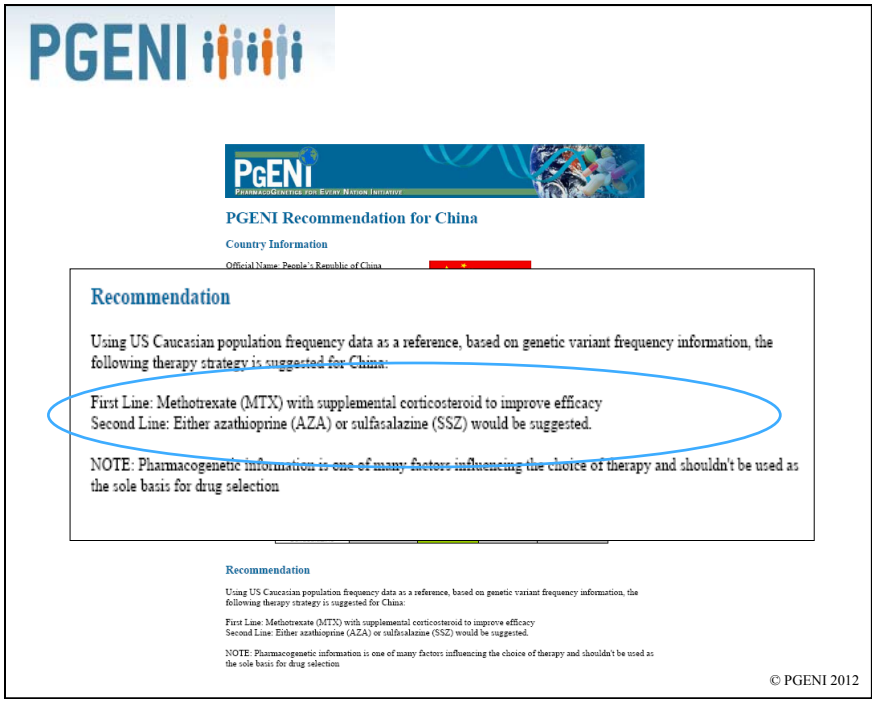
PGENI
PGENI Recommendation for China
Country Information
Recommendation
Using US Caucasian population frequency data as a reference, based on genetic variant frequency information, the following therapy strategy is suggested for China:
First Line: Methotrexate (MTX) with supplemental corticosteroid to improve efficacy
Second Line: Either azathioprine (AZA) or sulfasalazine (SSZ) would be suggested.
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
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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*

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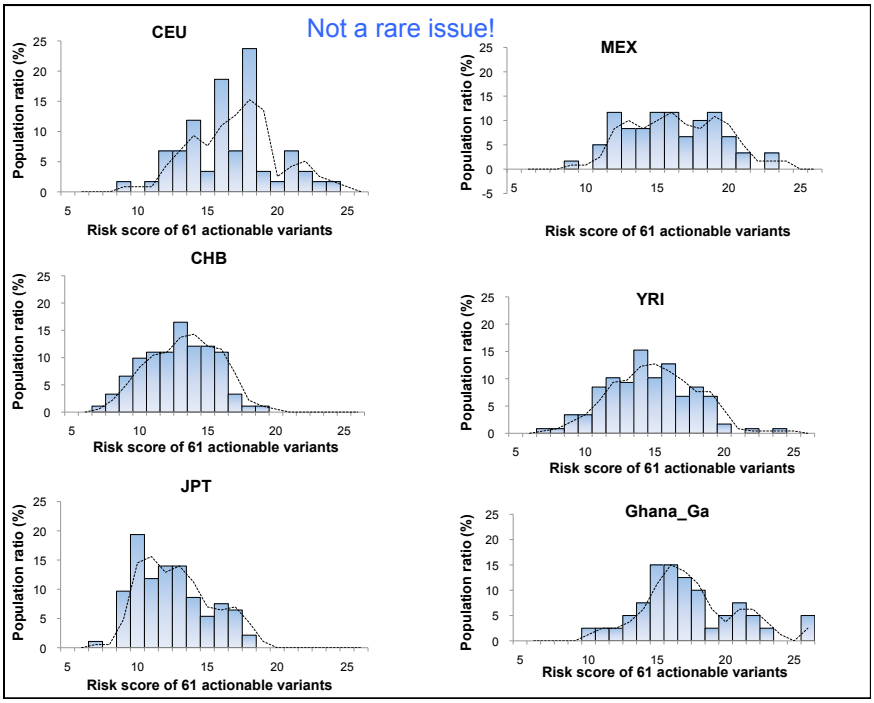
Not a rare issue!



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)

Applications of pharmacogenetics

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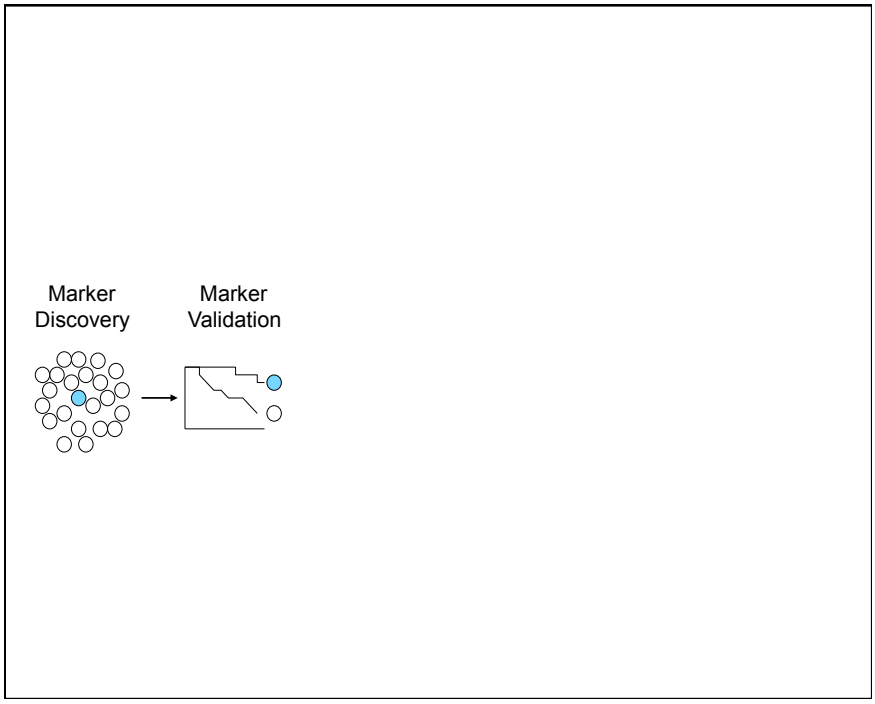
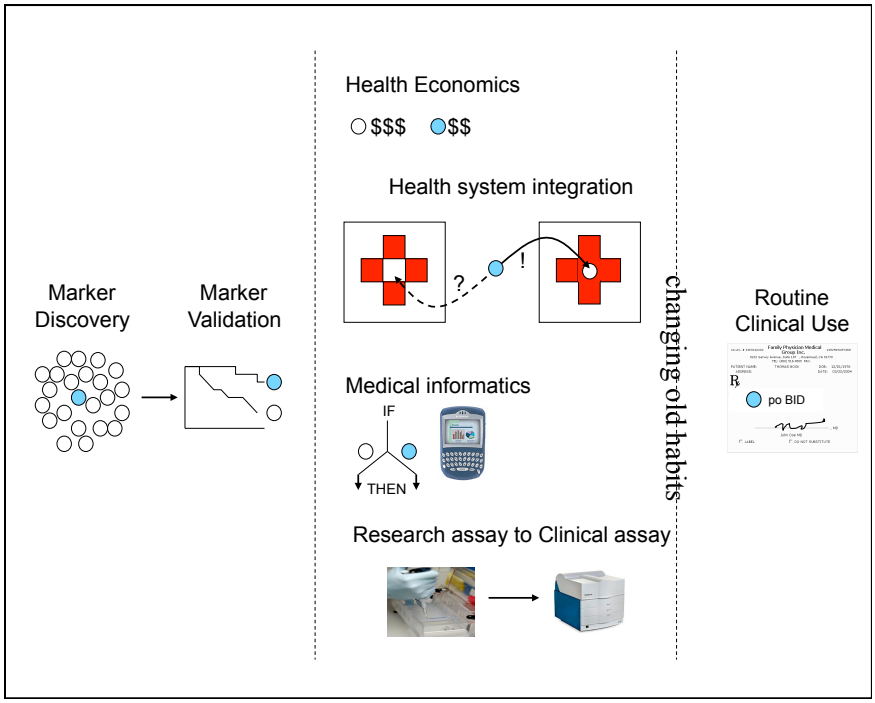
- Therapy selection (CYP2C19)
- Preemptive prediction (HLA-B*5701)

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

} Boring!


Marker Discovery
Marker Validation


Marker Discovery
Marker Validation
Health Economics
$$$
$$
Health system integration
?
!
Medical informatics
IF
THEN
Research assay to Clinical assay
changing old habits
Routine Clinical Use
po BID

Warfarin Package Insert

Table 5: Range of Expected Therapeutic Warfarin Doses Based on CYP2C9 and VKORC1 Genotypes†

VKORC1	CYP2C9					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
GG	5-7 mg	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg
AG	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg
AA	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg

†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
WARFARINDOSING
www.WarfarinDosing.org
Warfarin Dosing
Outcomes
Hemorrhage Risk
Patient Education
Contact Us
References
Glossary
About Us
Age:
Sex:
Ethnicity:
Race:
Weight:
Height:
Smokes:
Liver Disease:
Indication:
Baseline INR:
Target INR:
CYP2C9 Genotype:
Randomize & Blind
VKORC1-1639/3673 Genotype:
Amiodarone/CordaroneÂ® Dose:
Statin/HMG CoA Reductase Inhibitor:
Any azole (eg. Fluconazole):
Sulfamethoxazole/Septra/Bactrim/Cotrim/Sulfatrim:
ESTIMATE WARFARIN DOSE
Carrier
11:07 AM
iWarfarin
UNC Pharmacogenomics
Warfarin Dosing - IWPC Algorithm
Instructions
Learn
ft/lbs
cm/kg
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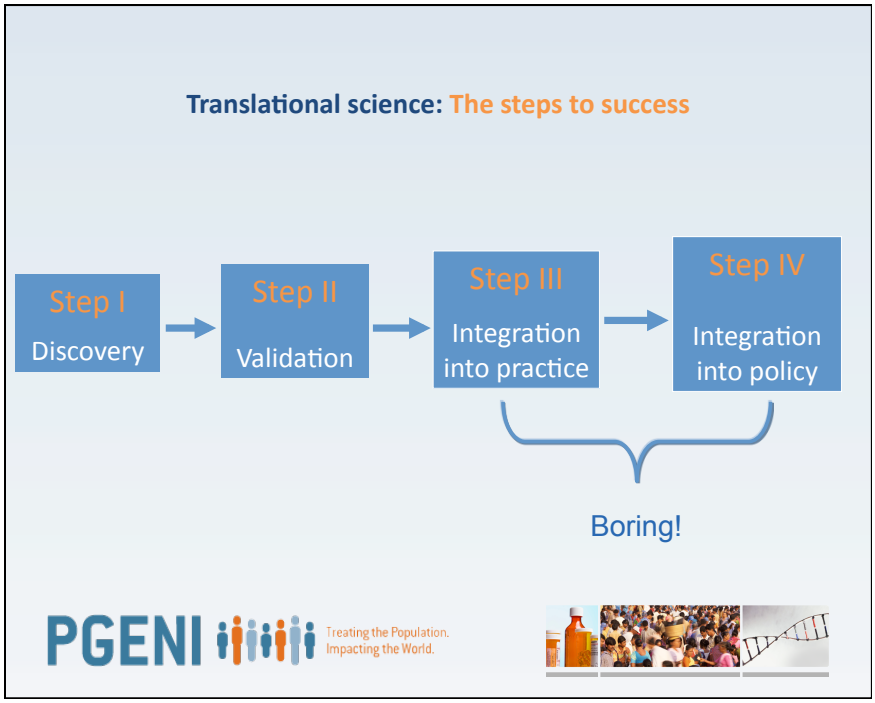
Translational science: The steps to success
Step I
Discovery
Step II
Validation
Step III
Integration into practice
Step IV
Integration into policy
Boring!
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We now have new audiences

- Past
  - Ourself
  - 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 2^0 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)

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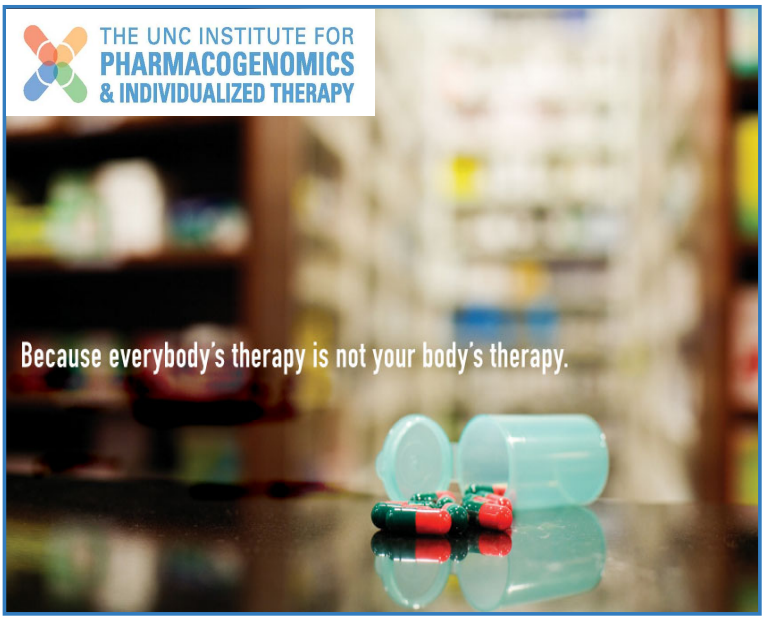
Thank you to the PGENIUSES!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& INDIVIDUALIZED THERAPY
Because everybody's therapy is not your body's therapy.