


Personalized Medicine and Pharmacogenomics: Moving from the Laboratory to Clinic

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Disclosure

I work for the US Government – so I have nothing to disclose, nor any conflicts of interest

Learning Objectives

- Understand the differences between genetic polymorphisms and somatic mutations
- Discuss when pharmacogenetics strategies are most likely to have an impact
- Review the 25 drugs that have some form of FDA recommendation or they have been incorporated into standard of clinical care utilizing personalized genotyping (pharmacogenetics)
- Review the differences between candidate gene analysis and high-throughput analysis of drug metabolism and transport genes
- Understand the barriers to implementing pharmacogenetics strategies into clinical care

"All things are poison, *only the dose permits something not to be poisonous*"



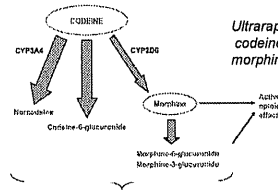
Paracelsus
(1493-1541)

...well that and inter-individual genetic variation...

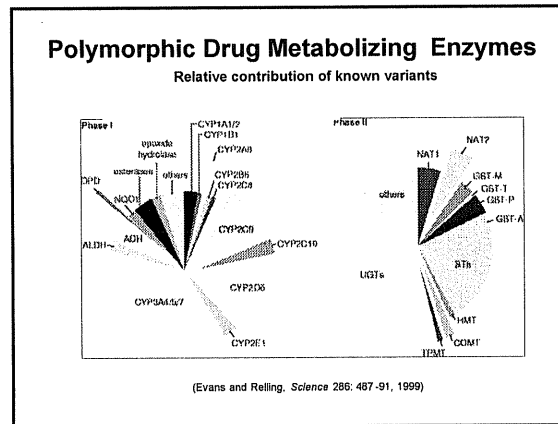
The NEW ENGLAND JOURNAL of MEDICINE Volume 361:827-828; August 20, 2009

Case report: Codeine, Ultrarapid-Metabolism Genotype, and Postoperative Death

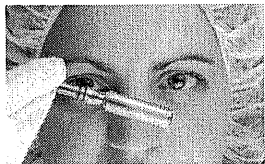
Healthy 2-yo boy, underwent outpatient elective adenotonsillectomy; After surgery, instructions to take 10-12.5mg of codeine + 120 mg APAP q 4-6 hr pm; 2 days post surgery, child died
 Autopsy results: Codeine (0.70 mg/L) & morphine (32 ng/ml) → toxic levels
 CYP2D6 genotyping → 3 copies of CYP2D6 allele → ultrarapid-metabolizer phenotype



Ultrarapid metabolizers may metabolize codeine too efficiently leading to morphine intoxication



What are the Reasons the Development of a new Drug is Halted?



Four Main Reasons the Development of a Drug is Halted

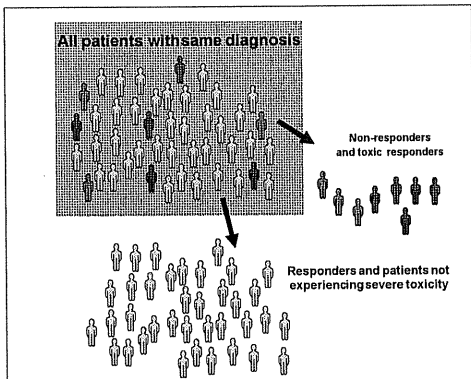
- Lack of Efficacy
- Side Effects
- Pharmacokinetics
- Pharmacogenetic Profile

34 FDA approved Agents have been Removed from the Market since 1990

- Annual economic value at the time of removal - \$31 billion

34 FDA approved Agents have been Removed from the Market since 1990

- Annual economic value at the time of removal - \$31 billion
- Twenty of these might have been saved or not developed if we had fully understood the pharmacogenetics



Pharmacogenomics and FDA

"Pharmacogenomics holds great promise to shed scientific light on the often risky and costly process of drug development, and to provide greater confidence about the risks and benefits of drugs in specific populations. Pharmacogenomics is a new field, but we intend to do all we can to use it to promote the development of medicines."

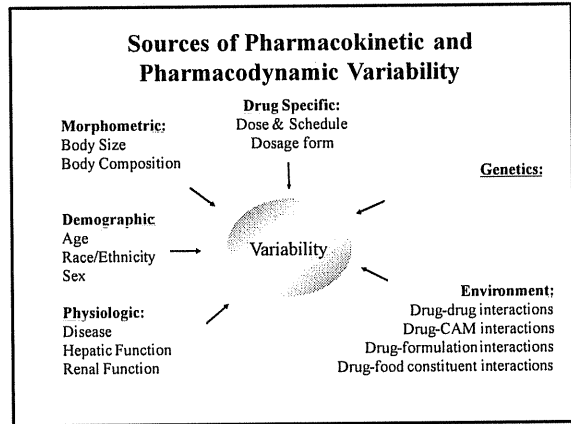
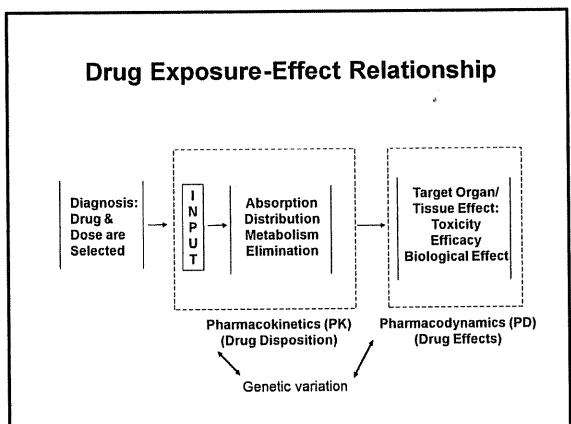
Mark McClennan, M.D.
FDA Commissioner Nov, 2003

Introduction - Pharmacogenetics

- Genetic polymorphisms in proteins involved in drug metabolism or transport might be of clinical relevance
- Variation in genes encoding for drug target proteins (e.g., receptors) may result in differences in efficacy
- The most commonly observed variants are single-nucleotide polymorphisms (SNPs; i.e., a variant with a population frequency of >1%)
- SNPs are responsible for >90% of all genetic variation in the human genome
- Goal of pharmacogenetics is to aid in individualized treatment with drugs

Introduction - Pharmacogenetics

Remember – this is germline DNA, not somatic mutations



Pharmacogenomics and Oncology

Pharmacogenomic Strategies Most Relevant When:

- Narrow therapeutic indices
- High degree of inter-individual variability in response
- Little or no available methods to monitor safety or efficacy
- Few alternative treatment options

Flowers and Veenstra 2004

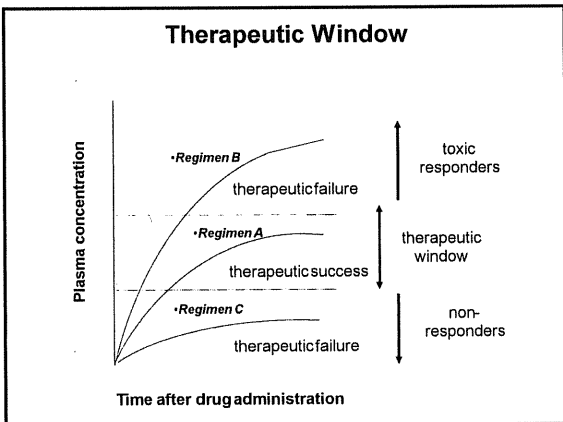
Pharmacogenomics and Oncology

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Flowers and Veenstra 2004

Anticancer agents meet all of these criteria



Pharmacogenetics

Implications of polymorphisms on Pharmacokinetics

- Drug Absorption
- Drug Metabolism
- Drug Elimination
- Drug Distribution
- Drug Activation

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Implications of polymorphisms on Drug Effect (Response and Toxicity)

- Receptors
- Target Proteins
- Resistant
- Toxicity

Genotyping Strategies in Medical Oncology

Example of anticancer drug metabolism by polymorphic enzymes

Drug	Pathway	Variability in CL
Amonafide	N-acetyl transferase (NAT)	>3-fold
Busulfan	Glutathione S-transferase (GST)	10-fold
Docetaxel	Cytochrome P-450 (CYP) 3A4 and 3A5	4 to 9-fold
5-Fluorouracil	Dihydropyrimidine dehydrogenase (DPD)	10-fold
Irinotecan	UDP glucuronosyltransferase (UGT)	50-fold
6-Mercaptopurine	Thiopurine methyltransferase (TPMT)	>30-fold

Evans and Relling, Science 286: 487-91, 1999

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TPMT and 6-Mercaptopurine

- 6-MP is administered to children with acute lymphoblastic leukemia (ALL), while related compounds (6-thioguanine and azathioprine) are also administered to individuals with ALL, inflammatory bowel disease, organ transplant and autoimmune disorders.
- MP drugs incorporate cytotoxic thioguanine nucleotides into DNA as their primary mechanism of action. May also inhibit *de novo* purine synthesis.

Azathioprine
6-Mercaptopurine
Thioguanine

TPMT and 6-Mercaptopurine

- TPMT is expressed in heart, blood cells, pancreas, and intestine.
- TPMT methylates mercaptopurine drugs, thus inactivating them.
- Metabolism of MP drugs is decreased with polymorphic TPMT variation up to 200-fold.
- Rapid metabolizers require higher dosing
- Slow metabolizers are at high risk for developing fatal neutropenia and require 7-15% of the normal dose of 6-MP due to accumulation of excessive thioguanine nucleotides in hematopoietic cells. Also risk secondary malignancies (i.e. brain tumors, and AML).

TPMT and 6-Mercaptopurine

- Rapid metabolizers ("wild-type" individuals) - require highest doses for efficacy (~ 80-98% of the population)
- Intermediate metabolizers (carry one copy of TPMT*2A, *3A, *3C) - require ~65% of normal dose, but have highest cure rate (~ 2-20% of the population)
- Slow metabolizers (carry two copies of TPMT*2A, *3A, *3C) - require 7-15% of original dose, and are at risk for secondary malignancies (~0.01 - 1% of the population)

Genetic variation in TPMT explains 95% of phenotype

Pharmacogenetics

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Implications of polymorphisms on Drug Effect

- Receptors (somatic)
- Target Proteins
- Resistant
- Toxicity

IGF and EGF Signaling Pathways

Response to Gefitinib (Iressa®)

- Somatic mutations identified in the tyrosine kinase domain of the EGFR gene in 8 of 9 patients with lung cancer responding to gefitinib
- No somatic mutations were identified in 7 patients not responding to gefitinib

Lynch et al. NEJM 2004; 350:2129

Tumors with a Major Clinical Response to EGFR Inhibitors

- ~ 10%–20% of tumors have a major response (dramatic shrinkage) to EGFR inhibitor
- Majority of responders have mutations in EGFR tyrosine kinase domain (exon 19 deletion, point mutation)
- Mutation led to constitutive activation of EGFR and increased sensitivity to EGFR inhibition
- Amplification of EGFR may also increase sensitivity
- Never smokers and patients of Asian origin had a high frequency of EGFR mutations

(Paez et al, Science 2003; Lynch et al, NEJM 2003; Tsao et al, NEJM 2005)

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ABCG2 (BCRP, ATP-binding cassette)

- Involved in intrinsic or acquired multidrug resistance (MDR) phenotype of tumor cells
- **ABCG2 encodes a half transporter**
- Located on chromosome 4q-22
- 66 kb; 16 exons; 15 introns
- 69 Known genetic polymorphisms including 65 SNPs; 13 SNPs in exons; 7 SNPs cause amino acid substitutions

ABCG2 421 C>A Genotype Frequencies

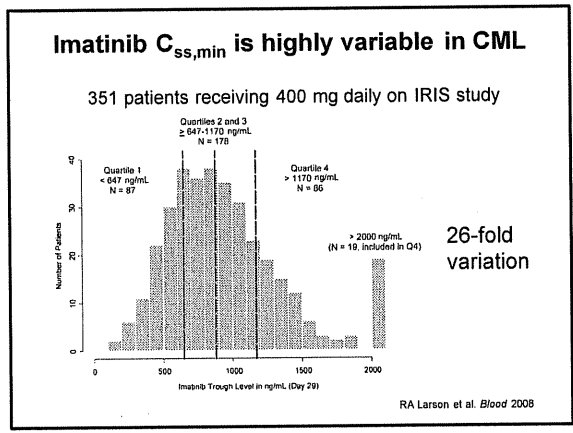
Population	WT	C/A	A/A
Caucasian	77%	22%	1%
African Am	90%	9%	1%
African	98.4%	1.5%	0.1%
Chinese	43%	45%	12%

Race is a social identification, and doesn't correspond to genetic differences (esp as it relates to pharmacogenetics). Furthermore, populations within the US are rather difficult to define by categories because of admixture.

Efficacy of Imatinib in Chronic Myelogenous Leukemia (CML)

(Druker et al, NEJM 2001)

- Imatinib inhibits BCR-ABL
- Of 54 patients treated with imatinib who had failed interferon-alpha (300 mg or higher bid), 53 complete hematological responses



Fluoropyrimidines (i.e. 5-FU, Capecitabine): DPYD

- Chemotherapeutic agent
- DPD deficiency in DPYD mutant heterozygotes and homozygotes at risk of rare but potentially fatal toxicities.
- Recommended
- Test: DMET

Interferon-alpha: IL28B

- Relevant to treatment of Hepatitis C Virus genotype 1 infections.
- One variant in IL28B gene predicts sustained virological response.
- A different variant predicts treatment inefficacy.
- Optional/Available
- Test: LabCorps, others in development

Irinotecan: UGT1A1*28

- Antineoplastic agent
- UGT1A1*28 homozygotes at increased risk of severe neutropenia.
- Package insert recommends lower dose for known mutant homozygotes.
- Recommended
- Test: DMET

Isoniazid: NAT2

- Used to treat tuberculosis.
- N-Acetyltransferase 2 variants confer Acetylator status.
- Slow Acetylator status associated with a 3-fold increase in drug induced liver injury (DILI).
- Optional/Available
- Test: DMET

Isoniazid: CYP2E1

- CYP2E1 wild-type homozygotes (with higher CYP2E1 activity) may be at increased risk of liver toxicity, regardless of Acetylator status.
- Optional/Available
- Test: DMET

Phenytoin: CYP2C9

- Anticonvulsant
- CYP2C9 *2 and *3 variants affect toxicity and efficacy.
- Optional/Available
- Test: DMET and others

Phenytoin: HLA-B*1502

- HLA-B*1502 variant is associated with SJS/TEN in Asian populations.
 - Package insert recommends avoiding use in HLA-B*1502 positive patients when other therapies are available.
 - Recommended
 - Test: TBD
-

Rasburicase: G6PD

- Used in the treatment of hyperuricemia in patients with tumor-lysis syndrome.
 - Patients with a G6PD deficiency are at a higher risk of hemolytic anemia.
 - Package insert recommends screening patients at high risk (e.g. of African or Mediterranean decent).
 - Recommended
 - Test: DMET
-

Statins: SLCO1B1

- SLCO1B1 variant confers risk of statin-induced myopathy.
 - Optional/Available
 - Test: DMET
-

HER2 Inhibitors (Trastuzumab, Lapatinib)

- Overexpression of HER2 receptor confers sensitivity to a HER2-inhibiting agent.
 - Optional/Available
 - Test: Molecular Pathology
-

BCR-ABL inhibitors (Dasatinib, Nilotinib, Imatinib)

- BCR-ABL is a fusion oncogene in hematologic malignancies.
 - BCR-ABL fusion gene is required for sensitivity to a BCR-ABL inhibitor.
 - Recommended
 - Available in Hematology/Oncology.
-

Imatinib: KIT

- GIST patients with KIT mutation show sensitivity to Imatinib.
 - Patients with a mutation in exon 9 of KIT may experience greater benefit with dose increase.
 - Recommended
 - Test: Molecular Pathology
-

BRAF inhibitors (i.e. Sorafenib) and EGFR inhibitors

- BRAF activating mutation predicts sensitivity to BRAF inhibitors.
- BRAF activating mutation associated with poor response to EGFR inhibitors, although a BRAF inhibitor can restore sensitivity.
- Recommended
- Test: Molecular Pathology

RET inhibitors (i.e. Vandetanib)

- RET mutation required for response to RET inhibitors.
- Recommended
- Test: to be available in Molecular Pathology

Alkylating Agents (i.e. Temozolomide, BCNU, Procarbazine): MGMT

- MGMT reverses DNA methylation. Expression may predict resistance in Glioblastoma Multiforme (GBM).
- MGMT promoter methylation (silencing) predicts response.
- Recommended
- Test: Molecular Pathology

One ultimate goal of pharmacogenetics is to provide a patient with individualized therapy (“getting the dose right”)

Using candidate gene approach - It will be virtually impossible to assign a patient to an unequivocal phenotype and especially to an unequivocal genotype

Current Genotyping Platforms

Manufacturer	Product	Genes	Total SNPs
Roche	AmpliChip P450	CYP2C19 and 2D6	23
TM Bioscience	P450-2C19	CYP2C19	7
	P450-2D6	CYP2D6	5
	P450-2D6	CYP2D6	14
GE Healthcare	Codelink P450	CYP2D6, CYP2C9, CYP2C19, CYP1A1, CYP1A2, CYP2E1, CYP2A4, CYP3A5, and CYP1B1	110
Junio	DrugMET	CYP2D6, CYP2C9, CYP2C19, CYP3A5, CYP2B6, NAT2, TPMT, and MDR1	27
AffymetrixParAllele	DMET	168	1248

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DMET Genotyping Platform

1936 variants (actual causative variants) in 235 PK/PD genes.
Useful for haplotype determination.

Captures the vast majority of SNPs involved in PK/PD.

Phase I Enzymes		Phase II Enzymes		Transporters		Other			
CYP1A1	CYP2D6	ADH1A	COMT	MAGB	ABCB1	SLC15A2	SLC7A5	ABPI	NR3C1
CYP1A2	CYP2F3	ADH1B	DYPD	NAT1	ABCB4	SLC18A1	SLC7A7	AHR	ORM1
CYP1B1	CYP2E1	ADH1C	FM01	NAT2	ABCB7	SLC39A1	SLC7A8	AKAP9	ORM2
CYP2A6	CYP2F1	ADH4	FM02	NNMT	ABCB11	SLC22A1	SLC01A2	ALB	PKMT
CYP2A7	CYP2F2	ADH5	FM03	ND01	ABCC1	SLC22A11	SLC01B1	ADX1	POM1
CYP2A13	CYP2F4	ADH8	FM04	TPMT	ABCC2	SLC22A12	SLC01B3	ARNT	PON2
CYP2B6	CYP2F7	ADH7	FM05	UGT1A1	ABCC3	SLC22A4	SLC02B1	AREA	PON3
CYP2B7	CYP2F8	ALDH1A1	FM06	UGT1A3	ABCC4	SLC22A2	SLC03A1	CBR1	POR
CYP2B11	CYP2F9	ALDH2	GSTA1	UGT1A4	ABCC5	SLC22A3	SLC04A1	CBR3	PP4R1
CYP2C9	CYP11B1	ALDH3A1	GSTA2	UGT1A5	ABCC8	SLC22A4	SLC05A1	CCA	PP4R2
CYP2C19	CYP11B2	CHST1	GSTA4	UGT1A7	ABCC9	SLC22A6	SLC12A2	CROT	PFUG
CYP2C8	CYP11B3	CHST2	GSTA5	UGT1A8	ABCC10	SLC22A7	SLC11A3	DCK	RAB11
CYP2C10	CYP11A1	CHST3	GSTM1	UGT1A9	ABCC12	SLC22A8	SLC11B1	EPH3	RPL13
CYP2E1	CYP2A1	CHST4	GSTM2	UGT1A10	AT7FA	SLC29A1	SLC11C1	EPH2	SEC15L1
CYP2F1	CYP2A2	CHST5	GSTM3	UGT2A1	AT7FB	SLC29A2	SLC11C2	FAAH	SEPPOR1
CYP2J2	CYP2A4	CHST6	GSTM4	UGT2B4	SLC13	SLC3A3	SLC11E1	GAPD	SETD4
CYP2D1	CYP2B1	CHST7	GSTM5	UGT2B7	SLC10A1	SLC3A1	SLC12A1	HMGCR	SFD7
CYP2A4	CYP2Z1	CHST8	GSTO1	UGT2B11	SLC10A2	SLC3A2	SLC12B1	HNMT	TBKAS1
CYP2A5	CYP2Z2	CHST9	GSTP1	UGT2B15	SLC13A1	SLC5A5	SLC14A1	MAT1A	TPSG1
CYP2A7	CYP2B3	CHST10	GSTT1	UGT2B17	SLC15A1	SLC3A6		METTL1	TYMS
CYP2A8	CYP2B4	CHST11	GSTT2	UGT2B28				NR1H2	VKORC1
CYP2A11	CYP2B5	CHST13	GSTZ1	UGT8				NR1H3	XDH
CYP2B1		MAGA							

Deeken [Figg] et al. (2009) Pharmacogenomics 1: [epub ahead of print]

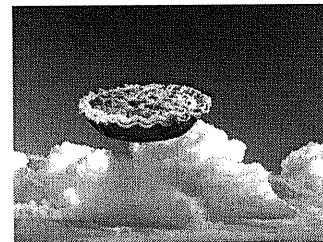
Where is Pharmacogenetics Going?



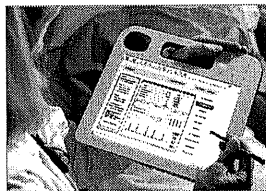
Where is Pharmacogenetics Going?



I can envision a day in which an infant is genotyped with a PG chip, from that day forward that information can aid in all prescriptions that individual receives



How much would you pay to know this information for your child or grandchild?



Barriers to Pharmacogenomics Progress

- Complexity of finding gene variation that affect drug outcome
- Limited drug alternatives for patients with gene variations that prevent them from using certain drugs
- Disincentives for pharmaceutical companies
- Educating healthcare providers
- Concerns about how personal genetic information would be stored and who would have access to this information

Difficulty Moving from Research to Routine Clinical Practice

Prospective validated data showing a clinical benefit for using PG data in patient care is needed

A cost savings data is needed – decrease hospitalization due to side effects or data showing prolong survival associated with PG testing

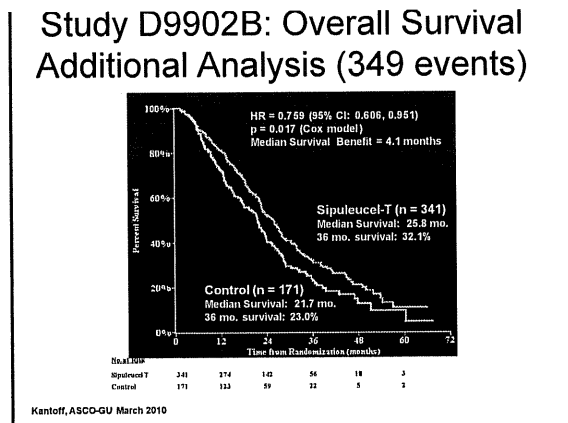
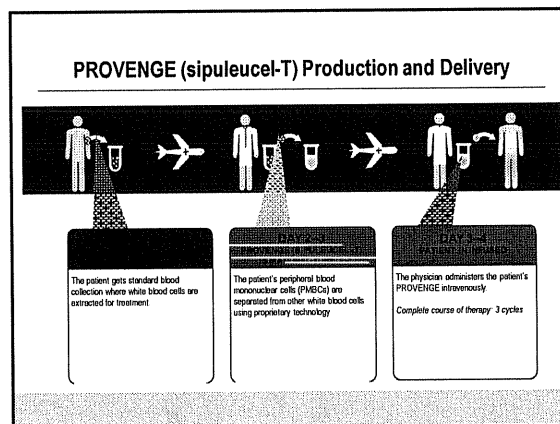
Difficulty Moving from Research to Routine Clinical Practice

The largest expense won't be the genotyping, but the bioinformatics to handle the data...
Where will the data be housed? Who has access?

The data needs to be accessible for all future physicians who are prescribing a drug to an individual patient that has been genotyped

The system needs to quickly determine if a drug is a problem for an individual patient

What about Truly Personalized Medicine?



Conclusion

- The era of personalized medicine has arrived – but one needs to understand the difference in target specific therapy, PG driven therapy and individualized therapy
- Pharmacogenetics hold tremendous promising in guiding clinical care, as well as drug development
- Difficult to predict pharmacogenetics based on simply on race
- The PG chip appears to provide important data in the understanding of pharmacogenetics
- The potential for the PG chip goes beyond drug development, to altering the patient care paradigm



Azathioprine, 6MP, 6TG: TPMT

- Purine analog
- Low levels of TPMT enzyme, or homozygous mutant genotype, predict severe myelosuppression.
- Package insert suggests TPMT genotyping or phenotyping of enzyme activity.
- Recommended
- Test: DMET platform

Codeine: CYP2D6

- Opioid
- CYP2D6 genotype determines poor, intermediate, or ultra metabolizer status.
- Poor metabolizers may experience reduced pain relief.
- Ultra metabolizers are at risk of CNS depression and death due to extensive conversion to morphine.
- Optional/Available
- Test: DMET, others

Tamoxifen: CYP2D6

- Used to treat breast cancer.
- CYP2D6 poor metabolizers may experience reduced efficacy and increased rates of recurrence.
- Recommended
- Test: DMET and others

Warfarin: VKORC1, CYP2C9

- Variants of both alleles increases sensitivity to warfarin and can cause over-anticoagulation and bleeding.
- Optional/Available (both)
- Test: DMET and others

EGFR inhibitors (Gefitinib, Erlotinib, Cetuximab, Panitumumab)

- EGFR mutation and copy number are predictors of response to these agents.
 - Recommended; available in Mol. Path.
- Activating KRAS mutations are associated with resistance to these agents.
 - Recommended; available in Mol. Path.
- PIK3CA and PTEN activating mutations may be related to Cetuximab and Panitumumab resistance.
 - Recommended; to be available in Mol. Path.