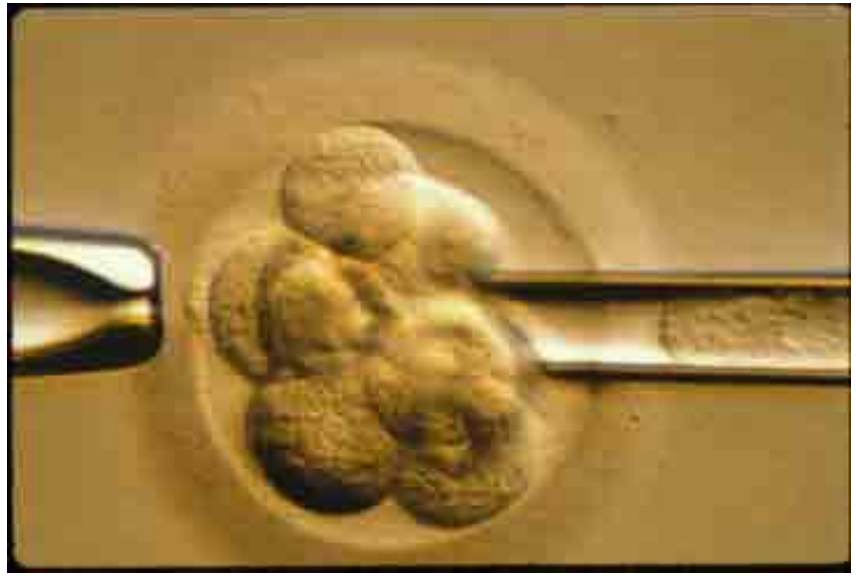
Pre-implantation Genetic Diagnosis (PGD)

Genetic analysis of a single cell from an eight-cell embryo done in conjunction with in vitro fertilization (IVF) to improve the chances of a “normal” pregnancy.

- Polar body
- Blastomere
- Trophectoderm



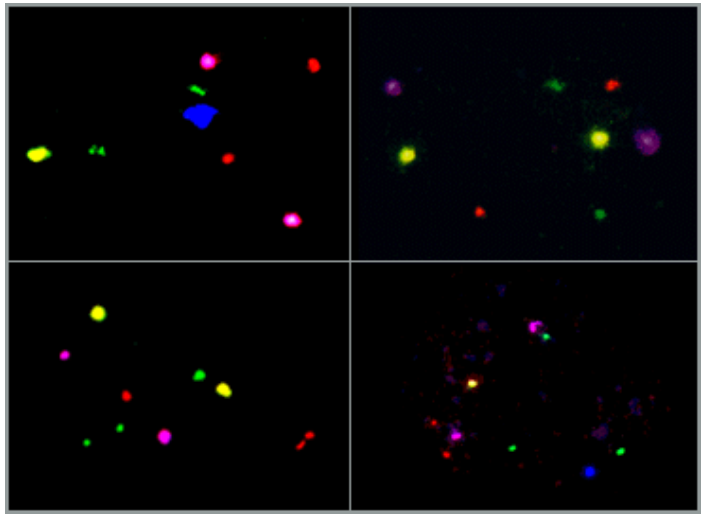
- Remove a single cell from the 6-8-cell embryo using a fine glass needle to puncture the zona pellucida and aspirate the cell

FISH

using fluorescent probes specific for each chromosome. These allow number and size of each chromosome to be checked.

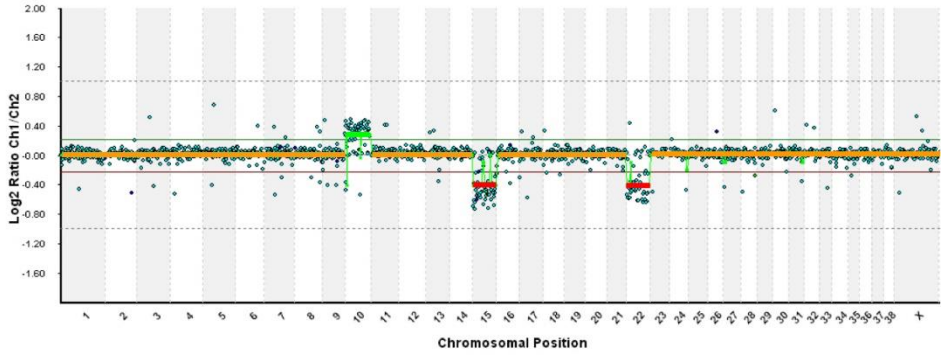
- useful for identifying aneuploidies (incorrect chromosome numbers) and translocations
- procedure destroys the tested cell
- limited number of chromosomes can be checked simultaneously; some abnormalites undetectable

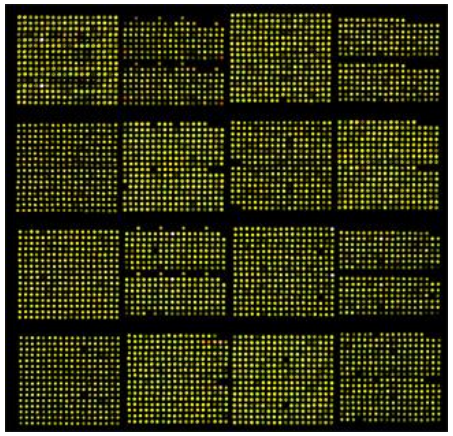
- Aneuploidy is the most frequent cause of spontaneous abortions.



genetic testing (PCR or gene chips)

- Array CGH (aCGH)





- PGD of monogenic diseases

based on indirect diagnostics using STR markers

direct diagnostics - sequencing

Limitations of PCR-based tests:

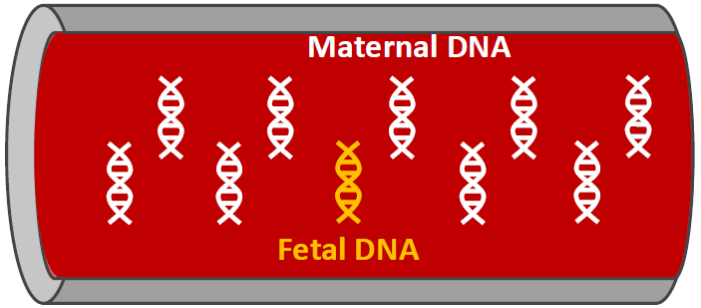
- Both alleles may not amplify equally, leading to misdiagnosis or inconclusive results

ADO (alelic drop out) – amplification of one alelle under the detection limit. Reasons unknown but influenced by cell lysis, PCR conditions, target DNA for sequencing, PCR product size

- PCR-based tests only detect disorders at target loci; other mutations may exist elsewhere

Cell free fetal DNA

- Short segments (<200 base pairs) of fetal DNA circulates in maternal plasma
- Origin is primarily **placenta**
- Placental cells undergoing apoptosis



- Reliably detected >7 weeks
- Increases throughout pregnancy (10-22 weeks constant)
- Cleared within hours of birth
  - Short half life (16 min), undetectable by 2 hours postpartum

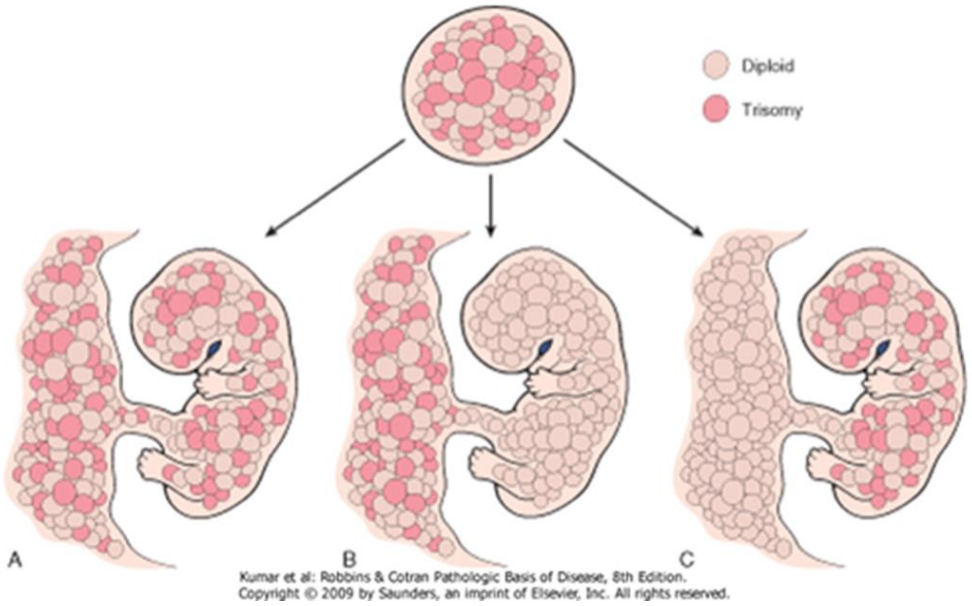
- Both cell-free ***fetal*** and cell-free ***maternal*** DNA circulate in maternal plasma.
- Cell-free fetal and maternal DNA circulate in maternal plasma as relatively short fragments (150-200 base pairs) and represent the entire genome.
- Fetal DNA comes primarily from the placenta.
- Maternal DNA comes primarily from maternal blood cells.
- Fetal DNA is 5-25% of the total cell-free DNA (~10% on average).

Cell free DNA Clinical Applications

- Sex Determination
- Single gene disorders – paternal origin
- Isoimmunization: noninvasively determine fetal Rh type
- **Aneuploidy: detect abnormal ratio of a particular chromosome;**
- **Does not detect nontargeted aneuploidies**

- Next generation sequencing
- Real-time PCR
- Many platforms and methods

- Why cell free fetal DNA fails?
- Confined placental mosaicism



Pharmacogenetics:
From DNA to Drug Treatment

- Pharmaco***genomics***
  - The science of how genes affect the way people people respond to drugs
  - How genes affect…

    …the way our body processes drugs (pharmacokinetics)

    …the interaction of drugs with receptors (pharmacodynamics)

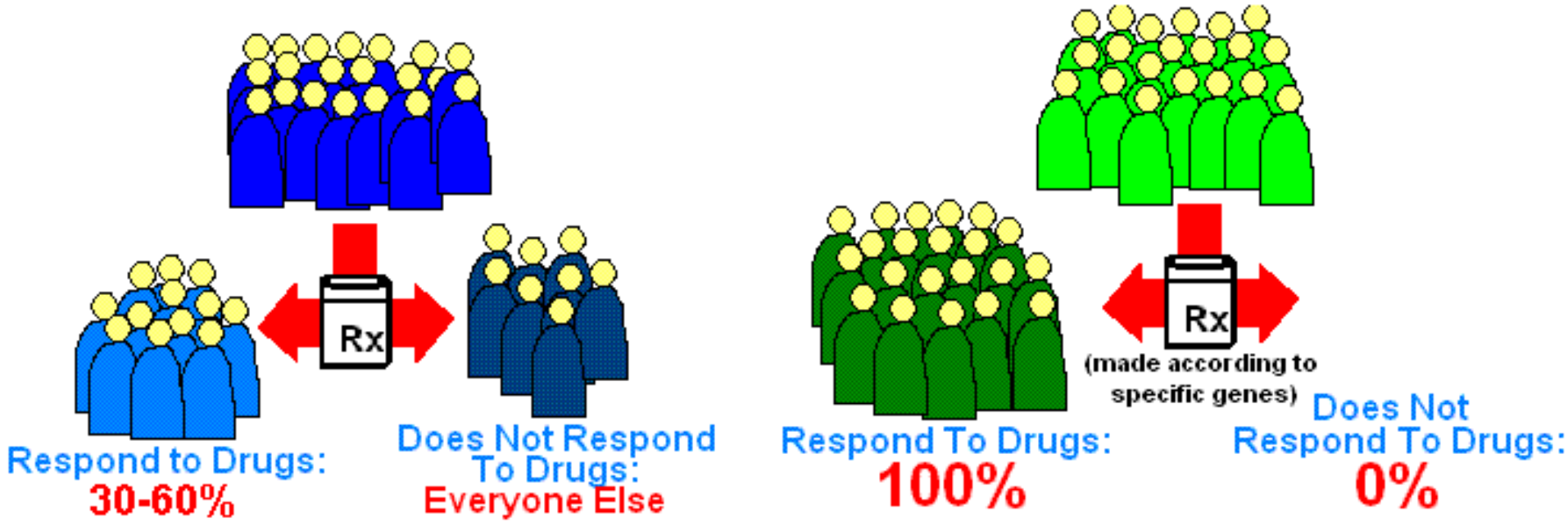
    …the treatment efficacy and adverse side effects

- Pharmaco***genetics***
  - A subset of ‘pharmacogenomics’
  - The study of how ***inherited variation*** affects drug response and metabolism

Why is this a good approach?

- Drugs can be dangerous
  - Many people have severe adverse reactions to drugs
  - Many people respond to drugs at different doses
  - Many drug treatments are horribly unpleasant, painful
- Drugs are expensive (to take and to make)
  - Ineffective drugs are a waste of money to take
  - Drug development needs to account for response variability
- Genetics provide *a priori* information
  - Genetics don’t change (except in cancer)
  - Genetics can point to the *cause* not just the symptom

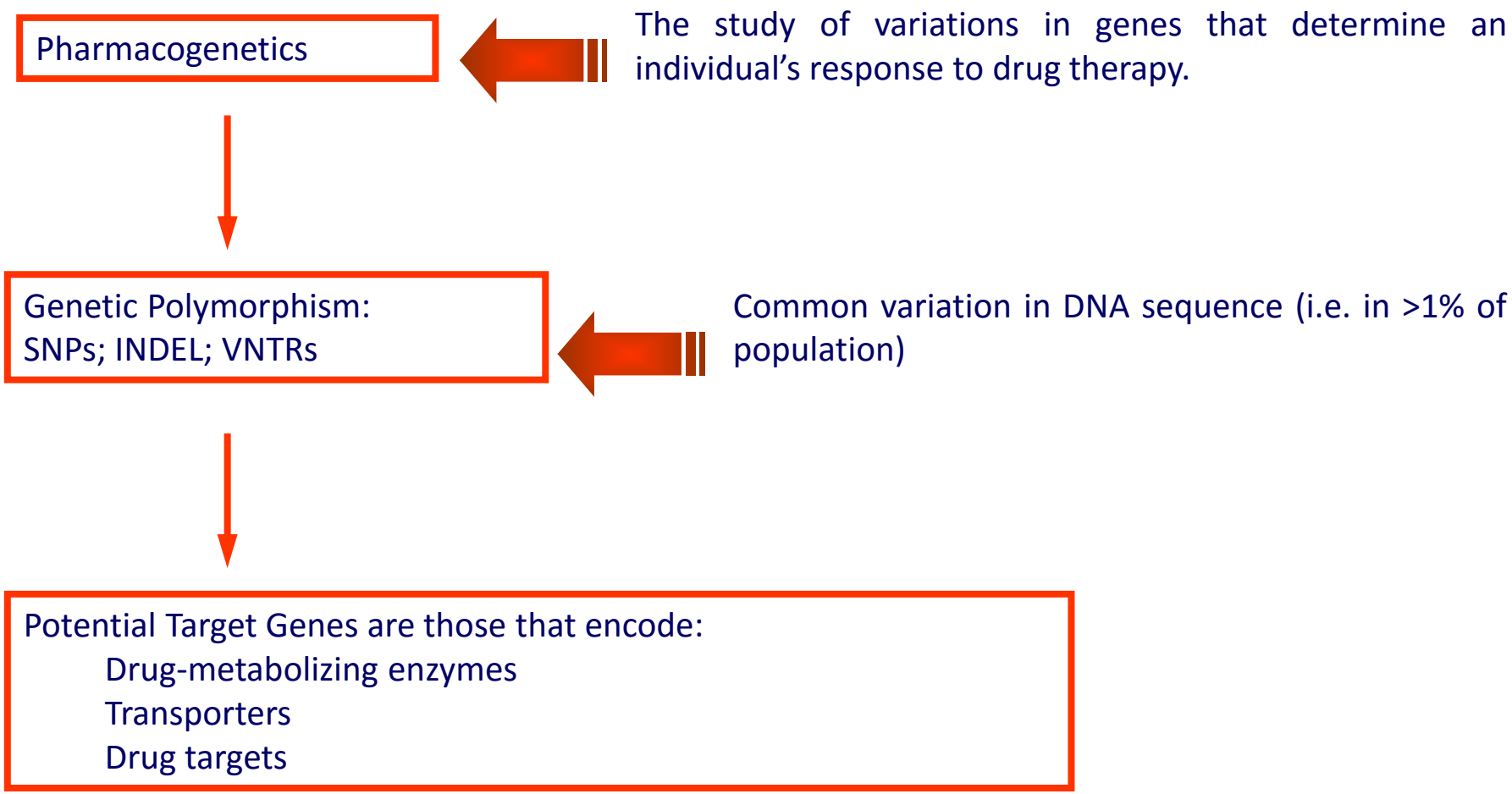
TODAY versus TOMORROW



The Goal of Personalized Medicine

- The Right Dose of
- The Right Drug for
- The Right Indication for
- The Right Patient at
- The Right Time.

Courtesy Felix W. Frueh


Pharmacogenetics
The study of variations in genes that determine an individual's response to drug therapy.
Genetic Polymorphism:
SNPs; INDEL; VNTRs
Common variation in DNA sequence (i.e. in >1% of population)
Potential Target Genes are those that encode:
Drug-metabolizing enzymes
Transporters
Drug targets

The Technology: Deep Sequencing

- Captures *every* base pair in the genome (3,000,000,000)
- (Currently) low throughput (slow)
- (Currently) Very expensive (> 10k)
- Captures common, rare, and personal variation
- New and hard to analyze

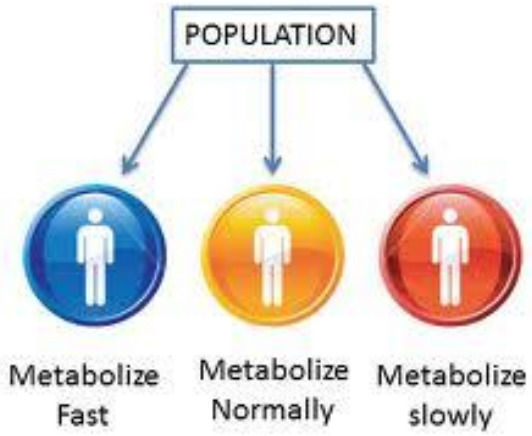
Sequencer

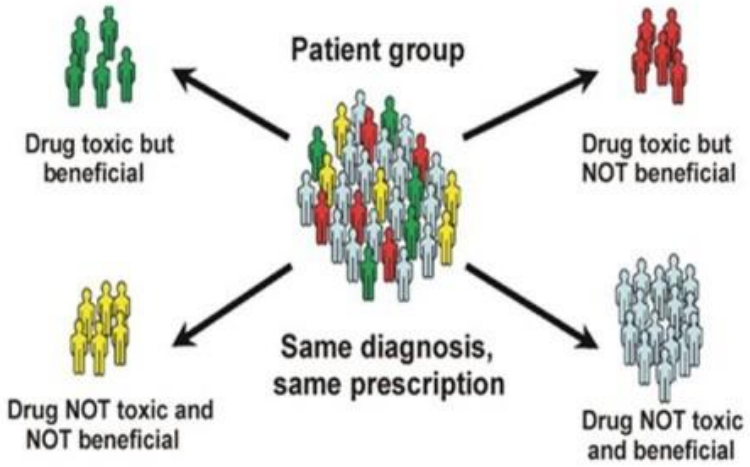
A T C G A A A T G C A T G A C C T T T G A T A T G A T C G G C T G C A G T C A G C
T T C G A A G T G C A T G A C T T T T G A C A T G A G C G G C G G C C C A C A G C

Common Variation **Rare Variation** **No Recorded Variation**

Back to the drugs…

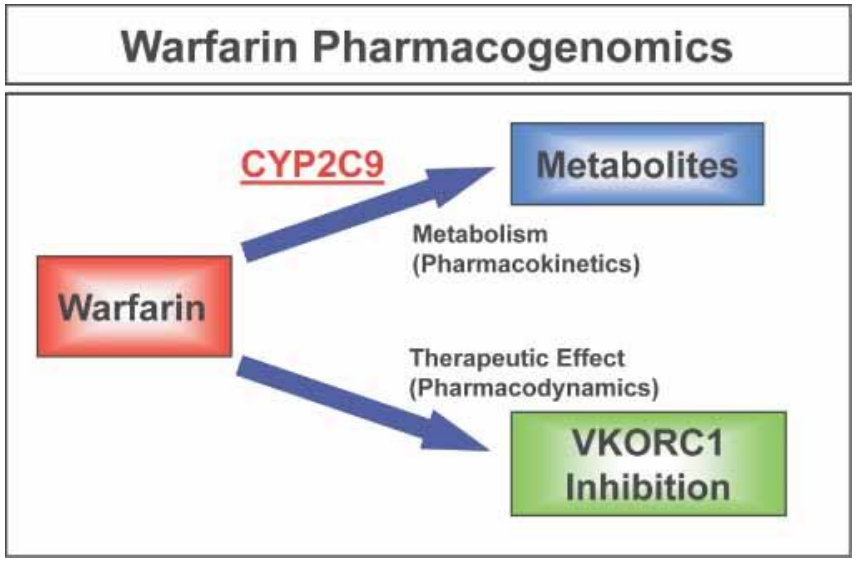
- The utility of pharmacogenetics:
  - Determining appropriate dosing
  - Avoiding unnecessary toxic treatments
  - Ensuring maximal efficacy
  - Reducing adverse side effects
  - Developing or choosing novel treatments
  - Can also explain variable response to illicit drugs

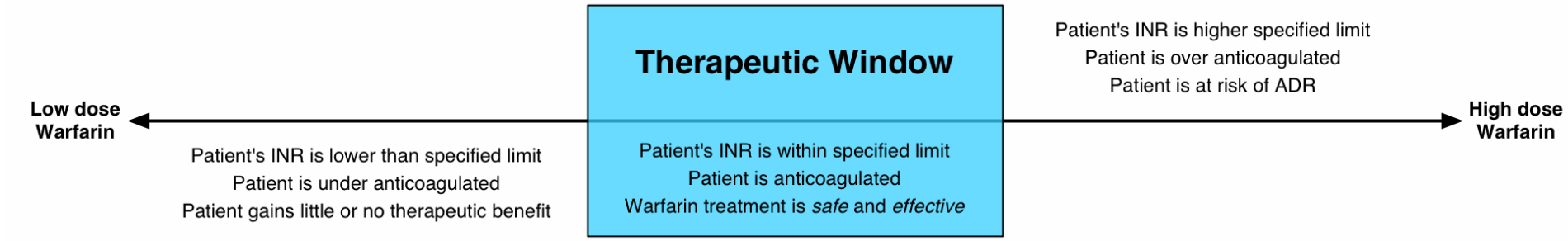




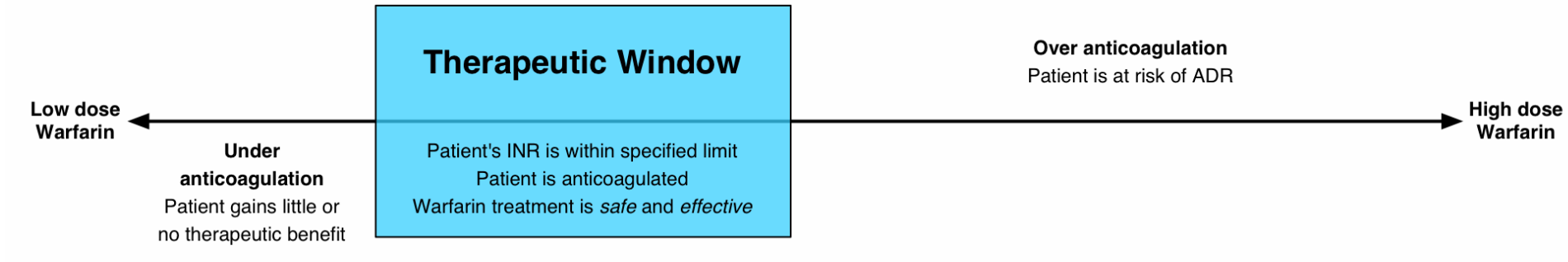
Warfarin: A dosage story

- Most widely used anticoagulant in the world
  - A “blood thinner”
- Prescribed doses vary widely (1-40mg / daily)
- Therapuetic index is very low
  - High risk of bleeding early in treatment
- Two genes involved in metabolism: *CYP2C9* and *VKORC1*

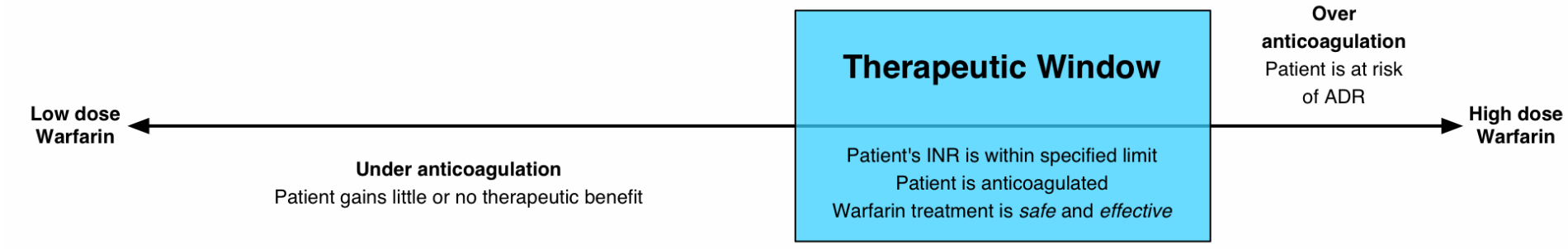




Homozygous wild-type *CYP2C9* and *VKORC1*

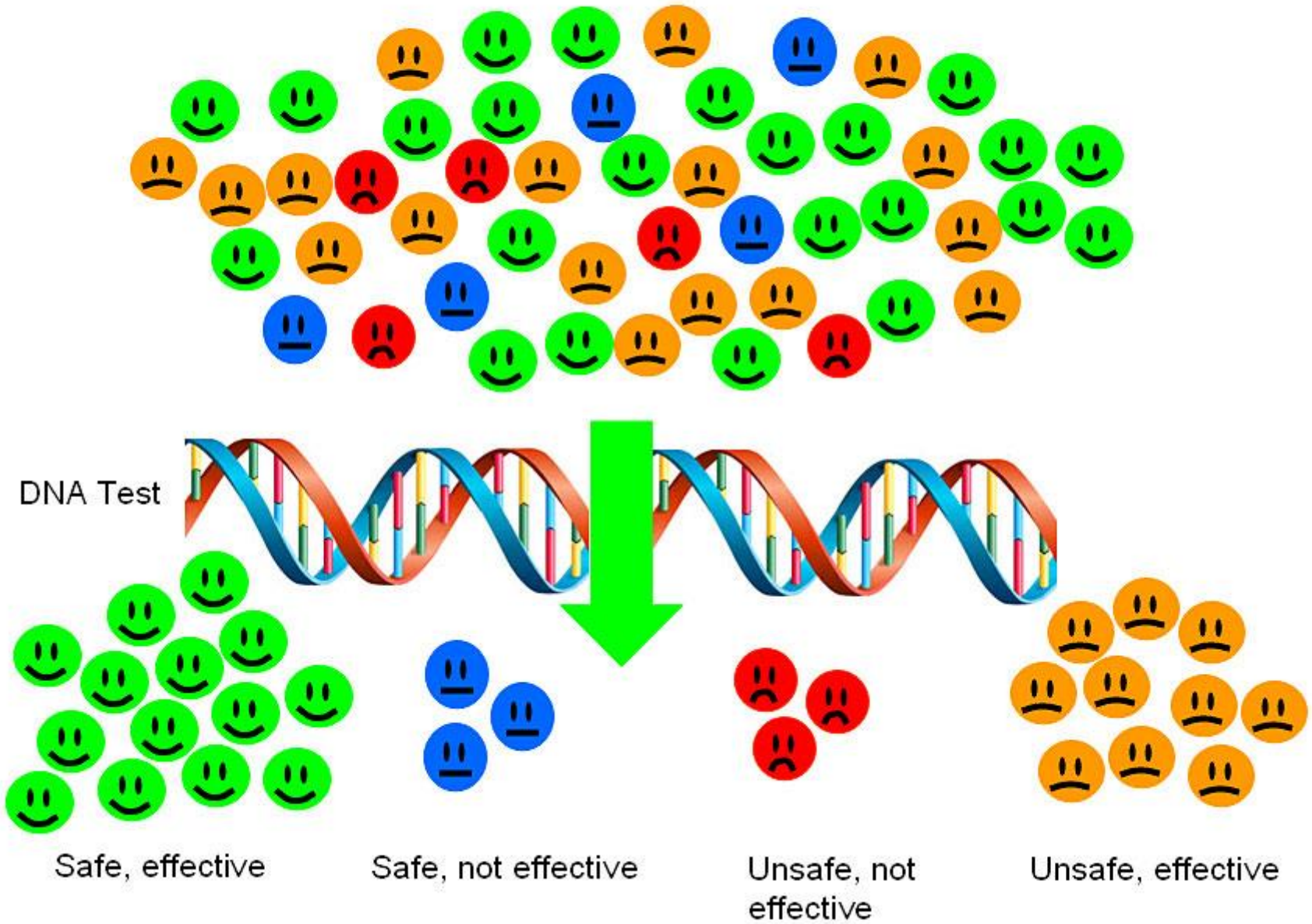


Carrier of *CYP2C9* mutant allele



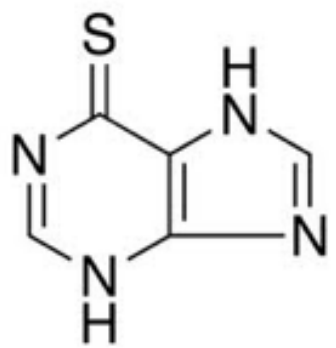
Carrier of *VKORC1* mutant allele

Your DNA Affects Your Response to Drugs

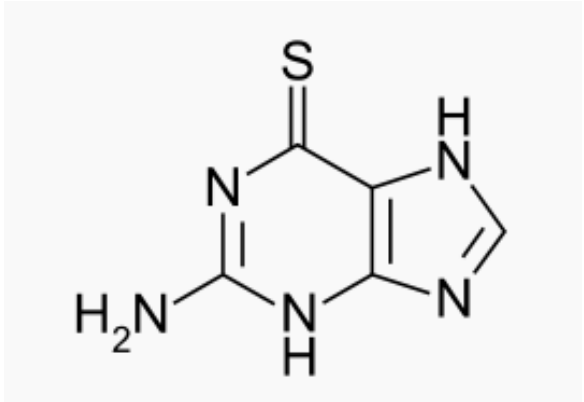


Purine Analogs: A Case Study in Pharmacogenetics

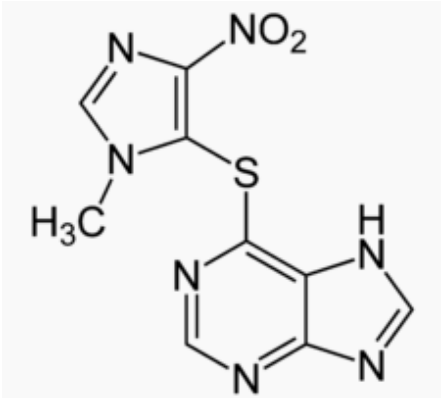
- 6-mercaptopurine, 6-thioguanine, azathioprine
- Used to treat lymphoblastic leukemia, autoimmune disease, inflammatory bowel disease, after transplant
- Interferes with nucleic acid synthesis
- Therapeutic index limited by myelosuppression
  (treatment limited by immune suppression side effect)



6-mercaptopurine

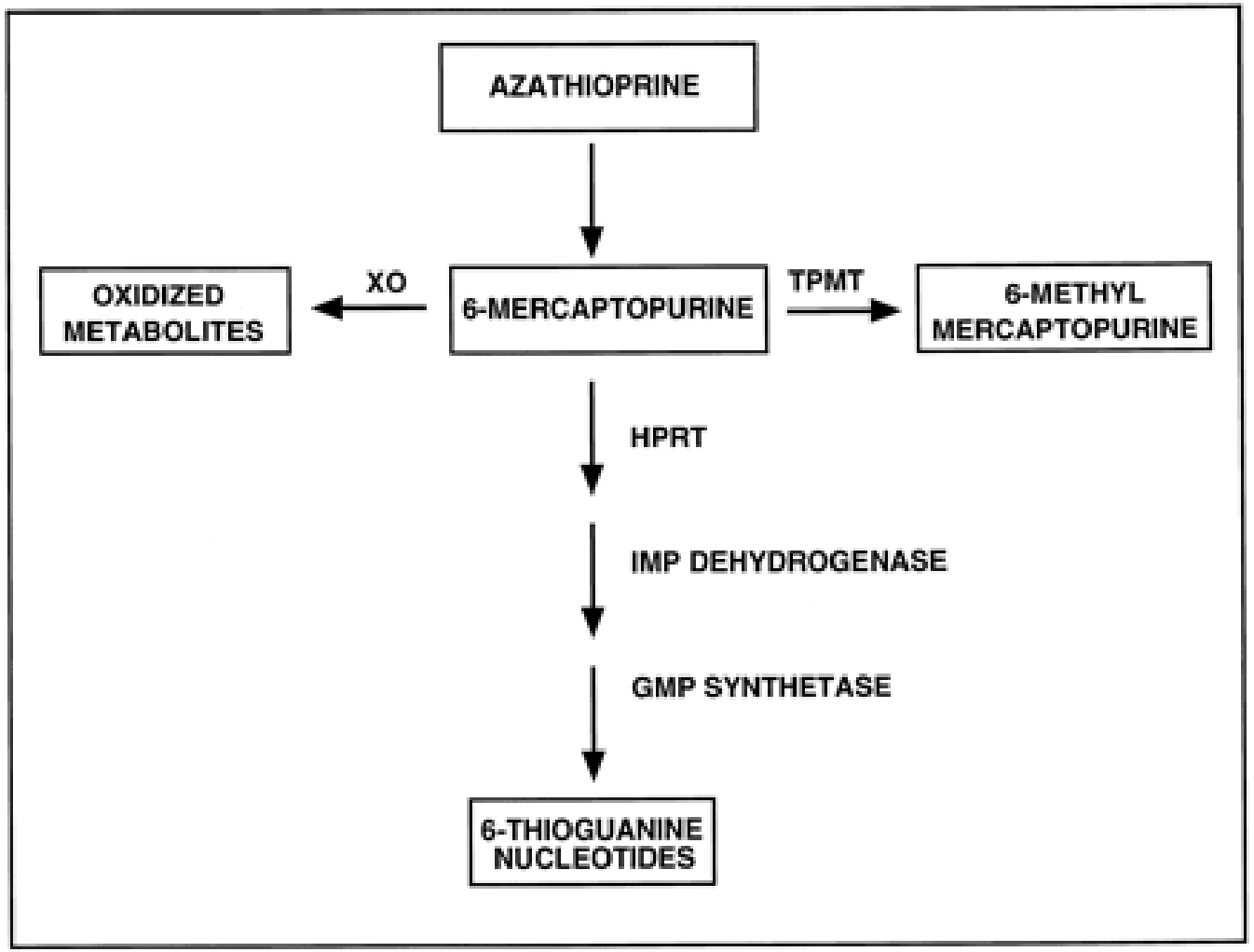


6-thioguanine



azathioprine

Metabolism of 6-MP



L Wang and R Weinshilboum, Oncogene 25, 1629-1638 (2006)

Pharmacogenetics: A Case Study

Ph

Individuals respond differently to the anti-leukemia drug 6-mercaptopurine.

Most people metabolize the drug quickly. Doses need to be high enough to treat leukemia and prevent relapses.

Others metabolize the drug slowly and need lower doses to avoid toxic side effects of the drug.

A small portion of people metabolize the drug so poorly that its effects can be fatal.

Courtesy of Michelle Whirl-Carillo

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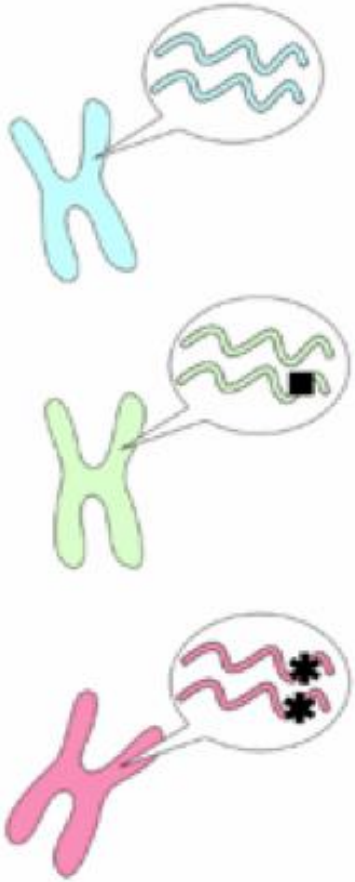
The diversity in responses is due to variations in the gene for an enzyme called TPMT, or thiopurine methyltransferase.



Courtesy of Michelle Whirl-Carillo

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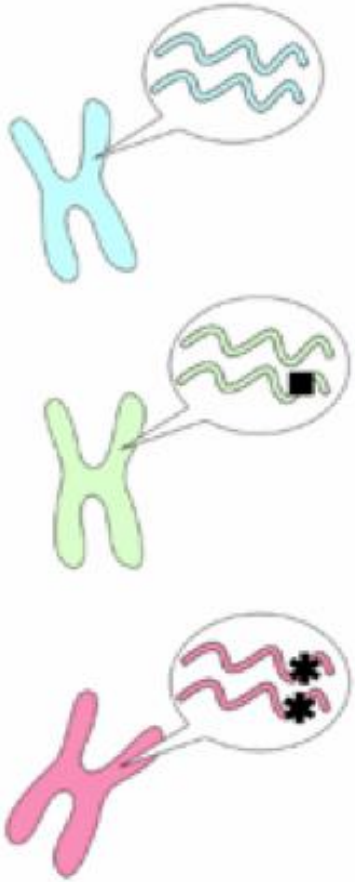
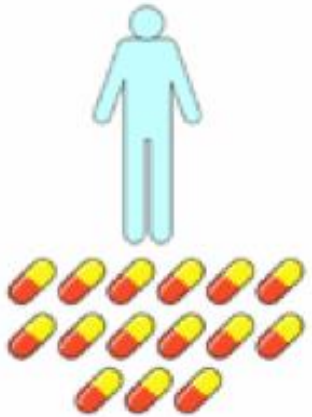


After a simple blood test, individuals can be given doses of medication that are tailored to their genetic profile.



Normal dose

Dose for an extra slow metabolizer (TPMT deficient)

Courtesy of Michelle Whirl-Carillo

Second Example: Codeine and Cytochrome P450 CYP2D6

- Codeine is a commonly used opioid
  - Codeine is a prodrug
  - It must be metabolized into morphine for activity
- Cytochrome P450 allele CYP2D6 is the metabolizing enzyme in the liver
- 7% of Caucasians are missing one copy of the Cytochrome P450 CYP2D6 gene
  - codeine does not work effectively in these individuals

Courtesy of Michelle Whirl-Carillo

Cytochrome Oxidase P450 Enzymes

- 57 Different active genes
- 17 Different families
- CYP1, CYP2 and CYP3 are primarily involved in drug metabolism.
- CYP2A6, CYP2B6, CYP2C9 ,CYP2C19, CYP2D6, CYP2E1 and CYP3A4 are responsible for metabolizing most clinically important drugs

Effect of Metabolic Rate on Drug Dosage

Drug	Poor Metabolizer Phenotype
Prodrug, needs metabolism to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Poor efficacy Possible accumulation of prodrug
Active drug, inactivated by metabolism (example is omeprazole)	Good efficacy Accumulation of active drug can produce adverse reactions May need lower dose

Drug	Ultra-rapid Metabolizer Phenotype
Prodrug, needs metabolism to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Good efficacy, rapid effect
Active drug, inactivated by metabolism (example is omeprazole)	Poor efficacy Need greater dose or slow release formulation

FDA Requires Genetic Tests for Certain Therapies

List of FDA Required or Recommended Biomarker Tests in Drug Labels

Biomarker	Test[13]	Drug Example	User Prevalence (%) (n=36.1 million)
CYP2C9	Recommended	Warfarin	2.0896
EGFR	Required	Cetuximab	0.0001
G6PD deficiency	Recommended	Dapsone	0.0257
G6PD deficiency	Recommended	Rasburicase	0.0000
HER2/neu overexpression	Required	Trastuzumab	0.0003
TPMT variants	Recommended	Azathioprine	0.1168
TPMT variants	Recommended	Mercaptopurine	0.0541
TPMT variants	Recommended	Thioguanine	0.0012
UGT1A1 variants	Recommended	Irinotecan	0.0002
Urea cycle enzyme deficiency	Recommended	Valproic acid	0.48
Total			2.768

CYP = cytochrome P450; EGFR = human epidermal growth factor receptor; G6PD = glucose-6-phosphate dehydrogenase; HER2/neu = human epidermal growth factor receptor 2; TPMT = thiopurine S-methyltransferase.