Pharmacogenomics

Michael Murray
Pharmacogenomics and Drug Development
Discipline of Pharmacology

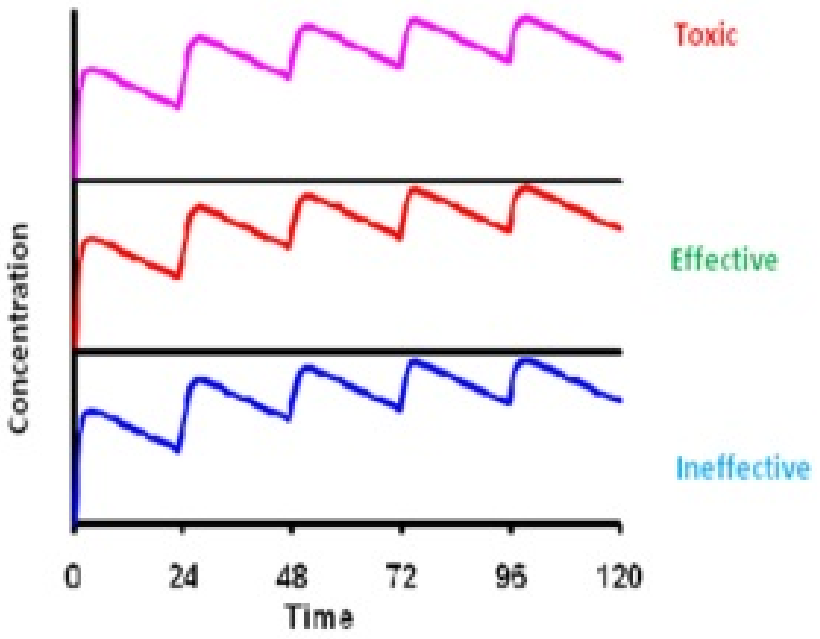
Focus of this lecture

- Drug toxicities and adverse drug reactions (ADRs)
- Type A (predictable) ADRs
  - *Genetic factors and mechanisms*
- Type B (drug hypersensitivity) ADRs
  - *Reactive metabolites and MHC molecules*
- Development of pharmacogenomic tests to prevent ADRs

All Chemicals are Potentially Toxic

- "All substances are poisonous; there are none that are not poisons. The right DOSE differentiates a poison and a remedy."
  - Paracelsus (1493-1541)
- This is true for most ADRs but some are unrelated to dose
- ADRs are responses to drugs that are unintended and that occur in man at near-normal therapeutic doses

Therapeutic window



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- Therapeutic index is the ratio of the toxic dose to the therapeutic dose
- Narrow for drugs like digoxin, warfarin, clozapine and lithium
- Therapeutic drug monitoring important for toxic drugs

Drugs and iatrogenic injury

- Drug treatment is itself a major cause of adverse events
- Recent estimate: up to 2 million patients are hospitalised annually in the US because of severe ADRs
- ADRs are considered to be the fourth to sixth leading cause of death in the US
- ~15% of courses of drug treatment may lead to an ADR

Types of ADRs

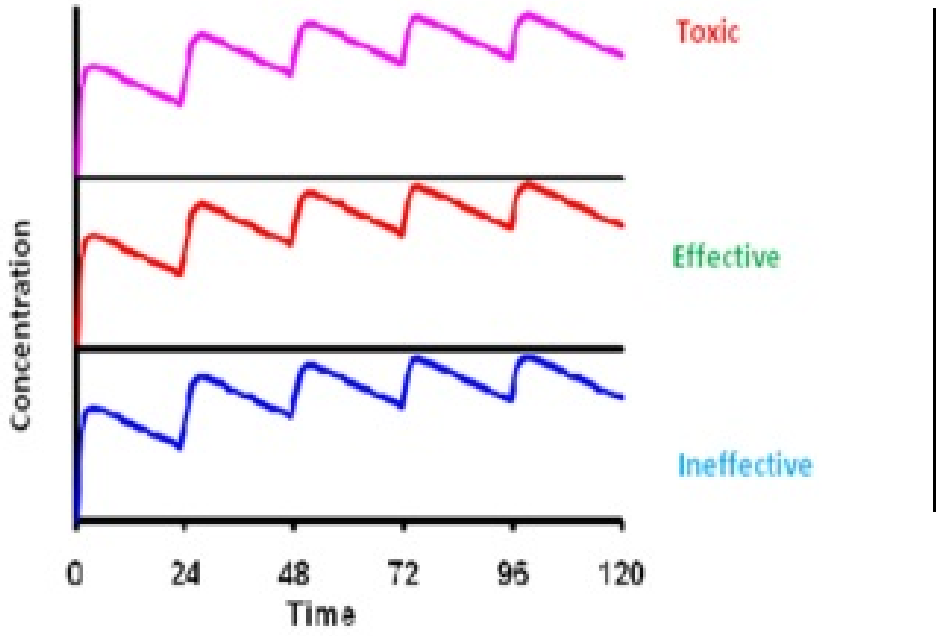
- **Type A**
  - Predictable
  - Usually related to the principal pharmacological actions of the drug
  - However, higher doses of some drugs cause predictable ADRs that are unrelated to their pharmacological actions
  - eg paracetamol hepatotoxicity and doxorubicin cardiotoxicity
- Treatment is to reduce dose or withhold drug

Types of ADRs

- **Type B**
  - Unpredictable or idiosyncratic
  - Less common than type A reactions
  - Unrelated to the principal pharmacological actions of the drug
  - High mortality
- Treatment is to withhold drug and avoid rechallenge, even with very low doses

ADRs and the therapeutic window

Type A



Type B

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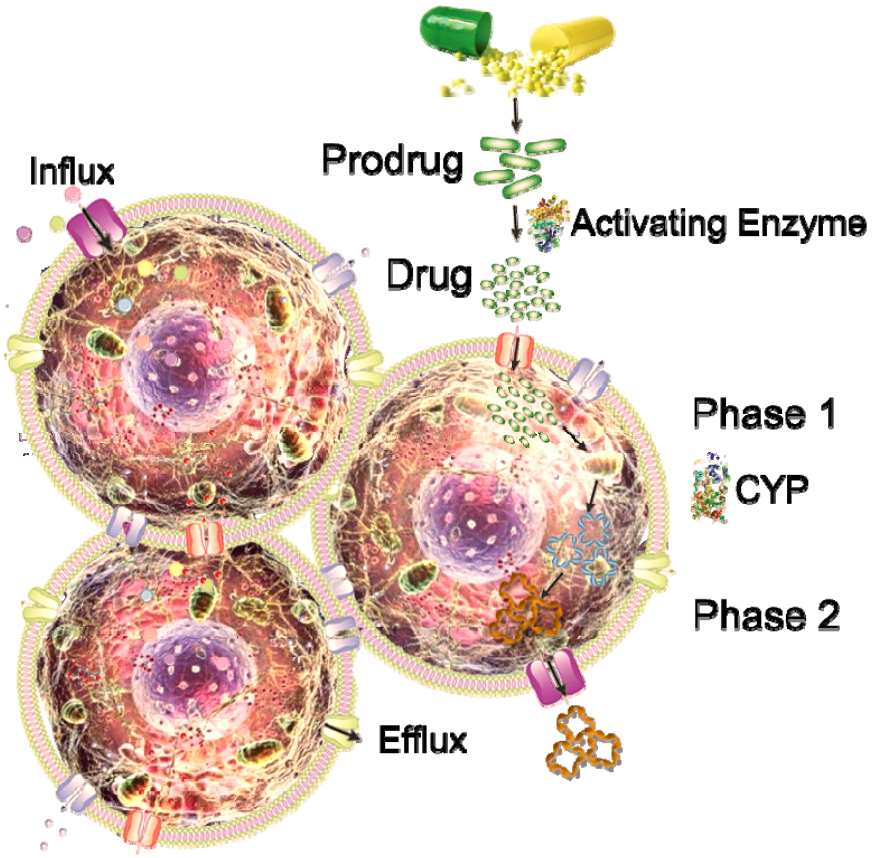
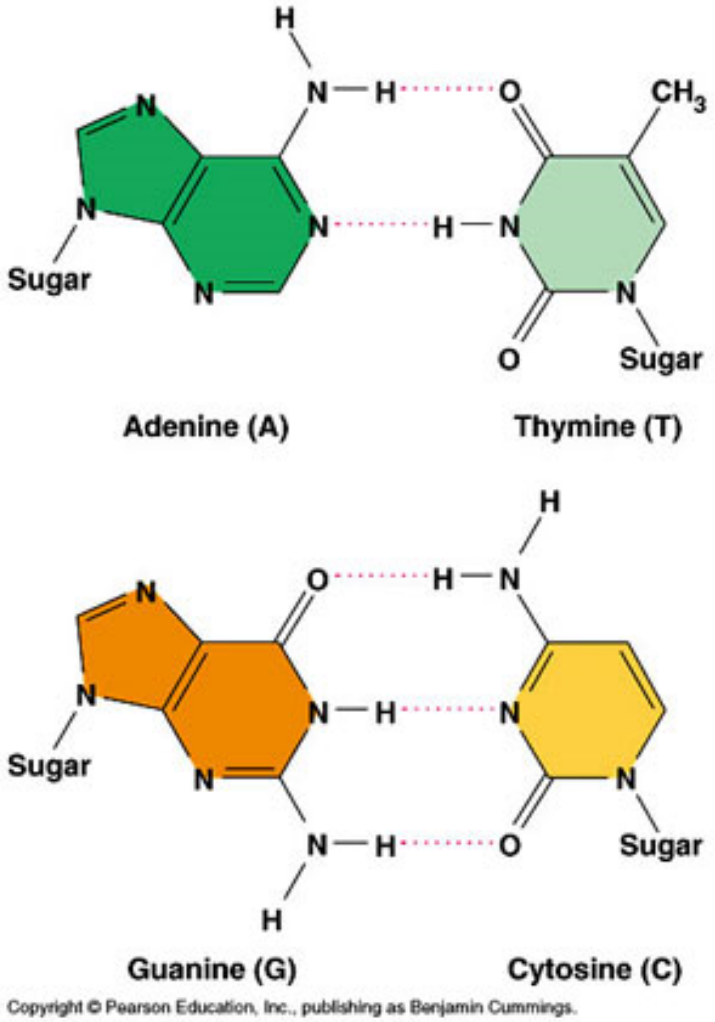
Pharmacogenetic variation (ADME factors)

- Transporter proteins and biotransformation enzymes determine drug influx into and efflux from cells and the duration of drug action
- Genes encoding these proteins are often highly polymorphic
- Defective proteins may promote drug accumulation and toxicity



Type A ADRs – pharmacogenetic background

- The human genome contains 3.1 billion nucleotide bases
- Estimated total number of genes between 20,000 and 25,000
- 99.9% of nucleotide bases are identical between individuals
- Alleles are alternate forms of the same gene or genetic locus



http://porpax.bio.miami.edu/~cmallery

Common polymorphisms - definitions

- Polymorphisms occur in at least 1% of population and exhibit a *stable inheritance pattern*
- Even though genomes are 99.9% identical this still allows 3 to 10 million sequence differences between any 2 genomes

Single Nucleotide Polymorphism (SNP):

GAATTTAAG
GAATTCAAG

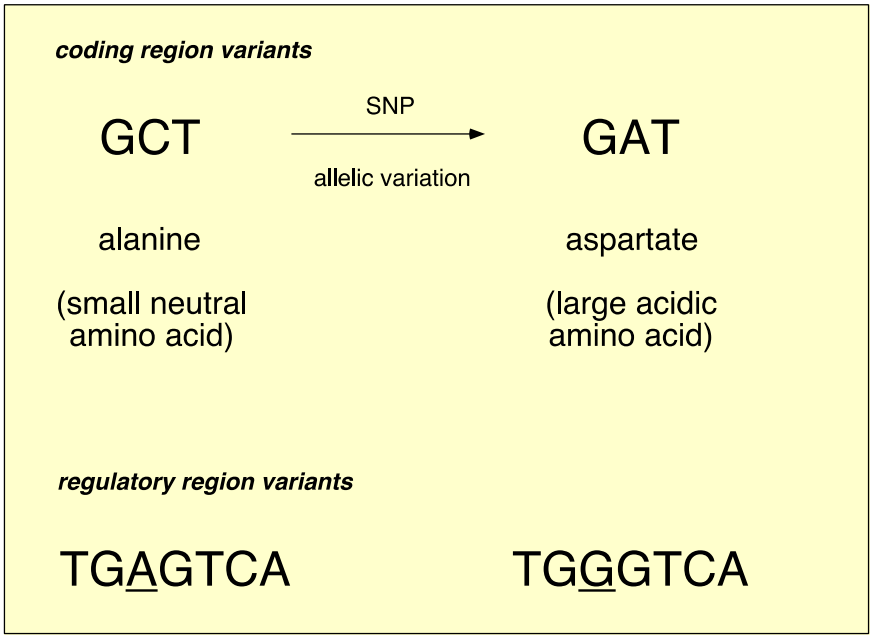
Variable number of tandem repeats (VNTR): (here repeat of 2bp-CA)

(4) CACACACA
(7) CACACACACACACA
(6) CACACACACACA

Insertions/Deletions:

GAAA -TCCAAG
GAAATTCCAAG

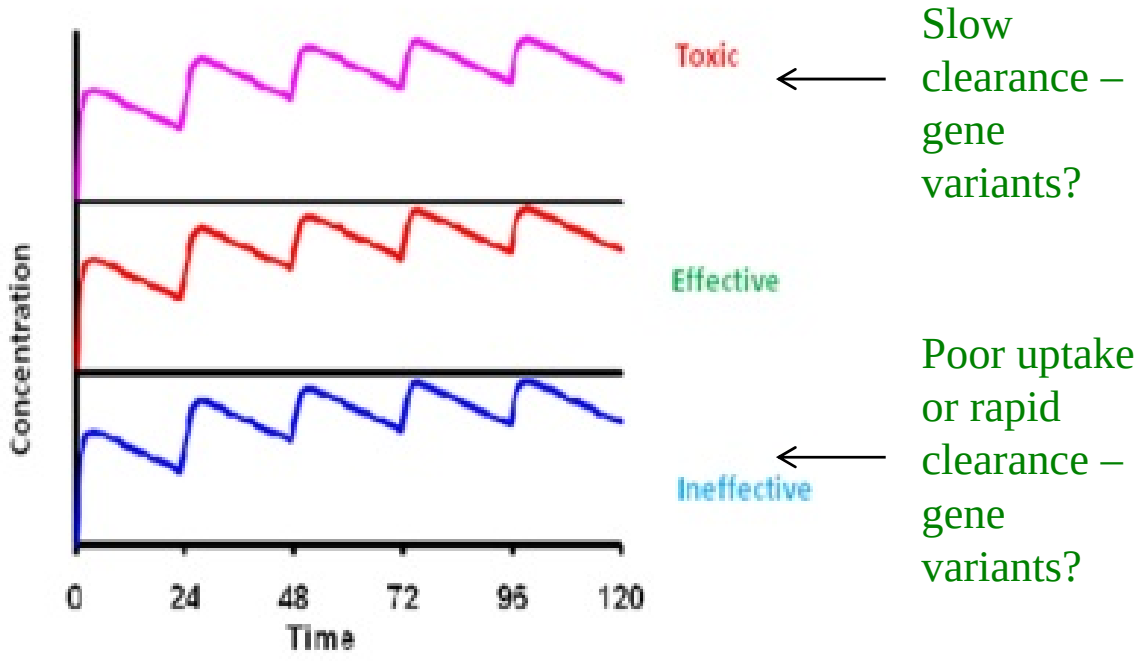
SNPs and their impact on gene function



- Codons are base triplets that encode an amino acid
- Coding region variants usually alter amino acid sequence of the encoded protein
- Regulatory region variants usually alter the amount of the protein produced

Pharmacogenomics and Type A ADRs: Predictable toxicity related to drug action – anticancer agents

- Most established anticancer agents have low therapeutic indexes
- 25-50% of cancer patients may not be treated effectively by chemotherapy
- Unpredictable toxicity in many patients, but also
- Under-treatment that leads to failure of therapy is quite common
- To date there has been a strong focus on variants of transporters and drug metabolising enzymes that affect drug disposition



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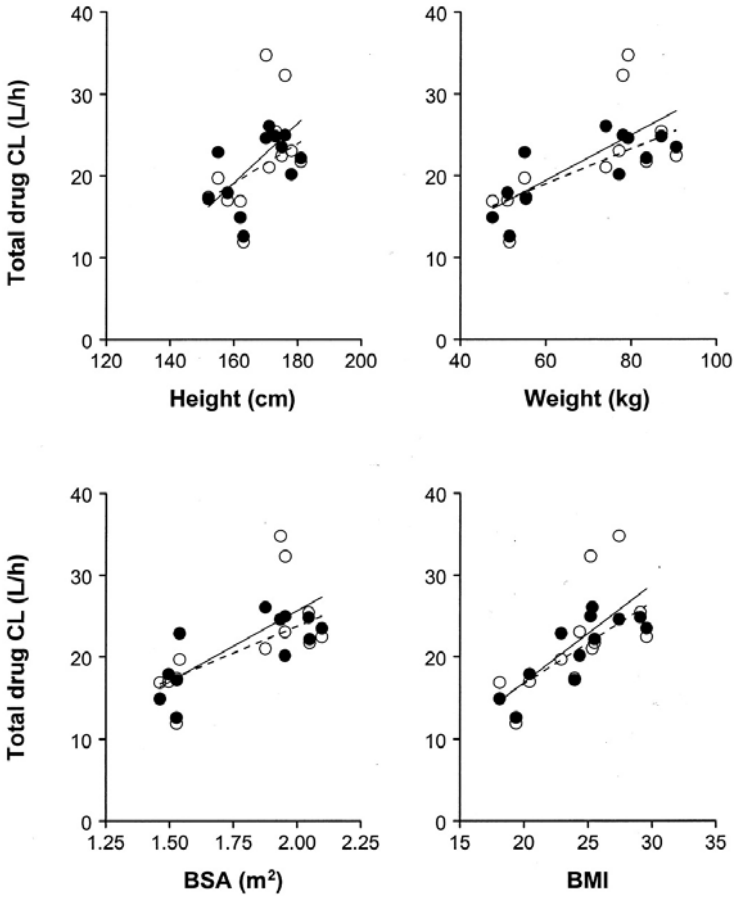
Dosing of anticancer agents: current approaches

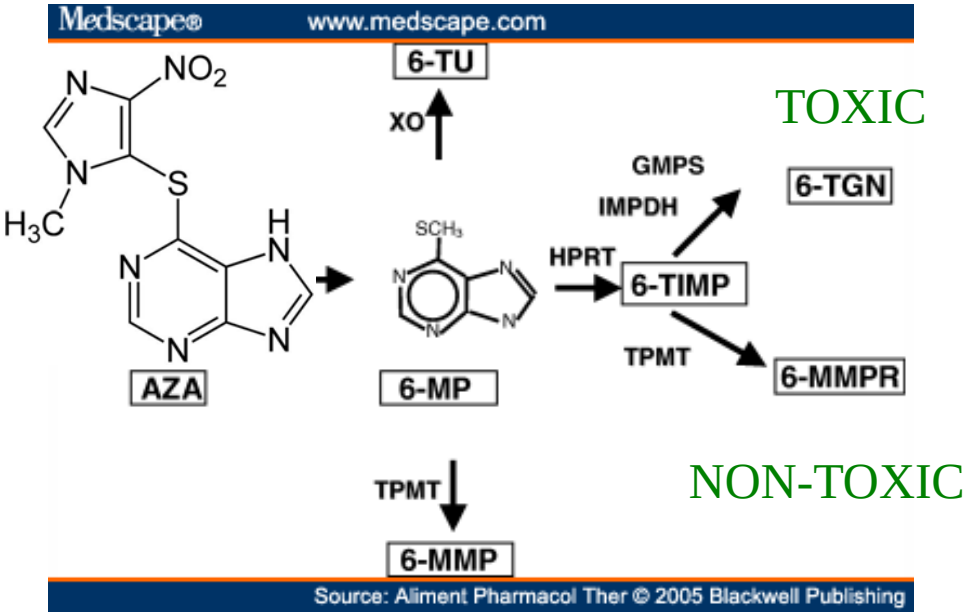
- Body surface area used to calculate initial dose
- Adjust dosage upwards until toxicity, then decrease
- If toxicity occurs at initial dose level adjust downwards
- Severe toxicity: dose interruption/cessation (risk of under-treatment and failure)
- NOTE: No patient- or tumour-specific factors considered in dose determination



Smorenburg C H et al. JCO 2003;21:197-202

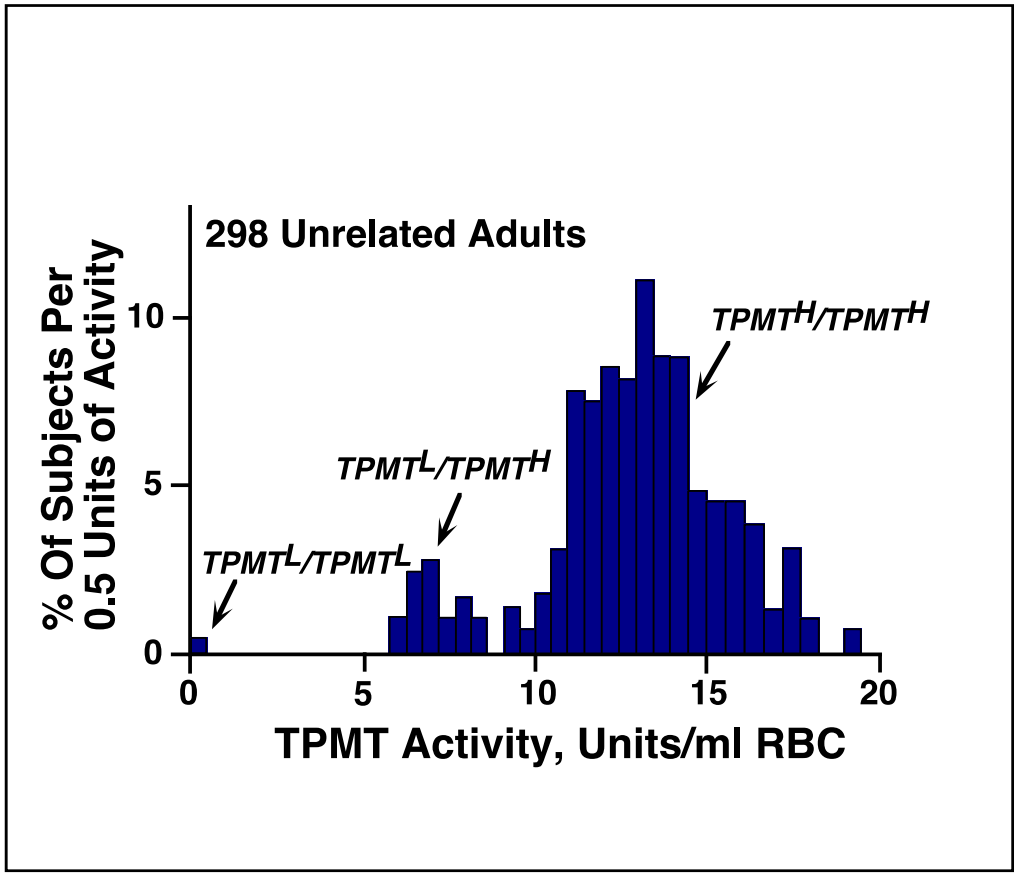
Thiopurine methyltransferase (TPMT)

- 6-mercaptopurine (6MP) used for treatment of ALL (acute lymphoblastic leukaemia)
- Azathioprine is a prodrug for 6MP used in immunosuppression
- Cytotoxic effect due to formation of 6-thioguanine nucleotides (6TGN) that inhibit gene replication
- TPMT deactivates 6MP so when it is deficient 6TGN are over-produced leading to ADRs



TPMT pharmacogenomics

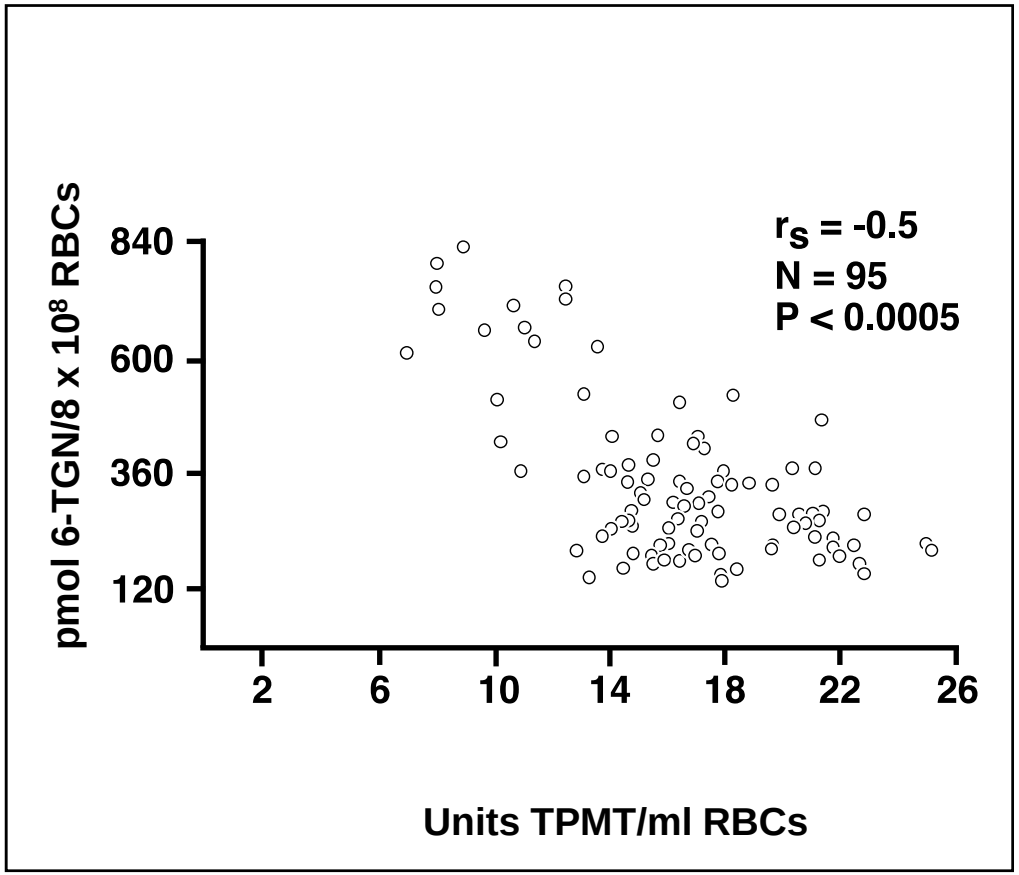
- Can measure 6TGN or TPMT activity in erythrocytes as a surrogate for overall TPMT status
- Wide inter-patient variation in activity
- 1 in 300 patients homozygous for low activity alleles
- Now genotyping for TPMT alleles is also possible



www.fda.gov/ohrms/dockets/ac/03/slides/3971S1_04_Weinshilboum.ppt+tpmt+fda

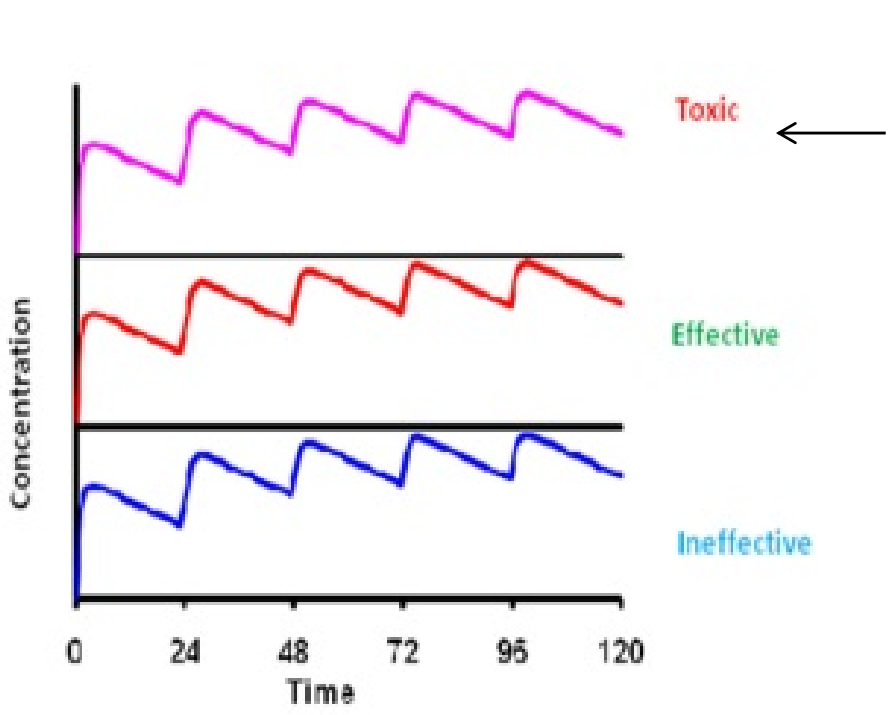
TPMT pharmacogenomics

- Patients who have low activity: frequent dose adjustments with AZA
- Those with normal activity: tolerate usual doses of AZA
- Those with high activity: low incidence of neutropenia, ?possible under-dosing



TPMT pharmacogenomics and therapeutic window

- Low TPMT activity
  - Defective alleles
  - Over-production of toxic 6TGN with standard doses of AZA
  - Lower production of non-toxic 6MMPR and 6MMP



Slow clearance – defective TPMT gene variants

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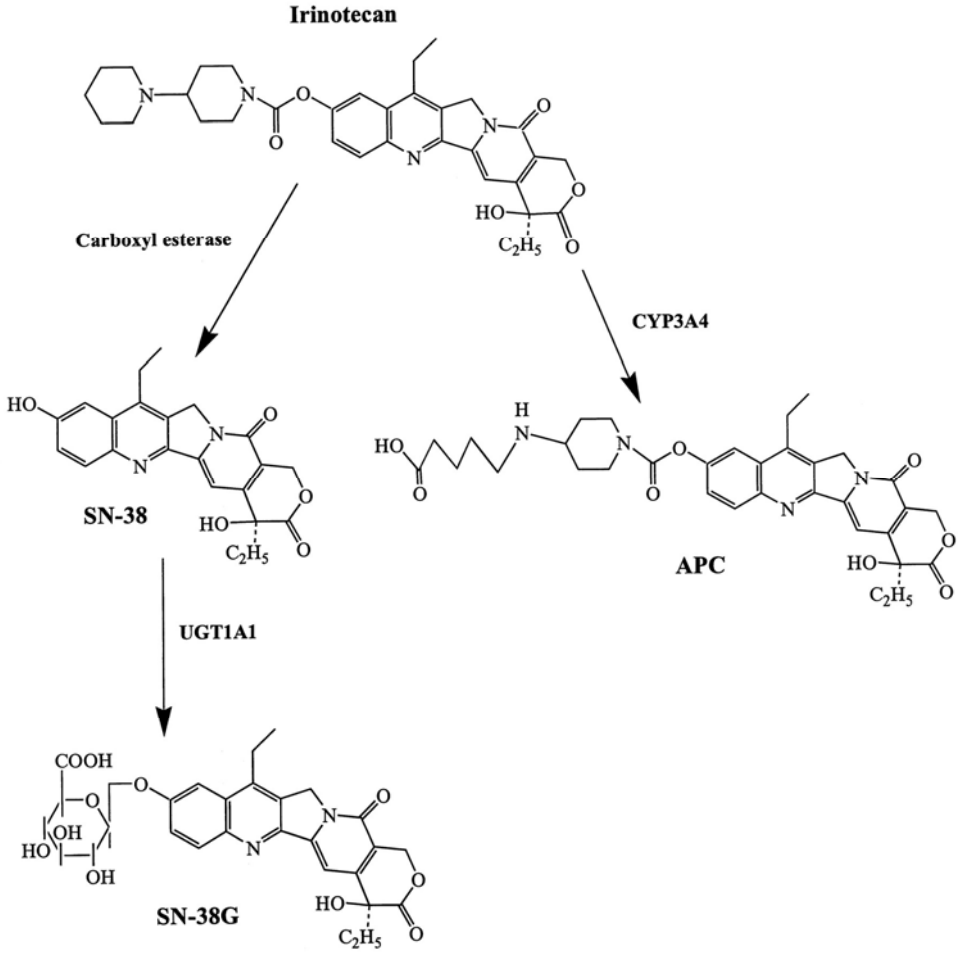
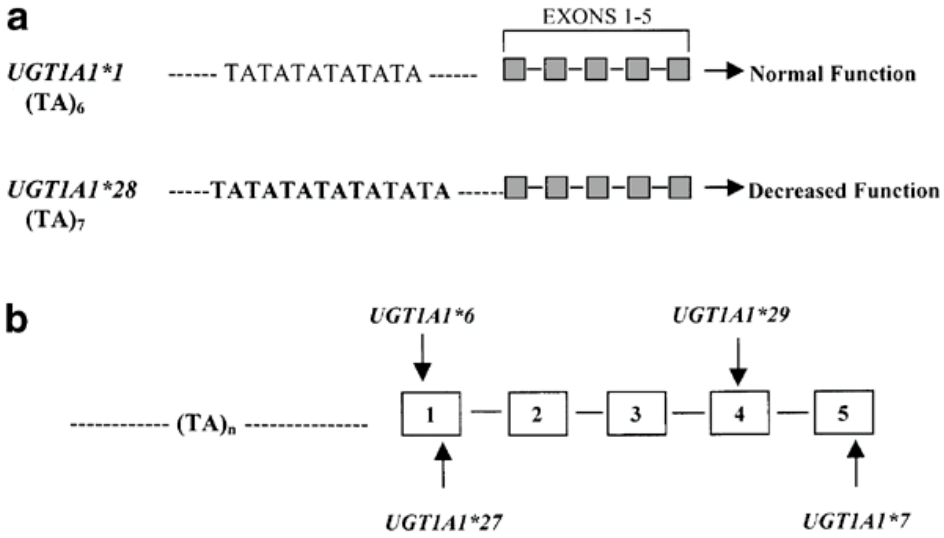
Irinotecan

- Topoisomerase inhibitor
- Topoisomerase repairs DNA that gets tangled in rapidly dividing cells
- Used in colorectal cancer but neutropaenia and delayed diarrhoea occurs in up to 40% of patients
- May require hospitalisation
- Irinotecan biotransformation is complex. The metabolite SN-38 is the active cytotoxic agent



UGT1A1 variants and SN-38 conjugation

- UGT1A1 mediates SN-38 conjugation
- Transcription is initiated at the TATA box in the UGT1A1 promoter
- **28* variant has 7 TA repeats instead of 6 (VNTR polymorphism)
- **28* variant – less transcription – less enzyme
- **28* homozygotes exhibit decreased SN-38 conjugation capacity and higher grade neutropaenia
- Another variant – the **6* allele – carries a coding region SNP – results in the amino acid substitution Gly71Arg – the encoded enzyme has decreased function



(Desai *et al*., Oncogene, 2002;
Tukey *et al*., Mol Pharmacol, 2002)

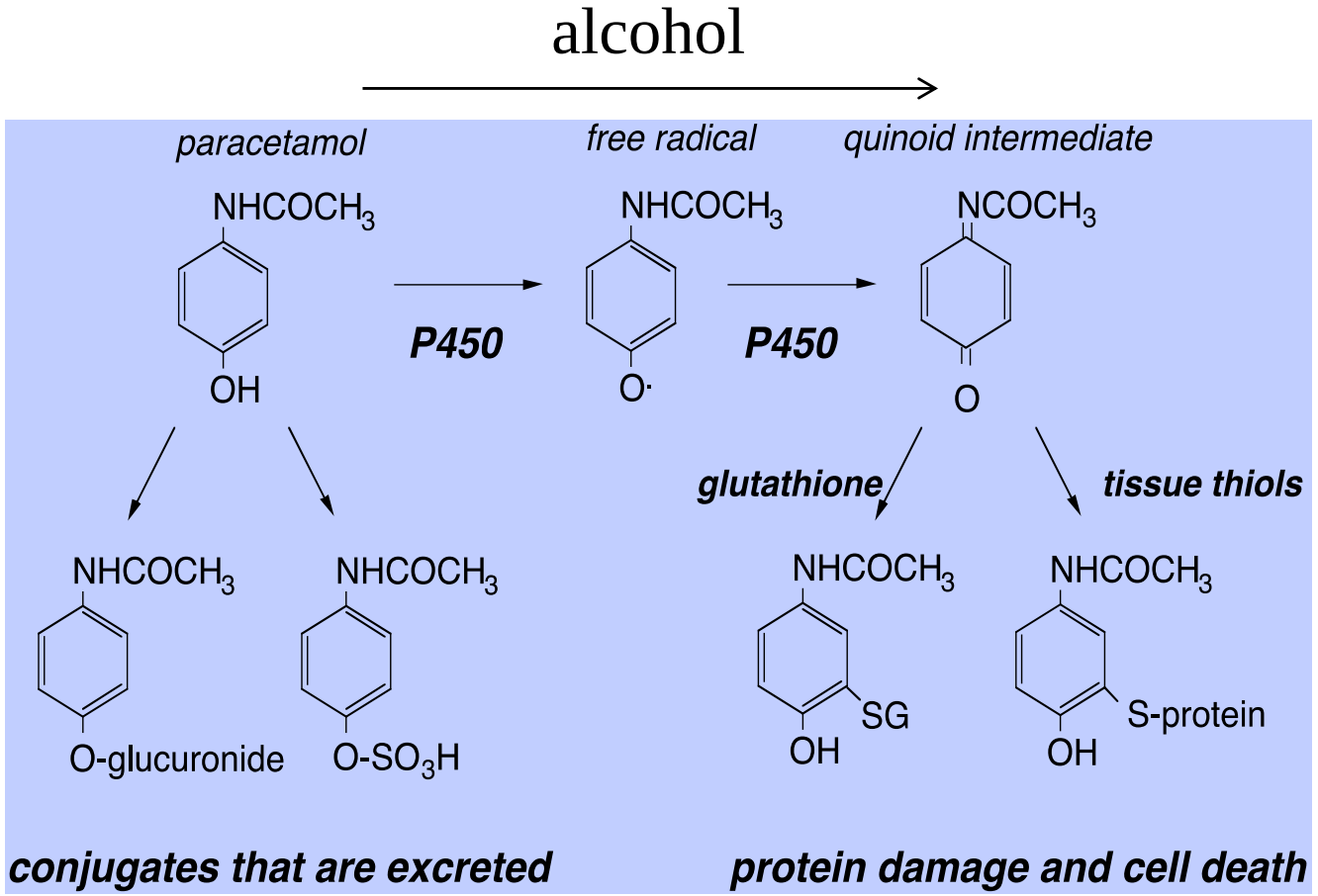
An exon is a DNA region that usually codes for protein (numbered above)

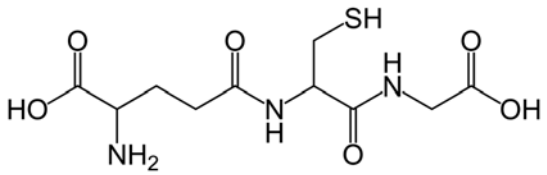
Ethnicity-related incidence of UGT1A1 variants

- Frequency of **28* allele >40% in sub-Saharan Africans and 30-40% in Caucasians, but lower in Asians (Haga *et al.*, Pharmacogenet Genom 2006)
- However, the low activity **6* allele is as frequent as **28* in Japanese, but is absent in Caucasians and Africans
- Whereas Caucasian **28* homozygotes are at risk from irinotecan toxicity susceptible Asians may carry either **28/*28, *6/*6* or **6/*28*
- The US FDA and Japanese/Singaporean regulatory bodies have different warnings for the use of irinotecan in relation to UGT1A1 variants and ADRs

Type A ADRs unrelated to drug action: paracetamol hepatotoxicity

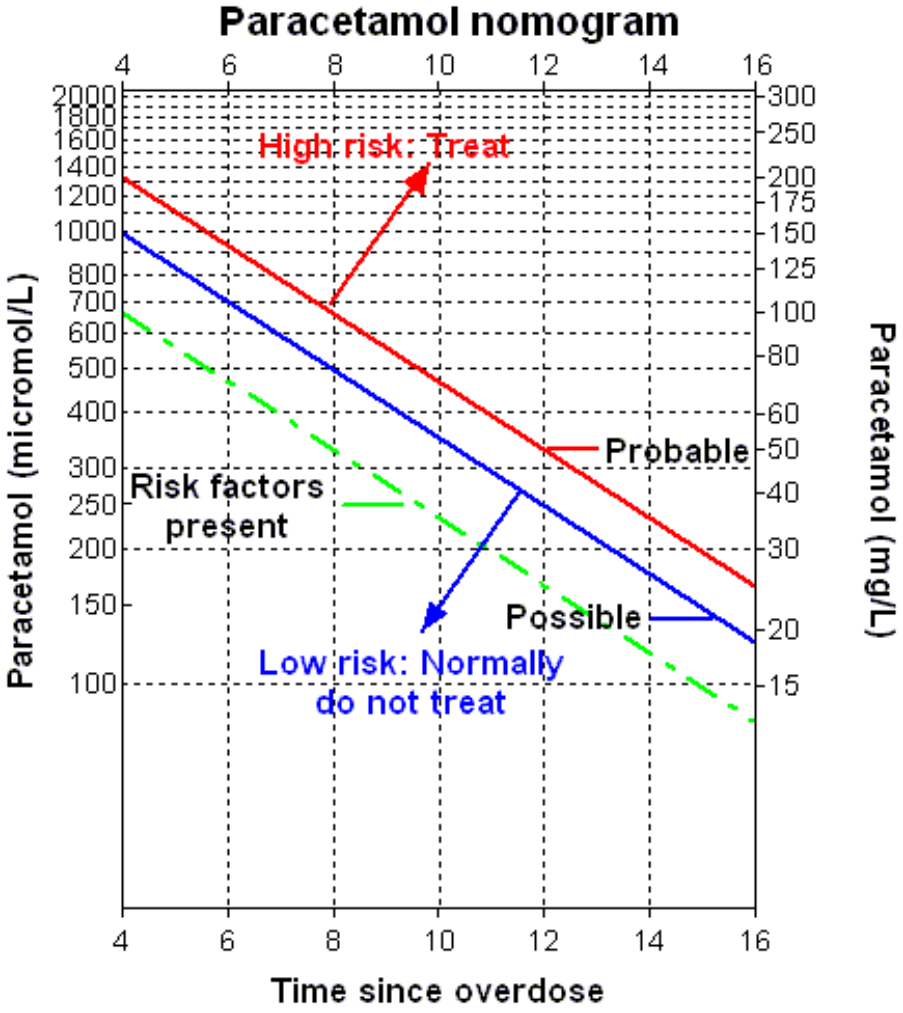
- Major metabolites are stable, non-toxic and readily eliminated (left side of diagram)
- Normally only ~5% of paracetamol is metabolized by CYP2E1 to the quinoid intermediate (right side)
- This amount of the intermediate is detoxified by glutathione.
- Alcohol increases formation of the toxic intermediate.
- High doses of paracetamol forms more of the toxic intermediate, depleting glutathione and causing hepatic injury.





glutathione

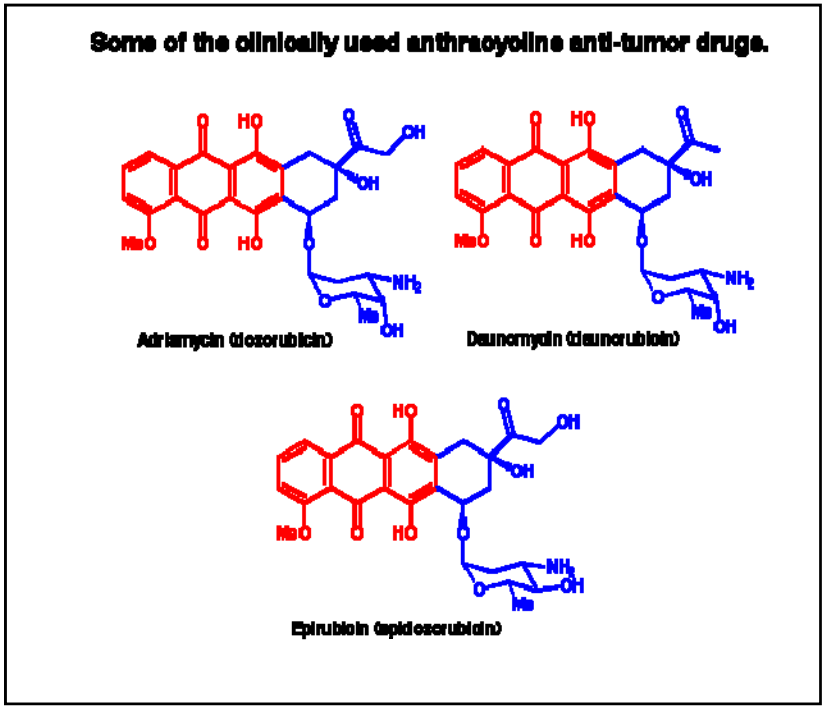
Nomogram to predict liver damage from plasma paracetamol concentrations



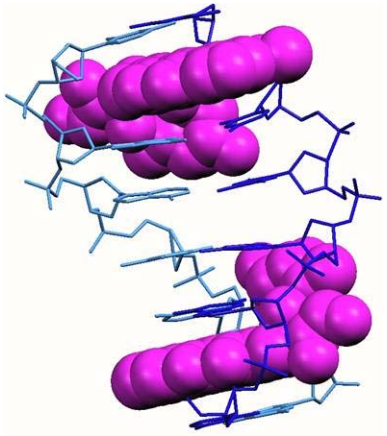
- Rumack-Matthew nomogram - doses greater than 150 mg/kg/24 hr (>10 g).
- Potential for hepatotoxicity after acute overdose is best predicted from serum paracetamol concentrations plotted against the time elapsed since ingestion.
- This is only a useful predictor if the person gets to hospital within 10 hrs of overdose.
- Treatment: N-acetylcysteine

http://members.ozemail.com.au/~ouad/doc/images/Paracetamol%20nomogram.gif

Type A ADRs unrelated to drug action: doxorubicin cardiotoxicity



http://stripe.colorado.edu/~kocht/Anthracycline.drugs.GIF

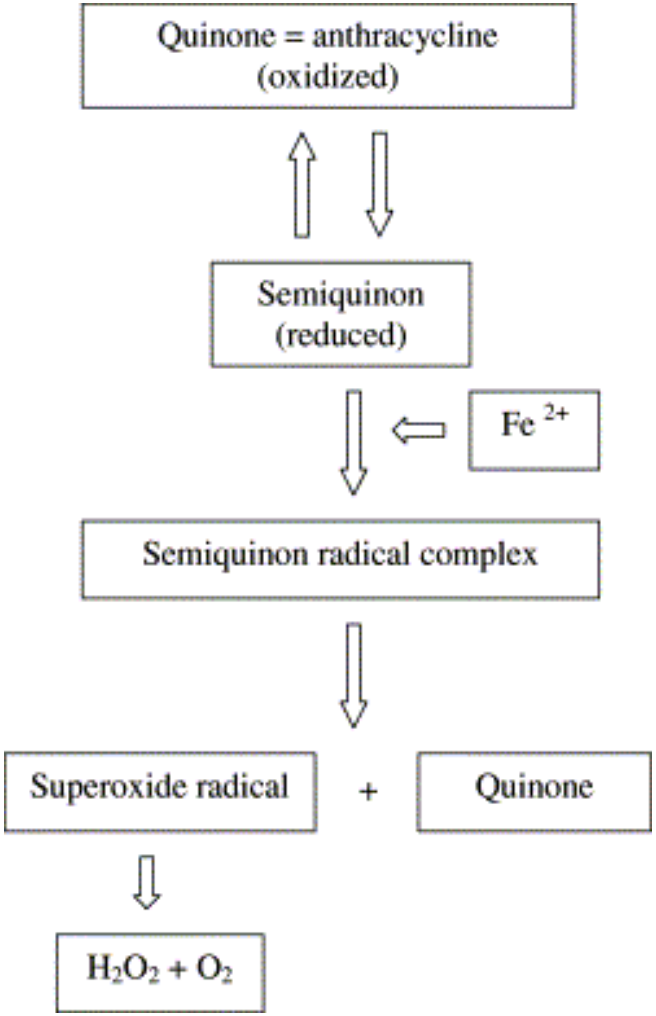


www.chem2.bham.ac.uk/ labs/ hannon/3wayjonction.htm

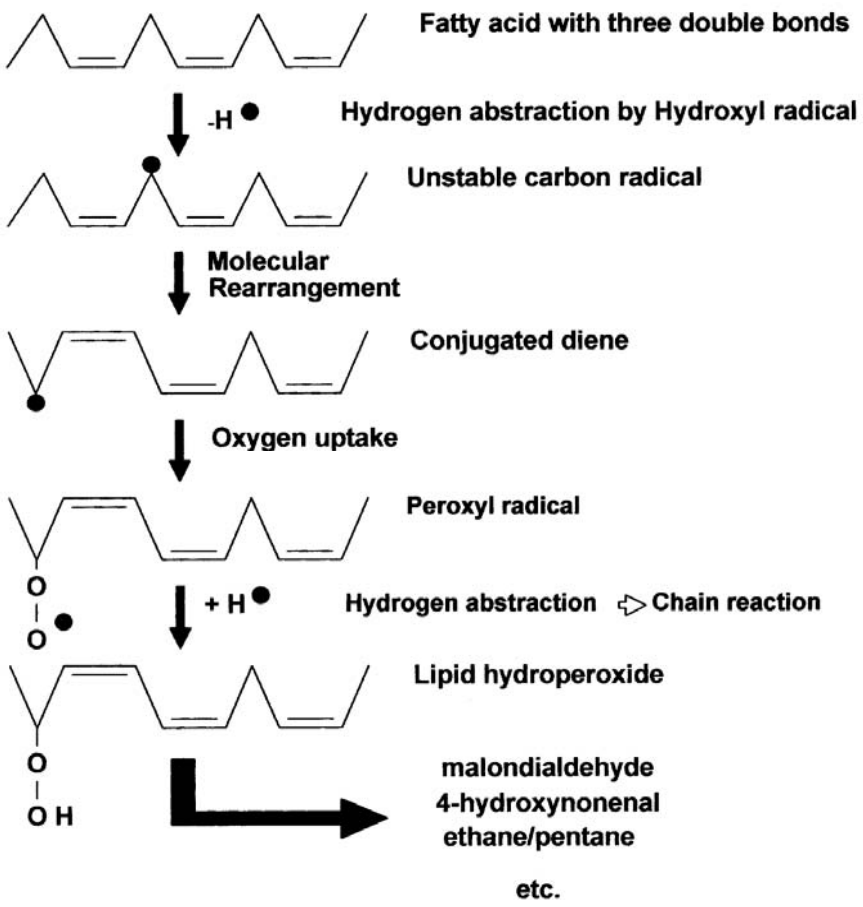
- Anthracycline type anticancer agents
- Bind to DNA and prevent replication
- Dose-related cardiotoxicity
- Heart failure

Doxorubicin toxicity

- Anthracyclines accumulate in the heart
- Accumulation produces oxidative stress and lipid peroxidation
- Heart tissue is very susceptible to oxidative stress
- Low levels of glutathione and antioxidants



Overview of lipid Peroxidation



http://www.biochemsoctrans.org/bst/029/0358/bst0290358a01.gif

- Radicals initiate lipid peroxidation
- After oxygen uptake reactive peroxyl radicals form
- Radicals are propagated
- Liberate reactive break-down products
- Also directly attack membranes and proteins

Predictability of Type A ADRs

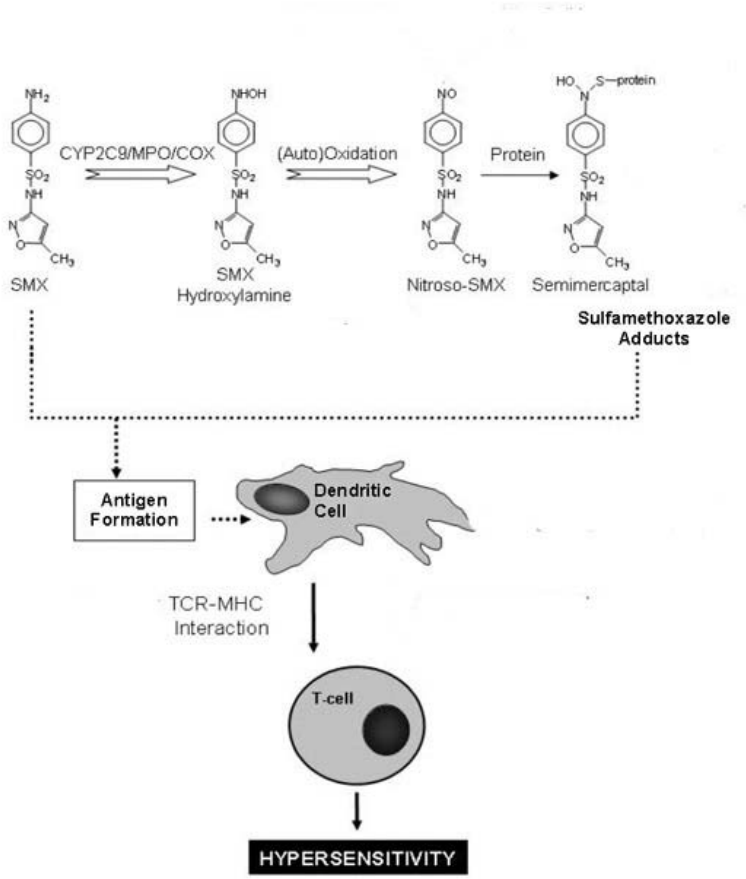
- Paracetamol: ingestion of high dose (range)
  - *Predictable hepatotoxicity*
- Doxorubicin: ingestion of cumulative dose (range)
  - *Predictable cardiotoxicity*
- Irinotecan: presence of UGT1A variants
  - *Not yet fully predictive of neutropaenia or other toxicities*

Types of ADRs

- **Type B**
  - Unpredictable or idiosyncratic
  - Less common than type A reactions
  - Unrelated to the principal pharmacological actions of the drug
  - High mortality
- Treatment is to withhold drug and avoid rechallenge, even with very low doses

Type B ADRs

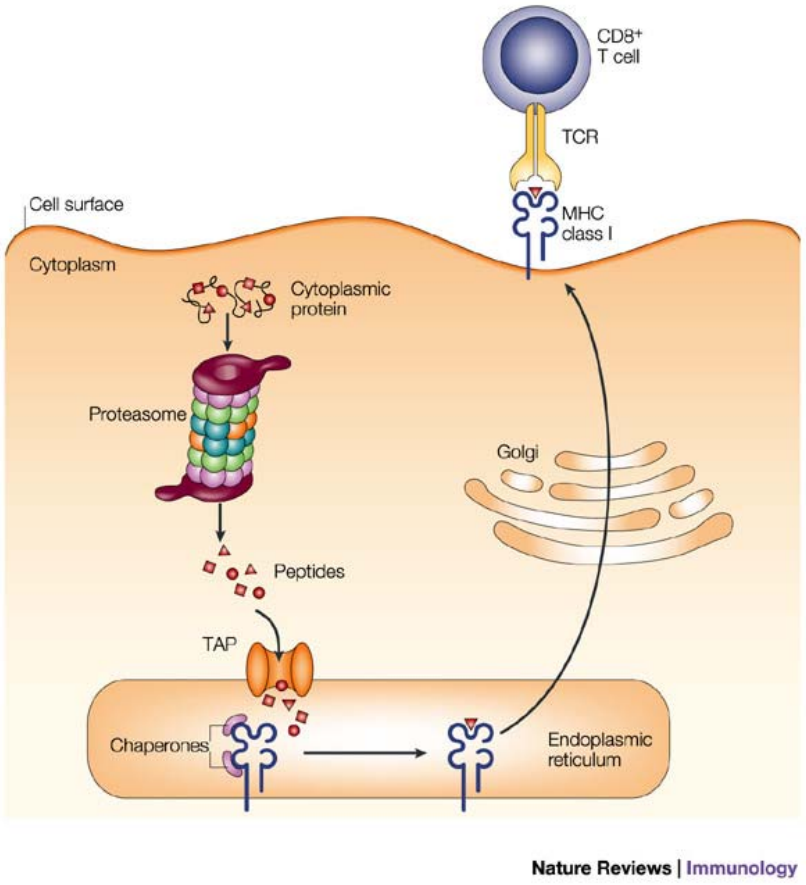
- Up to one-third of ADRs are due to unpredictable hypersensitivities
- Concept: drugs are not themselves immunogenic
- Instead a drug or metabolite interacts as a hapten with a carrier protein to form a stable conjugate
- Produces a modified protein no longer recognised as “self”
- Production of drug-specific cytotoxic T-cells



Sanderson AAPS Journal. 2006;8:E55-E64

The major histocompatibility complex (MHC)

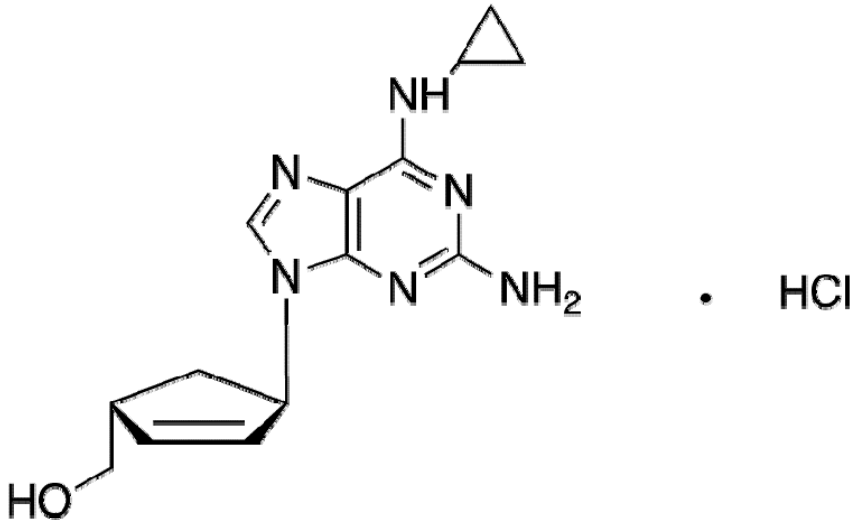
- The MHC system is a family of highly polymorphic genes that encode the human lymphocyte antigens (HLA)
- One of these genes (HLA-B) alone has >1000 alleles
- HLA molecules bind short peptides formed by intracellular proteolysis and present them on the surface of specialised cells to be detected by cytotoxic T-cells
- Peptide binding specificity is dictated by the amino acid sequence of the HLA binding groove
- So some peptides will only bind if the individual carries the appropriate HLA allele



Yewdell *et al.*, Nat Rev Immunol, 2003

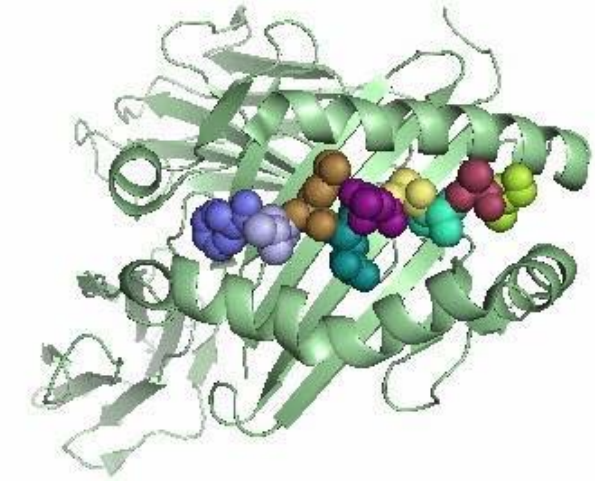
Abacavir

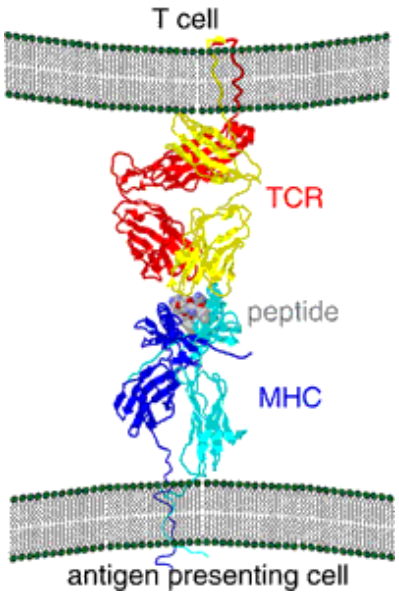
- A reverse transcriptase inhibitor used to treat HIV-AIDs
- Has a number of common toxicities that are not severe
- However, it also produces a hypersensitivity reaction in 5-10% of patients in the first few weeks of treatment
- Fever, dermatological, GI and respiratory symptoms that eventually resolve on removal but return if drug is resumed
- Occasionally fatal



Abacavir hypersensitivity syndrome

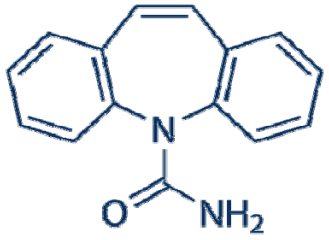
- associated with an HLA-B allele - several studies have demonstrated that testing for this allele is ***predictive*** (Mallal *et al.*, Lancet, 2002)
- Abacavir binds and distorts the binding cleft of the HLA-B molecule – which alters the peptides that can bind
- This exposes the self-tolerant T-cell compartment to modified peptides (Illing *et al.*, Nature, 2012)

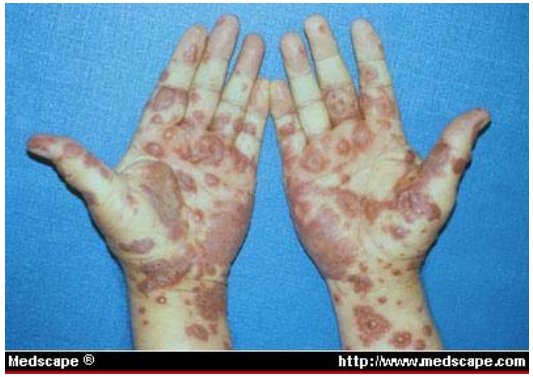




Stern, MIT 2013

Carbamazepine and severe cutaneous reactions





- Carbamazepine is an anticonvulsant used to treat seizures
- Stevens-Johnson-Syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe cutaneous reactions induced by carbamazepine that have significant mortalities
- Strong association with an HLA-B allele in Chinese subjects (Chung *et al.*, Nature, 2004) - prior genotyping recommended
- In Europe the incidence of SJS/TEN from carbamazepine is ~5-6% (~30% in Chinese) but incidence of the HLA-B allele is low
- Instead an HLA-A allele has been implicated in Japanese and Europeans
- Note: the HLA-B alleles implicated in abacavir and carbamazepine hypersensitivities are different

Whether to develop a pharmacogenetic test

(Based on Maitland *et al.*, TIPS, 2006)

- If there is unexplained variability in drug toxicity or efficacy
- Toxicity seems to be associated with a polymorphism that is common in the population
- A pharmacogenomic mechanism is implicated in toxicity
- Finally, in order to change practice, evidence from prospective studies is required
- The test should predict ADRs when the polymorphism is present and should predict no ADR if it is absent

Key points

- Simple ADRs (predictable) are often related to drug concentrations in serum
- Usually, but not always, an extension of pharmacological actions
- Defective ADME genes may account for many such ADRs (possible genotyping to detect susceptible individuals)
- Drug hypersensitivities usually have an immune basis
- Genetic variation in specific HLA genes now associated with type B ADRs with certain drugs
- Genetic tests now being developed to identify at risk individuals before therapy with certain drugs that have major toxicity profiles
- In future the unpredictable nature of Type B reactions may be modified

References

- Casarett and Doull's Toxicology, Ch 12 and Ch 13
- TPMT p277

- Drug hypersensitivity: *Curr Opin Allerg Clin Immunol* 7: 317, 2007 and *Curr Opin Immunol* 25: 81, 2013 (more detailed)
- UGT1A1 variants: Ann Oncol 19: 2089, 2008 and Mol Pharmacol 62: 446, 2002