Therapeutic drug monitoring (TDM) of thiopurine drugs

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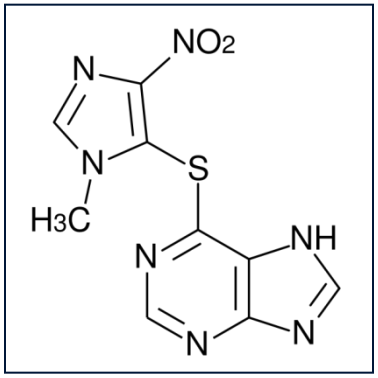
Topics

- Discuss the function of thiopurine drugs
- Thiopurine Drug Metabolism
- Pharmacogenetics of Thiopurine methyltransferase (TPMT)
- Describe analytical methods to support therapeutic drug management

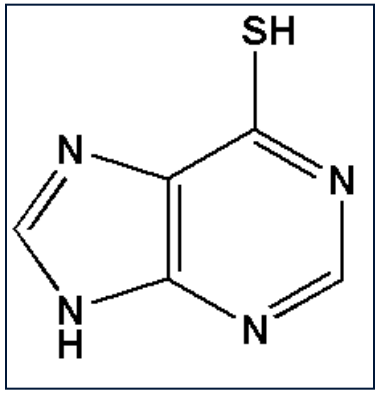
Thiopurine drugs

Immunosuppressive antimetabolites

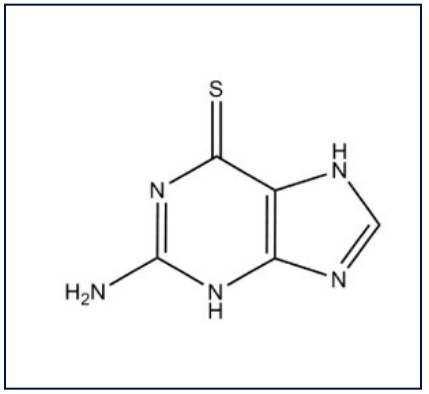
Azathioprine (Imuran®)



6-Mercaptopurine (Purinethol®)



Thioguanine (Tabloid®)



Analog of 6-Mercaptopurine

These compounds are analogs of hypoxanthine and guanine

Thiopurine Drugs - Clinical Use

Acute lymphoblastic leukemia –

- Cancer in WBC
- Most common cancer in children
- 3,000 new cases / year (3.7-4.9 cases / 100,000) in children
- 98% remission after treatment

- Reference: www.stjude.org

Thiopurine Drugs - Clinical Use

Inflammatory Bowel Disease

- Autoimmune Disease
- 1.6 million Americans (80,000 children)
- 70,000 new cases / year
- Maintain remission 70-75%

- Reference: www.ccfa.org

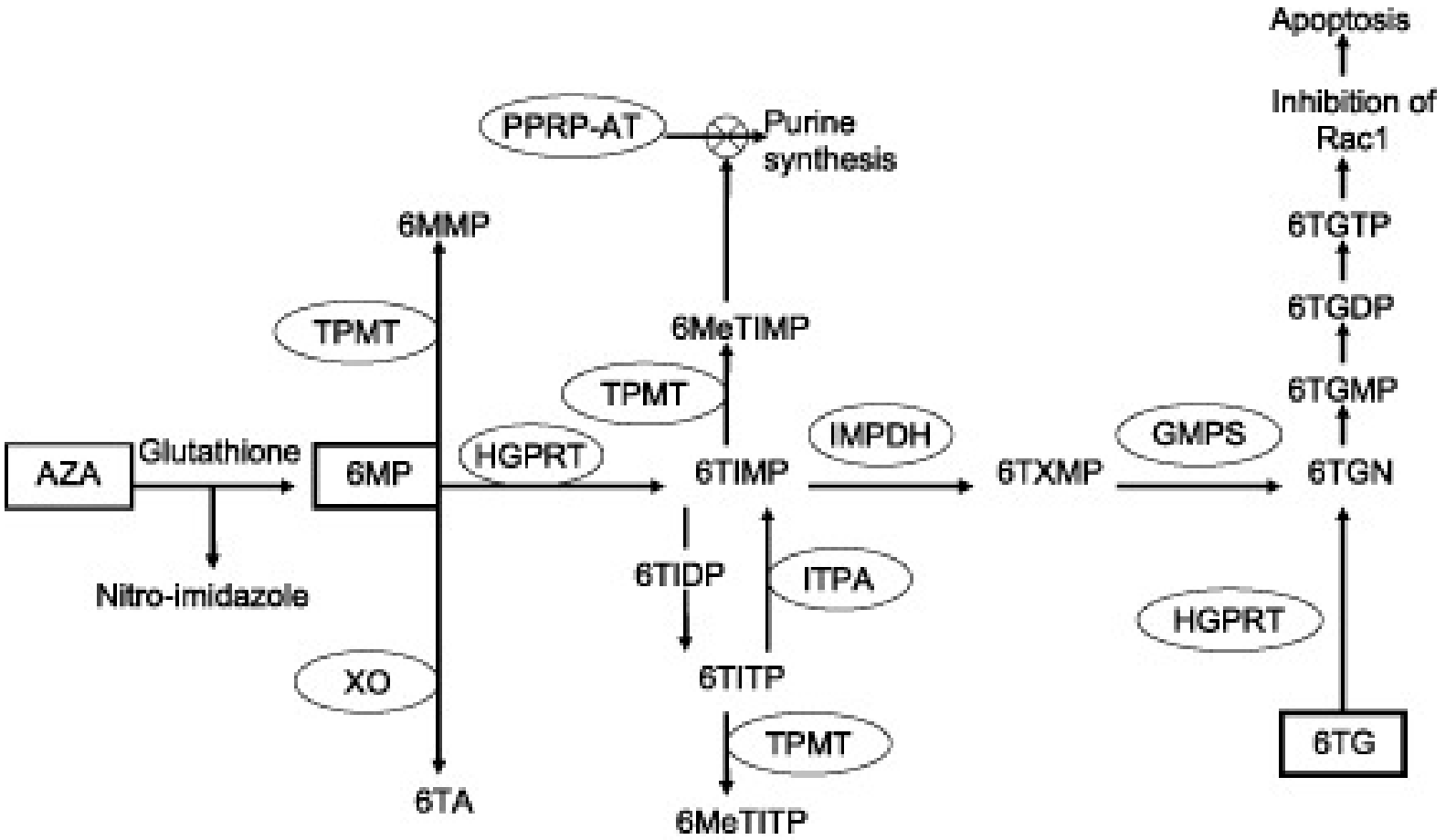
Thiopurine Drugs - Clinical Use

Rheumatoid Arthritis

- Autoimmune Disease – tissues near joints
- 1.3 million adults (>18 yr)
- Incidence – 89 / 100,000 (65 – 74 yr);
  41 / 100,000 (30 – 65 yr)
- 50 – 60% remission after treatment

- Reference: www.cdc.gov

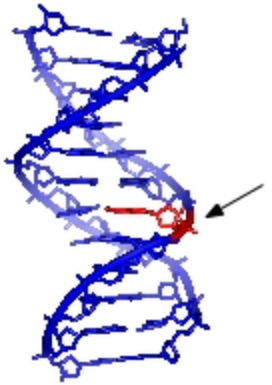
Metabolism of Thiopurine Drugs

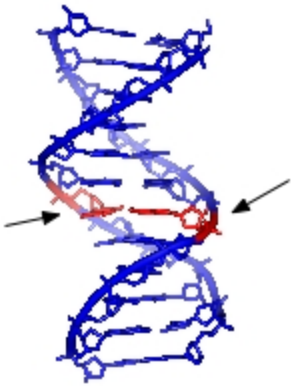


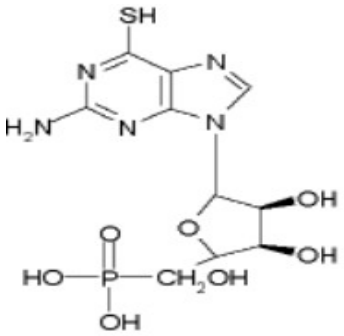
Kennedy et al. 2013 Intern Med J. 43(3):278-286.

Thiopurine Drugs - cytotoxicity

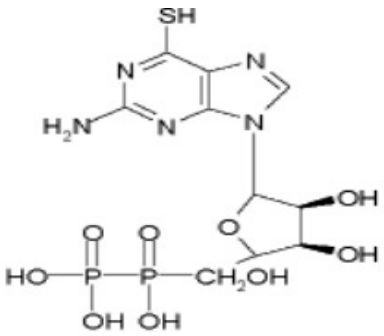
- 6-TGN resembles DNA nucleotides
- Incorporates into replicating DNA
  - Blocks proliferation (T-cells, B-cells)
  - Inhibits purine de novo synthesis (6-MMPN)
  - Promote immunosuppression
  - Induce T-cell apoptosis



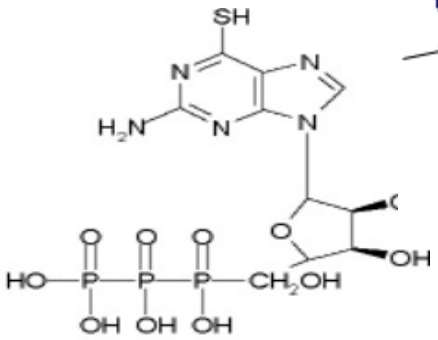




6-TGMP



6-TGDP



6-TGTP

Is Thiopurine Therapy Effective in All Patients?

Table 2. Azathioprine tolerance: estimated proportions of patients

Please estimate the percentage of your patient population in which azathioprine is tolerated well, reasonably well and poorly.

Reported tolerance	Derma (n = 95) mean% (SD)	Gastro (n = 92) mean% (SD)	Rheum (n = 96) mean% (SD)	All (n = 283) mean% (SD)	P* (significant)
Well	69 (21)	75 (12)	59 (19)	67 (19)	<0·001 (yes)
Reasonably well	17 (17)	13 (9)	22 (12)	17 (14)	<0·001 (yes)
Poorly	14 (10)	13 (7)	19 (11)	15 (10)	<0·001 (yes)
Missing responses	4	1	6	11	

*ANOVA; $P < 0·05$.

Table 3. Estimated proportions of patients experiencing azathioprine side-effects

Which of the following side-effects have you had experience of in your patient population? Please estimate the percentage.

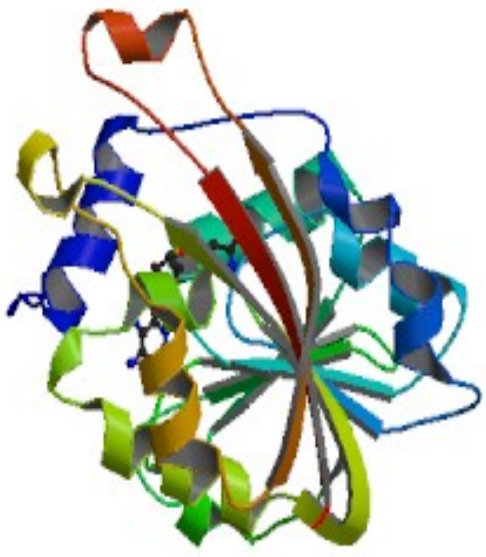
Side-effect	Derma (n = 95) mean% (SD)	Gastro (n = 92) mean% (SD)	Rheum (n = 96) mean% (SD)	All (n = 283) mean% (SD)	P* (significant)
Nausea	16 (16)	11 (10)	20 (18)	16 (15)	<0·001 (yes)
Pancreatitis	0·1 (0·5)	2 (2)	0·3 (1·3)	0·6 (1·7)	<0·001 (yes)
Neutropaenia	4 (6)	5 (4)	8 (11)	6 (8)	0·003 (yes)
Abnormal LFTs	9 (8)	5 (4)	10 (11)	8 (9)	<0·001 (yes)
Missing responses	2	8	7	17	

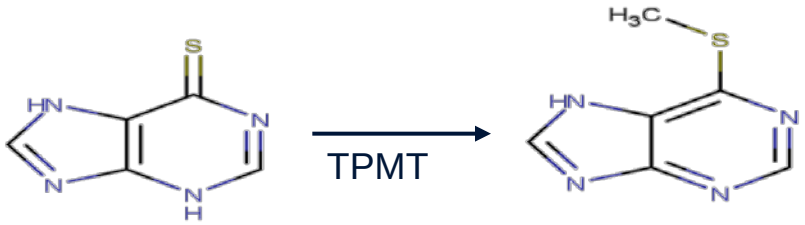
*ANOVA; $P < 0·05$.

Fargher et al. (2007) J. Clin Pharm Ther 32: 187-195

Thiopurine S-methyltransferase (TPMT)

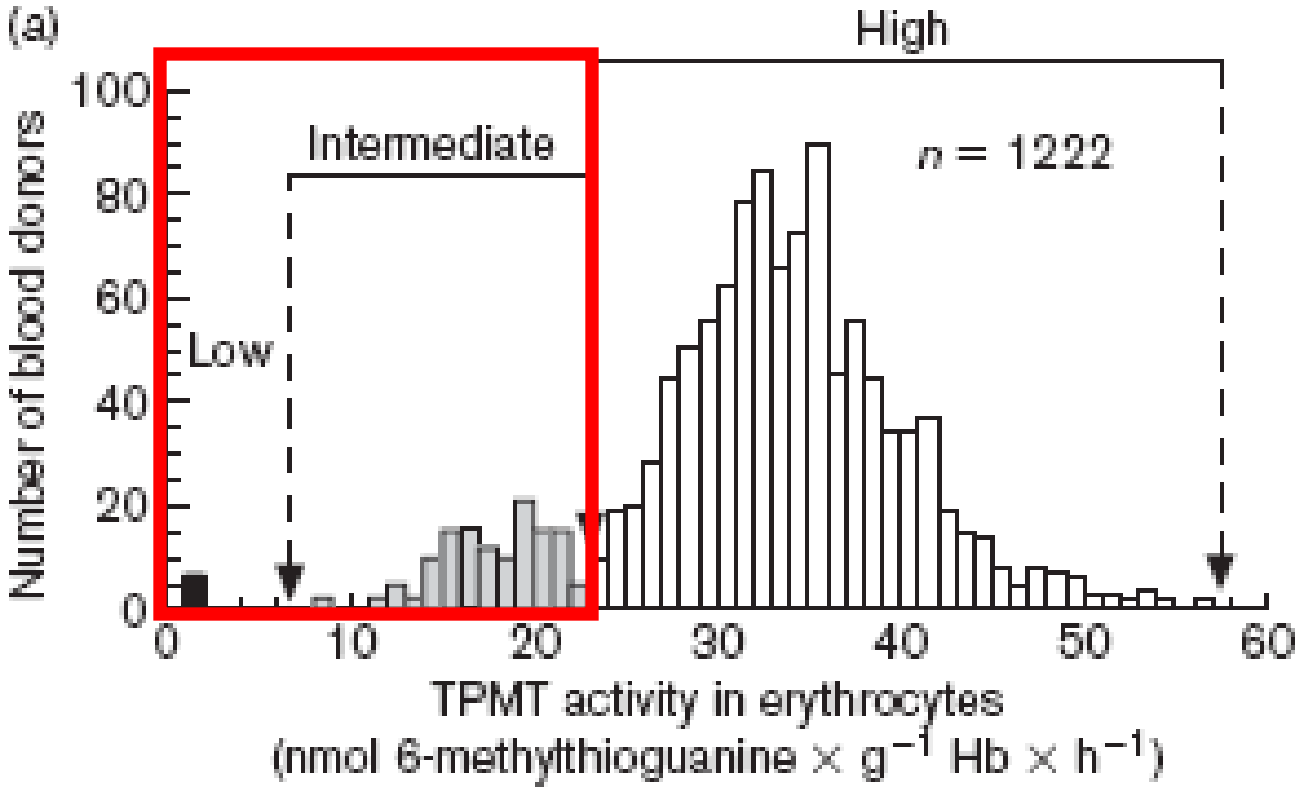
- Cytoplasmic s-methylation of thiopurines
  - 28 kDa, soluble protein; 245 amino acids
- Sites of Metabolism
  - Heart, Liver, Kidney, Intestines, Pancreas
  - RBC and WBC
- Exhibits genetic polymorphisms
  - May lead to adverse effects from thiopurine drug use





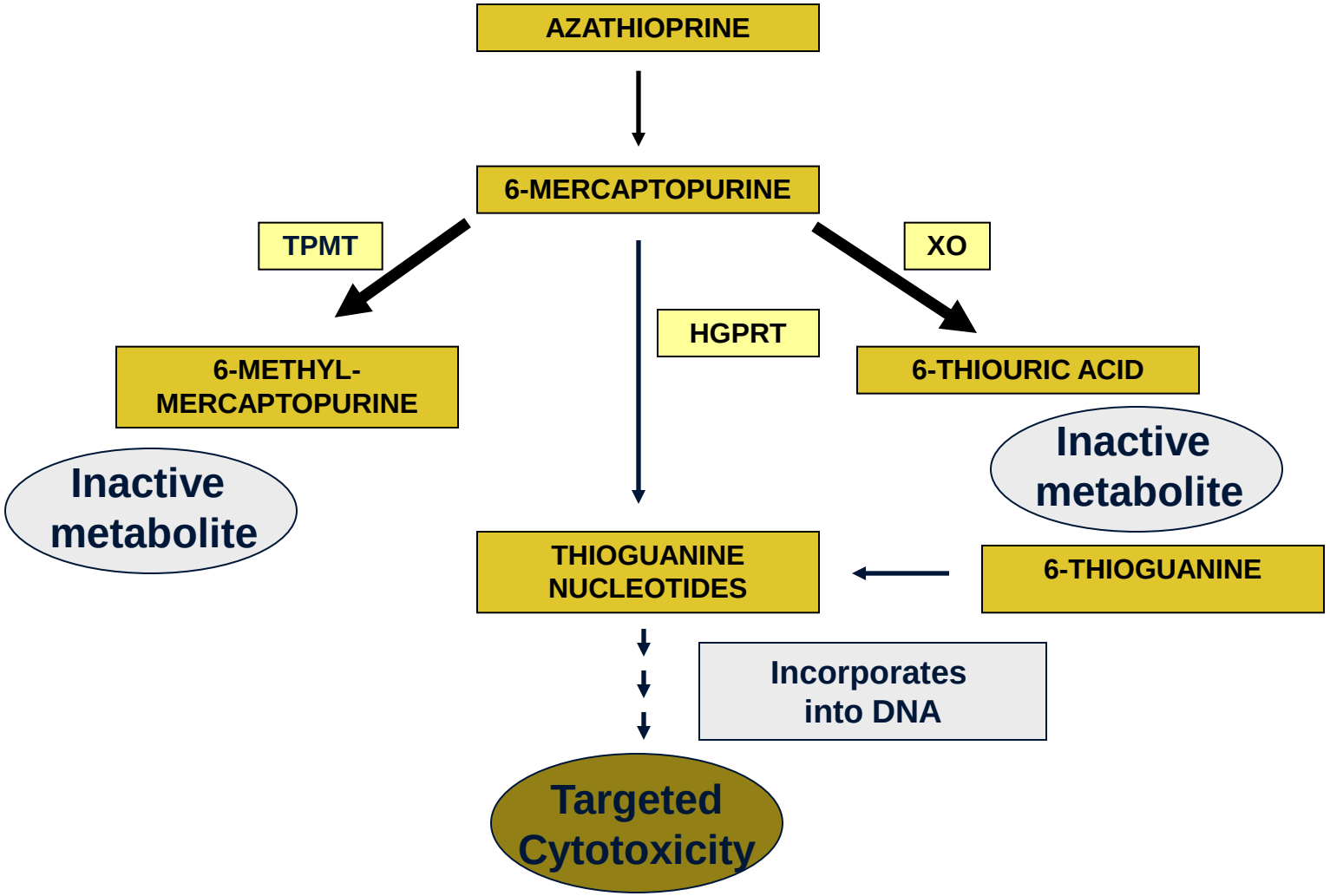
6-MP 6-MMP

Distribution of TPMT Activity – Caucasian Population

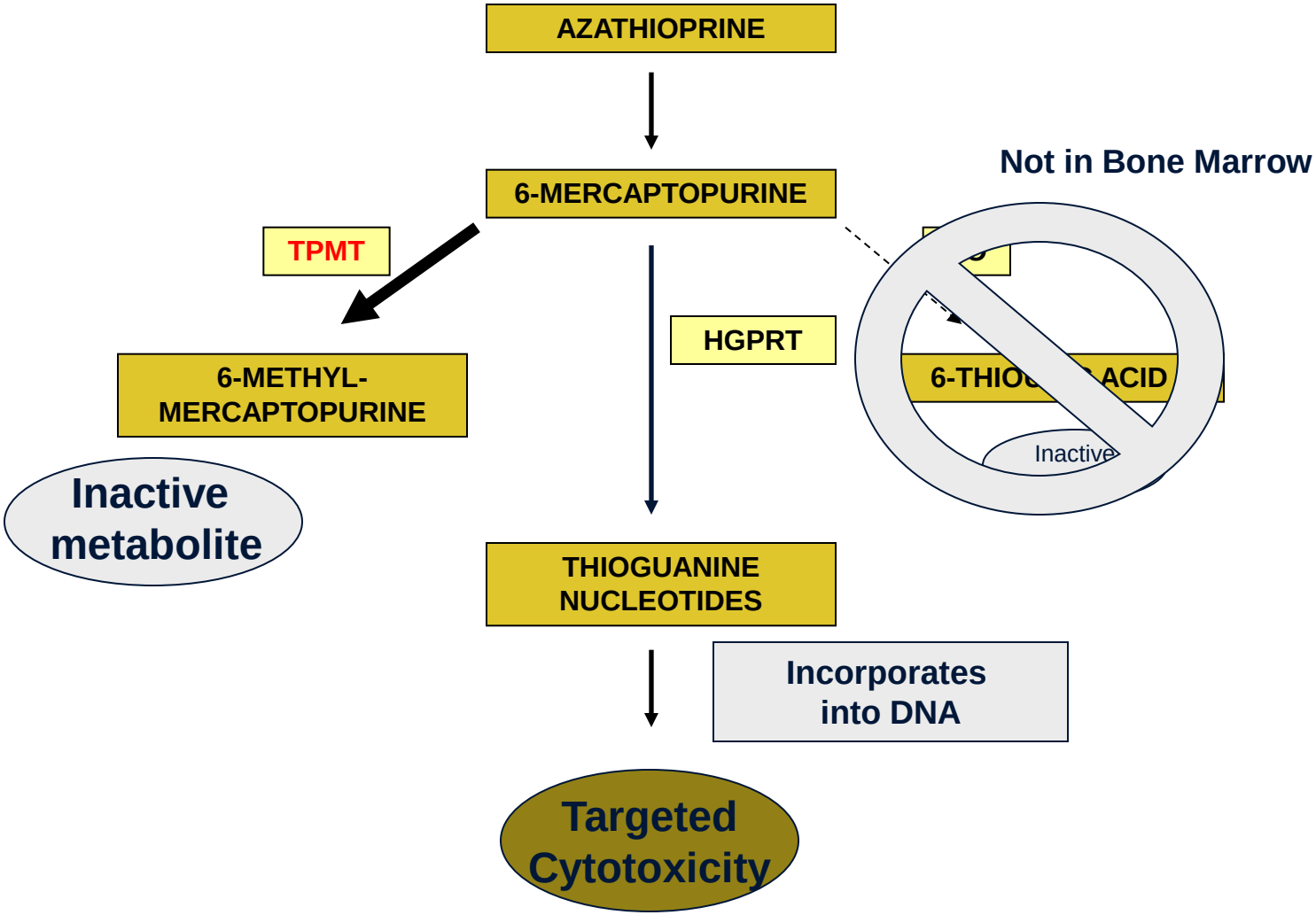


Shaeffeler E et al, Pharmacogenetics 2004

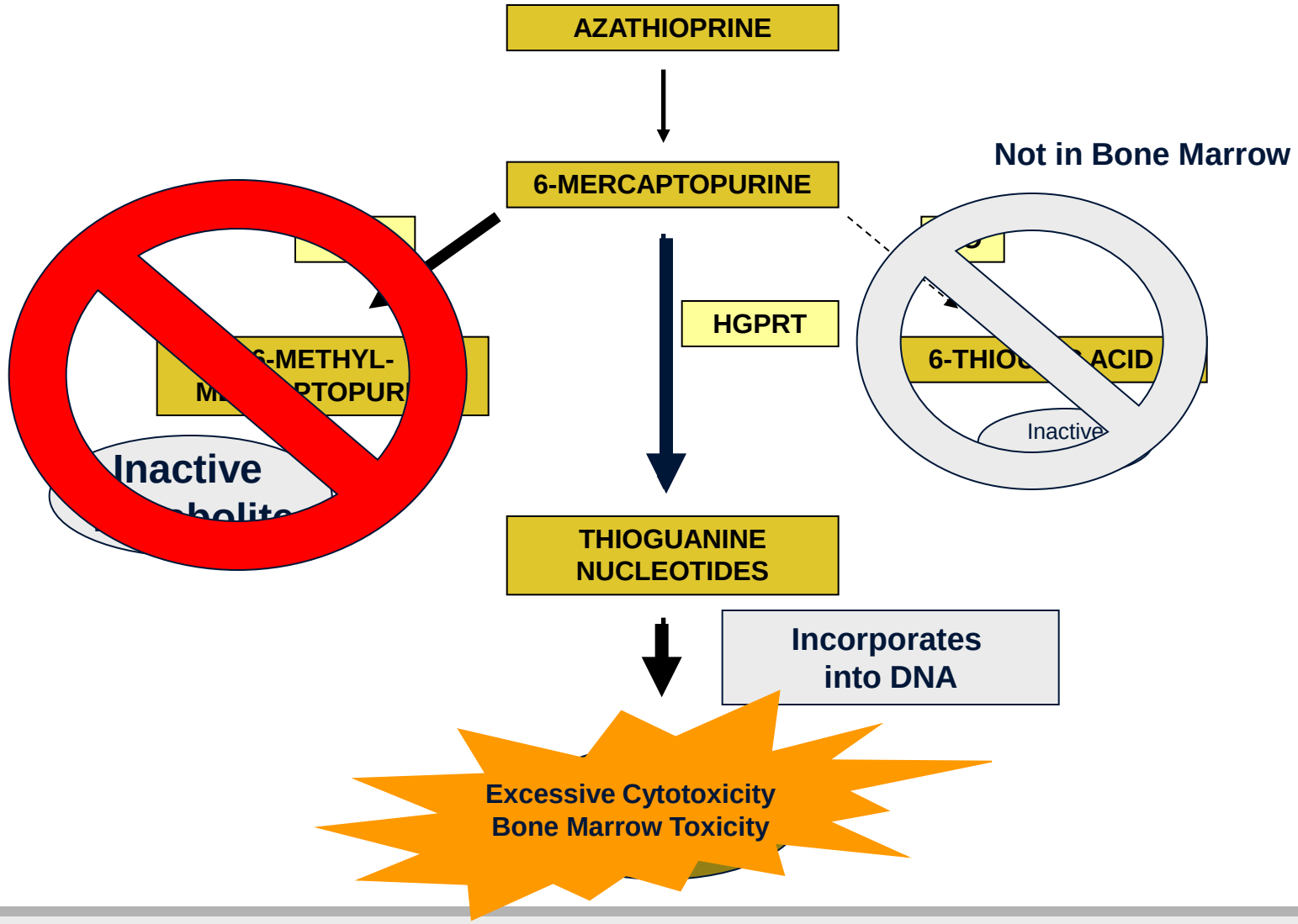
Metabolism of Thiopurine Drugs



Metabolism of Thiopurine Drugs



Metabolism of Thiopurine Drugs



TPMT Allele

Normal TPMT Allele

TPMT*1

- TPMT is ~30 kb long
- Consists of 10 exons
  - 8 of which encode for protein
- Patients with 2 normal TPMT alleles
  - normal high activity

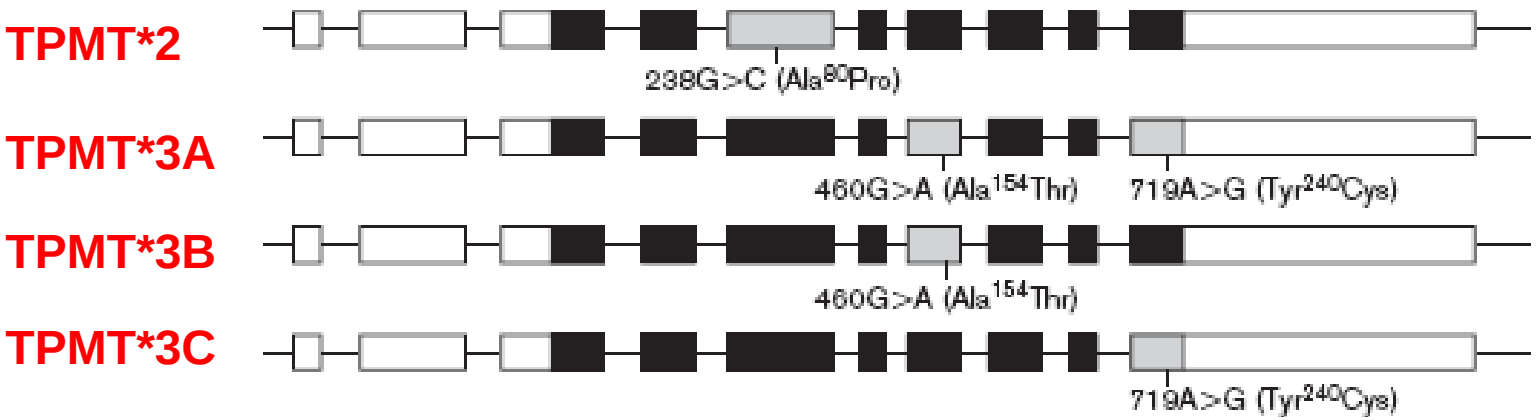
Shaeffeler E et al. 2004, Pharmacogenetics

Genetic Mutations in TPMT Alleles

- Single Nucleotide Polymorphisms (SNPs)
  - Occur in the coding region of the gene
  - Occur at exon-intron splice sites
  - Can decrease the function of the enzyme

- Variable number tandem repeats (VNTRs)
  - Occur in the promotor region of the gene
  - Decrease function of the enzyme
  - Most common – VNTR*3, *4, *5
    - Spire-Vayron et al, 1999; Roberts et al., 2008

Most Common Nonfunctional TPMT Alleles



- The four most common nonfunctional TPMT alleles account for >95% of low TPMT activity
- The most common allele variant found in the Caucasian population is the *TPMT *3A* allele
- The most common allele variant found in the Asian and African population is the *TPMT *3C* allele

Shaeffeler E et al. 2004, Pharmacogenetics

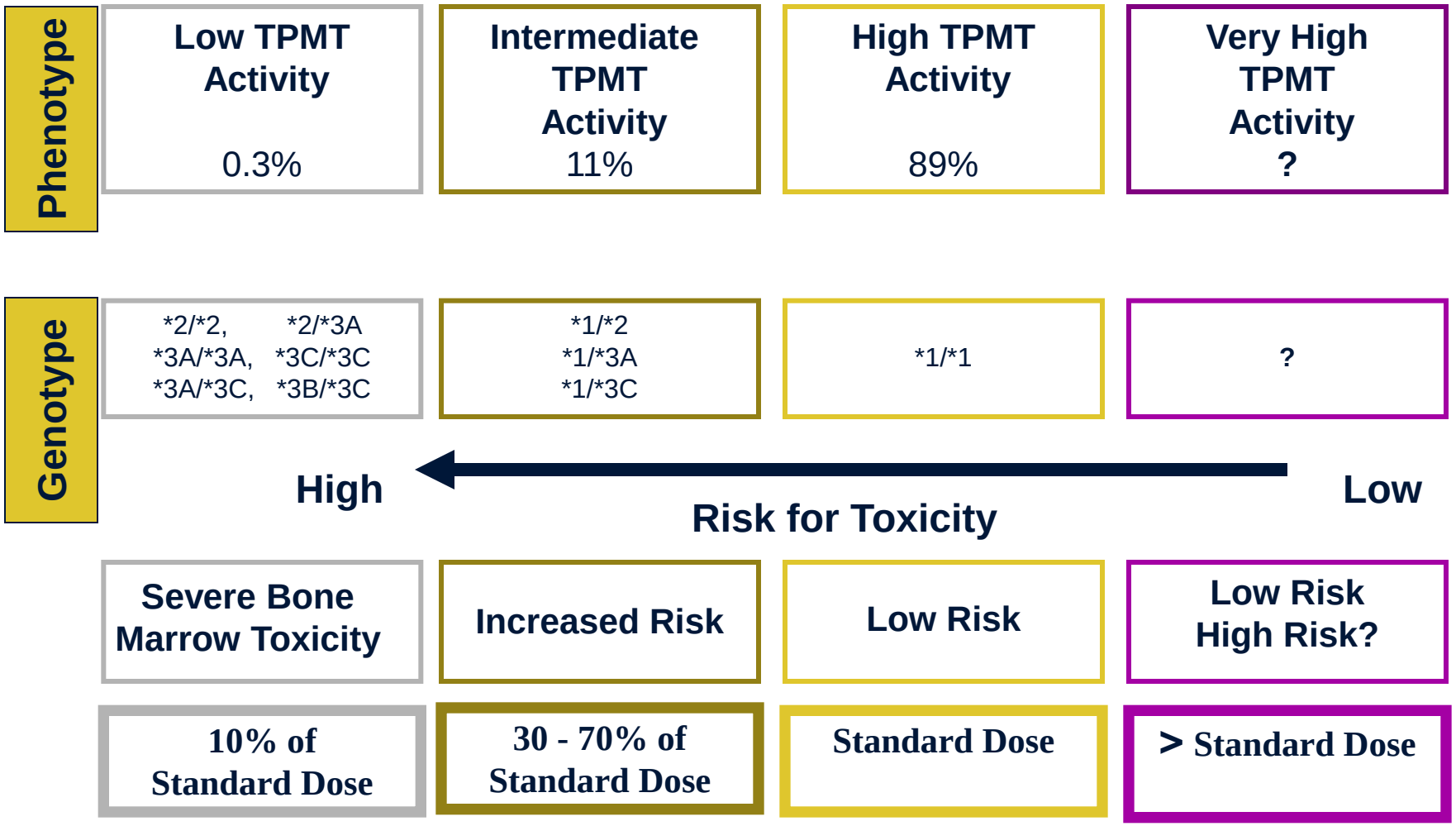
Mutations affect TPMT Activity

- Protein becomes unstable and undergo degradation
  - *TPMT*2, *3A, *3B, *3C* have enhanced proteolysis of variant proteins
    - Tai et al., (1999) Pharmacogenetics 9:641-50.

- Decreased enzyme half-life
  - *TPMT*1* – **18 h** vs. *TPMT*2* and *TPMT*3* – **15 min**
    - Tai et al., (1997) Nalt Acad Sci USA 94:6444-6449.

- Variant alleles cause decreased enzyme activity
    - Krynetski et al., (1995) Proc. Natl. Acad. Sci. 92:949-953.

Dose Adjustments for TPMT Activity



Relling MV et al. 2011 Clin Pharmcol Ther. 89(3):387-391.

Analytical Methods to support testing for thiopurine drugs

Testing Strategies to Access TPMT Activity

Phenotype

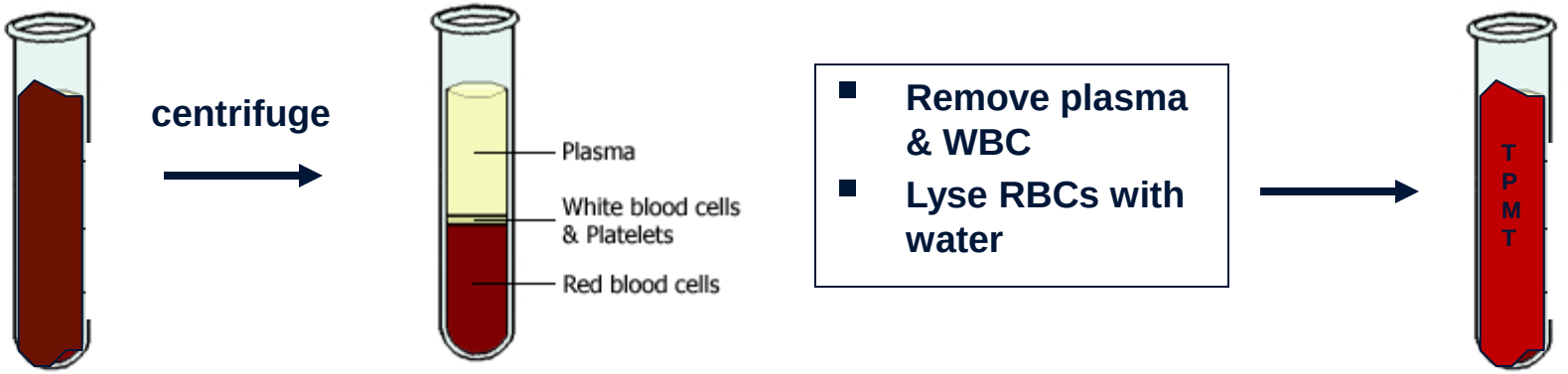
- Enzyme reaction – assess inactivation of thiopurine drug
- Quantification of thiopurine drug metabolites

Genotype

- Determines the allele pattern of a patient
- Primarily tests for 4 variant TPMT alleles –
  - *TPMT*2, *3A, *3B, *3C*

Phenotype Assay – TPMT Enzyme

6-MP + SAM ⟷ (TPMT) 6-MMP

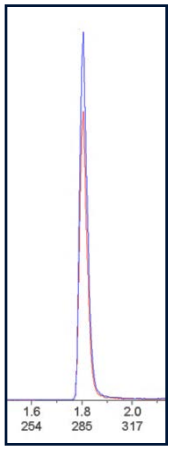


- **Remove plasma & WBC**
- **Lyse RBCs with water**

- **Add cosubstrates:** 6-mercaptopurine & S-adenosylmethionine
- **Incubate reaction @ 37 °C for 1 h**
- **Stop reaction with acid or heat:**
- **Prepare sample for HPLC or LC/MS/MS**

HPLC

Triple-Quad MS



U/mL = nanomoles 6MMP

Result Interpretation

Low (< 17 U/mL) – high risk of bone marrow toxicity with standard dosing regimens; dose reduction of 80-90% is recommended

Intermediate (17.0 - 23.9) – risk of bone marrow toxicity with standard dosing regimens; dose reduction by 20-50% may be necessary

Normal (24 - 44 U/mL) – low risk of bone marrow toxicity with standard dosing regimens

High (>44 U/mL) – risk of therapeutic failure due to excessive inactivation of these drugs. Patients may require a higher dose

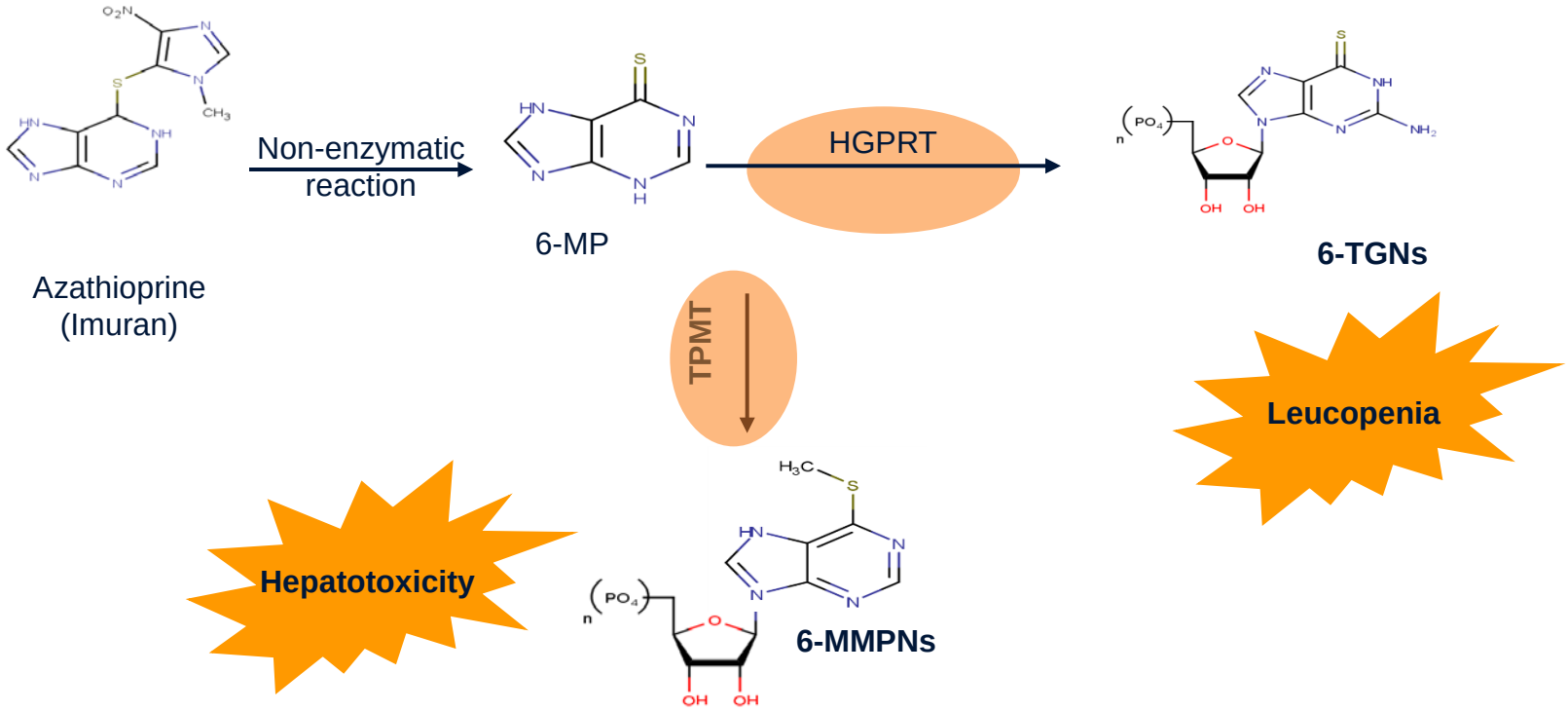
Phenotype test - Thiopurine Drug Metabolites

- Test quantifies 6-TGN and 6-MMPN levels in whole blood

Normal Reference Ranges

6-TGN: 230-400 pmol/8x10^8 red blood cells

6-MMPN: <5700 pmol/8x10^8 red blood cells



Phenotype Test

Pros

- Less expensive than genotype test
- May be more informative than Genotype

Cons

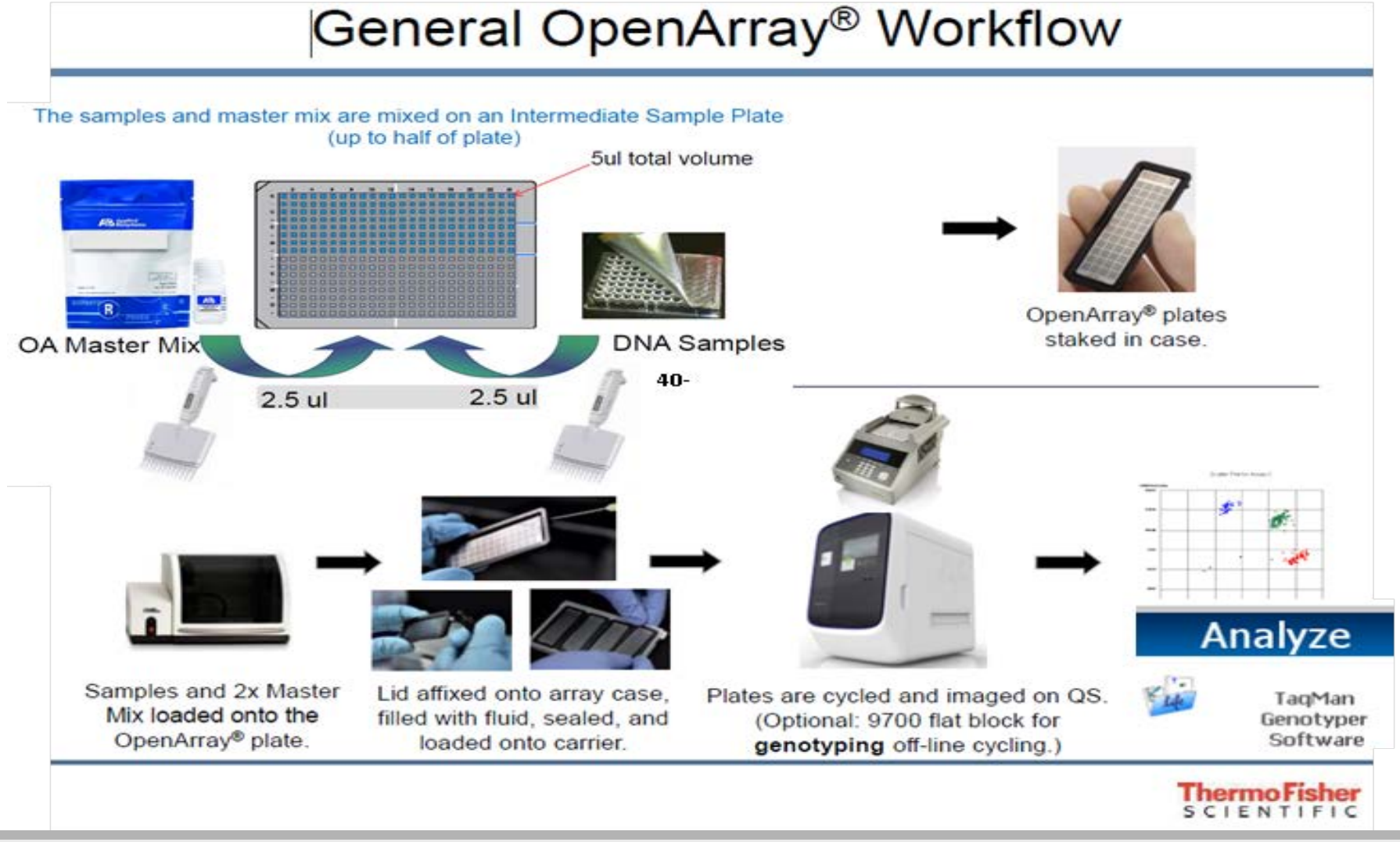
- Enzyme function can be altered by:
  - NSAIDS can inhibit enzyme activity
  - Specimen mishandling can impact TPMT enzyme activity
  - Blood transfusion
    - Not accurately assessed in patients who receive RBC transfusion within 60 days of testing

Genotype TPMT assay

- PCR
  - Mini-sequencing assay
  - Fluorescence detection

- Detect the most common alleles
  - *TPMT**2 [238G>C]
  - *TPMT**3A [460G>A and 719A>G]
  - *TPMT**3B [460G>A]
  - *TPMT**3C [719A>G]

Genetic Test - PCR



Genotype Test

Pros

- Genotype test is not affected by –
  - Blood transfusions
  - Concomitant medications

Cons

- Assay cannot detect all of the nonfunctional TPMT alleles (>30)
- Wildtype or Normal alleles are inferred if none of the four non-functional alleles are detected
- Cannot distinguish between a heterozygote or a compound heterozygote
  - *1/*3A or *3A/*3C, *3A/*3B, *3B/*3C

Summary

- Phenotype assay may provide more clinical information
  - Detects rapid metabolizer phenotypes and impaired enzyme activity from rare genetic variants
  - Genotype test may not detect all rare mutations
- Genetic testing can be performed before and after thiopurine drug therapy
  - Consider testing if erythrocyte TPMT activity is abnormal

Take Home Message

- Test patients for TPMT status prior to therapy
  - Phenotype
  - Genotype
- Patients with low or absent TPMT activity
  - Avoid Thiopurine therapy or reduce the standard dose
- Genotype/phenotype tests to assess TPMT activity do not replace routine clinical monitoring

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