NATIONAL CENTER FOR
CASE STUDY TEACHING IN SCIENCE
UNIVERSITY AT BUFFALO, STATE UNIVERSITY OF NEW YORK

Pharmacogenetics

How Genetic Information Is Used to Treat Disease

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West Chester, PA

At Children's Hospital, Two 14-yr-old Girls Meet in the Children's Ward

- Laura loves sports, is an excellent student, and plays soccer. The last few months she has been very tired and bruises easily.
- Beth enjoys animals and the theater. She seems to pick up colds easily and recently suffered from a high fever and swollen lymph nodes.
- After a visit to the doctor, they have blood tests performed.

Blood Test Results

Here are their results:

	Laura	Beth	Units
RBC count	2.6	3.5	million/mm^3
Hemoglobin	8.2	11.1	g/dl
Hematocrit	23	32	%
WBC count	6.5	2.0	thousand/mm^3
Platelet count	50	120	thousand/mm^3

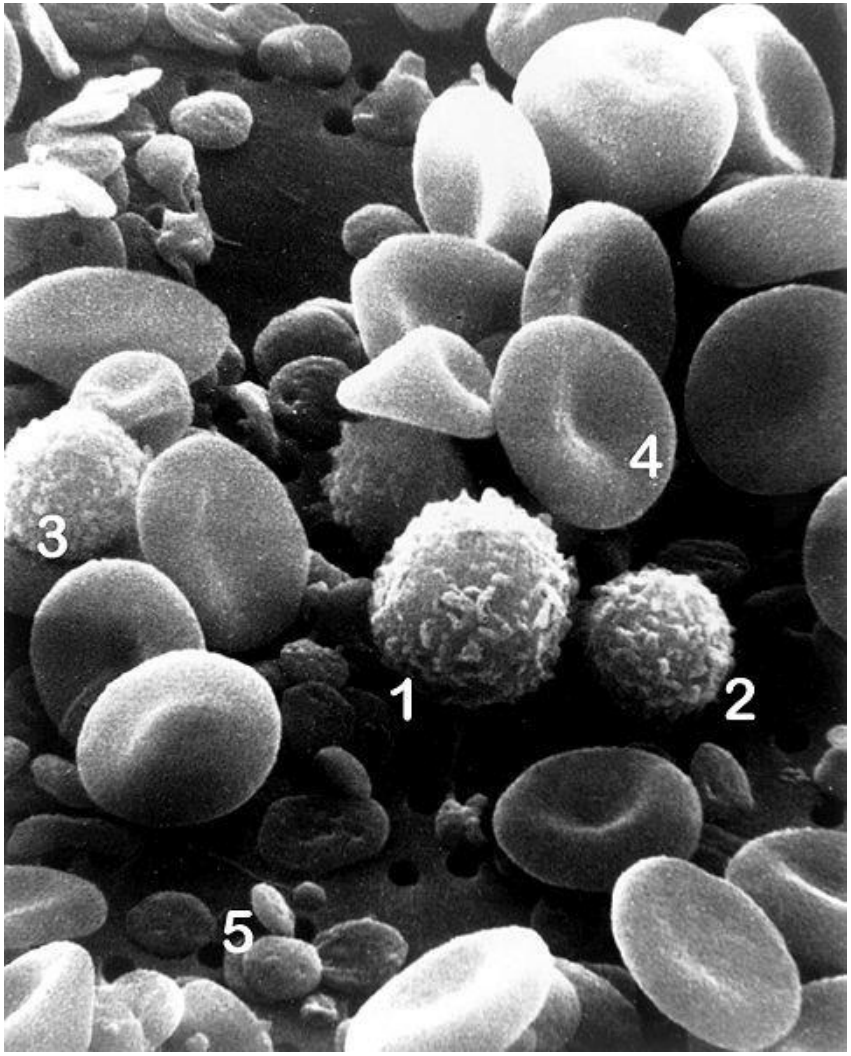
Turn to your neighbor and discuss these results. What differences do you see in the results between the two girls? Do you think that they have the same disease or a different disease?

Blood Cell Review

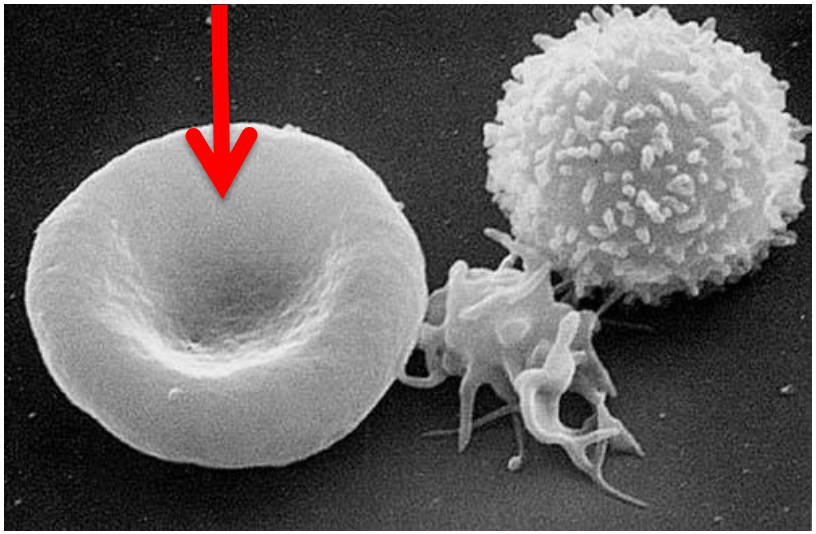
Why are the girls having these symptoms?

- Red Blood Cells = Erythrocytes
- White Blood Cells = Leukocytes
- Platelets = Thrombocytes

Turn to your neighbor and discuss the structural similarities and differences that you see in the cells labeled 1-5.



Red Blood Cells (RBCs)



A. Structure
- Biconcave disc
- Lack nucleus and organelles

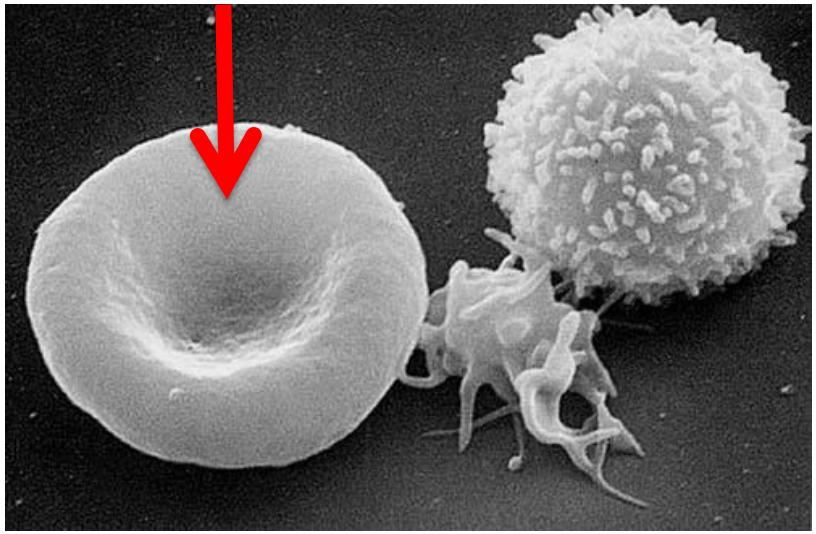
B. Function
- Transport O_2 via hemoglobin

C. Normal values
- RBC count = 4.0-5.2 million/ mm^3
- Hemoglobin = 11.8-15.5 g/dl
- Hematocrit = 36-46 %

Red Blood Cells (RBCs)

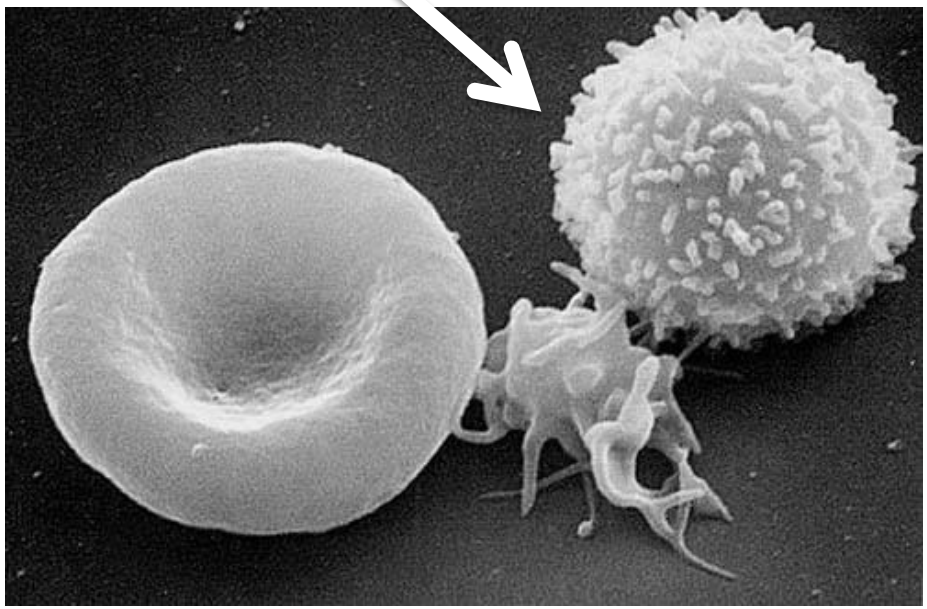
D. Abnormal values

- Low = anemia
  - Weakness
  - Fatigue
  - Shortness of breath
- High = polycythemia
  - Can lead to blood flow difficulty



White Blood Cells (WBCs)

A. Types
- Neutrophil
- Eosinophil
- Basophil
- Monocyte
- Lymphocyte (shown here)



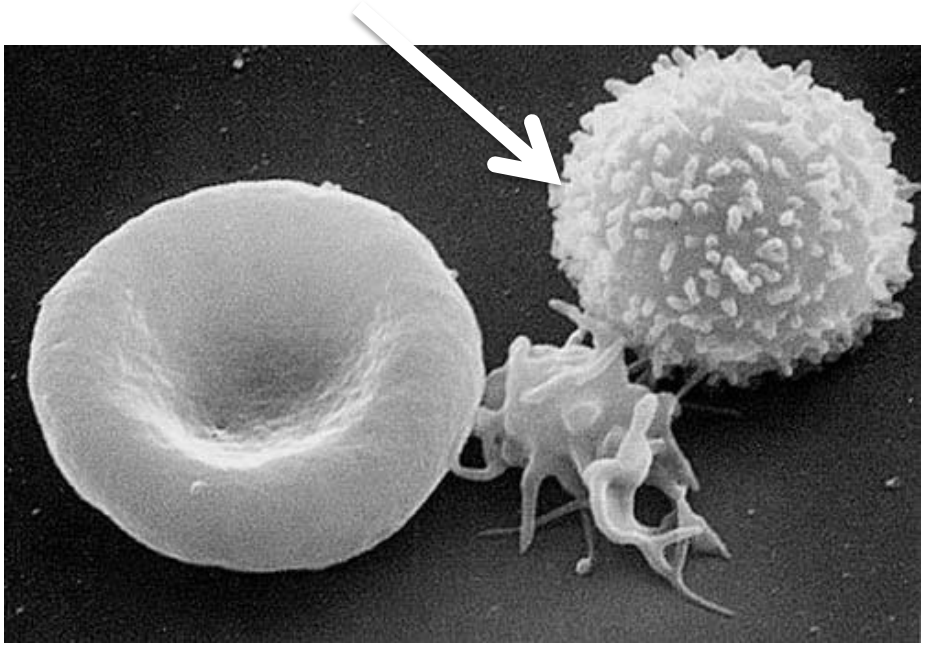
B. Function
- Combat infection

C. Normal values
- WBC count = 4.5-13.2 thousand/ mm^3

White Blood Cells (WBCs)

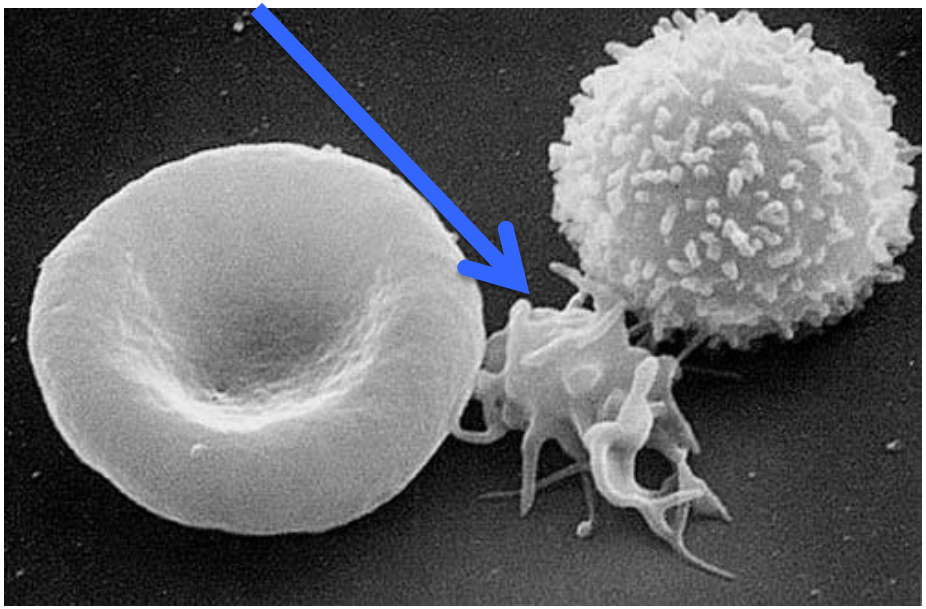
D. Abnormal values

- Low
  - Immunodeficiency
  - Failure to make WBCs in the bone marrow
  - Leads to increased susceptibility to infection
- High
  - Infection
  - Leukemia



Platelets

A. Structure
- Small cell fragments
- Lack nucleus
- Contain granules



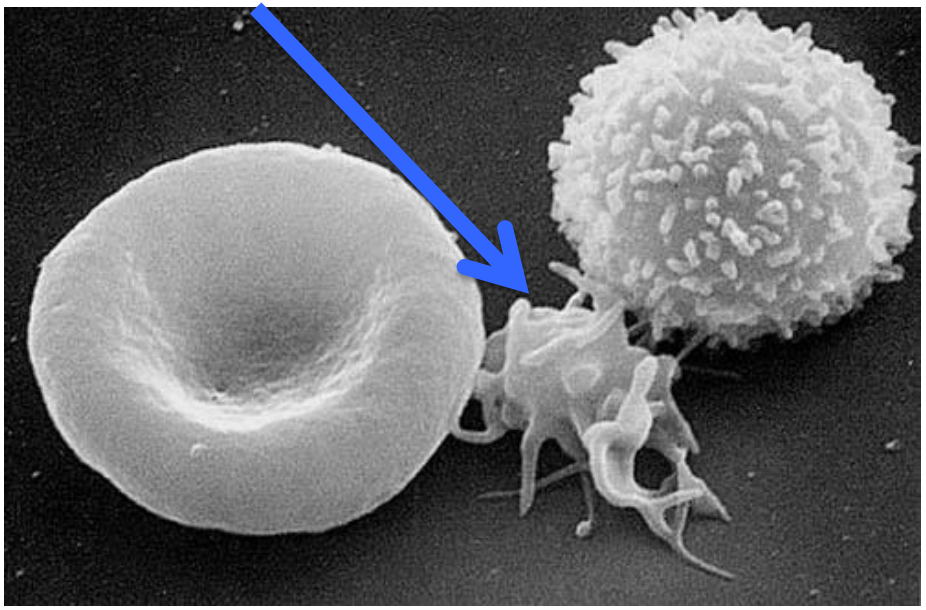
B. Function
- Blood clotting

C. Normal values
- Platelet count = 140-450 thousand/mm^3

Platelets

D. Abnormal values

- Low
  - Excessive bleeding
  - Bruising
- High
  - Blood clots



CQ1: The blood test result(s) that explain Laura's fatigue is (are) _____.

A) Low RBC count

B) Low hemoglobin concentration

C) Low hematocrit

D) All of the above

	Laura	Beth	Normal range (14 yr old F)
RBC count	2.6	3.5	4.0-5.2 million/ mm3
Hemoglobin	8.2	11.1	11.8-15.5 g/dl
Hematocrit	23	32	36-46 %
WBC count	6.5	2.0	4.5-13.2 thousand/ mm3
Platelet count	50	120	140-450 thousand/mm3

CQ2: Laura bruises easily because she has a ____ .

A) Low RBC count

B) Low hemoglobin concentration

C) Low hematocrit

D) Low WBC count

E) Low platelet count

	Laura	Beth	Normal range (14 yr old F)
RBC count	2.6	3.5	4.0-5.2 million/ mm3
Hemoglobin	8.2	11.1	11.8-15.5 g/dl
Hematocrit	23	32	36-46 %
WBC count	6.5	2.0	4.5-13.2 thousand/ mm3
Platelet count	50	120	140-450 thousand/mm3

CQ3: The blood test result for Beth related to swollen lymph nodes and frequent infections is _____ .

A) Low RBC count

B) Low hemoglobin concentration

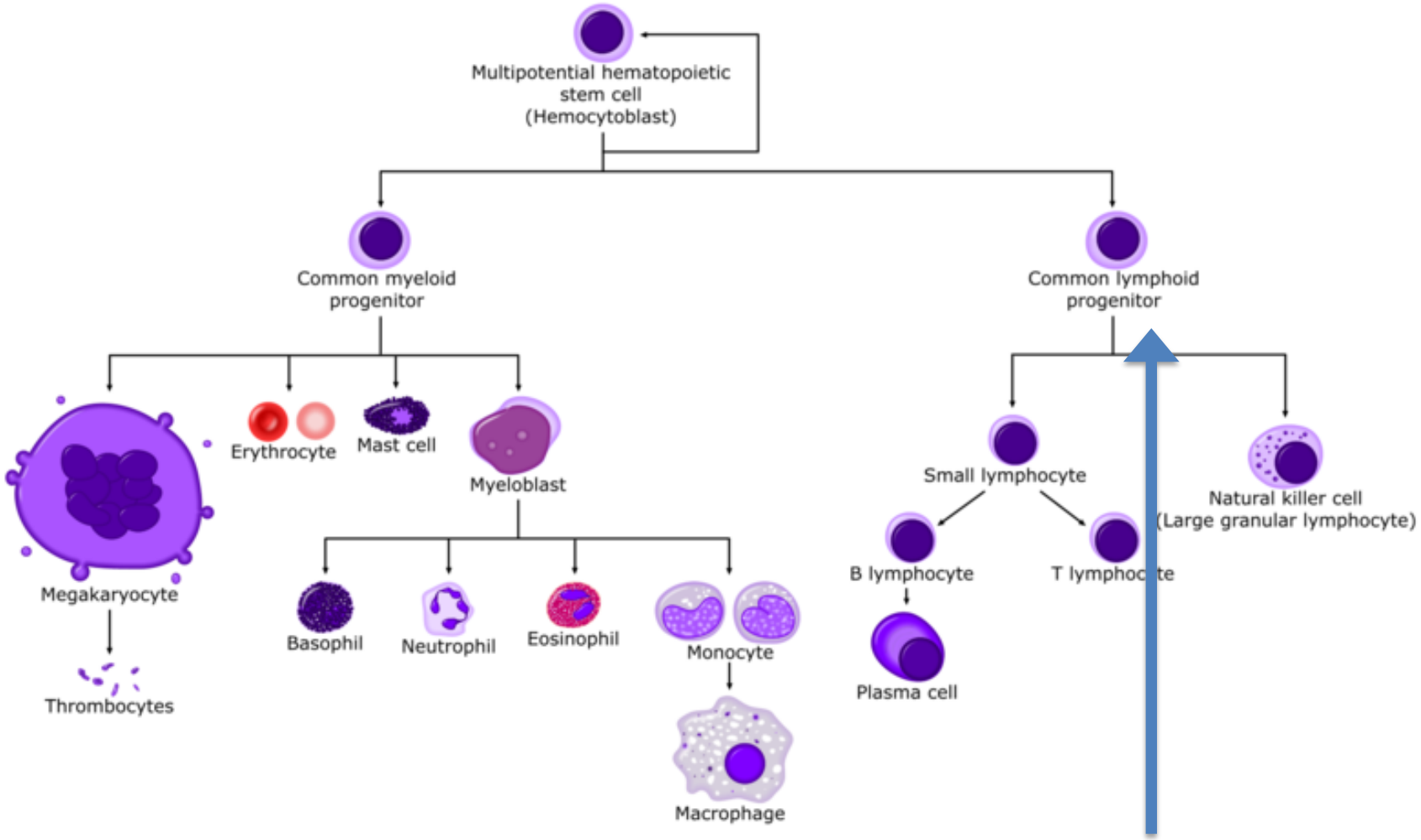
C) Low hematocrit

D) Low WBC count

E) Low platelet count

	Laura	Beth	Normal range (14 yr old F)
RBC count	2.6	3.5	4.0-5.2 million/ mm3
Hemoglobin	8.2	11.1	11.8-15.5 g/dl
Hematocrit	23	32	36-46 %
WBC count	6.5	2.0	4.5-13.2 thousand/ mm3
Platelet count	50	120	140-450 thousand/mm3

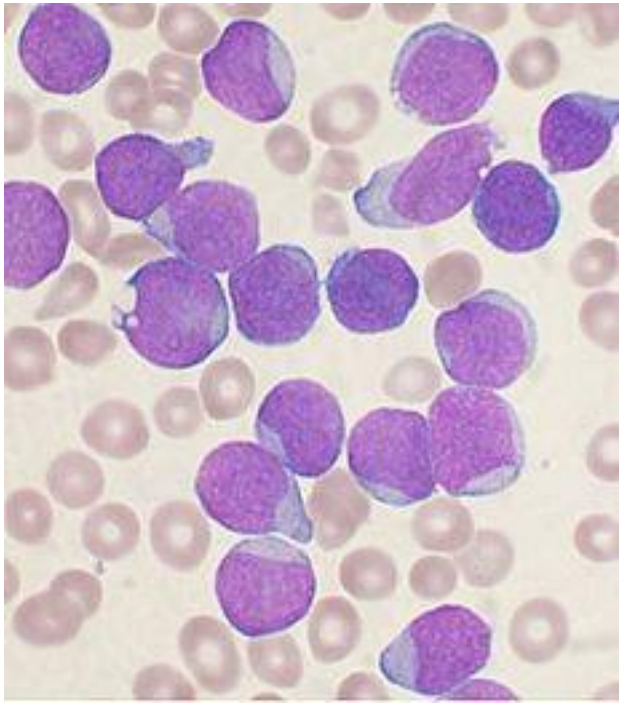
A Bone Marrow Biopsy Is Performed



Both girls are diagnosed with acute lymphoblastic leukemia, an abnormal production of immature lymphocytes.

What is Acute Lymphoblastic Leukemia (ALL)?

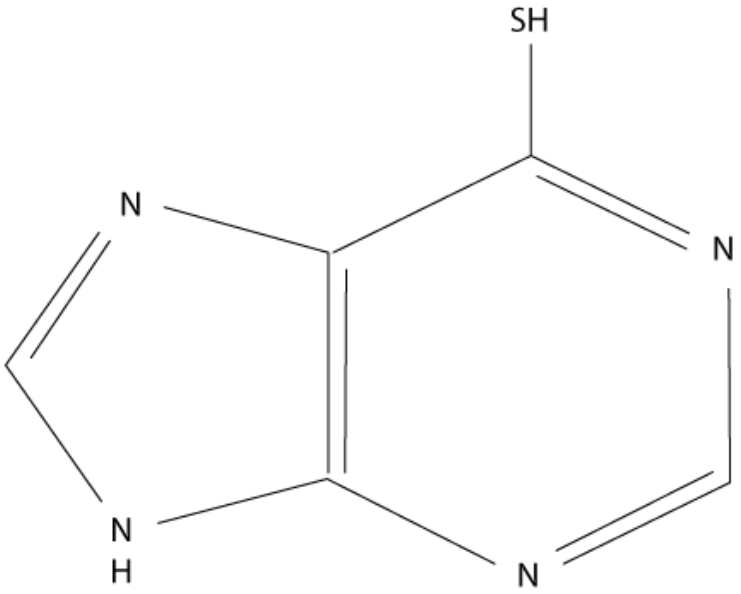
- Cancer of the white blood cells characterized by excess lymphoblasts.
- Most common in childhood age 2-5.
- Symptoms of the disease include anemia, sensitivity to infection and bleeding due to the overcrowding of the bone marrow with the cancer cells.



Bone marrow biopsy of patient with ALL

How Is ALL Treated?

- Thiopurine drugs
  - 6-mercaptopurine (shown here)
- Prodrugs
  - Must be converted to the active form in the body
- Guanine analogs
  - Act like guanine but disrupts DNA and RNA synthesis
  - Acts on rapidly dividing (cancer) cells but also GI, skin, hair follicles, bone marrow
- Narrow therapeutic index
  - Dose to affect cancer cells is not much higher than toxic dose
  - Toxic dose = decrease ability of bone marrow to make blood cells
    - myelosuppression



CQ4: After 3 days, Beth's condition is deteriorating while Laura is feeling better. What could cause this difference in response to the treatment?

A) Beth is more sensitive to the toxic effects of the drug.

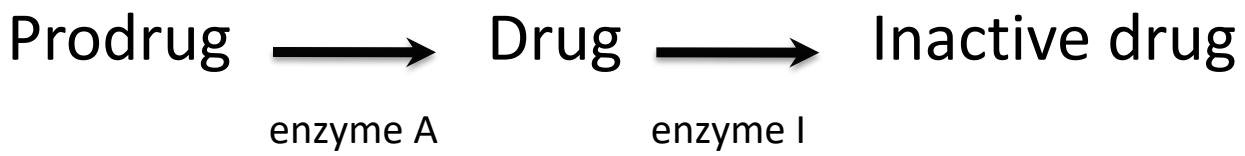
B) More drug is converted to the active form in Beth, leading to toxic levels.

C) The drug is not excreted in Beth, leading to toxic levels.

D) The drug is not inactivated in Beth, leading to toxic levels.

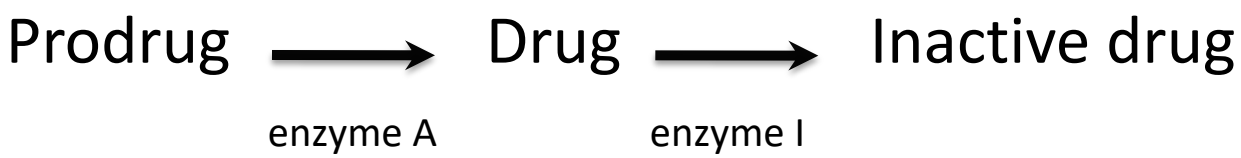
E) All of the above.

Drug Metabolism Basics



- Prodrug needs to be metabolized by enzyme A to be active
  - Poor metabolizers (low A activity) will need higher dose
  - High metabolizers (high A activity) will need lower dose
- Drug needs to be metabolized to be inactivated
  - Poor metabolizers (low I activity) will need lower dose
  - High metabolizers (high I activity) will need higher dose

CQ5: Which of the following mechanisms will lead to higher active drug dose?



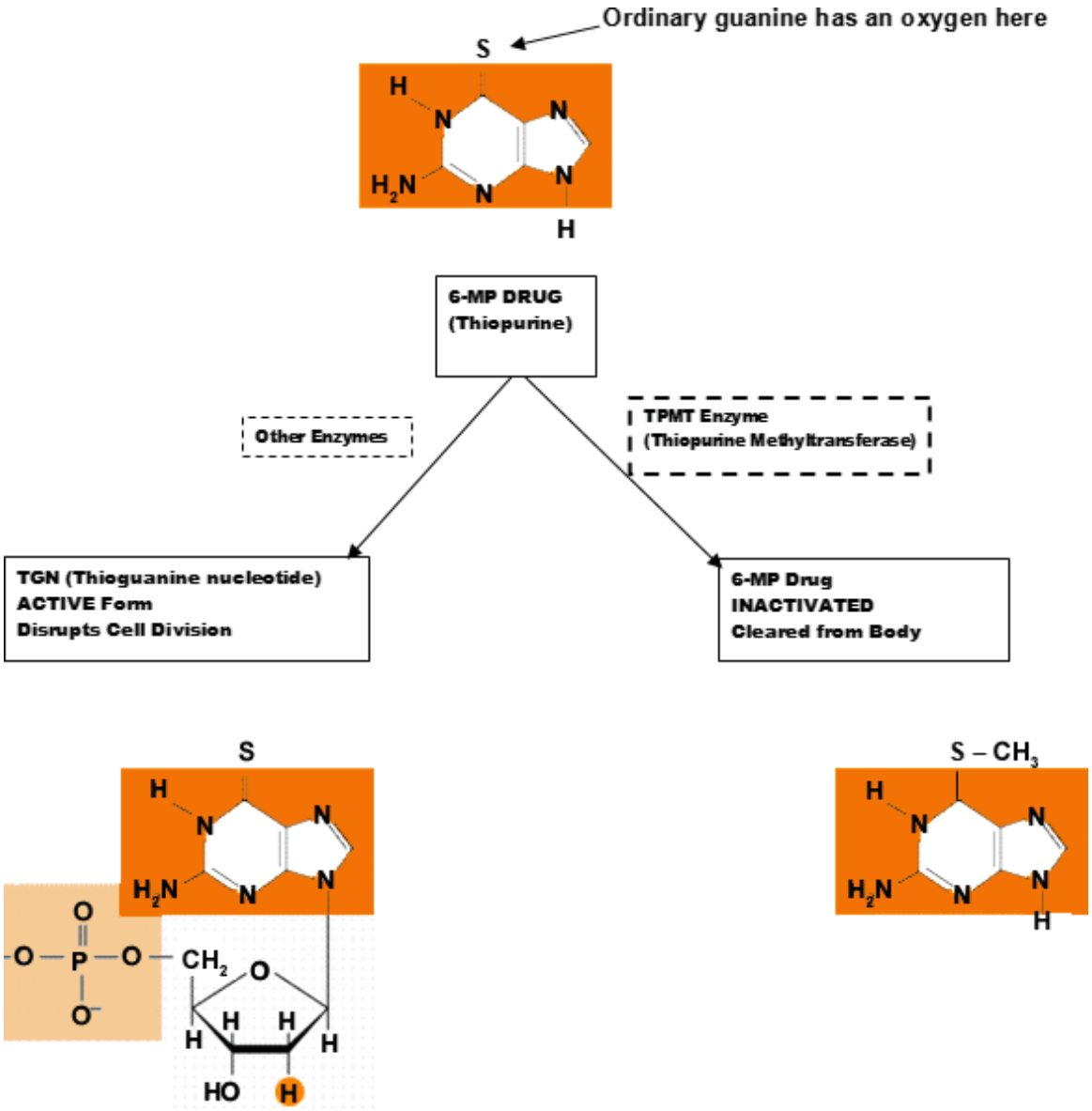
A) Increase activity of activating enzyme, decrease activity of inactivating enzyme

B) Decrease activity of activating enzyme, decrease activity of inactivating enzyme

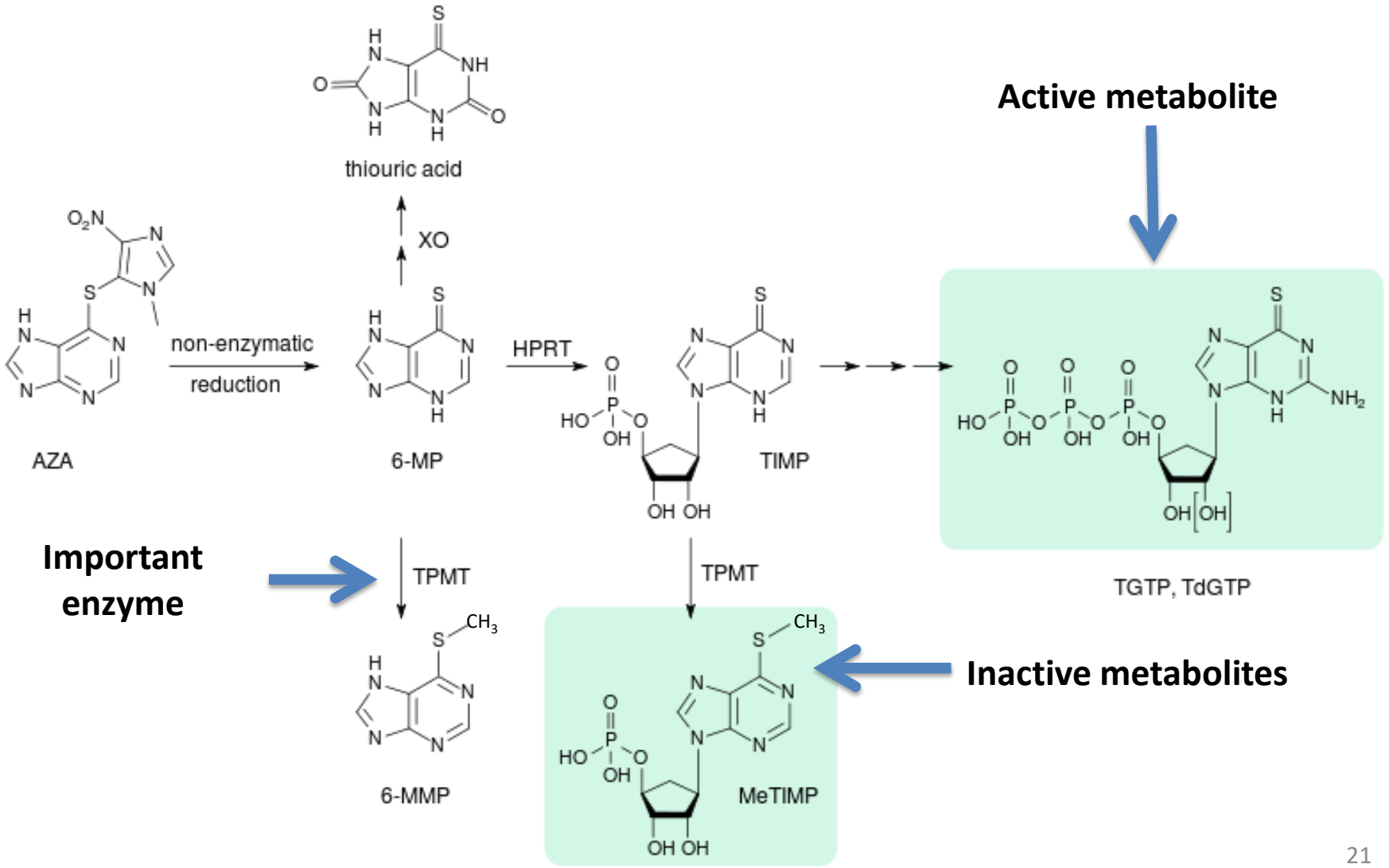
C) Increase activity of activating enzyme, increase activity of inactivating enzyme

D) Decrease activity of activating enzyme, increase activity of inactivating enzyme

How Are Thiopurines Metabolized?

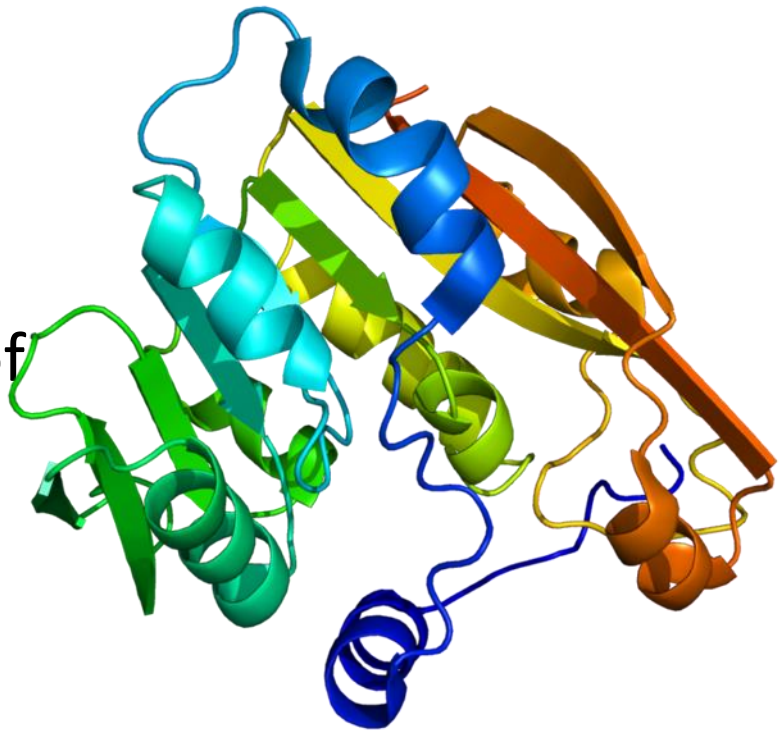


Thiopurine Metabolism

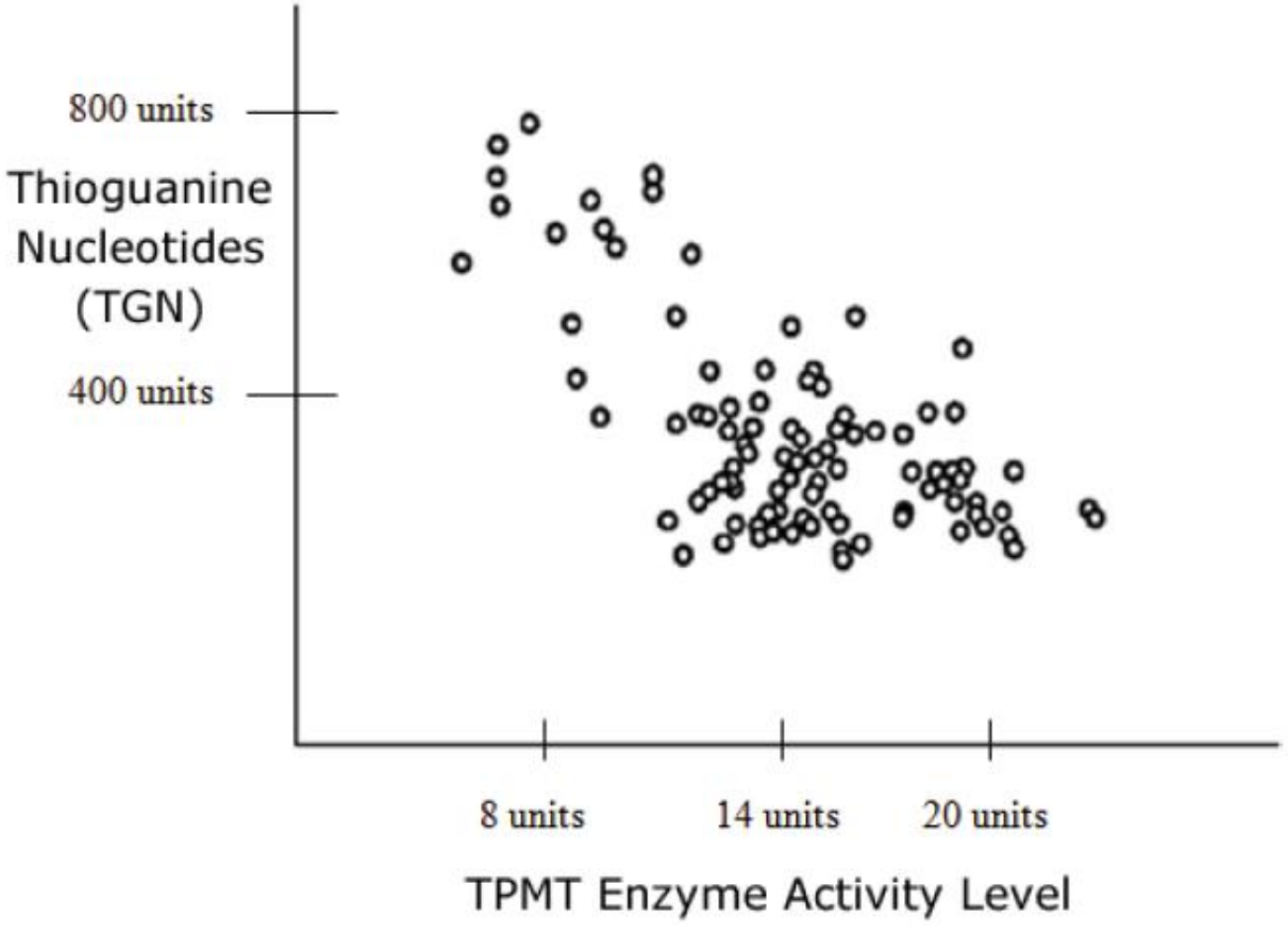


What Does the TPMT Enzyme Do?

- TPMT adds a methyl group (CH_3) to the sulfhydryl group (SH) on the drug or its metabolites
- Decreases the concentration of the active drug metabolites, thioguanine nucleotides
  - Thio-GTP
  - Thio-dGTP
- Acts indirectly to decrease the effective dose of the drug



What Is the Relationship between Drug Dose and TPMT Activity?



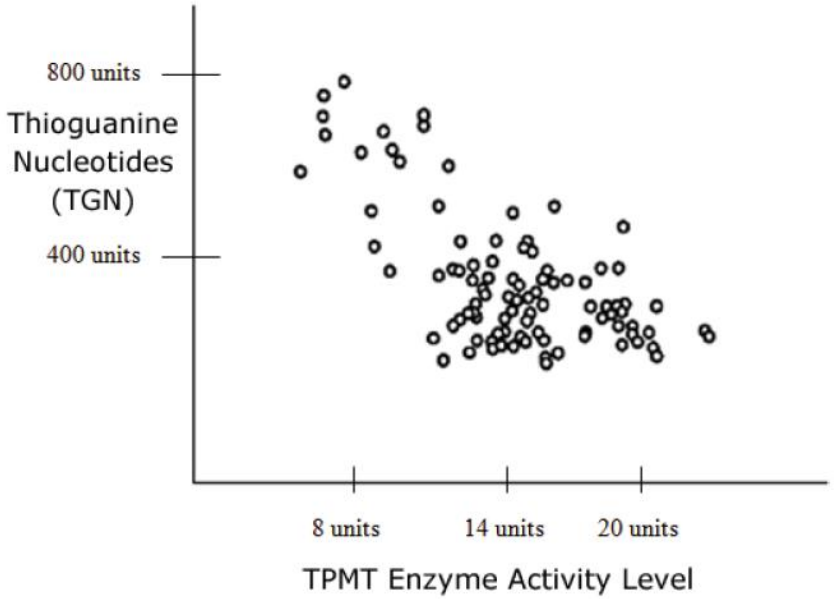
CQ6: Individuals with ___ TPMT activity would show _____ TGN levels, leading to toxicity.

A) low, low

B) high, high

C) low, high

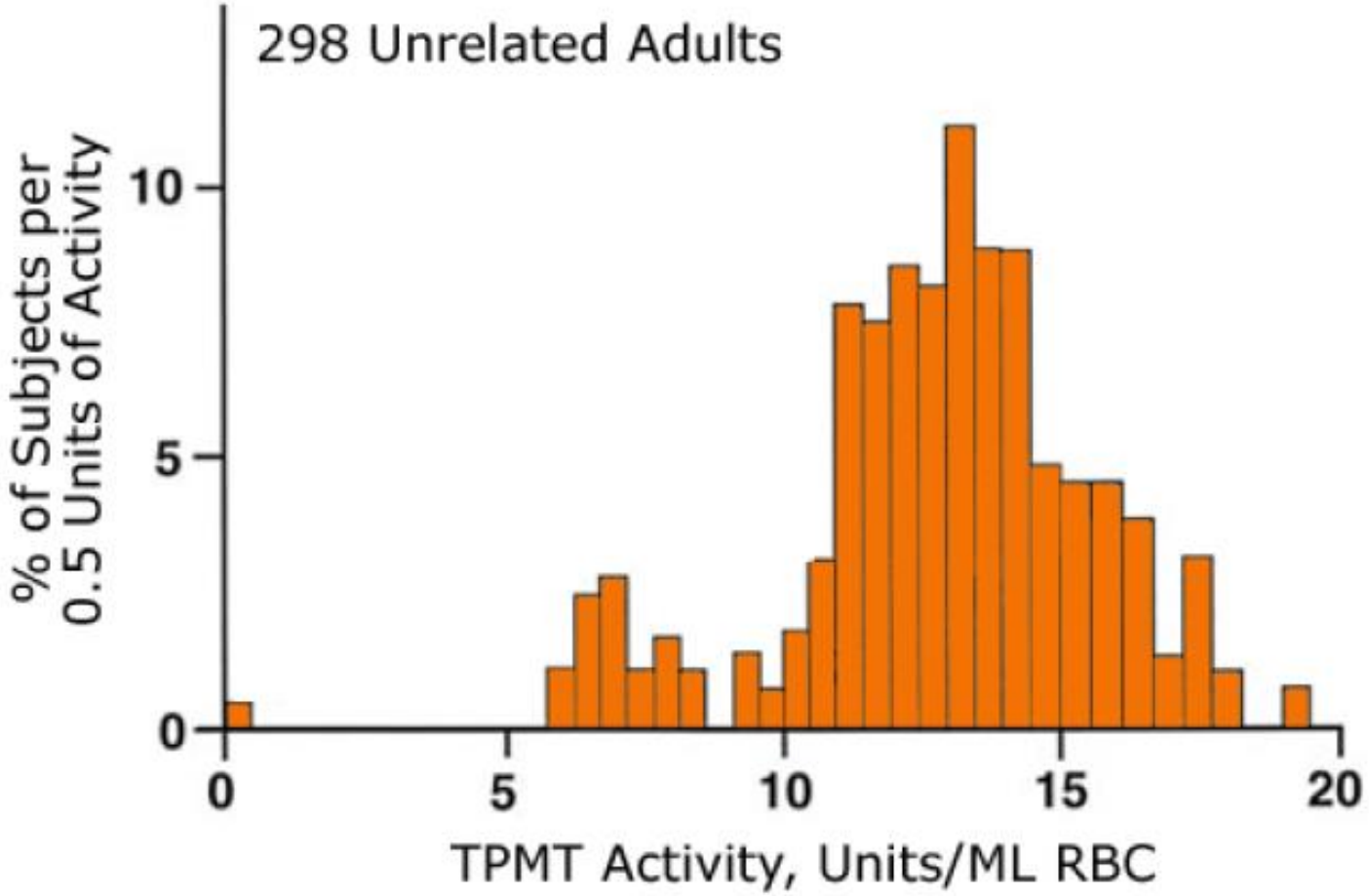
D) high, no change



TPMT Gene Has Different Forms (Alleles)

- High enzyme activity
  - Homozygous dominant (wild type)
- Medium enzyme activity
  - Heterozygous
- Low enzyme activity
  - Homozygous recessive

Distribution of TPMT Activity in 298 Caucasian Adults

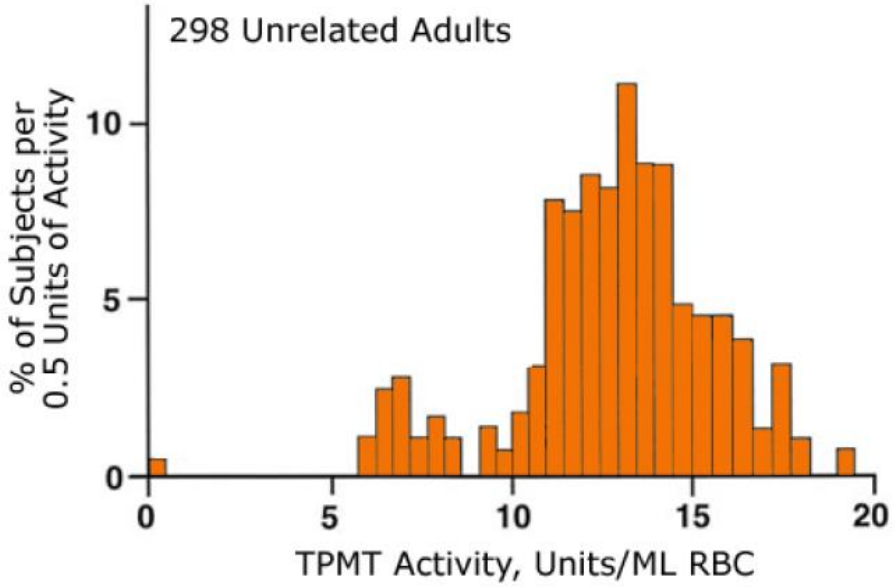


CQ7: Based on the graph, how many Caucasian patients out of 300 would possess the low activity form (less than 5 U/ml) of the TPMT enzyme?

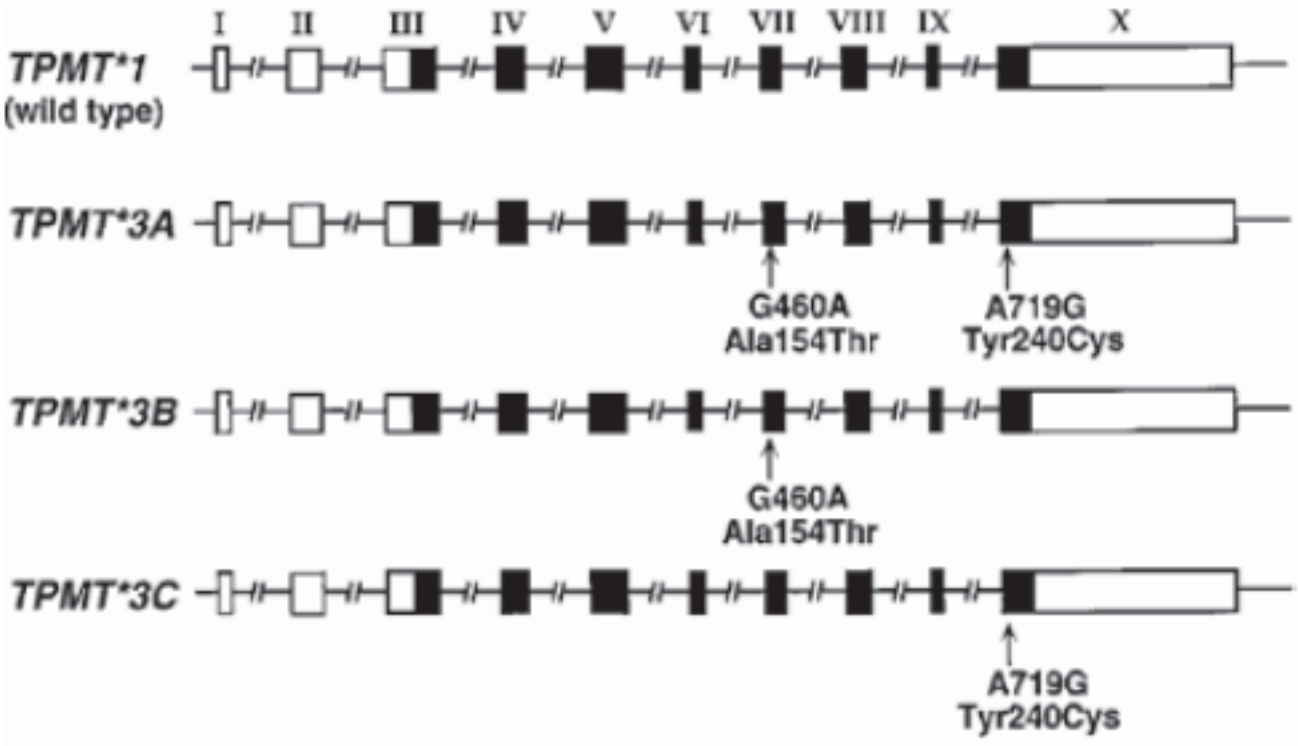
A) Approximately 1 out of 300

B) Approximately 10 out of 300

C) Approximately 290 out of 300



Common Mutations of the TPMT Gene



How Does the TPMT Mutation Decrease Enzyme Activity?

TPMT parameter	Wild type	*3A allele	*3B allele	*3C allele
Formation (fmol/ mg/ hr)	335	268	349	220
Degradation t1/2 (hr)	18	0.25**	6.1**	18

** significantly different than wild type protein

Turn to your neighbor and try to determine which mutation is more important for the change in degradation, exon 7 or exon 10?

CQ8: Beth has been diagnosed with the TPMT* 3a gene. Her deterioration following treatment is due to:

A) Decreased TPMT activity due to increased enzyme degradation.

B) Decreased TPMT activity due to decreased enzyme formation.

C) Decreased TPMT activity due to decreased enzyme degradation.

D) Increased TPMT activity due to increased enzyme formation.

TPMT parameter	Wild type	*3A allele	*3B allele	*3C allele
Formation (fmol/ mg/ hr)	335	268	349	220
Degradation t1/2 (hr)	18	0.25**	6.1**	18

CQ9: Effective treatment of individuals like Beth require:

A) Increased dose of drug

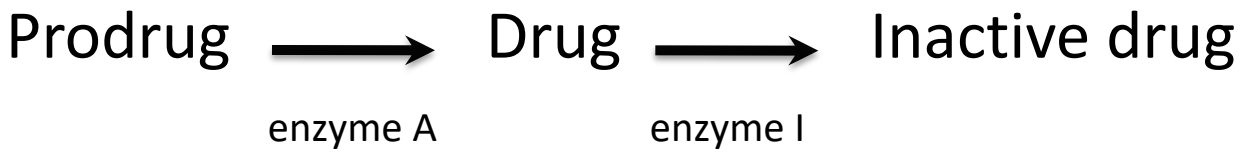
B) Decreased dose of drug

C) No change in drug dose

What Is “Pharmacogenomics”?

- The study of how genome-wide variation affects the body's response to drugs.
- Benefits for patients include better drug selection for initial treatment and more accurate dosing.
- Benefits for drug companies include genetic targeting of clinical trials for specific groups.
- The terms “pharmacogenetics” and “pharmacogenomics” are often used interchangeably

Another Example: Clopidogrel (Plavix)



- Taken by about 40 million people in the world to prevent blood clotting.
- CYP2C19 is responsible for its metabolic activation (see enzyme A in the diagram above).
- At least one loss-of-function allele is carried by 24% of the white non-Hispanic population, 18% of Mexicans, 33% of African Americans, and 50% of Asians.
- Homozygous carriers, who are poor CYP2C19 metabolizers, make up 3% to 4% of the population.

CQ10: Poor metabolizers of clopidogrel require _____ doses of drug to achieve an effective dose because the CYP2C19 enzyme does not_____ the drug.

A) Higher, activate

B) Lower, activate

C) Higher, inactivate

D) Lower, inactivate

The Future of Pharmacogenomics

- Pharmacogenomics is slowly being integrated into medical practice.
- Understanding the consequences of metabolizer status and the frequency of variants in a given population will be helpful when advising patients about treatment options.
- See the FDA Pharmacogenomic Biomarkers in Drug labels for a list of drugs and their associated genetic biomarkers.

Potential Barriers to Genetic Testing

- Complexity of finding gene variations that affect drug response
- Limited drug alternatives
- Disincentives for drug companies to make multiple pharmacogenomic products
- Educating healthcare providers
- Fear of discrimination based on genetic test results

CQ11: Which of the following do you think would be the greatest potential barrier for genetic testing?

A) Complexity of finding gene variations that affect drug response

B) Limited drug alternatives

C) Disincentives for drug companies to make multiple pharmacogenomic products

D) Educating healthcare providers

E) Fear of discrimination based on genetic test results

More Information about Pharmacogenomics

- The Pharmacogenomic Knowledge base
- The Pharmacogenomics Education Program
- Pharmacogenomics interactive tutorial

NOTE: Click on the links in full screen mode

Image Credits

Slide 4

Description: This is a scanning electron microscope image from normal circulating human blood. One can see red blood cells, several white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.Labels : (1) Monocyte, (2) Lymphocyte, (3) Neutrophil (4) Red Blood Cell (RBC), and (5) A few platelets (seen as small disc-shaped pellets)

Author: Bruce Wetzel (photographer). Harry Schaefer (photographer)

Source: http://commons.wikimedia.org/wiki/File:SEM_blood_cells.jpg

Clearance: This work is in the public domain in the United States because it is a work prepared by an officer or employee of the United States Government as part of that person's official duties under the terms of Title 17, Chapter 1, Section 105 of the US Code.

Slide 5-10

Description: A three-dimensional ultrastructural image analysis of a T-lymphocyte (right), a platelet (center) and a red blood cell (left), using a Hitachi S-570 scanning electron microscope (SEM) equipped with a GW Backscatter Detector.

Author: Electron Microscopy Facility at The National Cancer Institute at Frederick (NCI-Frederick)

Source: http://commons.wikimedia.org/wiki/File:Red_White_Blood_cells.jpg

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Slide 14

Description: Simplified hematopoiesis

Author: from original by A. Rad

Source: http://en.wikipedia.org/wiki/File:Hematopoiesis_simple.svg

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Slide 15

Description: A Wright's stained bone marrow aspirate smear of patient with precursor B-cell acute lymphoblastic leukemia

Author: VashiDonsk

Source: http://commons.wikimedia.org/wiki/File:Acute_leukemia-ALL.jpg

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Slide 16
Description: Structure of 6-mercaptopurine
Source: National Center for Case Study Teaching

Slide 20
Description: Flow chart showing activation and inactivation pathway of the drug 6-mercaptopurine (6-MP).
Source: Figure 1 in "Pharmacogenetics: Using Genetics to Treat Disease"
Author: Jeanne Ting Chowning, Director of Education, Northwest Association for Biomedical Research
Clearance: National Center for Case Study Teaching

Slide 21
*Description: M*etabolism of thiopurine drugs. XO, xanthine oxidase; 6-MP, 6-mercaptopurine; TPMT, thiopurine methyltransferase; 6-MMP, 6-methylmercaptopurine; HPRT, hypoxanthine-guanine phosphoribosyltransferase; TIMP, thioinosine monophosphate thioinosinic acid; MeTIMP, methyl-thioinosine monophosphate; TGTP, thioguanosine triphosphate; and TdGTP, thio-deoxyguanosine triphosphate.
Source: http://commons.wikimedia.org/wiki/File:AZA_metabolism.svg
Author: Karran, P. (2008). "Thiopurines in current medical practice: Molecular mechanisms and contributions to therapy-related cancer". *Nature Reviews Cancer 8 (1): 24–36. DOI:10.1038/nrc2292. PMID 18097462.*
Clearance: This image of a simple structural formula is ineligible for copyright and therefore in the public domain, because it consists entirely of information that is common property and contains no original authorship.

Slide 22
Description: Structure of the TPMT protein.
Source: http://commons.wikimedia.org/wiki/File:Protein_TPMT_PDB_2bzg.png
Author: Based on PyMOL rendering of PDB 2bzg
Clearance: This file is licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

Slide 23-24
Description: Scatter plot of TGN versus enzyme activity
Primary Author: Lennard L., J.S. Lilleyman, J. Van Loon, and R.M. Weinshilboum (1990) Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia*. Lancet*, 336, 225-229, modified by Jeanne Chowning.
Secondary Author: Jeanne Ting Chowning, Director of Education, Northwest Association for Biomedical Research.
Source: Figure 4 in "Pharmacogenetics: Using Genetics to Treat Disease"
Clearance: National Center for Case Study Teaching

Slide 26-27
Description: RBC TPMT frequency distribution histogram for 298 randomly selected Caucasian subjects.
Primary Author: Weinshilboum, R.M., and S. Sladek (1980) Mercaptopurine pharmacogenetics: Monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *American Journal of Human Genetics*, 32: 651-662. Modified by Jeanne Chowning
Secondary Author: Jeanne Ting Chowning, Director of Education, Northwest Association for Biomedical Research
Source: Figure 3 in "Pharmacogenetics: Using Genetics to Treat Disease"
Clearance: National Center for Case Study Teaching

Slide 28
Description: Examples of TPMT alleles.
Primary Author: Weinshilboum, R. (2001) Thiopurine pharmacogenetics: Clinical and molecular studies of Thiopurine Methyltransferase, American Society for Pharmacology and Experimental Therapeutics 29: 601-605. Available online at http://dmd.aspetjournals.org/. Modified by Jeanne Chowning
Secondary Author: Jeanne Ting Chowning, Director of Education, Northwest Association for Biomedical Research
Source: Figure 5 in "Pharmacogenetics: Using Genetics to Treat Disease"
Clearance: National Center for Case Study Teaching

Slide 29-30
Description: Half-lives and synthesis rates of wild-type and mutant TPMT proteins in yeast.
Original author: Hung-Liang Tai, Eugene Y. Krynetski, Erin G. Schuetz, Yuri Yanishevski, and William E. Evans. Enhanced proteolysis of thiopurine S-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT3A, TPMT2): Mechanisms for the genetic polymorphism of TPMT activity *Proc Natl Acad Sc*i U S A. 1997 June 10; 94(12): 6444–6449.PMCID: PMC21069.
Secondary Author: Table 1 modified by Maureen Knabb
Clearance: National Center for Case Study Teaching