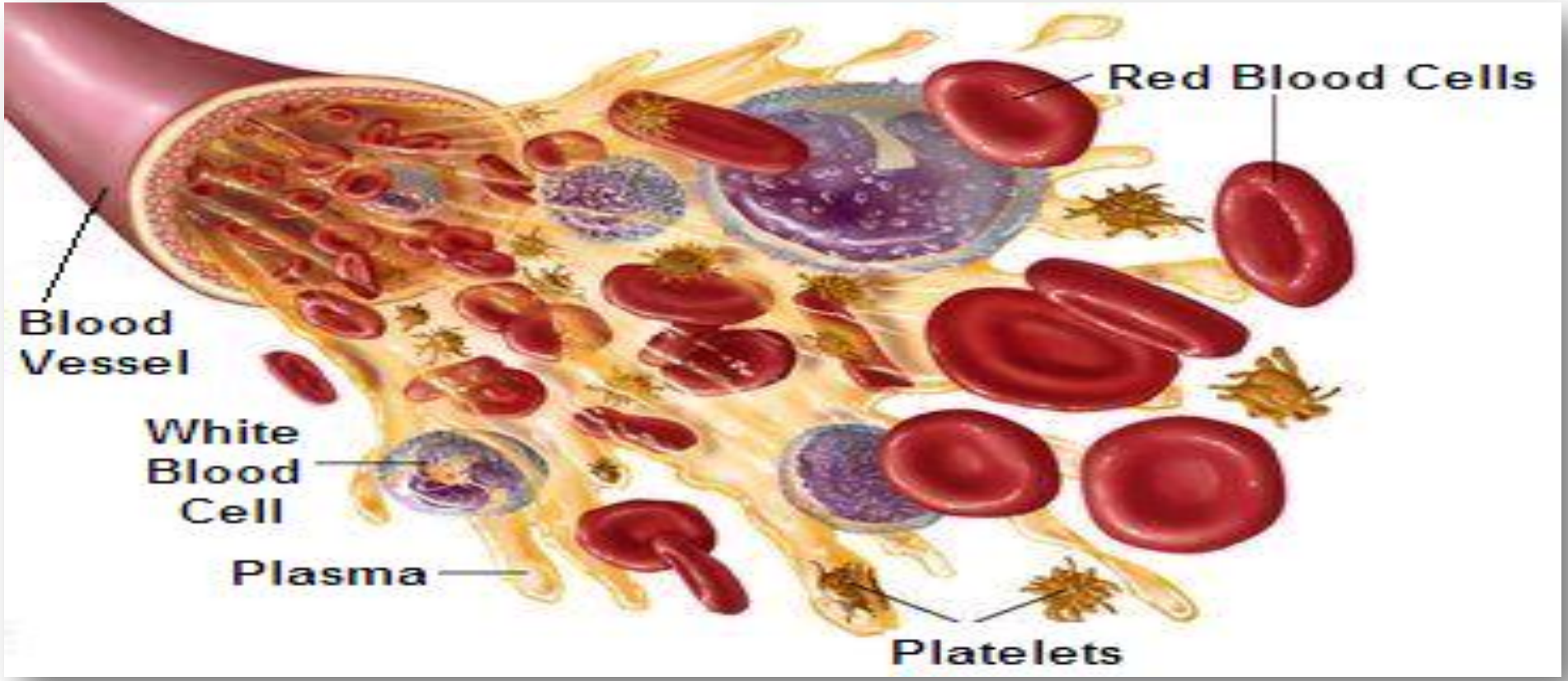
Measuring Thrombopoietin - 2012

A New Tool for Hematologists?



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Disclosure

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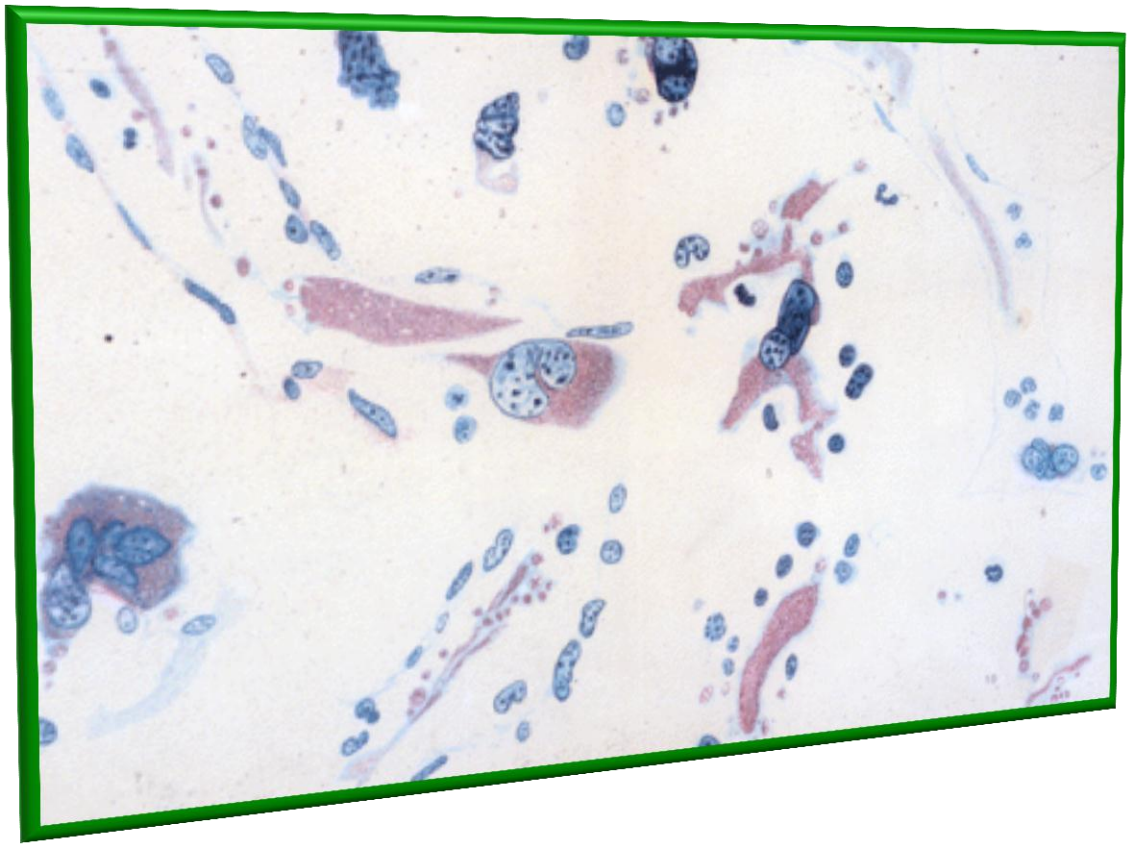
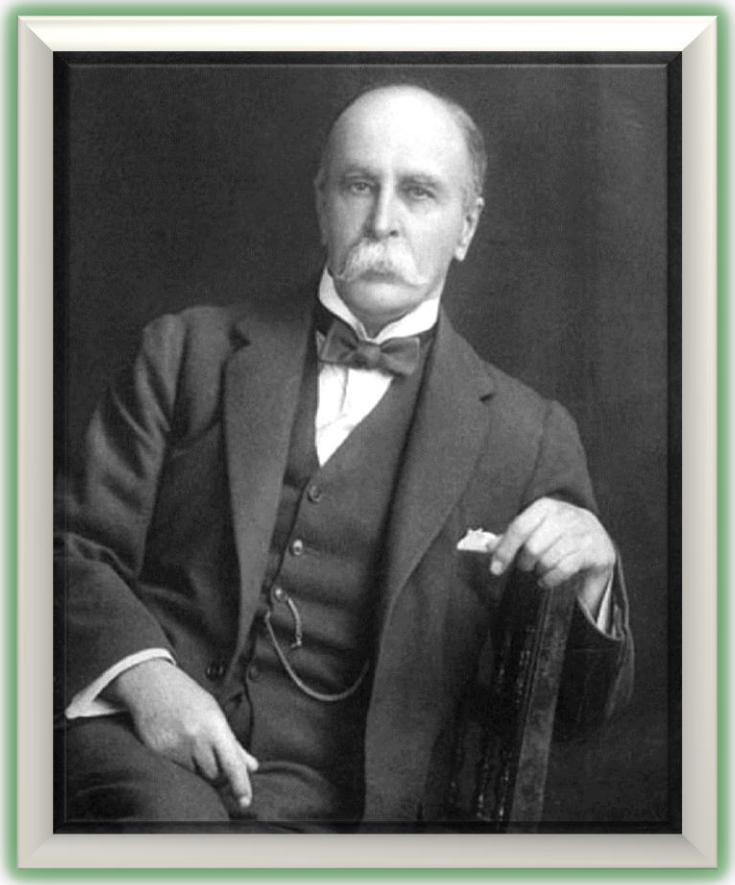
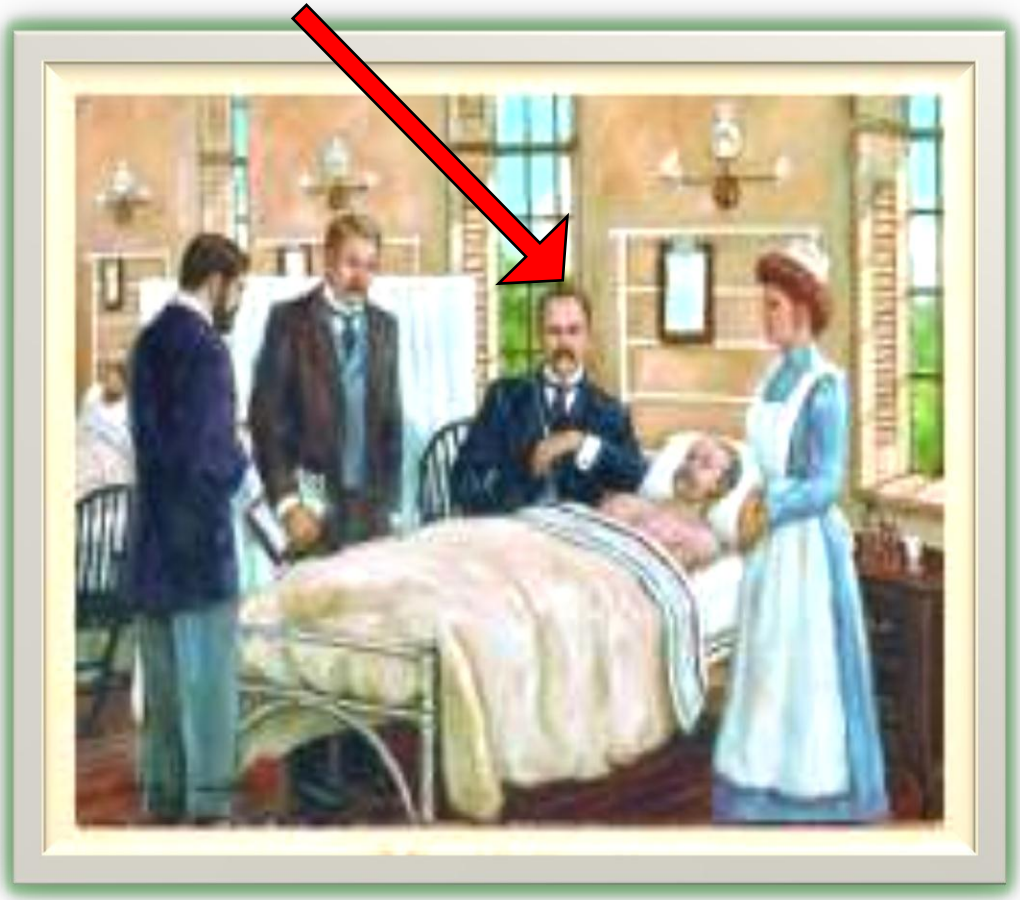
Doctor James Homer Wright
(1869 – 1928)



James Homer Wright Established The Basic Elements Of Thrombopoiesis In <u>1906</u>

Sir William Osler
(1849 – 1919)





Medicine at the bedside

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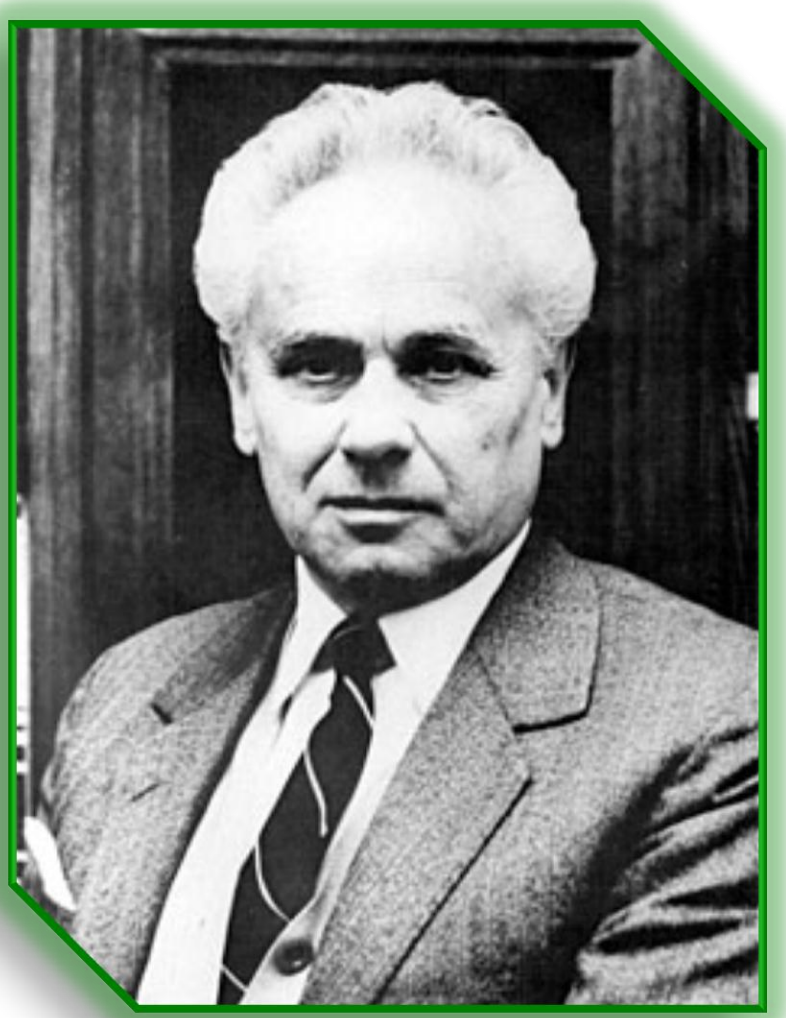
Doctor Endre Keleman (1921-2000)



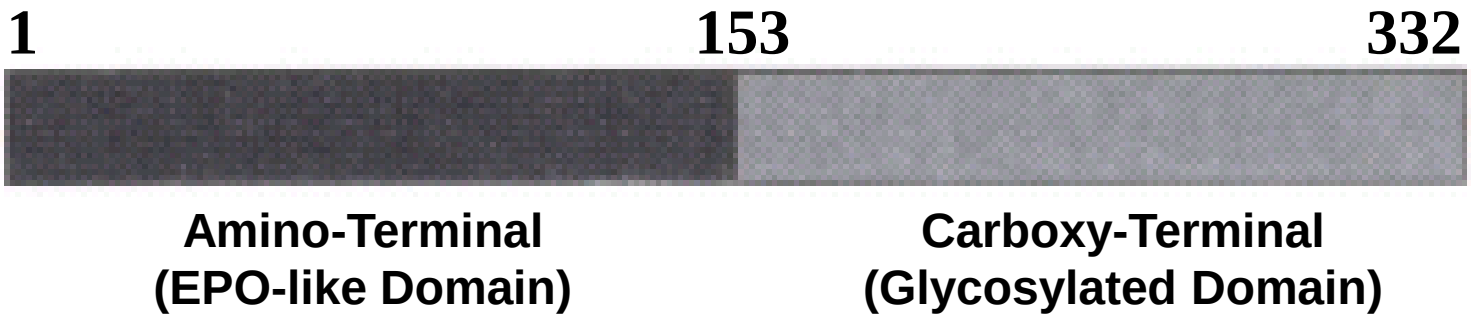
Dr. Endre Kelemen Described Human Thrombopoietin In 1958

Dr. Kenneth Kaushaunsky

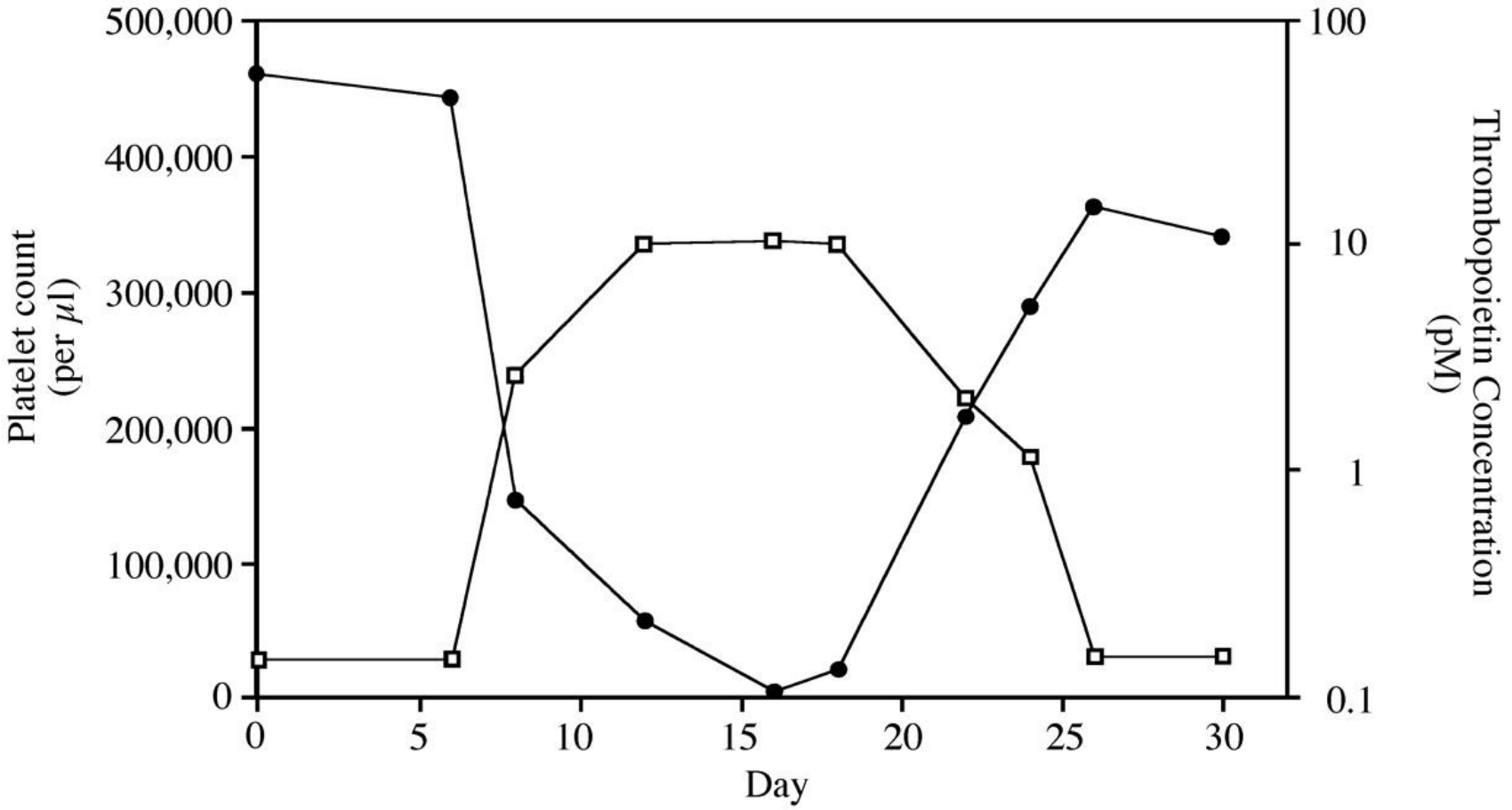
Dr. David Kuter

Thrombopoietin Structure

The Structure of Human Thrombopoietin



Relationship Between The Platelet Count (•) and The Thrombopoietin Concentration (□).



A rabbit was made thrombocytopenic by the administration of busulfan on Day 0 and platelet counts and thrombopoietin levels measured thereafter.

Kuter D J The Oncologist 1996;1:98-106

Early Studies on Thrombopoiesis

BLOOD The Journal of Hematology

JULY, 1960 VOL. XVI, NO. 1

Studies on Thrombopoiesis. I. A Factor in Normal Human Plasma Required for Platelet Production; Chronic Thrombocytopenia Due to its Deficiency

By Irving Schulman, Mila Pierce, Abby Lukens and Zinet Currimbhoy

SUMMARY

- **A case of chronic thrombocytopenic purpura has been presented in which the pathogenesis appears to be due to congenital deficiency of a platelet-stimulating factor.**
- **The factor exists in normal plasma and is stable on storage under normal blood banking conditions and on freezing.**
- **The factor appears to act by stimulating megakaryocyte maturation and platelet production in an orderly and sequential manner.**

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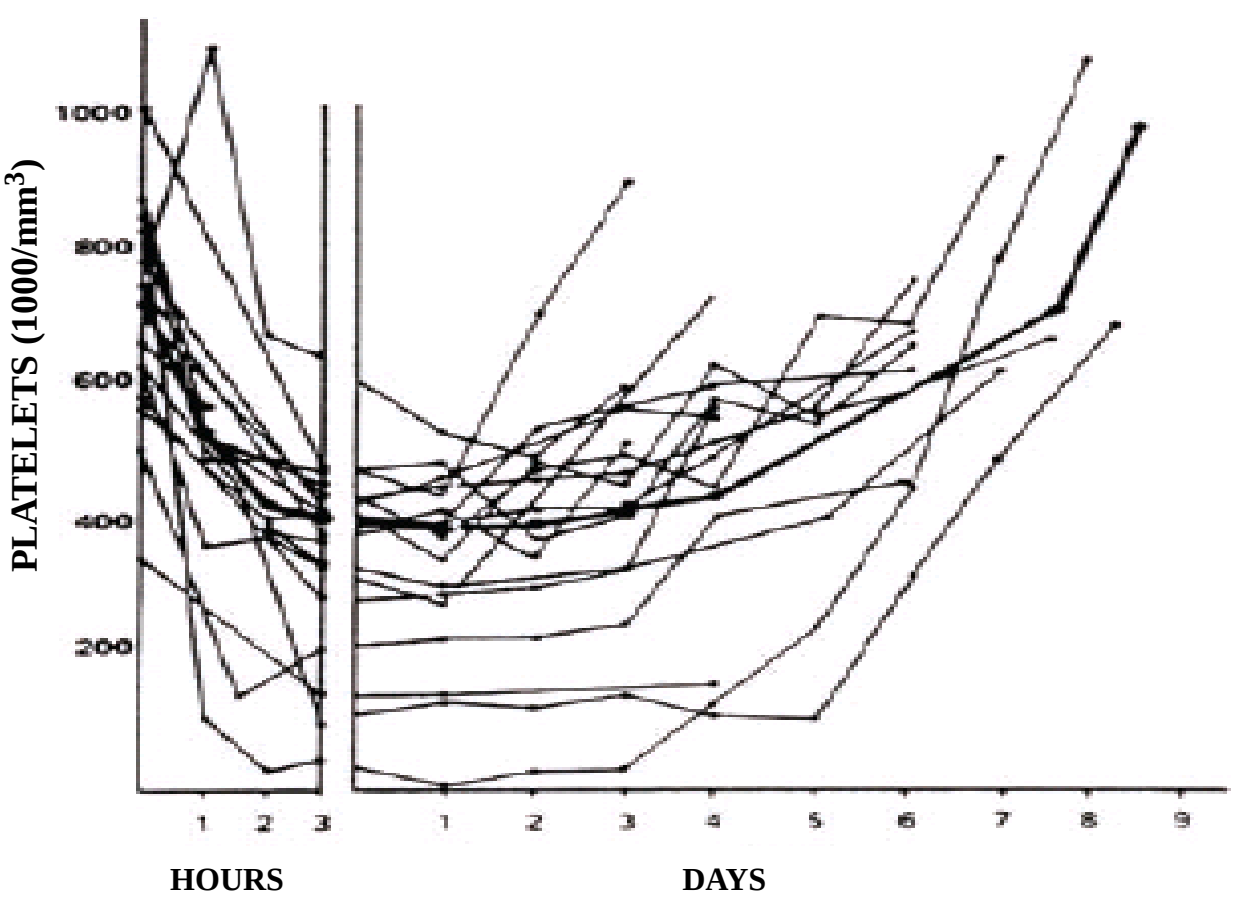
The Harrington–Hollingsworth Experiment



William J. Harrington (1924–1992)

Graph shows rapid development of thrombocytopenia, followed by a return to normal platelet levels, in healthy volunteers who received plasma from patients with idiopathic thrombocytopenic purpura

Case Study # 1

A 42 year - old woman with refractory immune thrombocytopenia (ITP) presents for a second opinion to a university hematologist after Undergoing an extensive treatment regimen including high dose dexamethasone, intravenous immunoglobulin and rituximab.

Lab Results:

- Platelet Count: 11,000 /μl^3
- Hemogram otherwise: Normal
- Mean Platelet Volume: 12.4 /fl (n. 7.5 – 11.5)
- Direct Glycoprotein Antibody (IIb/IIIa): Strongly Positive
- Blood Smear: No Schistocytes
- Immunoglobulins: Normal
- Serology for Epstein-Bar: Negative
- Hepatitis C: Negative
- Helicopter Pylori: Negative
- Protein Electrophoresis: Negative
- Splenectomy offered: Patient declined

Case Study # 1

Clinical Course:

The hematologist offers her a T.P.O. mimetic, Eltrombopag or Romiplastin which stimulates "Megakaryocytopoiesis". A serum thrombopoietin level is drawn and comes back 621 pg/ml (n. < 99 pg/ml).

After 3 weeks of Romiplastin (1 mcg/kg), the platelet count remains low at 14,000 μl^3 (n. 150 – 400 $x10^3$). The dosage of Romiplastin is increased to 10 mcg/kg without response at week # 12.

Final Decision:

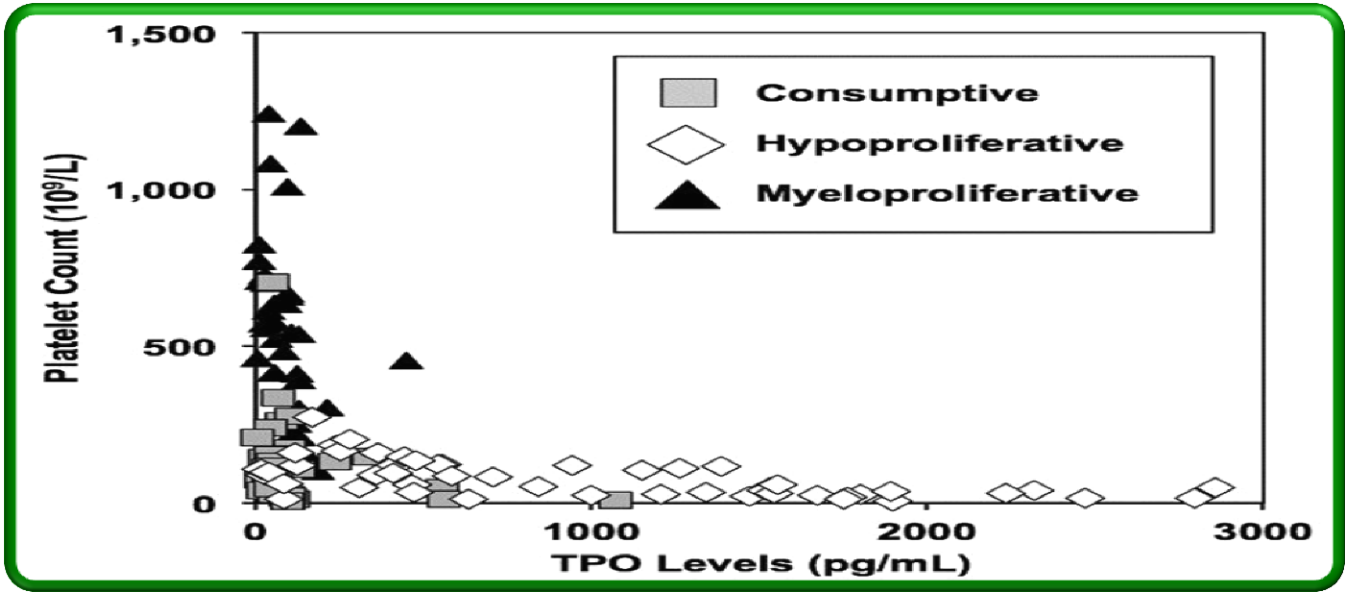
A splenectomy is performed without incident and the platelet count is 151,000 / μl^3 at month 9 after surgery.

Case Study # 1

What is the value of the
elevated TPO level in this patient?

- The TPO level has no value in this case
- The patient has a thrombopoietin – producing tumor
- The assay for TPO is faulty due to poor Quality Control
- The patient has a marked elevation of erythropoietin (EPO) which is cross reacting with the TPO assay

Thrombopoietin Levels in Blood Disorders



Category (n)	Mean Age (yrs.)	Female (%)	Specific Diagnoses (n)
Consumptive Thrombocytopenia (39)	51 (21-83)	24 (62%)	Primary or Secondary ITP (36) Thrombotic Thrombocytopenic Purpura (2) Antiphospholipid Antibody Syndrome (1)
Hypoproliferative Thrombocytopenia (49)	58 (31-87)	22 (45%)	Chemotherapy-Related (29) Primary or Secondary Bone Marrow Failure Syndromes (20)
Myeloproliferative Disorders (34)	65 (28-88)	20 (59%)	Essential Thrombocytosis (20) Polycythemia Vera (10) Myeloproliferative Disorder NOS (4)

Makar, R., Zhukov, O., Sahud, M. and Kuter, D. submitted for publication 2012

Case Study # 1

ANSWER…..

- Elevated TPO levels found in patients with ITP are less likely to respond to TPO mimetics drugs.
- Some ITP patients have elevated TPO levels suggesting that inadequate megakaryopoiesis is the predominant pathological feature

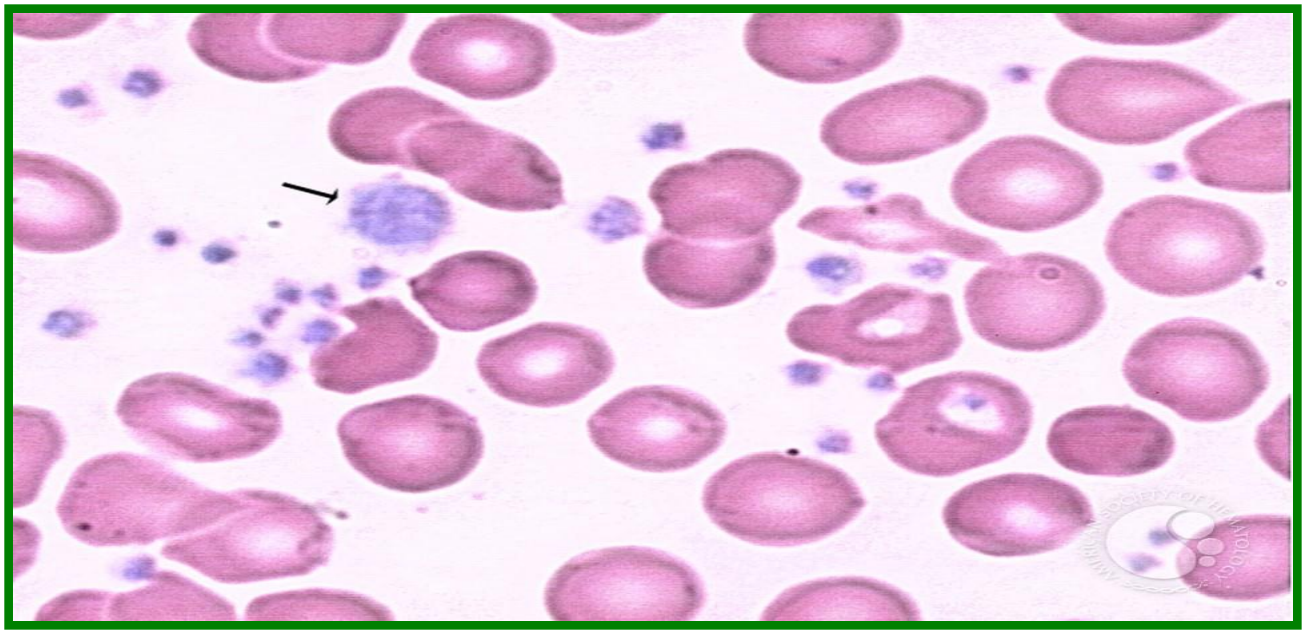
		Clinical Response †	
		YES	**NO**
TPO Level	**≤ 95 pg/mL**	14	1
	> 95 pg/mL	1	8
	Median (IQR)	49 (34 -66)	1001 (110 -1752)

Thrombopoietin: Why Should We Measure It?

- **Patients with high TPO levels do not respond to TPO mimetic's**
- **Reimbursement for TPO mimetics may hinge on “normal” TPO levels prior to treatment**
- **Methods of Measuring TPO:**
  - Home brew assay
  - C-MPL responsive assay
  - First market advantage

Case Study # 2

- RL, a 73 year - old man has a history of Thrombocytosis, with an initial platelet count of 1,730,000 with normal Hct and WBC. He is treated with Hydrea. The diagnosis is Essential Thrombocythemia (JAK2-neg). He remains on Hydrea at a dose of 500-1000 mg /day for over 3 months. His platelet count falls to 450,000/µl^3.



Case Study # 2

- Four months into therapy his platelet count is now 150,000/µl^3. The Hydrea is discontinued, but over the next 3 months the platelet count continues to fall to 3,000 /µl^3.
- Patient receives platelet transfusions and bleeding is reduced.
- Diagnosis is uncertain. Treatment includes steroids, IVIG and Winrho.
- Referred to University Hospital for consideration of splenectomy or Thrombopoietin mimetic.

Case Study # 2

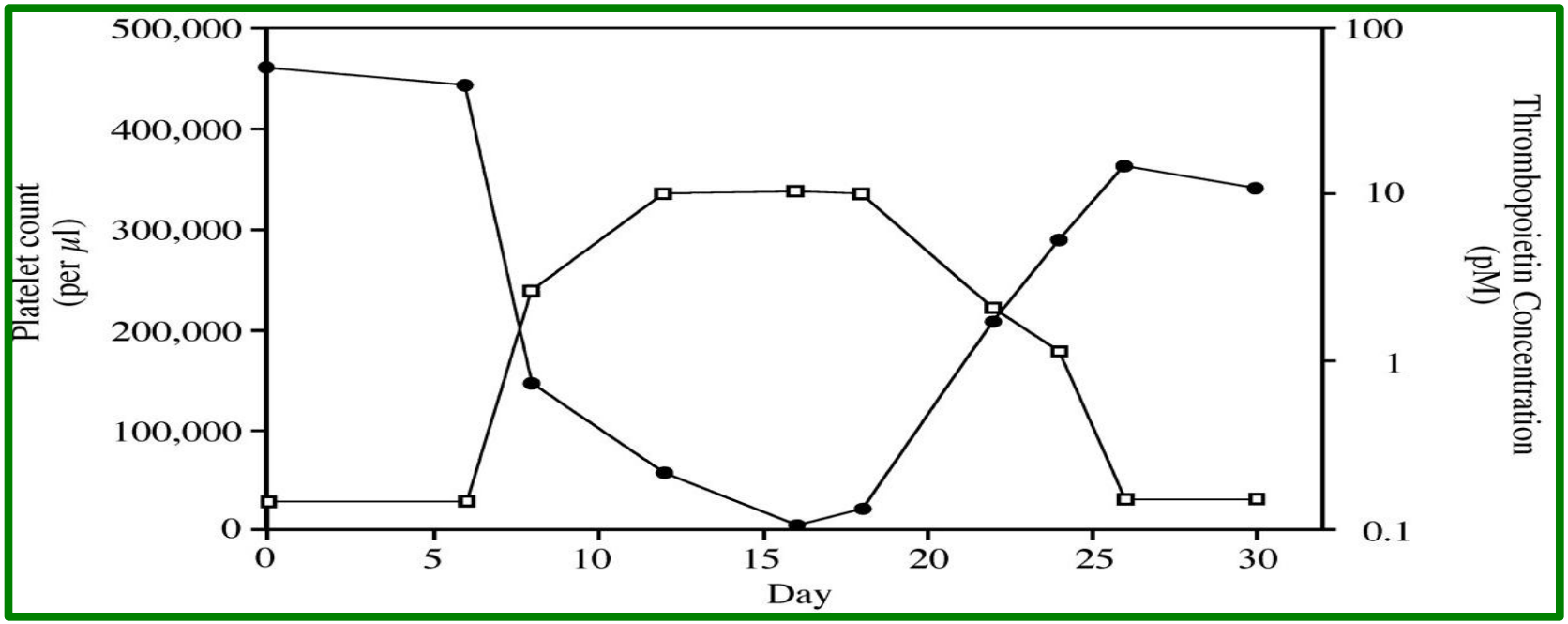
Lab Results

- Exam / diffuse ecchymoses, no splenomegaly, normal WBC count 3,900 and Hematocrit 41%
- Platelet count - 5,000 /µl^3 and many large forms noted on smear.
- Would a TPO level be of value in this patient?
  - No, the patient requires a splenectomy as soon as possible
  - No, the patient is surreptitiously taking Hydrea:
    - *Obtain Plasma Hydrea level*
  - No, the patient is septic: draw 3 blood cultures

Case Study # 2

ANSWER:

- TPO level is 1,500 pg/ml (N. ≤ 75): **Suggesting Bone marrow failure**



- Bone marrow is deferred and Romiplastin is not given
- 3 months later the platelet count has slowly returned to 115,000 /μl^3

DIAGNOSIS:

- Idiosyncratic reaction to Hydrea…..?

Case Study # 3

A 39 year-old woman presents for hematologic evaluation at SMC with elevated platelet count.

History

- An elevated platelet count was detected at age 19
- Transient ischemic attack 4 years ago (Platelet count 1.4 million μl^3)
- Treatment for the last 18 months previously included:
  - Anegrelide, Interferon-α and Hydrea 500 mg
- Denies fever, sweating , weight loss, early satiety or vasomotor symptoms
- P.E. - No splenomegaly or bruising

Lab Results (at Stanford in March 2010):

- Platelet count - 1,015,000/ μl^3 (without other abnormalities)
- WBC – 3,700: 42% neutrophils
- Hemoglobin – 11.6 gm/dl
- Peripheral blood smear - occasional large, hypogranular platelets.
- Reactive causes of thrombocytosis - excluded
- Examination - normal
- *JAK2* V617F mutation analysis - negative

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Case Study # 3

THIS CASE REPRESENTS A TYPICAL
PRE-FIBROTIC STAGE
OF ESSENTIAL THROMBOCYTHEMIA

EXCEPT… IT ISN'T!!

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Case Study # 3: The Art of History Taking

Clinical Course:

A first year medical resident comes in to take a thorough history.

Doctor:

"Are there any blood disorders in your family?"

Patient:

"No, but my sister has high platelets also."

Doctor:

"Do you have children?"

Patient:

"Yes, I have a boy age 9 and a girl age 11. They both have elevated platelet counts….so it must be catching!"

Doctor:

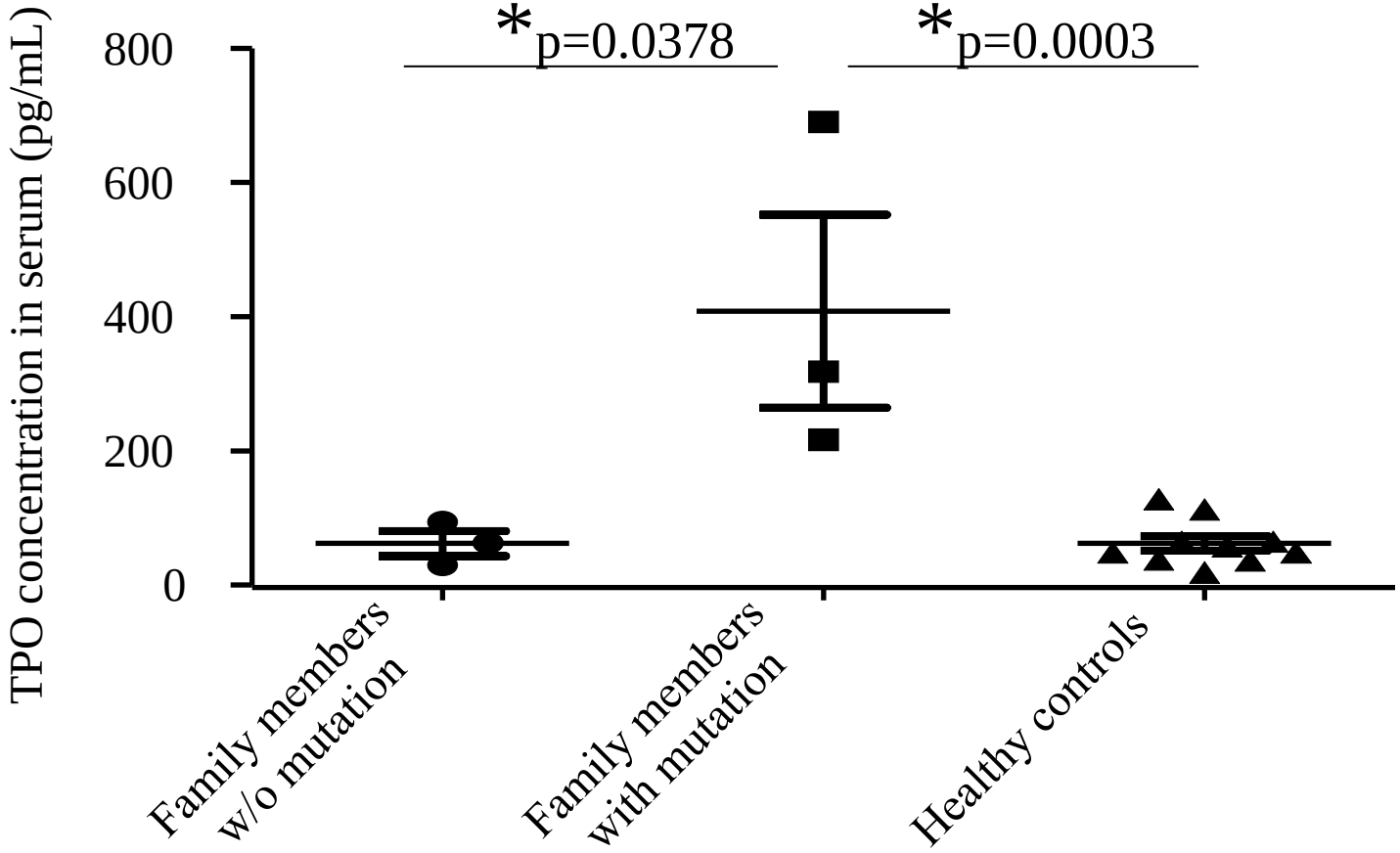
"Well, does your husband have elevated platelets?

Patient:

"Oh No! and no other family members have it, except my mother"

Case Study # 3

- Hereditary Thrombocythemia (HT) is suspected
- THPO or MPL mutations are investigated
- Serum TPO Levels are drawn in family members

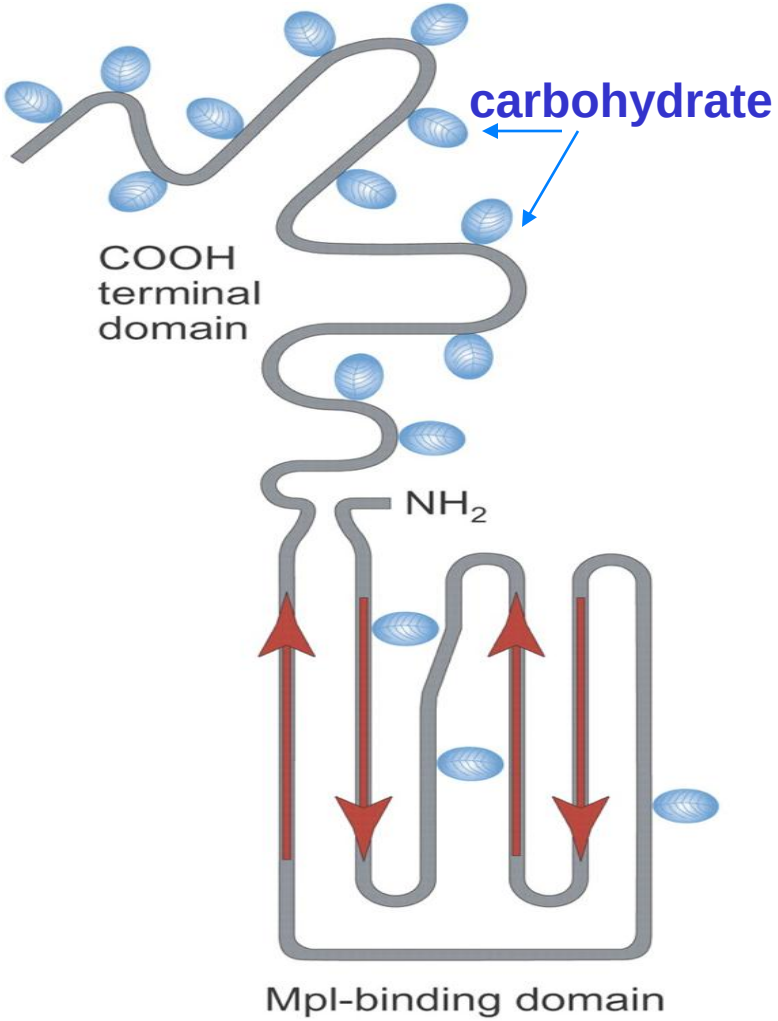


Jason Gotlib, et al

Thrombopoietin Signaling

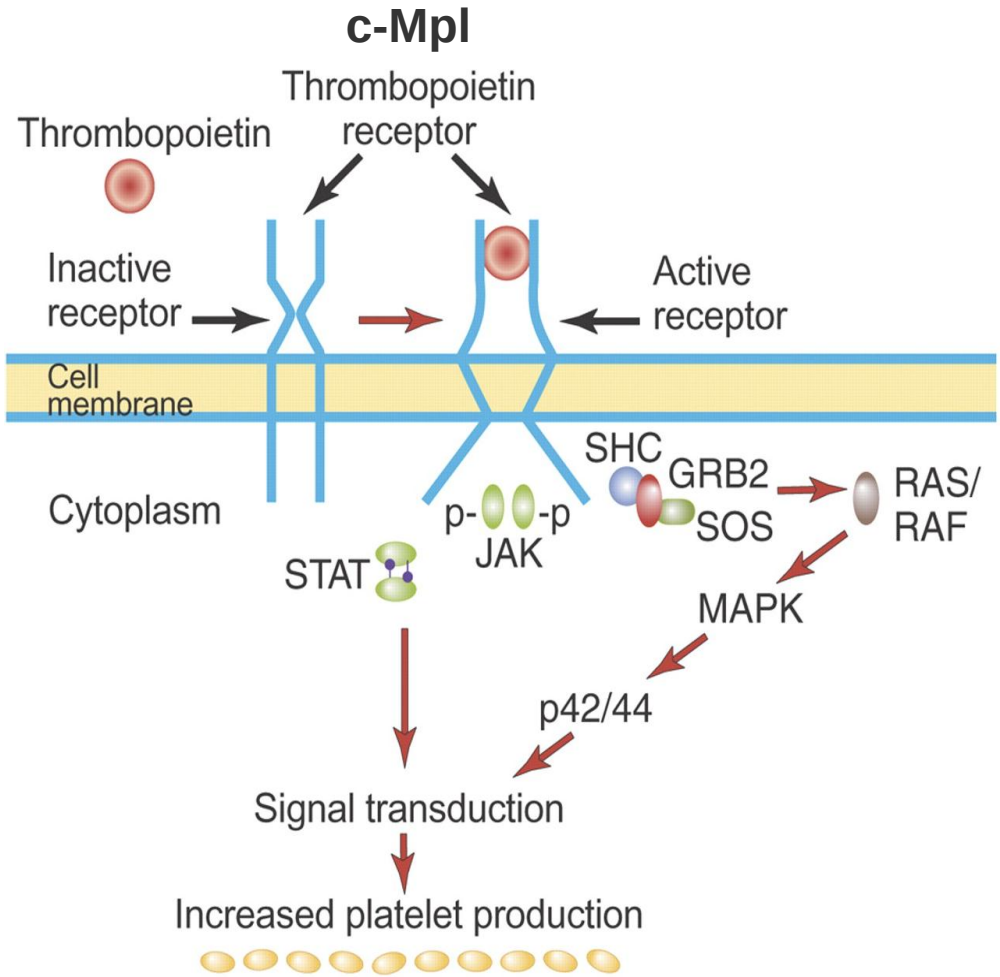
Glycoprotein:
332 amino acids, 95 kDa Synthesized mainly in the liver

Thrombopoietin



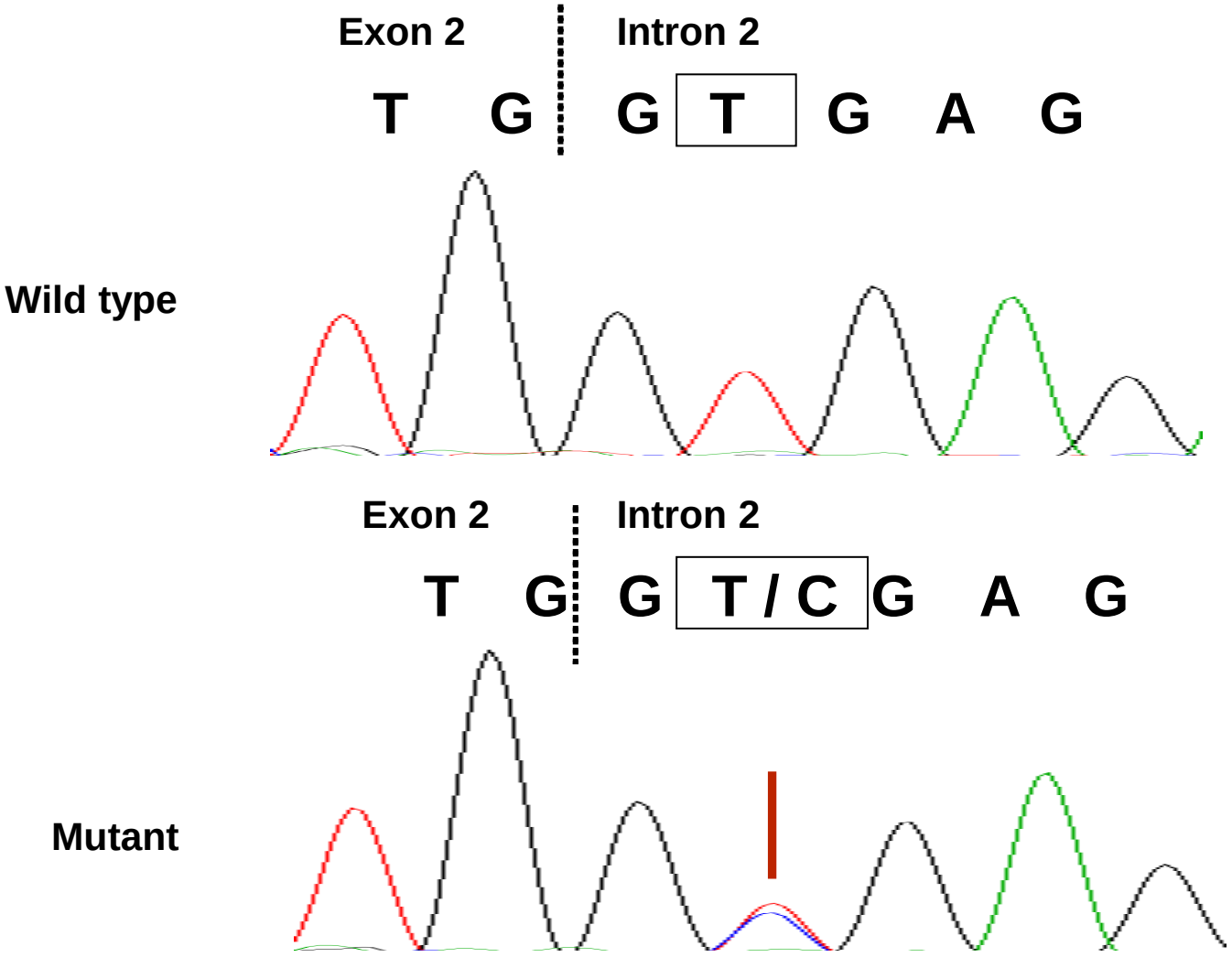
Cytokine Receptor:
Homologous to the oncogene in murine Myeloproliferative leukemia virus Present on megakaryocytes and platelets

Receptor



(Kuter DJ. New thrombopoietic growth factors. Blood. 2007;109:4607-4616)

Case # Study 3: THPO Germline Gene Mutation



Case Study # 3: Summary of THPO in HT

- To date, five HT families with three distinct *THPO* mutations have been published, including Dutch, Japanese, and Polish pedigrees.

- No consistency in reports of thrombosis or clinical outcomes; our proband maintained on ASA

- In all cases, the mechanism of overproduction of platelets is related to alteration of the 5' UTR of the *THPO* gene which results in enhanced translation of thrombopoietin (TPO) mRNA.

Case Study # 3: MPL Mutations in HT

MPL W515L/K Mutation Frequency in Acquired MPNs	
Essential thrombocythemia	~1-5%
Primary myelofibrosis	~5-10%

MPL Mutations in Hereditary Thrombocythemia	
MPL Ser505Asn (S505N)*	Japanese[1], Italian[2,3]
MPL Pro106Leu (P106L)	Arab[4]

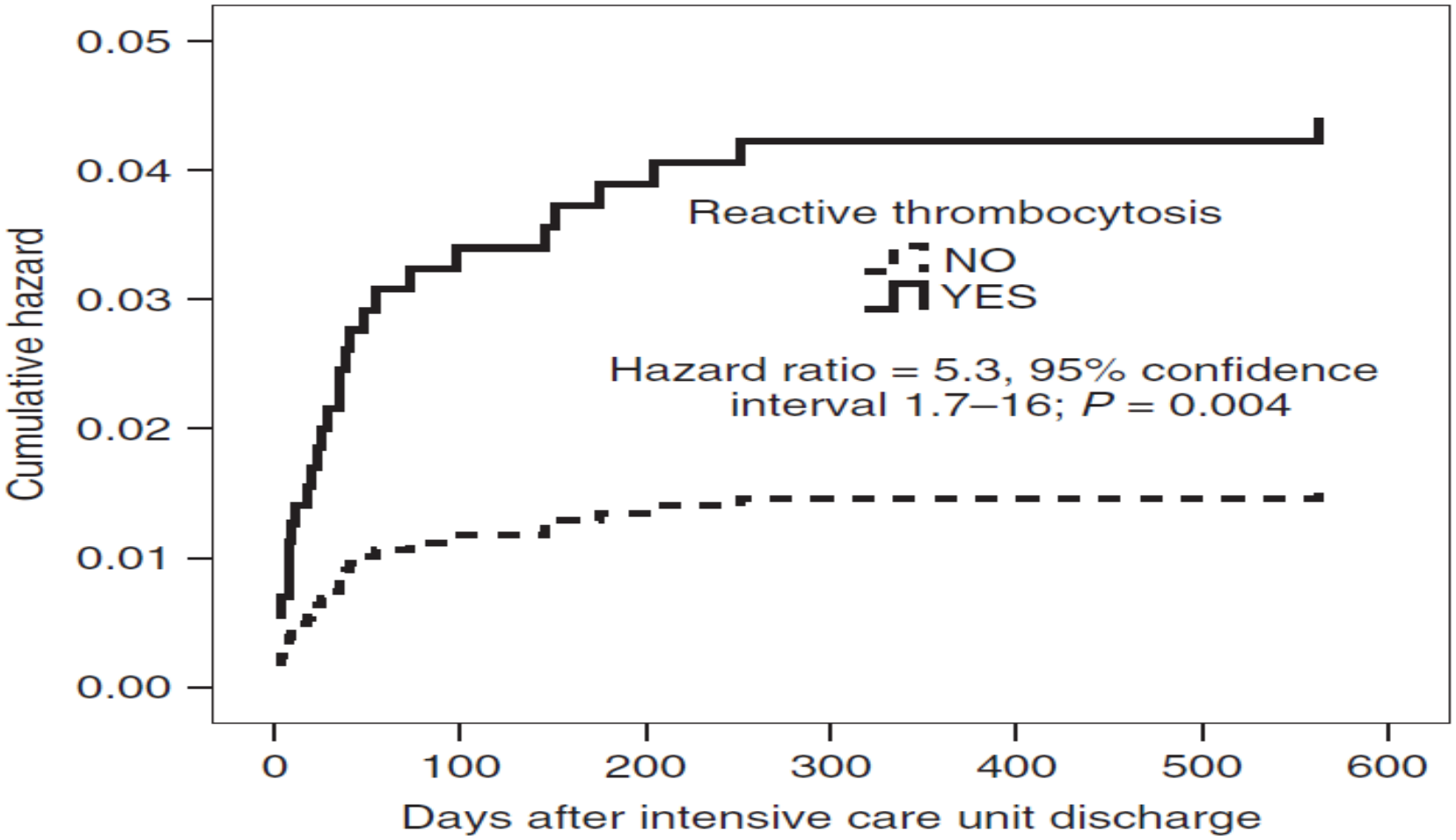
*Rare frequency in PT-1 Cohort

[1] Ding J, et al, Blood. 2004.
[2] Teofili L, et al. J Clin Oncol. 2007
[3] Liu K, et al. Haematol. 2009.
[4] El-Harith HA, et al. Haematol. 2009.

Gene Mutations

Authors	Gene mutation	Consequence
Wiestner[29]	*TPO*, G>C in intron3 position +1	Loss of uORF-mediated repression[29]
Kondo,[30] Ghilardi[32]	*TPO*, deletion of G in 5'-UTR	Loss of uORF-mediated repression[32]
Ghilardi[31]	*TPO*, G>T in 5'-UTR	Loss of uORF-mediated repression[31]
Jorgensen[33]	*TPO*, A>G in intron3 position +5	Not studied
Ding[80]	*MPL*, G>A in exon 10 resulting in S505N in Mpl protein	Constitutively active Mpl protein
Moliterno[53]	*MPL*-K39N	Co-dominant, mild thrombocytosis in homozygotes, function uncertain
El-Harith[55]	*MPL*-P106L	Co-dominant, elevated Tpo serum levels
Kawamata[51]	*MPL*-S204F	Found in uniparental disomy 1p, function uncertain
Williams [49]	*MPL*-S204P	Function uncertain
Komatsu[41]	*MPL*-S505N	Constitutive activation of Mpl protein, autosomal dominant thrombocytosis
Chaligne[50]	*MPL*-A506T	Function uncertain
Chaligne[50]	*MPL*-L510P	Function uncertain
Pikman[44]	*MPL*-W515L	Constitutive activation of Mpl protein, sporadic ET or PMF
Pardanani[44]	*MPL*-W515K	Constitutive activation of Mpl protein, sporadic ET or PMF
Vannucchi[46]	*MPL*-W515A	Constitutive activation of Mpl protein, sporadic ET or PMF
Chaligne[50]	*MPL*-A519Y	Function uncertain
Kawamata[51]	*MPL*-Y591D	Found in uniparental disomy 1p, function uncertain

Reactive Thrombosis



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What Do These Case Studies Tell Us Today?

- **In case #1:**
  - **We learned that serum thrombopoietin may give further insight into the nature of I.T.P. and perhaps save $1200 per month for a treatment that is unlikely to yield results.**
- **In case #2**
  - **We find out that serum thrombopoietin may allow one to predict imminent platelet recovery in a patient with a Hydroxyuea-induced hypoplastic marrow.**
- **In case #3**
  - **The features of Essential Thrombocythemia suggesting a Myeloproliferative Disorder may in fact be hereditary In nature and that data is emerging that high platelets are risk factors for D.V.T. and P.E.**

THANK YOU

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