HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

1 - Biology of Hematopoiesis

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Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- None

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Hematopoiesis

- Hematopoiesis is the orderly continuous process by which hematopoietic stem and progenitor cells give rise to the mature circulating blood cells responsible for oxygen transport, host defense and hemostasis

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Requirements of Hematopoiesis

Cell Type	Life Span (days)	Turnover Rate (cells/day)
Erythrocytes	120	10^{12}
Granulocytes	0.5	10^{11}
Platelets	9	10^{11}

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Hematopoiesis is not merely a process but a unique organ system with specific characteristics

- Hematopoiesis has a distinct ontogeny, anatomy and physiology
- Hematopoietic ontogeny repeats its phylogeny
- Hematopoiesis is hierarchical
- Hematopoiesis is clonal and normally polyclonal
- Hematopoiesis is both deterministic and random in behavior

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Ontogeny of Hematopoiesis

	% Body Weight	Site	Mature Cell	Hemoglobins
Embryonic	-	Yolk sac Intravascular	Nucleated red cells	Embryonic
Fetus	1.5	Liver, spleen Extravascular (Intravascular) Appendicular	Enucleate Red cells	Fetal
Adult	4.5	Bone marrow Extravascular (Intravascular) Axial	Enucleate Red cells	Adult

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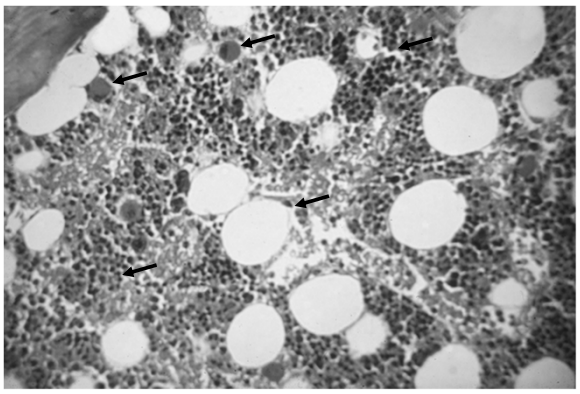
Normal Bone Marrow Biopsy (H&E)



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The Hematopoietic Microenvironment

- Mammalian hematopoiesis is normally extravascular after birth
- Within the marrow, hematopoietic progenitor cells differ in their location according to their lineage
- Stromal cells essential for promoting hematopoiesis include: fibroblasts, osteoblasts, adipocytes, endothelial cells, reticular cells, and macrophages

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The Hematopoietic Microenvironment (Continued)

- Stromal elements essential for promoting hematopoiesis include: the various collagens, fibronectin, laminin and the glycosoaminoglycans
- Stromal cells synthesize soluble and membrane-bound growth factors, matrix proteins and glycosoaminoglycans that tether growth factors
- Hematopoietic progenitor cells express adhesion receptors (integrins) and homing proteins for cell-cell and cell-matrix interactions.

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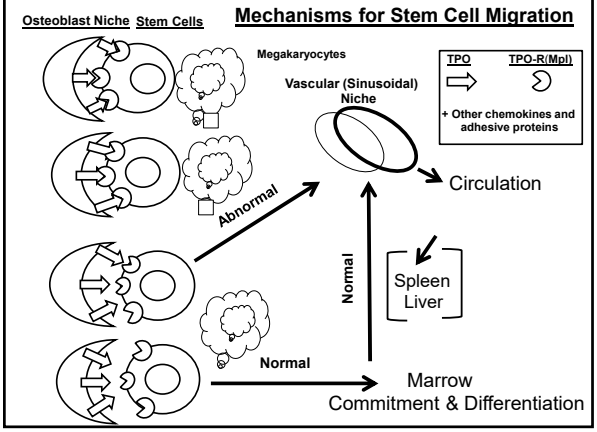
Mechanisms for Stem Cell Migration



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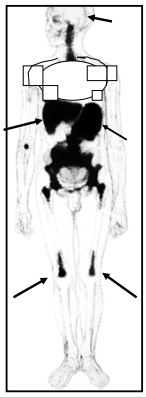
Expansion of Hematopoiesis with Increased Demand



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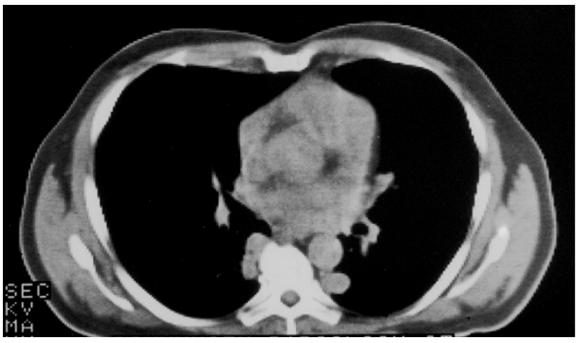
Paravertebral Extramedullary Hematopoiesis



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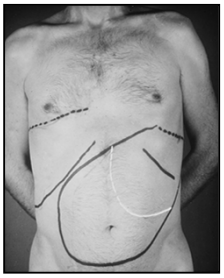
Polycythemia Vera: Extramedullary Hematopoiesis



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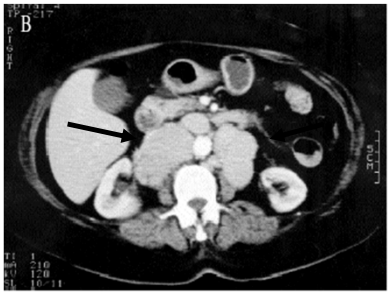
Primary Myelofibrosis: Extramedullary Hematopoiesis



Haematologica 86:784, 2001

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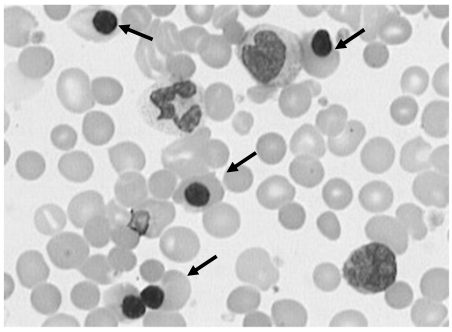
Leukoerythroblastic Reaction



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Causes of Extramedullary Hematopoiesis and Leukoerythroblastic Reactions

- Carcinoma metastatic to the bone marrow
  - (prostate, breast, lung, stomach)
- Lymphoma involving the bone marrow
  - (Hairy cell leukemia, CLL)
- Primary myelofibrosis
- Polycythemia vera
- Chronic myelogenous leukemia
- Myelodysplasia
- **Acute hepatic injury**
- Chronic hemolysis
- Recombinant hematopoietic growth factor therapy (EPO; G-CSF)

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Hematopoietic Growth Factors

- Hematopoietic growth factors (except for erythropoietin) exhibit redundancy, pleiotrophy, and synergy
  - Growth factor production is redundant since stromal cells can synthesize more than one type of growth factor
  - Some have multiple functions and stimulate more than one type of progenitor cell
  - Most have overlapping functions
  - Combinations of growth factors can be more effective than individual ones (Epo +G-CSF)

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Hematopoietic Growth Factors (Continued)

- Growth factor synthesis is highly localized with growth factor tethering
- Myeloid growth factors influence both primitive progenitor cells and their mature progeny
- Growth factors act to:
  - Maintain target cell viability
  - Initiate cell cycle activity
  - Activate effector functions

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Hematopoietic Growth Factors

Factor	Source	Function
Erythropoietin	Kidney, Liver	Stimulates erythroid progenitor cell proliferation
Granulocyte colony Stimulating factor	Monocytes Mesenchymal cells Neutrophils	Stimulates granulocyte progenitor cell proliferation and activation
Thrombopoietin	Liver, Kidney	Stimulates megakaryocytopoiesis and thrombopoiesis and HSC quiescence and activation

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Essential Factors in Erythropoiesis

- Intensity of the stimulus for red cell production
- Functional capacity of the bone marrow
- Available nutrients
- Red cell survival

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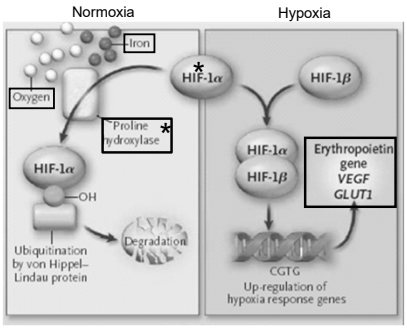
Hypoxic Regulation of Erythropoietin Production



N Engl J Med 348:1282, 2003

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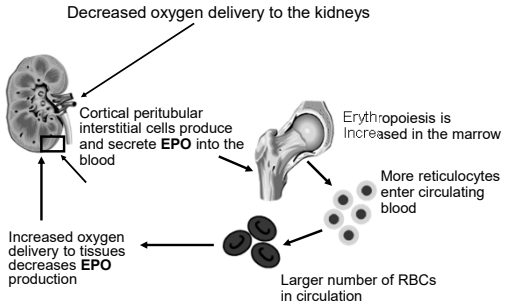
Erythropoietin Production



Semin Oncol. 2001;28(Suppl 8):19.

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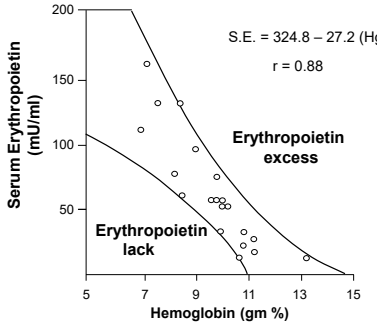
Confidence Intervals for Serum Immunoreactive Erythropoietin



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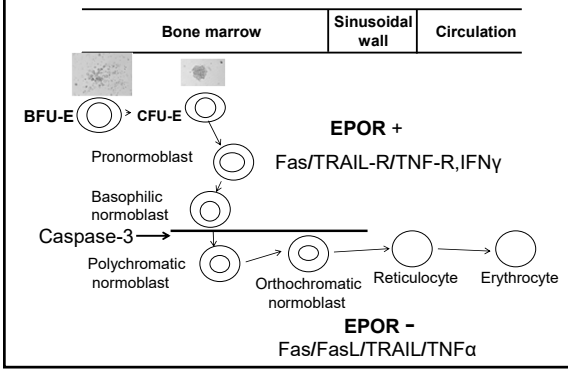
Erythropoiesis



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The Major Functions of EPO are Reflected in Its Plasma Level

	Production	Plasma Level
Erythroid cell viability factor	Constitutive	Constant
Erythroid cell mitogen	Inducible	Variable

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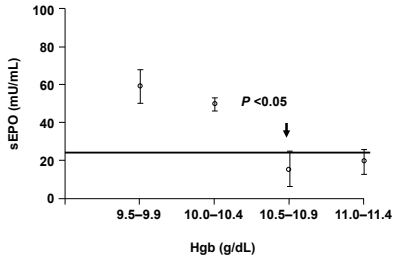
Serum Immunoreactive Erythropoietin in Iron Deficiency Anemia



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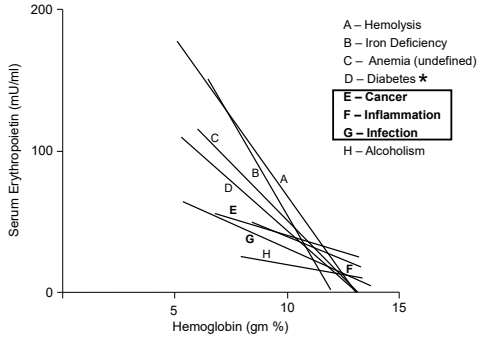
Association Between Hemoglobin and Serum Erythropoietin Levels in Various Disease States



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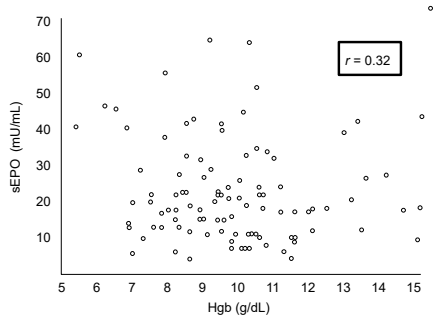
Loss of the Hgb-Serum EPO Correlation When the Serum Creatinine is Greater than 1.5 mg %



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Change in Risk with the Recombinant Erythropoietins Over Time

	RR	CI
2001		
Hematologic Response	3.43	(3.07 - 3.84)
Reduction in transfusions	0.64	(0.60 – 0.68)
Risk of Thromboembolism	1.58	(0.94 - 2.66)
Overall Survival	0.81	(0.67 – 0.99)
2007		
Risk of Thromboembolism	1.67	(1.35 - 2.06)
Overall Survival	1.08	(0.59 – 1.18)

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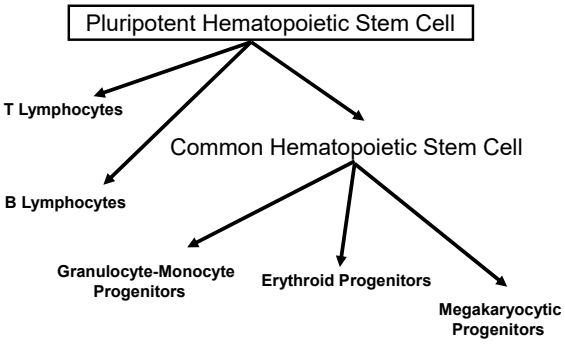
Pluripotent Hematopoietic Stem Cell



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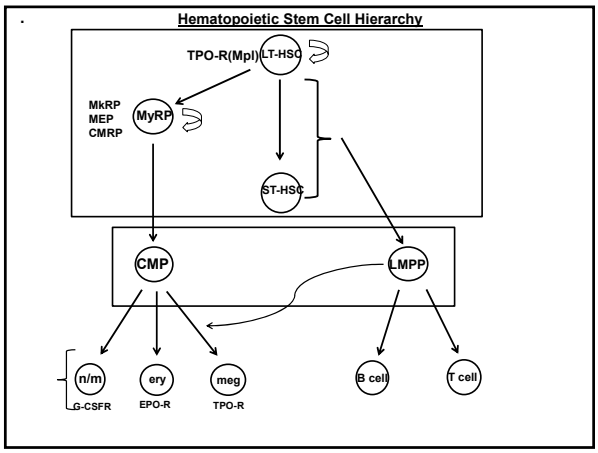

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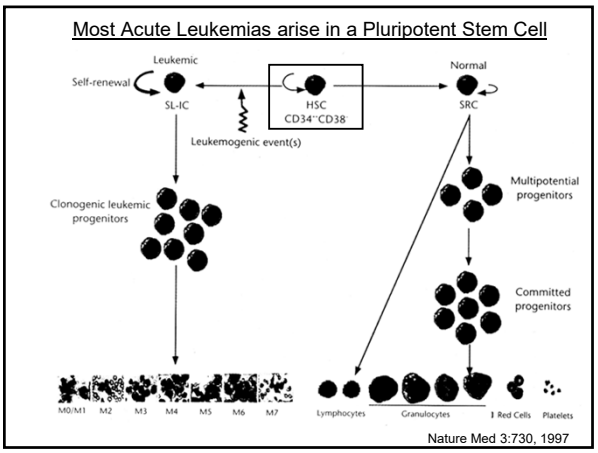

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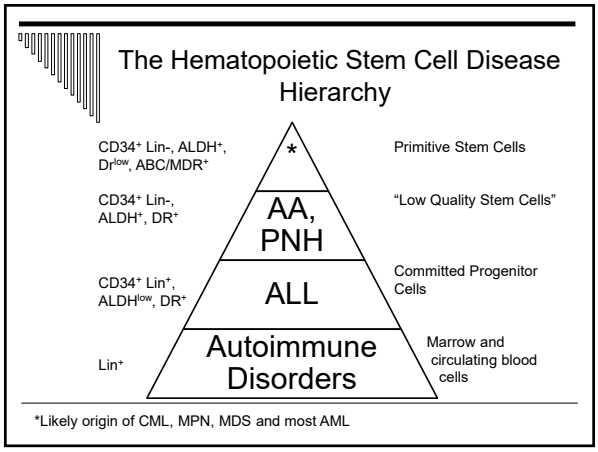

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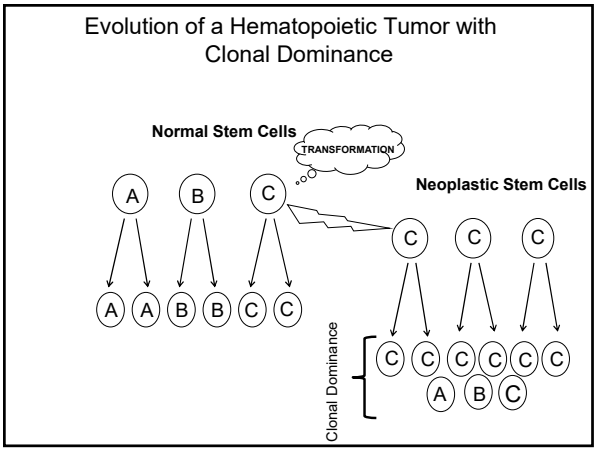

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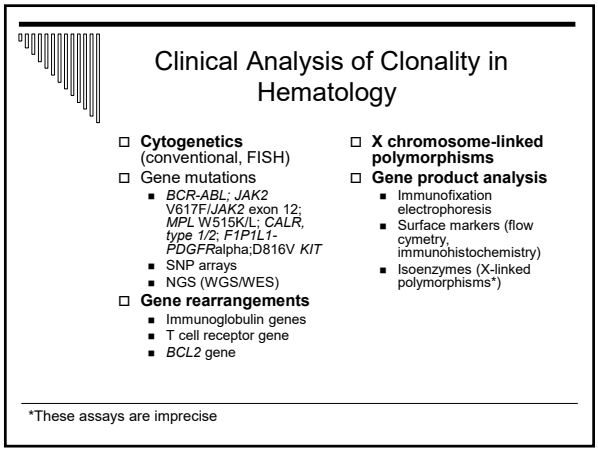

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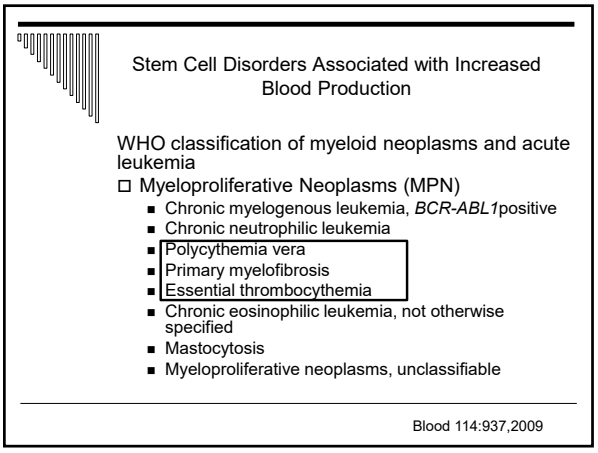

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Distribution of *JAK2*, *CALR* and *MPL* driver mutation in the MPN

	PV	PMF	ET
***JAK2* V617F**	92%	~ 55%	~ 50%
***JAK2* Exon12**	5%	0%	0%
CALR	~1%	~36%	~30%
MPL	0%	~ 4%	~ 8%
Unknown	3%	~ 5%	~ 12%

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Stem Cell Disorders Associated with Increased Blood Production (Continued)

- Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalites of *PDGFRA, PDGFRB, or FGFR1*
  - Myeloid and lymphoid neoplasms associated with *PDGFRA* rearrangement
  - Myeloid neoplasms associated with *PDGFRB* rearrangement
  - Myeloid and lymphoid neoplasms associated with *FGFR1* abnormalities

Blood 114:937,2009

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Stem Cell Disorders Associated with Increased Blood Production (Continued)

- Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
  - Chronic myelomonocytic leukemia
  - Chronic neutrophilic leukemia (*CSFR2* mutations)
  - Atypical chronic myeloid leukemia, *BCR-ABL1*-negative (*SETBP1*, *CSFR2* mutations)
  - Juvenile myelomonocytic leukemia (7del;*NF*-1)
  - Myelodysplastic/myeloproliferative neoplasm, unclassifiable
    - Refractory anemia with ring sideroblasts and thrombocytosis (*SF3B1*)

Blood 114:937,2009

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The Chronic Myeloproliferative Neoplasms

The chronic myeloproliferative neoplasms are **clonal hematopoietic stem cell disorders**, in which there is **overproduction** of one or more of the **normal** formed elements of the blood in the absence of a definable stimulus, **extramedullary hematopoiesis** and **transformation** to **myelofibrosis** or **acute leukemia** at variable but low rates.

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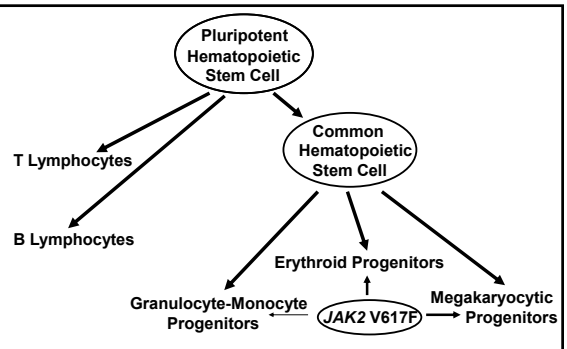

Polycythemia vera is the ultimate phenotypic consequence of the *JAK2* V617F mutation and most common MPN

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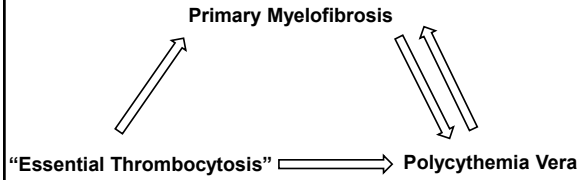
The Interrelationships Between the Chronic Myeloproliferative Disorders



"All pathways lead to polycythemia vera"

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Features "Unique" to Specific "Chronic Myeloproliferative Disorders"

Polycythemia vera	Erythrocytosis
Idiopathic Myelofibrosis	Elevated circulating CD34+ cells (early only)
"Essential Thrombocytosis"	None

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Splenomegaly has been omitted as a diagnostic criterion as have the red cell, leukocyte and platelet counts and the JAK2 V617F allele burden

		Polycythemia Vera (PV)	Essential Thrombocythemia (ET)
Major criteria	1	Hemoglobin (Hgb) >16.5 g/dL (men) >16 g/dL (women) or Hematocrit >49% (men) >48% (women) or ↑ red cell mass >25% above mean	1 Platelet count ≥450 x 10⁹/L
	2	Bone marrow (BM) tri-lineage myeloproliferation with pleomorphic mature megakaryocytes*	2 BM megakaryocyte proliferation with large and mature morphology and hyper-lobulated nuclei. Reticulin fibrosis grade should be ≤1
	3	Presence of *JAK2* mutation	3 Not meeting WHO criteria for other myeloid neoplasms
			4 Presence of *JAK2*, *CALR* or *MPL* mutation

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Microcytic erythrocytosis:a clue to polycythemia vera

HEMOGLOBIN (13.9-16.3)	9.3 gm %	13.2 gm %
HEMATOCRIT (41-53%)	31.9 %	42 %
RED CELL COUNT (4.5 – 5.9 x 10^6/μL)	5.53 x 10^6/μL	6.02 10^6/μL
MCV (80-100 fL)	57.7 fL	65.1 fL
RDW (11.5-14.5)	36.4	18.6
	Thalassemia Minor	Polycythemia Vera

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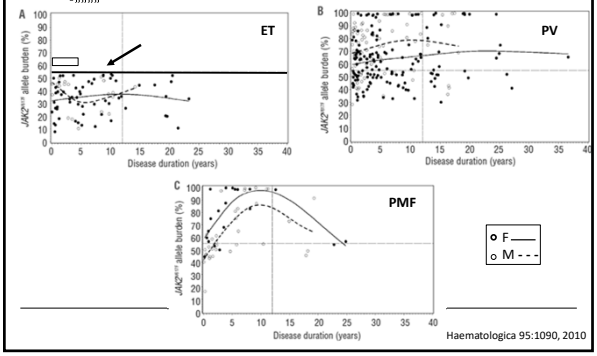
JAK2 V617F Quantitative Allele Burdens in the MPN



Haematologica 95:1090, 2010

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Causes of Absolute Erythrocytosis

- ☐ **Hypoxia**
  - Carbon monoxide intoxication (tobacco abuse, environmental)
  - High O_2 affinity hemoglobins
  - High altitude
  - Pulmonary disease
  - Right to left shunts
  - Sleep apnea
- ☐ **Neurologic Diseases**
- ☐ **Renal Disease**
  - Renal artery stenosis
  - Focal sclerosing or membranous glomerulonephritis
  - Renal transplantation

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Causes of Absolute Erythrocytosis (Continued)

- ☐ **Tumors**
  - Hypernephroma
  - Hepatoma
  - Cerebellar hemangioblastoma
  - Uterine fibromyoma
  - Adrenal tumors
  - Meningioma
  - Pheochromocytoma
- ☐ **Drugs**
  - Androgenic steroids
  - Recombinant EPO
- ☐ **Familial**
  - (with normal hemoglobin function; Chuvash(vHL mutations); EPO receptor mutations; 2, 3 BPG mutations;EPAS1(HIF2a) and EGLN1(PHD) mutations)
- ☐ **Polycythemia vera***
  - ***JAK2* V617F**
  - ***JAK2* exon 12 mutations**
  - **Rarely CALR**

*Only ~5-10% of erythrocytosis patients are likely to have polycythemia vera

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Causes of Relative Erythrocytosis

- **Loss of Fluid from the Vascular Space**
  - Emesis, diarrhea, diuretics, sweating, polyuria, hypodipsia, hypoalbuminemia, capillary leak syndromes, burns, peritonitis
- **Chronic Plasma Volume Contraction**
  - Hypoxia from any cause
  - Androgen therapy
  - Recombinant erythropoietin therapy
  - Hypertension
  - Tobacco use
  - Pheochromocytoma
  - Ethanol abuse
  - Sleep apnea

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Causes of Thrombocytosis

- Tissue Inflammation
  - Collagen vascular disease, inflammatory bowel disease
- Malignancy
- Infection
- Myeloproliferative Disorders
  Polycythemia vera, Primary myelofibrosis, Essential thrombocytosis, Chronic myelogenous leukemia
- Myelodysplastic Disorders
  5q-syndrome, Idiopathic refractory sideroblastic anemia

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Causes of Thrombocytosis (Continued)

- Postsplenectomy or hyposplenism
- Hemorrhage
- Iron deficiency anemia
- Surgery
- Rebound
  - Correction of vitamin B12 or folate deficiency, post ethanol abuse
- Hemolysis
- Familial
  - Thrombopoietin overproduction, constitutive Mpl activation(*MPL* S505N), *MPL* K39N, *MPL* P106L

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Causes of Myelofibrosis

Malignant
- Acute Leukemia
  - lymphocytic, myelogenous, megakaryocytic
- Chronic Myelogenous Leukemia
- Hairy Cell Leukemia
- Hodgkin's Disease
- **Primary Myelofibrosis**
- Lymphoma
- Multiple Myeloma
- Myelodysplasia
- Metastatic carcinoma
- Polycythemia Vera
- Systemic Mastocytosis

Non Malignant
- HIV infection
- Hyperparathyroidism
- Renal osteodystrophy
- Systemic Lupus Erythematosus
- Tuberculosis
- Vitamin D deficiency
- Thorium Dioxide exposure
- Gray Platelet Syndrome
- Drugs
  - Thrombopoietin analogs

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Differential Diagnosis of Primary Myelofibrosis

- Chronic myelogenous leukemia
- Polycythemia vera
- Acute myelofibrosis
- Myelodysplasia
- Hairy cell leukemia
- Primary bone marrow lymphoma
- Systemic mastocytosis
- Metastatic carcinoma

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Causes of Leukocytosis

- Infection
- Inflammation
- Chronic myeloproliferative disorders (clonal)
  Chronic myelogenous leukemia
  Polycythemia vera
  Primary myelofibrosis
  Hypereosinophilia
  Myelodysplasia
  CMMoL
- Acute leukemias (clonal)

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Causes of Leukocytosis (Continued)

- Drugs
  - Corticosteroids
  - Lithium
  - G-CSF, GM-CSF
- Tobacco
- Obesity
- Exercise/Seizures
- Postsplenectomy/hyposplenism
- Rebound from myelosuppression
- Sweet's syndrome
- Heat stroke
- Artifact
  - Cryoproteins

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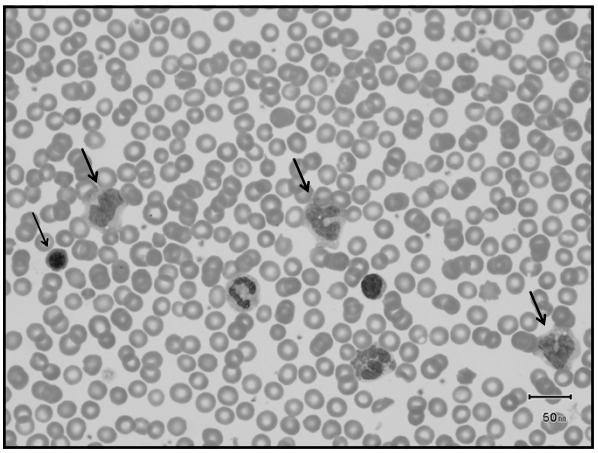

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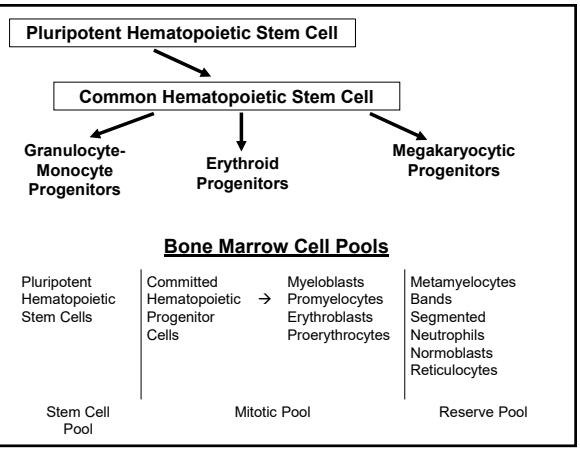

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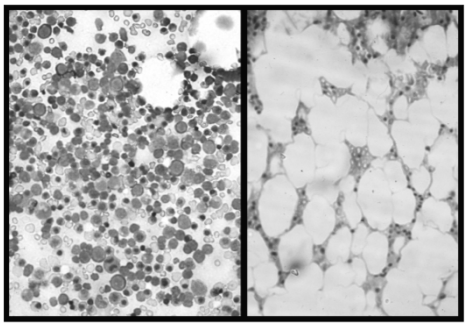
Severe Aplastic Anemia: Marrow Aspirate and Biopsy



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Diseases Causing Bone Marrow Aplasia or Hypoplasia

Inherited

- ☐ Fanconi Anemia
- ☐ Schachman-Diamond syndrome
- ☐ Dyskeratosis Congenita
- ☐ Amegakaryocytic thrombocytopenia

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Diseases Causing Bone Marrow Aplasia or Hypoplasia (Continued)

Acquired

- ☐ Idiopathic Aplastic Anemia*
- ☐ Drug-induced Aplastic Anemia
- ☐ Direct toxicity or idiosyncratic reaction
- ☐ Myelodysplasia*
- ☐ Paroxysmal Nocturnal Hemoglobinuria*
- ☐ Large granular lymphocyte syndrome (neutropenia, red cell aplasia, thrombocytopenia, aplastic anemia)
- ☐ Thymoma (red cell aplasia, aplastic anemia)
- ☐ Pregnancy (red cell aplasia, aplastic anemia)
- ☐ Thiopurine S-Methyltransferase deficiency (pancytopenia)

*Acquired clonal disorders

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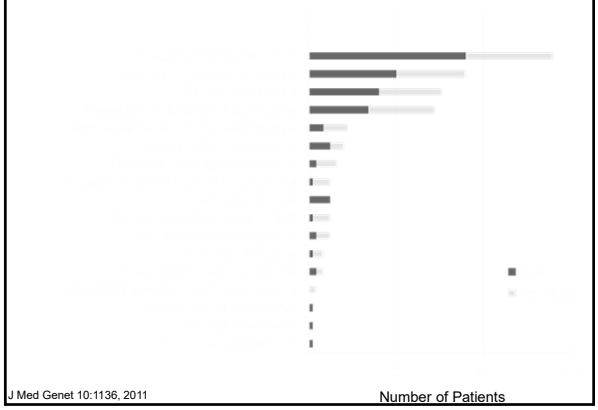
Distribution of Primary Inherited Bone Marrow Failure Syndromes



J Med Genet 10:1136, 2011

Number of Patients

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Inherited Marrow Failure Syndromes in Adults

Fanconi Anemia	Dyskeratosis Congenita	Diamond-Blackfan
Pancytopenia	Pancytopenia	Anemia
Aplastic Anemia	Aplastic Anemia	-
Leukemia/MDS	Leukemia/MDS	Leukemia/MDS
Cancer (HN, Gyn, Brain)	Cancer (HN)	Osteosarcoma
Café au Lait spots	Pigmentation, Gray hair Oral leukoplakia	-
Skeletal abnormalities	Nail dysplasia Pulmonary fibrosis	Short neck
FANC gene mutations	**Telomerase gene mutations** **Dyskerin gene mutations**	**RP S17, 19 and 24 loss**

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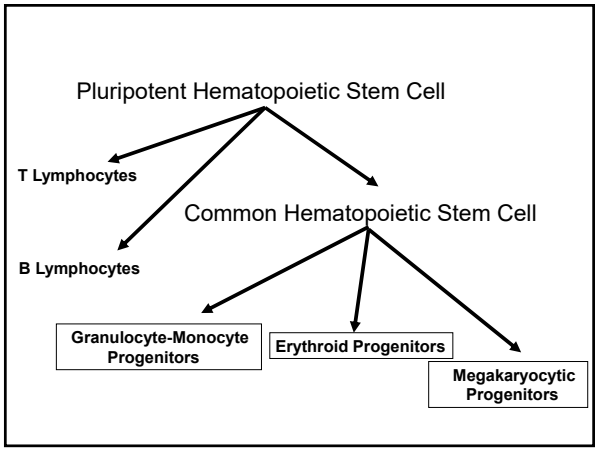

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Conditions Causing Single Lineage Bone Marrow Aplasia

- Pure Red Cell Aplasia or Hypoplasia
  - Congenital
    - Diamond Black-Fan Syndrome*
  - Acquired
    - Autoimmune
    - Thymoma, T-cell mediated (LGL)
    - Drug-induced
    - Solid tumors
    - **Hematological malignancies***
      (Myelodysplasia, CML lymphoma)
    - Infection (Parovirus B19)
    - Collagen-vascular disease
    - Pregnancy
    - Drugs
    - Erythropoietin antibodies

*Clonal disorders

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Conditions Causing Single Lineage Bone Marrow Aplasia (Continued)

- Pure White Cell Aplasia
  - Congenital (Kostmann's syndrome)*
  - Autoimmune, T-cell mediated (LGL)
  - Drugs
- Pure Megakaryocytic Aplasia
  - Congenital* (CAMT)
  - Thymoma, T-cell mediated (LCL)
  - Autoimmune
  - **Hematological Malignancies***

*Clonal disorders

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Stem Cell Defects Causing Monocytopenias

Disease	Clinical Phenotype	Genetic Location
Diamond-Blackfan syndrome	Red Cell Hypoplasia	RP mutations (S17; S19; S24)
Kostmann's syndrome	Neutropenia (Acute Leukemia)	? *G-CSFR* mutations
Congenital amegakaryocytic thrombocytopenia	Thrombocytopenia (Pancytopenia)	*TPO-R (MPL)* mutations
Myelodysplasia	Red Cell Aplasia; 5q-, Aplastic Anemia, Thrombocytopenia	RP mutation (S14)

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A 42-Year-Old Man

July	• Resection of brain tumor • Postoperative seizures	• Dilantin • Phenobarbital Carbamazepine • Steroids • 4 blood transfusions • HCT 42
Aug	• Abnormal liver function tests	• Dilantin • Carbamazepine • Steroids discontinued • HCT 37
Sept	• Diffuse skin rash • Abnormal liver function tests • Fever	• Phenobarbital discontinued • HCT 26 • WBC 11,500 • Eos 31% • PL 634,000 • Retic 0.2%

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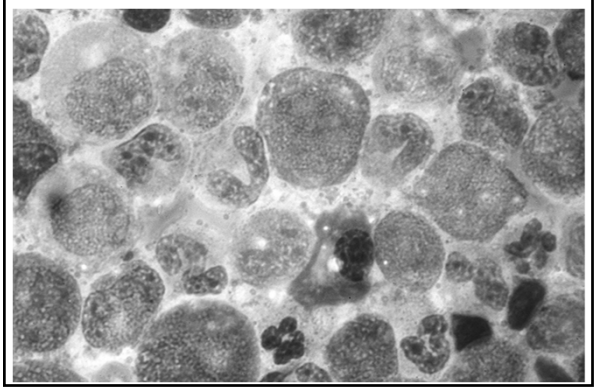
Bone Marrow Aspirate in Pure Red Cell Aplasia



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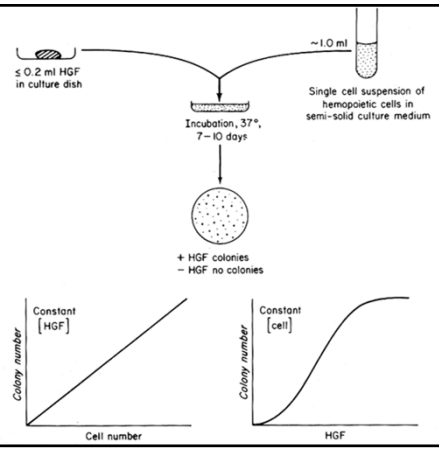

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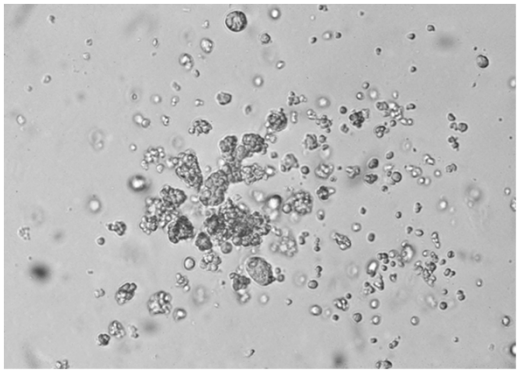
Erythroid Colony Grown In Vitro



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Erythroid Colony Growth in PRCA

Category	Colony Growth	Response to Therapy		
		C.R.	P.R.	None
I	Normal	70%	30%	-
II	Reduced	25%	-	75%
III	Undetectable	-	-	100%

Adapted from Blood 64:71, 1984

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Classification of Red Cell Aplasia or Hypoplasia

Congenital
- Diamond-Blackfan syndrome

Acquired
- Idiopathic
- Secondary
  - **Hematologic Malignancies**
    - (AL, MDS, CLL, NHL, HD, AILD, CML, PMF)
  - Solid Tumors
    - (Thymona, Lung, Stomach, Breast)
- Immunologic Disorders
  - (LGL syndrome, SLE, RA, AIHA, Pregnancy, BMT, HIV, Polyglandular syndromes I and II)
- Infectious Diseases
  - (Parvovirus B19, EBV, Hepatitis A, B, C)
- Drugs
- Anti-erythropoietin antibodies

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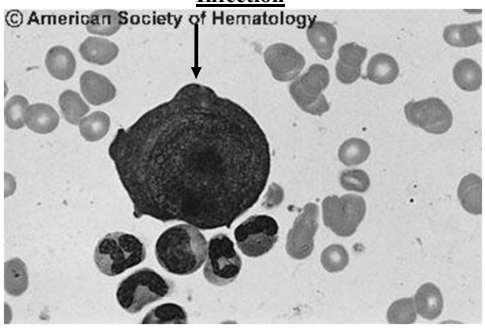
Giant Pronormoblast Seen in Parvovirus B19 Infection



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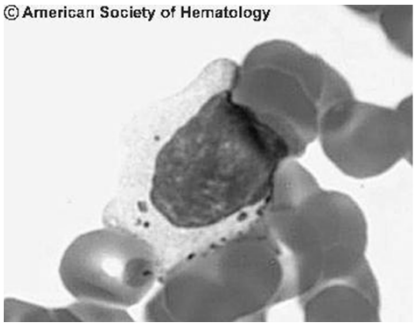
Large Granular Lymphocyte



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Drugs Associated with Red Cell Aplasia

Confirmed
- Phenytoin
- Azathioprine
- Isoniazid
- Mycophenolate mofetil
- Recombinant erythropoietin

Suspected
- Allopurinol
- D-penicillamine
- Interferon alpha
- FK506
- Lamivuudine
- Rifampicin
- Valproate
- Sulfonamide derivatives
- Halothane
- Rituximab*
- Fludarbine*
- Chloramphenicol**

*probably secondary to immunosuppression leading to B19 infection
**The effect is dose-dependent

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Classification of Adult Hematopoietic Disorders

	Clonal	Nonclonal
Decreased Production	Aplastic anemia Red cell aplasia Megakaryocytic aplasia MDS PNH Sideroblastic anemia*	Aplastic anemia Red cell aplasia White cell aplasia Megakaryocytic aplasia Anemia due to renal disease
Increased Production	Polycythemia vera* Essential thrombocytosis* Primary myelofibrosis* MDS (thrombocytosis; *JAK2* V617F) CML CMMoL; CNL	2^0 Erythrocytosis 2^0 Thrombocytosis Leukemoid reactions
Increased Destruction	PNH	Hemolytic anemia Immune thrombocytopenia Agranulocytosis

*Can be *JAK2* V617F+

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Summary

- Hematopoiesis is hierarchical
- Hematopoiesis is clonal but stem cell defects can mimic polyclonal (single cell line) disorders
- Hematopoiesis is governed by both intrinsic and extrinsic signals and thus its behavior is both nonrandom and random
- An explanation for the molecular basis of both the acute leukemias and the chronic myeloproliferative disorders will be found at the level of the hematopoietic stem cell
- Clonal disorders of hematopoiesis are often phenotypically similar to nonclonal disorders of hematopoiesis

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