

Myeloproliferative Disorders: Genetic Trailblazers

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Important Hematologic Mutations

• Objectives

- Review the FAB Classification of the Myeloproliferative Disorders
- List WHO Classification of the Myeloproliferative Disorders
- Discuss the mutation, the corresponding molecular defect, pathophysiologic result, and treatment resulting from the mutation for each of the following hematologic conditions:
 - 1. CML
 - 2. PV
 - 3. ET
 - 4. MMM (PMF or CIMF)

Myeloproliferative Disorders

• Definition

- Four Classic FAB Myeloproliferative Disorders
 - Polycythemia vera
 - Chronic Myelocytic Leukemia
 - Essential Thrombocythemia
 - Myelofibrosis with Myeloid Metaplasia
- Atypical Myeloproliferative Disorders under WHO
 - Chronic Neutrophilic Leukemia (absent bcr-abl)
 - Chronic Eosinophilic Leukemia
 - Hypereosinophilic Syndrome
 - Chronic basophilic Leukemia
 - MPD/MDS
 - Systemic mastocytosis
 - Unclassified Myeloproliferative Disorder

Myeloproliferative Disorders

Lab Tests	PV	CML	ET	MMM
RBC, Hgb, Hct	↑↑↑	N-↓	N-↓	↓
WBC	N-↑	↑↑↑	N-↑	↑to ↑↑
Platelets	↑	↑or↓	↑↑↑	↑or↓or N
Immature Neut	None	↑↑↑	Rare	↑↑
LAP	N-↑	↓	N-↑	↑or↓or N
Ph1	Absent	Present	Absent	Absent
Spleen Size	↑	↑	N-↑	↑
BM Fibrosis	Absent to ↑	Absent to ↑	Absent to ↑	↑↑↑
JAK2	85-97%	0%	50-60%	50-60%

Chronic Myelocytic Leukemia

• Chronic Myelocytic Leukemia (CML)

- Incidence
 - 1 new case/150,000 people = 3500-5000 new cases/year in US
 - 15-20% of adult leukemias
 - Middle ages (median = 53yo) men (3:2)
- Pathogenesis
 - Ionizing radiation
 - Alkylating agents
 - Toxins
 - Acquired mutation
 - Philadelphia Chromosome

Chronic Myelocytic Leukemia

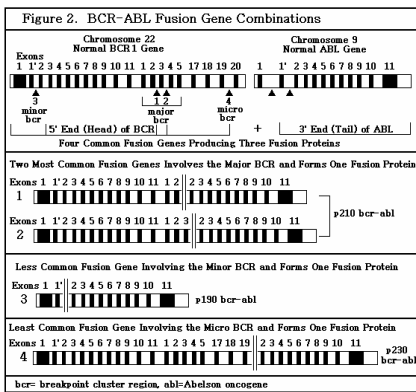
• Mutation

- Chromosomal translocation t(9:22)
 - Philadelphia chromosome
 - Discovered in 1960 by Nowell & Hungerford
 - Characterized in 1973 by Janet Rowley
- Molecular Defect
 - Fusion gene (BCR/ABL)
 - Required for diagnosis by WHO
 - Fusion Protein (BCR/ABL)
 - Increased tyrosine kinase activity
 - Treated with Gleevec, a tyrosine kinase inhibitor
- Mutation is diagnostic, prognostic, and indicates treatment

Molecular Biology of t(9;22)

• BCR/ABL Fusion Gene

- Four possible transcripts with three protein products
 - e1a2 = P185/p190 (Minor)
 - BCR exon 1 + abl exons 2-11
 - Found in 50% of adults and 80% of children with Ph1 + ALL
 - e13a2 or e14a2 = p210 (Major)
 - BCR exons 1-13/14 + abl exons 2-11
 - Found in all chronic phase CML by WHO criteria
 - Sometimes found in Ph1 positive ALL
 - e19a2 = p230 (Micro)
 - BCR exons 1-19 + abl exons 2-11
 - Found in a smoldering subgroup of CML patients
 - Associated with CNL with thrombocytosis



Leukemogenic Mechanisms in CML

- Aberrant signal transduction
 - Constitutive tyrosine kinase activity
 - Activation of RAS
 - Independent of cytokine control
 - Protect against apoptosis
 - Decreased integrins (adhesion) (FAK pathway)
 - Reduced contact inhibition – Decreased cytokine contact
 - Increased release into circulation
 - Activation of other signal transduction pathways
 - JAK-STAT pathway
 - PI-3 kinase pathway
 - » Increased proliferation

Imatinib – Phase III Clinical Trial June 2000				
First Line Imatinib – IRIS Study				
DATA	CHR	MCR	CCR	PFS
Months follow-up	24 42 54	24 42 54	24 42 54	24 42
Estimated Rate (%)	-- 98 97 90%/3m	88 91 88	78 84 82	96 94

Second Generation Tyrosine Kinase Inhibitors	
<ul style="list-style-type: none"> • Dasatinib (BMS-354825) <ul style="list-style-type: none"> - Recently FDA approved <ul style="list-style-type: none"> • all phases of CML & Ph1 ALL - Thiazolcarboxamide (different than imatinib) - Binds kinase domain in active & inactive conformation - 325 fold more potent than imatinib - Effective on nearly all imatinib resistant mutants <ul style="list-style-type: none"> • Except T315I - Inhibits 5 Oncogenic enzymes <ul style="list-style-type: none"> • BCR/ABL • SRC family kinases • C-Kit • PDGFR • Ephrin A receptor kinase 	<ul style="list-style-type: none"> • Nilotinib (AMN107) <ul style="list-style-type: none"> - Aminopyrimidine - Structural derivative of imatinib - Binds ABL kinase domain in inactive conformation - 30-50 fold increased potency over Imatinib for CML - Does not inhibit SRC family kinases - Phase II Clinical Trial • Bosutinib (SKI-606) <ul style="list-style-type: none"> - In Phase I Clinical Trials - Inhibits bcr-abl & induces apoptosis in cell lines - 10x more potent than imatinib - Effective against most bcr-abl mutations except T315I - Dual activity against abl and src kinases - Phase II trials are underway at a dose of 400mg daily

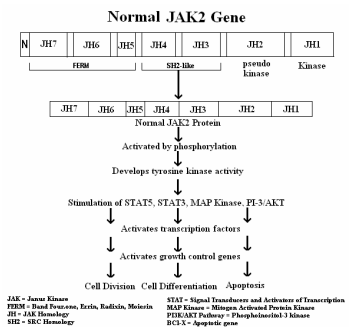
Other Tyrosine Kinase Inhibitors	
<ul style="list-style-type: none"> • ONO12380 (binds A-loop) <ul style="list-style-type: none"> - Inhibits bcr/-abl & Lyn Kinases (10x greater potency) • ONO1910 (Phase I Trials) <ul style="list-style-type: none"> - Similar to ONO12380 - Also inhibits PI3K kinase <ul style="list-style-type: none"> • A cell cycle kinase • MK 0457 (VX 680) <ul style="list-style-type: none"> - Inhibits aurora kinases - Inhibits other kinases <ul style="list-style-type: none"> • Flt-3 Kinase - Effective against T315I mutation • CGP76030 	<ul style="list-style-type: none"> • AT9283 • KW2449 • INNO-406 (NS-187) • AP23464 (AP23884) • PP1 • PD166326 (Multikinase) • PD180970 <ul style="list-style-type: none"> - Not effective against T315I • PD173955 <ul style="list-style-type: none"> - 100x more potent • BIRB-796 (Inhibits T315I) <ul style="list-style-type: none"> - Bcr-abl, p38, MAP Kinases • Adaphostin

Polycythemia vera (PV)

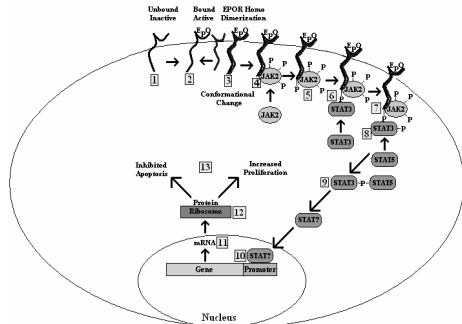
Janus Kinase (JAK) Family of Proteins

JAK Protein	Cytokines	Functions
JAK1	IF- α , β , γ and IL-2, 4, 7, 9, 13, 15	Defective lymphoid & neural development & response to INF
JAK2	β -chain GM-CSF, IL-3, IL-5, EPO, TPO, INF γ	Defective erythropoiesis, β -chain cytokines & TPO, INF γ
JAK3	γ -chain IL-2, 4, 7, 9, 13, 15, 21	SCID
TYK2	INF- α & INF- β	Defective INF- α , LPS resistance

Polycythemia vera (PV)



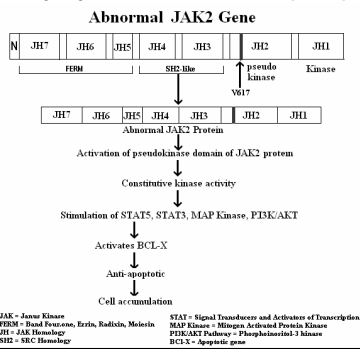
Normal JAK2 Function



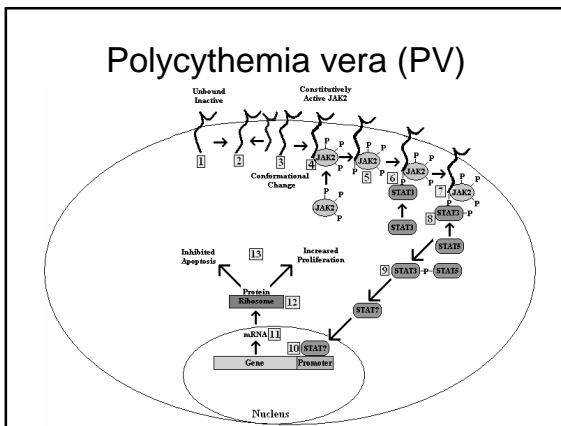
Polycythemia vera (PV)

- JAK2 Mutation (JAK2V617F)
 - Somatic mutation in hematopoietic stem cell
 - Replacement of valine for phenylalanine at codon 617
 - Increased tyrosine kinase activity
 - Hypersensitivity to EPO bound EPOR
 - Observed in 85-97% of PV
 - Only one other rare JAK2 mutation found
 - JAK2T875N

Polycythemia vera (PV)



Polycythemia vera (PV)



Other Hematological Disorders

- JAK2 Mutation
 - Seen in 50-60% of MMM and ET
 - Can also stimulate GCSFR and MPL (TpoR-Plt)
 - MMM and ET
 - Occasionally in CMML, myelodysplasia, and AML

Phenotype Specificity of JAK2

- Pre-JAK2 Mutation (Class I)
 - BCR/ABL and MPL mutations (MPLW515)
 - Creates hyperproliferative clone
- JAK2 Dosage Effect
 - MPL (TpoR) is high on megas so little JAK2
 - EpoR is low on RBC progenitors so much JAK2
 - MPL oversignaling stimulates fibroblasts
- Hypothesized pathogenesis
 - Mutation > abnormal clone > 1 JAK2 and/or MPLW515 > MPL stimulation > ET > 2 JAK2 > EpoR stimulation > PV > continued MPL > Fibroblast stimulation > MMM

Treatment for JAK2+ MPD

- ICNB018424
 - Phase I trials in MMM patients
 - Inhibits JAK1 and JAK2, not JAK3 or TYK2
 - Reductions in splenomegaly and others
 - No reduction in JAK2 burden
 - Developed thrombocytopenia
 - Reversed with drug discontinuation

THANK YOU

QUESTIONS ????????
