Payvand
Clinical Specialty Lab.
Pathology, Medical Genetics & Molecular

Islamic Azad University

Session: Laboratory & Cancer Genetics (Diagnosis, Prognosis, Monitoring)

Molecular- Genetic Testing in Diagnosis, Prognosis & Treatment of Hematopoietic Malignancies

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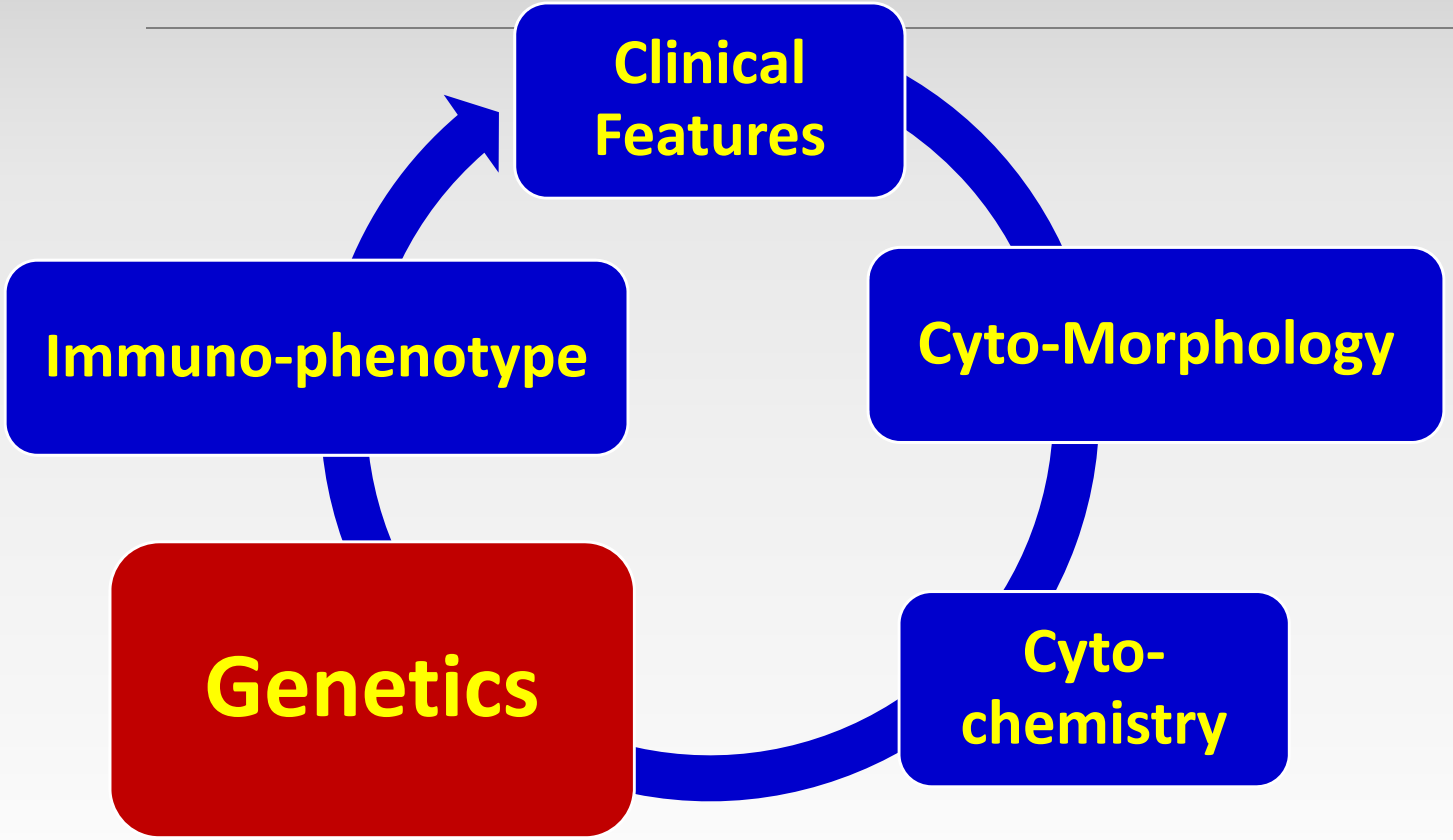
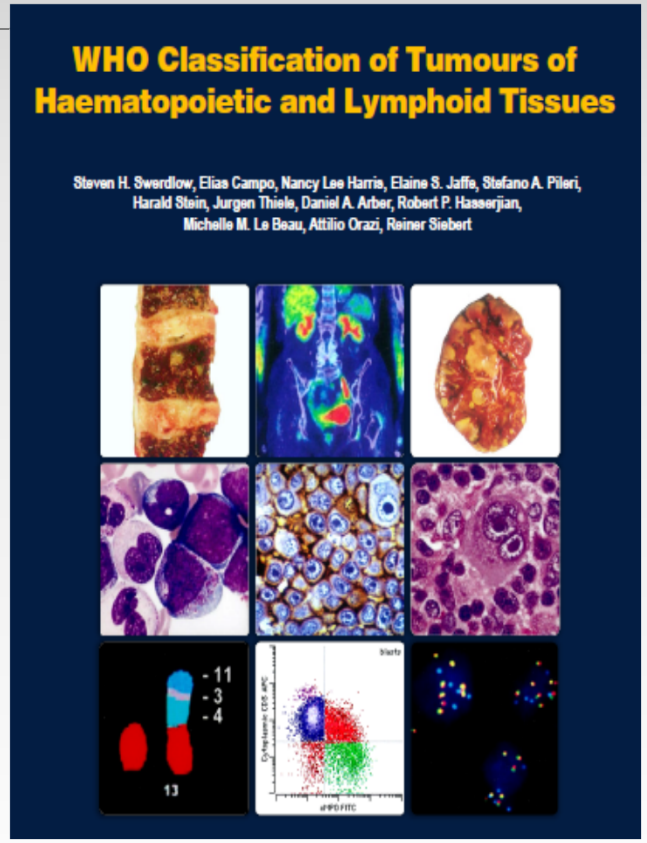
Objectives

My objective is to brief review of Molecular & Cytogenetic findings in Hematopoietic malignancies with focus on:

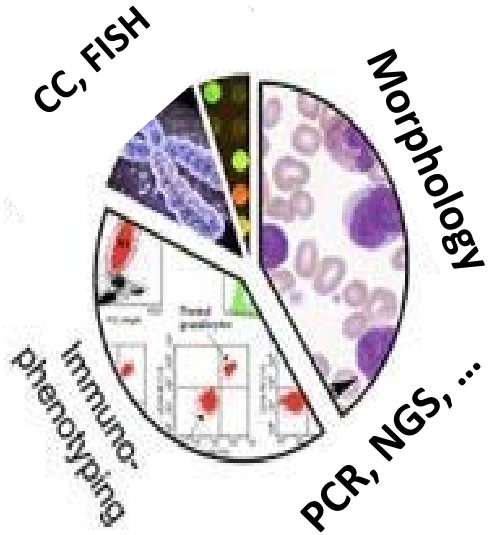
- ALL & AML with focus on new subgroups
- Molecular Markers of NHLs
- Molecular monitoring & resistance of CML
- Molecular studies on CLL
- Braf mutation in HCL
- Molecular markers of MPNs
- Cytogenetics and Multiple Myeloma
- …,..

The Updated WHO Classification of Hematologic Malignancies



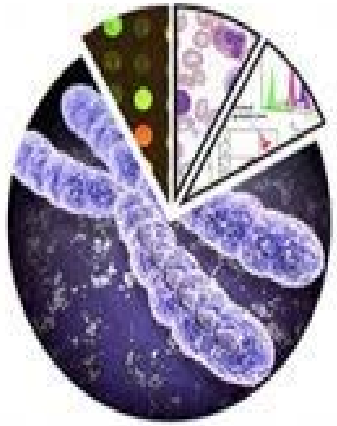


Specialized Diagnostic Testing



- Ensure accurate diagnosis and and classification according to WHO criteria
- Identify markers for risk assessment and potential therapeutic target:
  - genotypic markers, e.g. t(15;17)/PML-RARA or complex karyotypes
  - immunophenotypic markers, e.g. CD33 expression
- Identify most appropriate and sensitive disease-associated marker for therapeutic monitoring

Prognosis & Therapeutic Targets

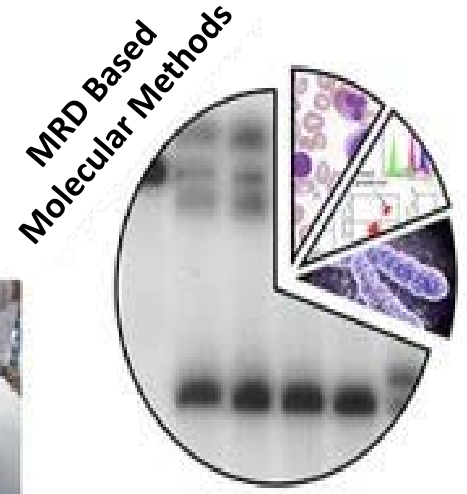


Treatment Types

Chemotherapy:
- Standard risk protocols
- Intermediary risk protocols
- High risk protocols

Targeted therapy:
- Genetic targets, e.g. ATRA
- Monoclonal antibodies, e.g. anti-CD33 therapy

Follow-up & Disease Monitoring



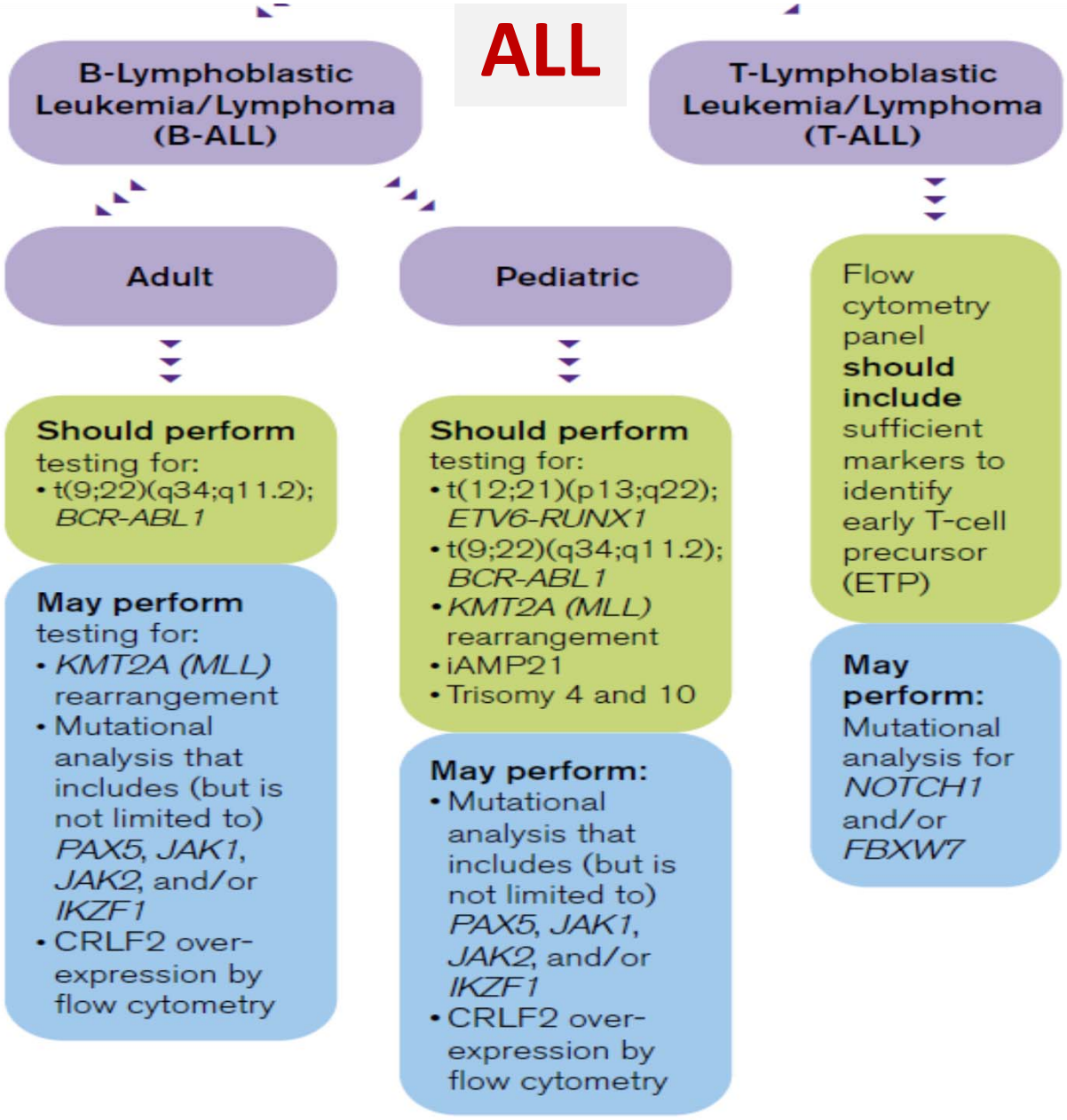
Complete remission, i.e. no evidence of disease by standard criteria, e.g. < 5% morphologically detectable blast cells

Minimal residual disease (MRD), i.e. persistence of small numbers of malignar cells undetectable by standard methods and requires additional treatment

Acute Lymphoblastic Leukemia
WHO Classification

B-lymphoblastic leukemia/lymphoma
B-lymphoblastic leukemia/lymphoma, NOS
B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukemia/lymphoma with hyperdiploidy
B-lymphoblastic leukemia/lymphoma with hypodiploidy
B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like
Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21
T-lymphoblastic leukemia/lymphoma
Provisional entity: Early T-cell precursor lymphoblastic leukemia
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

Initial Diagnostic Workup of ALL



Strong Recommendation

Recommendation

Guideline from the CAP & ASH

Acute Lymphoblastic Leukemia with t(9;22) / BCR-ABL

The t(9;22): **25%** & **5%** of adult & pediatric B-ALLs,

In CML & ALL, the cluster region in **ABL gene** is somewhat constant but variable for BCR gene:

major-BCR [M-BCR], p210

minor-BCR [m-BCR], p190, (p190 is detected only in 1% of patients with CML)

micro-BCR [mu-BCR]), p230

The t(9;22) can be detected by several methods including CC, Southern blotting, FISH, & RT-PCR.

The ***RT-PCR method*** is the most commonly used test when looking for Measurable Residual Disease, **MRD**.

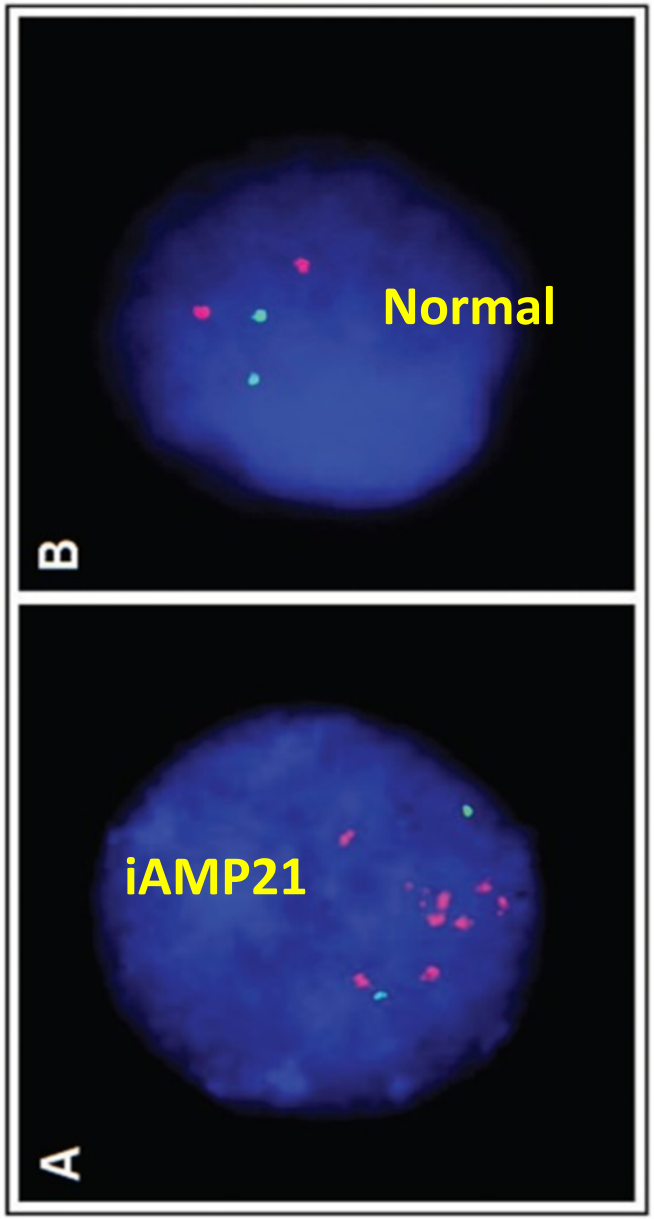
BCR-ABL1–Like B Lymphoblastic Leukemia

- A **high risk ALL** with a gene expression profile similar to that of *BCR-ABL1*+ ALL
- Account for **10% of pediatric** & **25% of adult ALL**
- Poor clinical outcomes, **potential response to TKI treatment**
- **The main molecular or genetic findings:**
  - ***IKZF1* deletions**
  - ***Jak* (*JAK2*) mutations**
  - ***CRLF2* translocations** (mainly with IgH) & **increase expression**

Next-generation sequencing is the gold standard method to detect this group of leukemia

ALL with Intra-Chromosomal Amplification of ch. 21, iAMP21

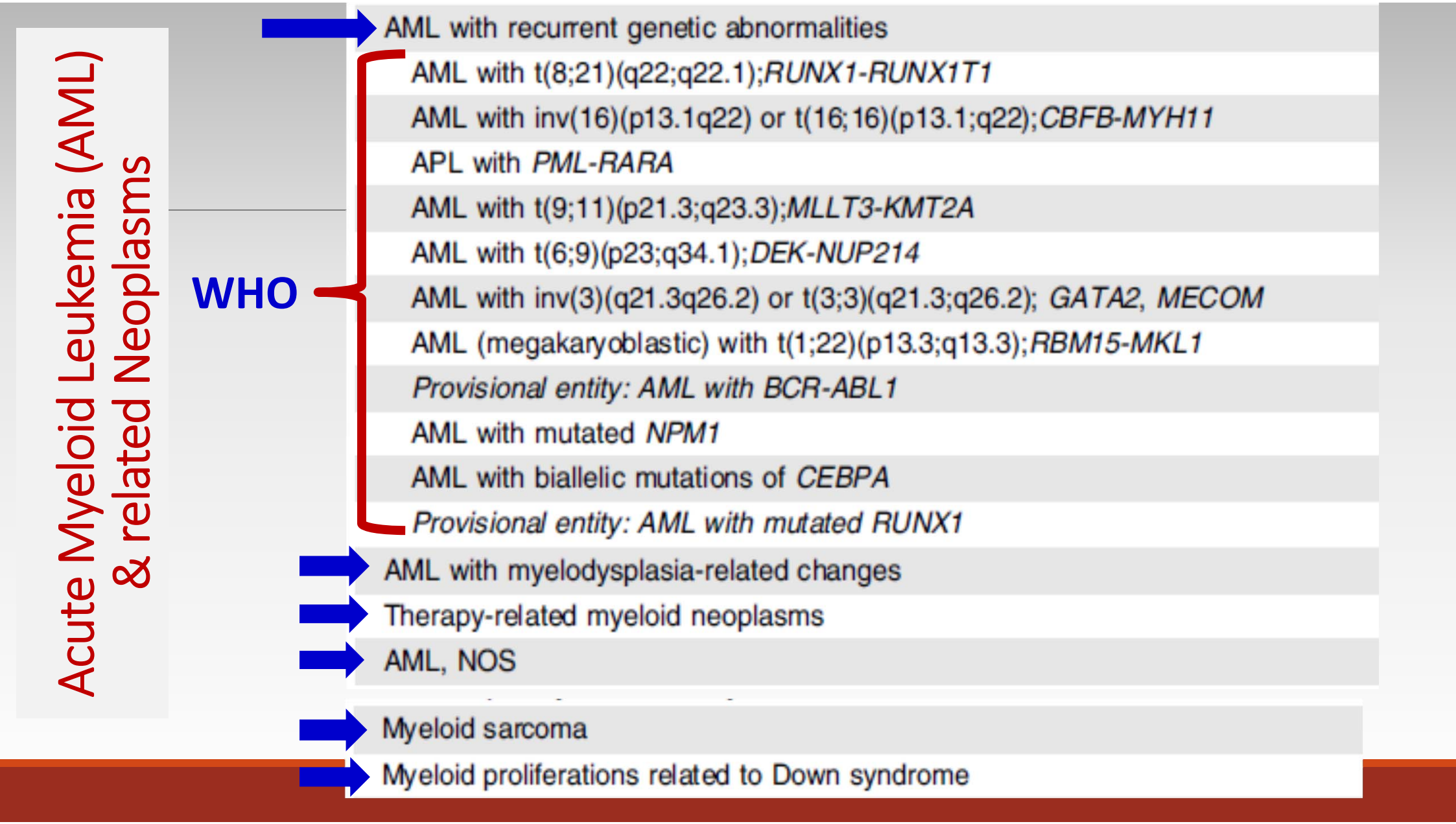
- iAMP21 accounts for about **2%** of B-ALL
- Generally in ***older children*** (median age 9 y/o) with **lower WBC**
- **Adverse outcomes** when treated with standard risk therapy; but improved when treated as a high risk ALL
- Presence of ***4-5 or more copies of RUNX1*** On a single cell
- Reliably detected by **FISH** for *RUNX1* & confirmed by cytogenetics, **t(12;21)(p13;q22) ETV6/RUNX1 probes**



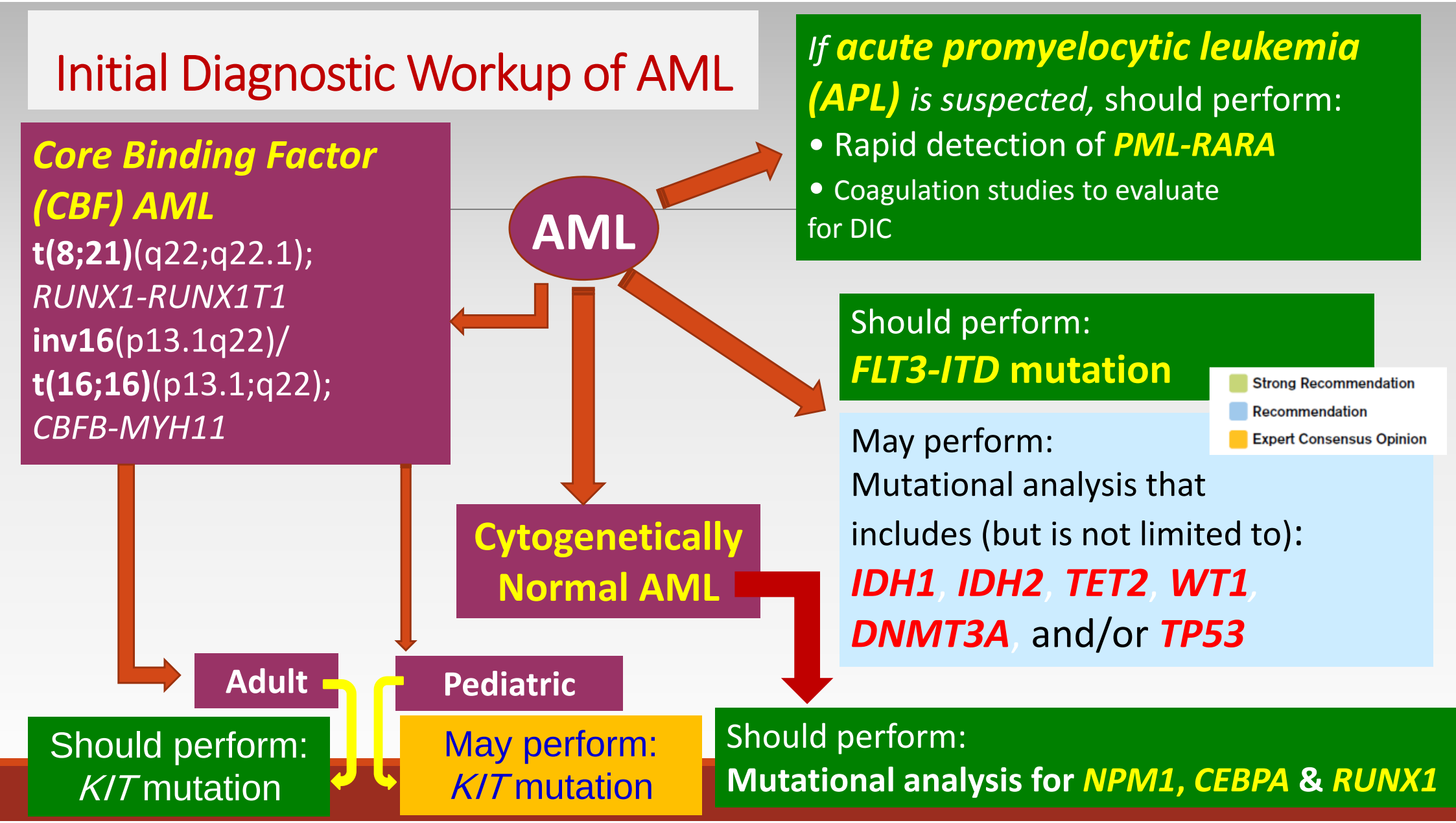
Am J Clin Pathol 2015;144:103-112

B-Lymphoblastic Leukemia/Lymphoma with Hypodiploidy

- **Low hypodiploid** (**32-39** ch.) & **near haploid** (**24-31** ch.) B-ALL have:
- ***worse prognosis*** than near diploid cases & are likely distinct entities
- Near haploid ALL is often associated with ***RAS*** & **receptor TK signaling mutations**
- **>90%** of low hypodiploid cases have **TP53 mutations** & often have alterations of ***IKZF1*** & ***RB1***
- **43%** of low hypodiploid ALL have **germline *TP53* mutations**


Acute Myeloid Leukemia (AML) & related Neoplasms
WHO
AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
APL with *PML-RARA*
AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
AML with t(6;9)(p23;q34.1);*DEK-NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
Provisional entity: AML with BCR-ABL1
AML with mutated *NPM1*
AML with biallelic mutations of *CEBPA*
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS
Myeloid sarcoma
Myeloid proliferations related to Down syndrome

Initial Diagnostic Workup of AML



Fms-like tyrosine kinase-3, FLT3,Gene–Mutated Acute Myeloid Leukemia

- FLT3 gene is a ***tyrosine kinase receptor***, involved in **cell maturation** & **inhibition of apoptosis**.
- Mutated FLT3 is found in **approx. 30%** of AMLs and is associated with **poor outcome**. FLT3 may undergo:

1. **Point mutation of the aspartic acid residue 835 (D835) on exon 20 .**
2. **Internal Tandem Duplication (ITD) of the juxtamembrane domain on exon 14/15.**

FLT3 mutations are detected by multiplex PCR amplification followed by capillary electrophoresis for length mutations (FLT3-ITD) and resistance to EcoRV digestion (D835). **High mutant/wild-type ratio** is associated with **worse prognosis.**

Non-Hodgkin Lymphoma, NHL
Identify Clonality: T-CELL & B-CELL Populations

1. Immunoglobulin Gene Rearrangement

2. T-Cell Gene Rearrangement

Most B-cell & T-cell NHLs show clonal rearrangement

Neoplastic T calls & B cells have the same rearranged variable region of the ***TCR*** or ***Heavy chain*** & of the ***Light chain*** genes

- B- & T-cell clonality tests should be limited to samples with ***diagnostic difficulties***
- Clonality is not the absolute test to determine B- or T-cell lineage & that **a positive** result is ***not systematically equivalent to malignancy***.

Non-Hodgkin Lymphoma, NHL
Translocations

B-Cell Leukemia/Lymphoma 2, BCL-2, t(14;18), Follicular lymphoma is the most common lymphoma to express BCL-2 (approx. 85%–90%), & approx. 20-30% of DLBCLs are positive for BCL-2.

Cyclin-D1 Translocation, t(11;14), hallmark of mantle cell lymphoma, **MCL >95%** of cases & a subset of plasma cell myeloma (**CD20** positive with the morphologic appearance of **small plasmcytoid lymphocytes**)

FISH is the golden standard for identification of the BCL2 or CCND1 rearrangement,

Non-Hodgkin Lymphoma, NHL
Translocations

B-Cell Lymphoma 6, *BCL-6* Translocation, 20-40% of **DLBCLs** & in **5-10%** of **follicular lymphomas,** FISH is common method.

Cellular Myelocytomatosis Translocation, c-myc, In virtually all cases of **BL**, high no. of **AIDS-related lymphoma** (**EBV^{pos}** and **EBV^{Neg}** cases).

t(8;14),**80%**, & less frequently **kappa t(2;8) (15%)** than **lambda t(8;22) (5%)** gene segments. FISH detection is the method most used

Mucosal-Associated Lymphoid Tissue Translocation, t(11;18)/API2-MALT1 is the most common translocation in **MALT lymphomas**, approx. **30-40%** of cases.

The **most common sites** for the t(11;18) are **stomach** & **lung**, Interphase FISH is becoming the main test to look for such translocations.

Gastric lymphomas with t(11;18)/API2-MALT1 respond **poorly** to treatment directed **against Helicobacter pylori** microorganism.

Non-Hodgkin Lymphoma, NHL
Translocations

Anaplastic Lymphoma Kinase, ALK Translocation, Approximately 85% of pediatric & 35% of adult ALCLs are associated with a recurrent cytogenetic abnormality that involves ALK in locus 2p23.

Lymphoplasmacytic Lymphoma / Waldenstrom Macroglobulinemia (LPL/WM), *MYD88* L265P with Reflex to *CXCR4,* Useful for Establishing the diagnosis of LPL/WM **(>90%)** in the setting of an elevated **IgM** serum monoclonal paraprotein.

CXCR4 mutations are identified in approximately **30-40%** of LPL/WM & highly associated with ***MYD88 L265P*** and are important determinants of **clinical presentation**, **overall survival** & **therapeutic response to ibrutinib**.

https://www.mayocliniclabs.com/test-catalog/Overview/61114

Chronic Myelogenous Leukemia with t(9;22)(q34;q11)/BCR-ABL1

Mutation	Treatment Recommendation[m]
Y253H, E255K/V, or *F359V/C/I*	Dasatinib
F317L/V/I/C, T315A, or *V299L*	Nilotinib
E255K/V, F317L/V/I/C, F359V/C/I, T315A, or *Y253H*	Bosutinib
T315I	Ponatinib,[n] Omacetaxine,[o] allogeneic HCT (CML-6*), or clinical trial

***BCR-ABL1* mutations** implicated in some resistant cases of CML & **Approx. 85%** are caused by a **single amino acid substitution**: **T315I**, **F359V**, **Y253F/H**, **M244V**, **G250E**, **E255K/V**, and **M351T**.

Additional Clonal Cytogenetic Abnormalities (ACA), or Cytogenetic Clonal Evolution (CE) in CML

NCCN National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2019
Chronic Myeloid Leukemia

In conclusion:

- the presence of clonal evolution per se does not signal worse prognosis at least in the chronic phase.

- The type of abnormality appears to have a minimal role on outcome, although the ***highest risk abnormalities*** (i.e., abnormalities in ch. 3 & i17q) are rarely if ever detected at diagnosis.

- Patients with ACA/Ph+ at diagnosis be watched carefully for evidence of therapy failure

Early Treatment Response Milestones

BCR-ABL1 (IS)	3 months	6 months	12 months[g]	>15 months
>10%[h]	YELLOW	RED		
>1%–10%	GREEN		YELLOW	RED
≤1%	GREEN			

COLOR	CONCERN	CLINICAL CONSIDERATIONS	SECOND-LINE TREATMENT
RED	TKI-resistant disease	• Evaluate patient compliance and drug interactions • Consider mutational analysis	Switch to alternate TKI (CML-5) and evaluate for allogeneic HCT (CML-6*)
YELLOW	Possible TKI resistance	• Evaluate patient compliance and drug interactions • Consider mutational analysis • Consider bone marrow cytogenetic analysis to assess for MCyR at 3 mo or CCyR at 12 mo	Switch to alternate TKI (CML-5) or Continue same TKI (other than imatinib) (CML-F*)[i] or Dose escalation of imatinib (to a max of 800 mg) and Consider evaluation for allogeneic HCT (CML-6*)
GREEN	TKI-sensitive disease	• Monitor response (CML-F*) and side effects	Continue same TKI (CML-F*)[i]

Baseline Evaluation of Patients with CLL

Diagnostic test	General practice	Clinical trial
Additional tests before treatment		
Molecular cytogenetics (FISH) for del(13q), del(11q), del(17p), add(12) in peripheral blood lymphocytes	Always	Always
Conventional karyotyping in peripheral blood lymphocytes (with specific stimulation)	NGI*	Desirable
TP53 mutation	Always	Always
IGHV mutational status	Always	Always
Serum β_2-microglobulin	Desirable	Always
CT scan of chest, abdomen, and pelvis	NGI	Desirable
MRI, PET scans	NGI	NGI
Abdominal ultrasound†	Possible	NGI

Blood. 2018;131(25):2745-2760

NGI, not generally indicated

CLL Chromosomal Aberrations

The initiating chromosomal aberrations comprise:

- Deletion of chromosome **13q** (del[13q]), about **55%** of cases,
- Acquisition of chromosome **12** (trisomy 12), **10–20%** of cases,
- Deletion of chromosome **11q** (del[11q]), about **10%** of cases,
- Deletion of chromosome **17p** (del[17p]), about **5–8%** of cases,

Del(13q) causes the loss of miRNAs (miR-15a and miR-16-1), which initiates leukemogenesis.

Del(11q) causes the loss of the ***ATM* gene**, which encodes a DNA damage response kinase ATM.

Del(17p) typically deletes the tumour suppressor gene *TP53*.

>80% of cases with a del(17p) also carry mutations in the **remaining *TP53* allele**, resulting in a functional disruption of the TP53 pathway. *TP53* mutations and del(17p) are therefore collectively categorised as **genetic *TP53* aberrations**, predict an **aggressive disease course**

www.thelancet.com Published online February 21, 2018 http://dx.doi.org/10.1016/S0140-6736(18)30422-7

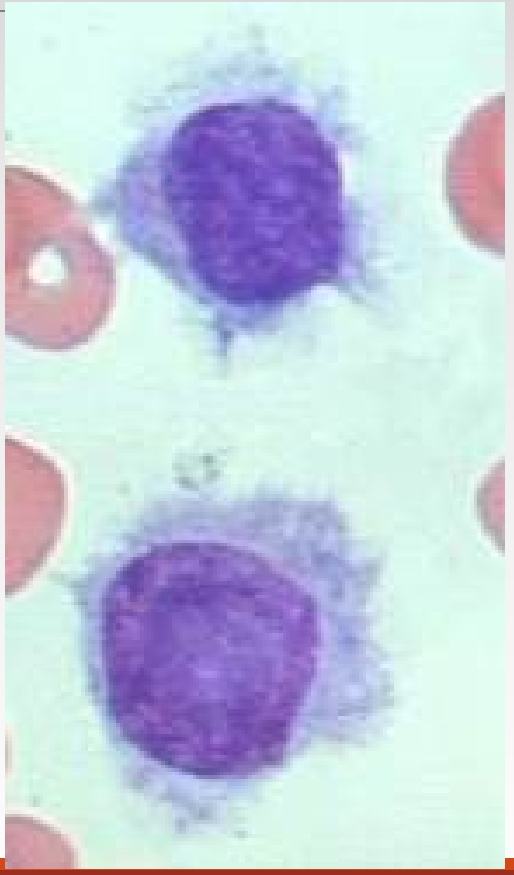
Mutational status of IGHV genes in CLL

	Unmutated	*Mutated*
Incidence	45%	55%
M:F ratio	11:1	1.1:1
Morphology	Atypical	Typical
Trisomy 12	Frequent	Infrequent
Abnormal 13q14	Rare	Common
Stage (BiNet)	B&C (2/3)	Stage A (2/3)
Disease course	Progressive	Stable
CD38/ZAP70	Expressed	Negative
Therapy	Required	Not needed
Response	Poor	Normal
Survival	Short	Long

B-raf V600E mutation

B-raf proto-oncogene (BRAF gene) Mutation

- The mutation occurs in **exon 15** at position **1799**, in which **thymine** & **adenine** are exchanged, leading to **valine** (V) being substituted by **glutamate** (E) at **codon 600** (V600E) of the BRAF protein,
- **Positive in up to 80–90% of Hairy Cell Leukemia** cases, hallmark of the HCL leading to increased expression of genes involved in **survival** & **proliferation**.
- Not been identified in other B-cell chronic lymphoproliferative disorders except a few cases of CLL and multiple myeloma.
- Absence of the BRAF gene mutation was reported in up to **10% - 20%** of patients with HCL and could constitute a subgroup of HCL patients with a poor prognosis.



Am J Hematol. 2017;92:1382–1390

International Myeloma Working Group Diagnostic Criteria for MM

Disorder	Disease definition

Multiple Myeloma	Both criteria must be met:
	• Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma
	• Any one or more of the following myeloma defining events:
	  • Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
	  • Hypercalcemia: serum calcium >0·25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2·75 mmol/L (>11 mg/dL)
	  • Renal insufficiency: creatinine clearance <40 mL per minute or serum creatinine >177 μmol/L (>2 mg/dL)
	  • Anemia: hemoglobin value of >2 g/dL below the lower limit of normal, or a hemoglobin value <10 g/dL
	  • Bone lesions: one or more osteolytic lesions on skeletal radiography, computed tomography (CT), or positron emission tomography-CT (PET-CT)
	• Clonal bone marrow plasma cell percentage ≥60%
	• Involved: uninvolved serum free light chain (FLC) ratio ≥100 (involved free light chain level must be ≥100 mg/L)
	• >1 focal lesions on magnetic resonance imaging (MRI) studies (at least 5 mm in size)

Am J Hematol. 2018;93:1091–1110.

Primary molecular cytogenetic classification of MM

Secondary cytogenetic abnormalities arise along the disease course of multiple myeloma including:

- Gain (1q),
- del (1p),
- del (17p),
- del (13),
- RAS mutations,
- Secondary translocations involving MYC.

Both primary & secondary cytogenetic abnormalities can influence: disease course, response to therapy, & prognosis.

Importantly, the interpretation and impact of cytogenetic abnormalities in MM vary depending on the disease phase in which they are detected.

Other cytogenetic abnormalities in absence of IgH translocations or trisomy or monosomy 14	*5.5*
Normal	*3*

Am J Hematol. 2018;93:1091–1110.

Cytogenetic abnormalities on clinical course and prognosis in MM

Cytogenetic abnormality	Multiple myeloma
Trisomies	Good prognosis, standard-risk MM, median OS 7–10 years Most have myeloma bone disease at diagnosis Excellent response to lenalidomide-based therapy
t(11;14) (q13;q32)	Good prognosis, standard-risk MM, median OS 7–10 years
t(6;14) (p21;q32)	Good prognosis, standard-risk MM, median OS 7–10 years
t(4;14) (p16;q32)	Intermediate-risk MM, median OS 5 years Needs bortezomib-based initial therapy, early ASCT (if eligible), followed by bortezomib-based consolidation/maintenance
t(14;16) (q32;q23)	High-risk MM, median OS 3 years Associated with high levels of FLC and 25% present with acute renal failure as initial MDE
t(14;20) (q32;q11)	High-risk MM, median OS 3 years
Gain(1q21)	Intermediate-risk MM, median OS 5 years
Del(17p)	High-risk MM, median OS 3 years
Trisomies plus any one of the IgH translocations	May ameliorate adverse prognosis conferred by high risk IgH translocations, and del 17p
Isolated Monosomy 13, or Isolated Monosomy 14	Effect on prognosis is not clear
Normal	Good prognosis, probably reflecting low tumor burden, median OS >7–10 years

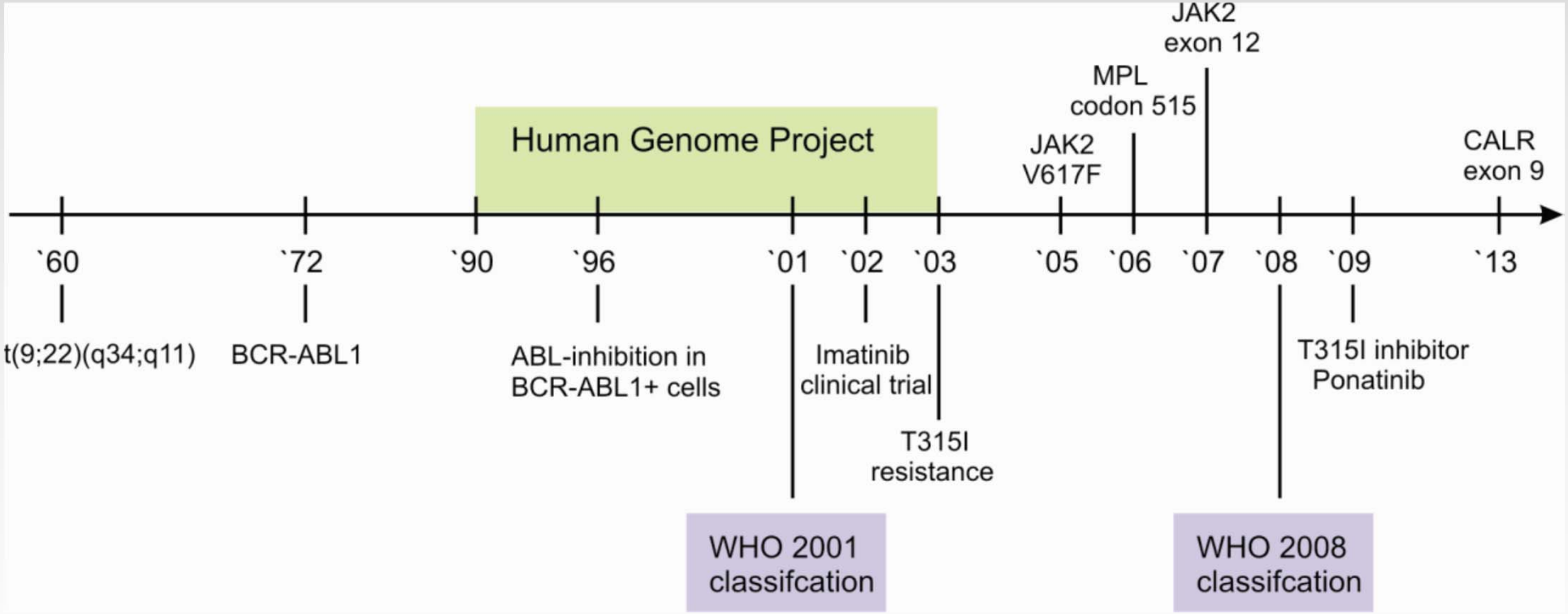
Am J Hematol. 2018;93:1091–1110.

MyeloProliferative Neoplasms, MPN

MPNs are a heterogeneous group of clonal diseases associated with excessive production of mature hematopoietic elements, including:

BCR-ABL1–positive CML,
Polycythemia vera (PV),
Primary Myelofibrosis (PMF)
Essential Thrombocythemia (ET),
Chronic Neutrophilic Leukemia,
Chronic Eosinophilic Leukemia (CEN) NOS,
MPN, NOS.

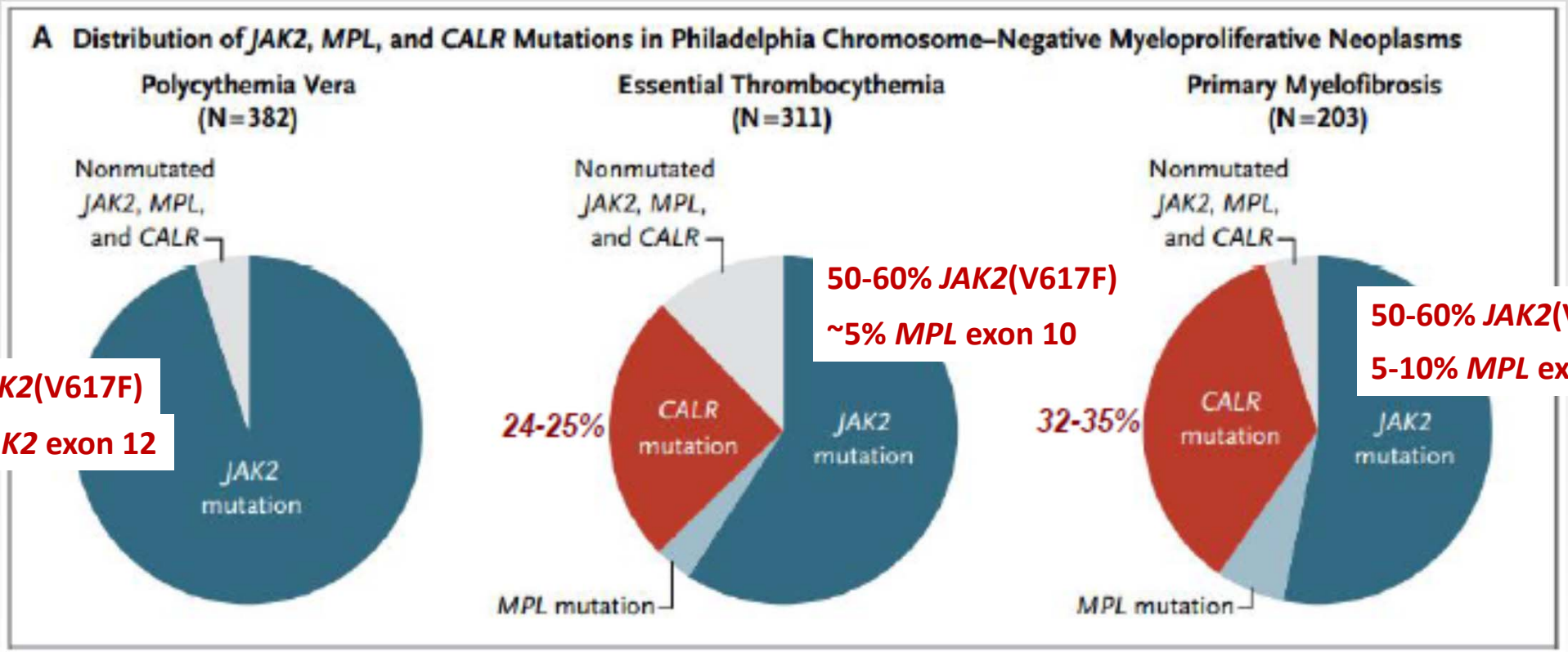
Timeline of the elucidation of genomic alterations in MPNs



Genes **2014**, *5*, 444-459; doi:10.3390/genes5020444

Philadelphia Negative MPNs
Frequency of Common Mutations

Signaling MPN driver: JAK2, MPL, CALR



~95% *JAK2*(V617F)

~2-3% *JAK2* exon 12

50-60% *JAK2*(V617F)

~5% *MPL* exon 10

50-60% *JAK2*(V617F)

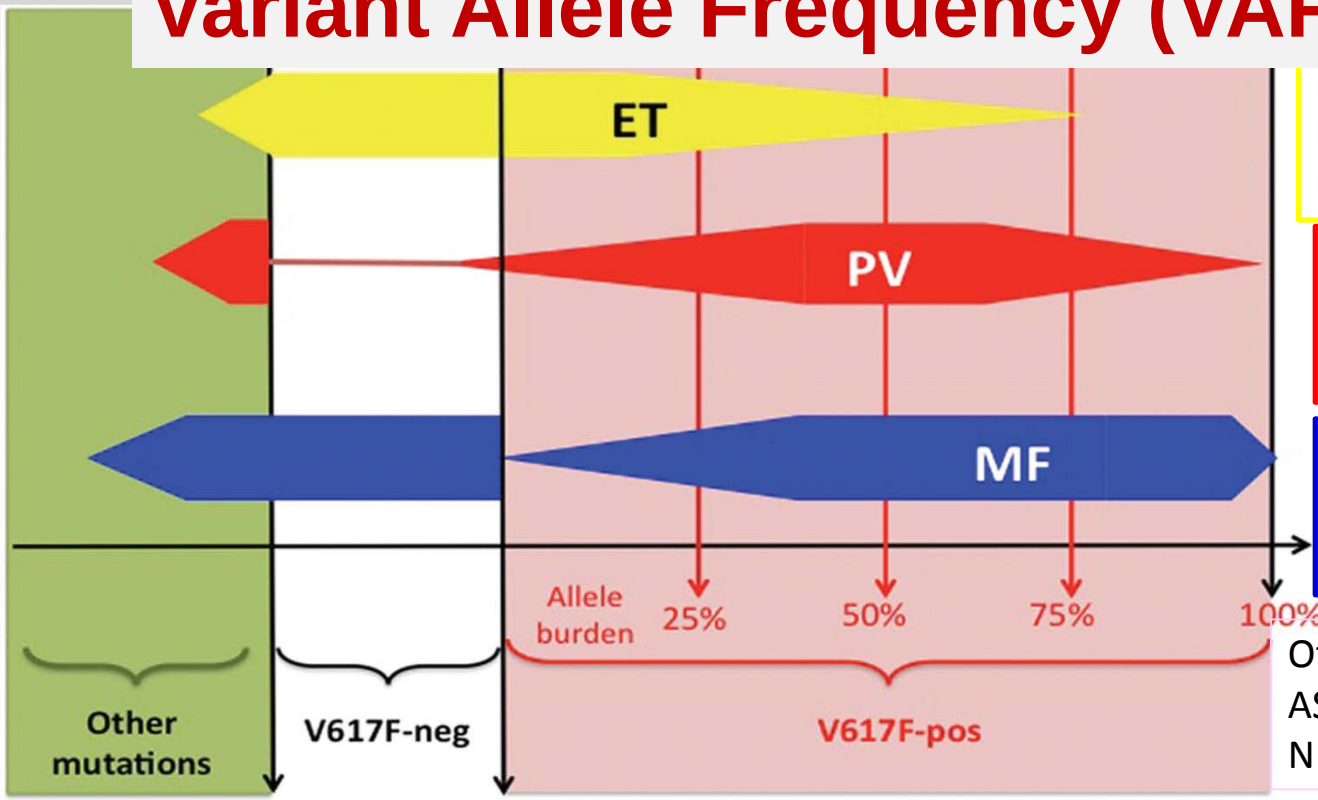
5-10% *MPL* exon 10

Klampfl T et al. N Engl J Med 2013;369:2379-90

Overlaps in molecular lesions

Schematic representation of the distribution of molecular lesions across diseases

Variant Allele Frequency (VAF) in granulocytes



splay a mutation. JAK2-positive ET patients, the majority have a **low allele burden (median around 20%),** but a few may have a higher allele burden

In **PV** the majority of patients are positive for the JAK2-V617F mutation, with a **higher median allele burden (approximately 50%).**

In **PMF**, approximately 60% of patients are JAK2-V617F positive, with a **high allele burden (often above 50%).**

Other mutations include: MPL, JAK2-exon 12, TET2, ASXL1, LNK, CBL, EZH2, SOCSs, IDHs, p53, NRAS, NF1, IKZF1, RUNX1, and RB

The spectrum of JAK2-positive myeloproliferative neoplasms, ASH 2012

Jean-Jacques Kiladjian1
1Saint-Louis Hospital and Paris Diderot University, Paris, France

Emerging Value of Other Mutations as Prognostic Markers

- ***ASXL1*, *SRSF2*, *IDH*1/2, and *EZH2* mutations identified as "*prognostically detrimental*" markers, associated with shortened survival in PMF**
- **Only *ASXL1* mutations remain significant independent of IPSS/DIPSS+**
- ***High risk molecular signature:***

  ≥2 mutations (*ASXL1, SRSF2, EZH2, IDH1/2*)

  CALR-ASXL1+

  Triple negative (*JAK2-CALR-MPL*-)

- **NRAS (G12V and G12D) mutation- association with highest DIPSS+ score in MPN**
- **TP53 somatic mutation with loss of heterozygosity -association with leukemic transformation**

Myeloid/Lymphoid Neoplasms with Eosinophilia & Rearrangement of PDGFRA, PDGFRB, or FGFR1, or with PCM1-JAK2

Myeloid/lymphoid neoplasms with:

- **PDGFRA rearrangement,** at 4q12 locus
- **PDGFRB rearrangement** at 5q33 locus
- **FGFR1 rearrangement** at 8p11.2 locus

Provisional entity:

Myeloid/lymphoid neoplasms with PCM1-JAK2

Cases with **PDGFRA** & **FGFR1** tend to have a ***lymphoid component*** & may present initially as **T** or **B-lymphoblastic leukemia/lymphoma** with **eosinophilia**, whereas cases with **PDGFRB** tend to present as **CMML with eosinophilia** or as **CEL**.

Patients with **PDGFRA** or **PDGFRB** rearrangements are sensitive to **imatinib (Gleevec)** therapy.

Rearrangement of PDGFRB & FGFR1 can be detected by CC , FISH & PCR amplification and sequencing.

Because of the cryptic nature of PDGFRA rearrangement, however, molecular testing through PCR amplification & sequencing PCR is usually necessary.

Cytogenetic Abnormalities Associated with MyeloDysplastic Syndrome, MDS

MDS is a group of diseases associated with **ineffective hematopoiesis**, **dysplastic changes in BM elements**, & **peripheral cytopenia**.

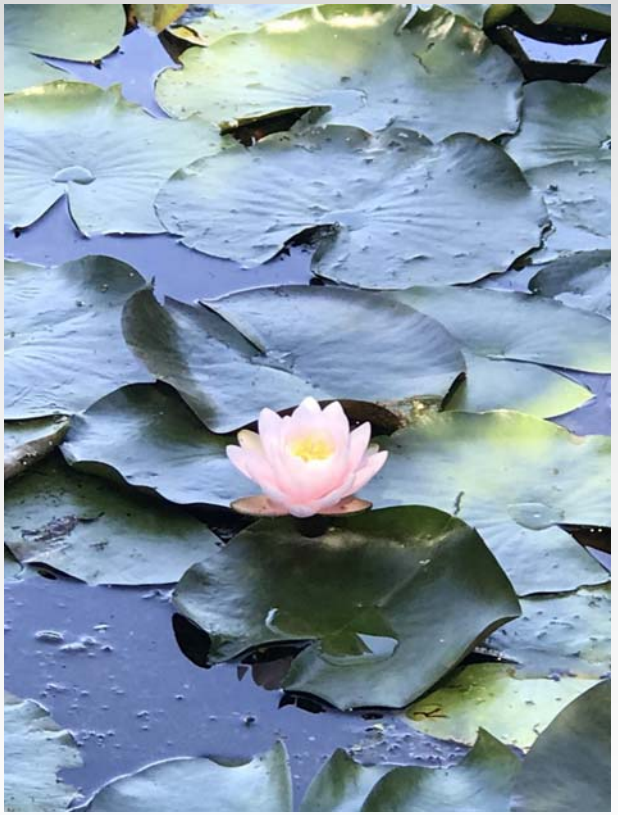
Most cytogenetic abnormalities are either **numerical (loss/gain)** or, less commonly, **unbalanced/balanced chromosomal abnormalities**.

They are seen in approx. **80%** of secondary MDS cases after chemotherapy or radiotherapy and in approx. 50% of de novo cases.

The most common abnormalities observed in MDS include monosomy 5 (5-) or deletions in the long arm of chromosome 7 (7q-), 7- or 7q=, 13- or 13q=, trisomy 8 ,and 20q-.

Approximately 33% & 40% of MDS cases show a single abnormality or are part of a monosomal karyotype, respectively. Complex karyotypes are seen in approximately 11% of MDS cases. Conventional karyotypes detect most of the chromosomal abnormalities associated with MDS. FISH analysis & spectral karyotyping also can be used; however, they do not show increased sensitivity over CC.

Thank you for your attention



Question?

- How can we diagnose ***BCR-ABL1*–Like B Lymphoblastic Leukemia?**