Molecular in My Pocket…

Hematopathology

Prepared by the Association for Molecular Pathology Training and Education Committee

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Acute Myeloid Leukemia (AML)

Good Prognosis
- Core Binding Factor (CBF) AML
  - t(8;21)(q22;q22); *RUNX1-RUNX1T1*
    - Blasts with salmon/pink granules
    - Predominant in younger patients
    - >70% of patients show additional chromosome abnormalities including sex chr loss, del(9q)
  - inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
    - Abnormal eosinophils
  - Worse prognosis in CBF AMLs when *KIT* is mutated
- Acute Promyelocytic Leukemia (APL)
  - t(15;17)(q22;q12); *PML-RARA*
    - Bilobed blasts with granules +/- Auer rods
    - Associated with disseminated intravascular coagulation
    - Sensitivity of APL cells to ATRA/arsenic treatment
      - APL variants like *ZBTB16-RARA* and *STAT5B-RARA* fusions are resistant to ATRA
- *NPM1* mutation without *FLT3*-ITD
- Biallelic mutations of *CEBPA*
  - *FLT3*-ITD mutations occur in 22-33% of cases (unclear prognosis)

Intermediate Prognosis
- t(9;11)(p22;q23); *MLLT3-KMT2A*
  - Blasts with monocytic differentiation and fine azurophilic granules
  - Associated with gingival myeloid sarcoma
  - More common in children (10% pediatric AML)
- Normal Karyotype, mutation status unknown (or rarely negative)

Poor Prognosis
- t(6;9)(p23;q34); *DEK-NUP214*
  - With or without monocytic features, often associated with basophilia and multilineage dysplasia
- inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM*
  - Abnormal megakaryocytes
  - Multilineage dysplasia
  - Common secondary karyotypic abnormalities include -7 (50% cases), del(5q) and complex karyotypes
- AML with myelodysplasia related changes (AML-MRC)
  - ≥50% dysplasia in ≥2 lineages (if no *NPM1* mutation)
  - History of MDS
  - MDS-defining cytogenetic abnormality (see MDS section)
- 11q23 (non t(9;11), many partners)
- t(9;22) (q34;q11.2); *BCR-ABL1*
- *FLT3*-ITD mutation
  - ~20% AML cases
- *ASXL1, TP53, RUNX1* mutation

Myelodysplastic Syndromes (MDS)

Cytogenetics
Very Good Prognosis
- del(11q)* or -Y

Good Prognosis
- Normal
- del(5q)*, del(12p)*, del(20q), double del(5q)

Intermediate Prognosis
- del(7q)
- Monosomy 5*
- Trisomy 8, trisomy 19
- i(17)(q10)*
- Monosomy 13* or del(13q)*
- 2+ independent clones
- Double any other abnormality

Poor Prognosis
- Monosomy 7*
- inv(3), t(3;3), del(3q), 3+ abnormalities

Very Poor Prognosis
- Complex (≥3 abnormalities)*

*MDS defining abnormality

Mutations
Good Prognosis
- *SF3B1* mutation (strongly correlated with ring sideroblasts)
  - With *SF3B1* mutation can diagnose MDS with ring sideroblasts (MDS-RS) with only 5% ring sideroblasts rather than 15% without the mutation

Poor Prognosis
- *TP53*

Other mutations may impart worse prognosis: *ASXL1, SRSF2, STAG2, EZH2, U2AF1, RUNX1, NRAS*

MDS-associated mutations may also occur in clonal hematopoiesis of indeterminate potential—particularly *DNMT3A, TET2, ASXL1*. Mutational findings alone are not diagnostic of MDS.

Myeloproliferative Neoplasms (MPN) and Mastocytosis

Chronic Myelogenous Leukemia (CML)
- t(9;22)(q34;q11.2);*BCR-ABL1*
  - Usually M-BCR (p210) breakpoint
  - Rarely m-BCR (p190) or µ-BCR (p230) breakpoints
  - *ABL1* kinase mutations confer TKI resistance
    - Particularly T315I
    - Transformation to accelerated phase (AP) or blast phase (BP) often accompanied by extra Ph chromosome, +8, +19, or i(17)(q10)

Polycythemia Vera (PV)
- *JAK2* V617F (~95% of cases)
- *JAK2* exon 12 mutation (~5% of cases)

Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF)
- *JAK2* V617F (~50% of cases)
- *CALR* exon 9 indel mutations (~30% of cases)
- *MPL* W515K/L (~5% of cases)

Chronic Neutrophilic Leukemia (CNL)
- Activating membrane proximal mutations in *CSF3R* at exon 14, especially T618I and T615A; present in 50-80% of CNL

Mastocytosis
- *KIT* D816V (~95% of cases)
- *TET2* mutations in ~25% of mastocytosis – correlate with more aggressive behavior
- Additional mutations: *SRSF2* (30-40%), *ASXL1* (24%), *IDH2 (7%)*

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Other Entities

Chronic Myelomonocytic Leukemia (CMML) (MDS/MPN "bridging" category)
- Frequent mutations: *TET2* (~50%), *SRSF2* (~30-50%), *ASXL1* (40-50%, poor prognosis if missense mutations are excluded), *KRAS* and *NRAS* (myeloproliferative phenotype), *SETBP1* (poor prognosis), *JAK2* (not specific)

Atypical Chronic Myeloid Leukemia (aCML)
- Overlap MDS/MPN neoplasm
- *BCR/ABL1* negative
- Cytogenetics: +8, i(17q), -7, del(7q), del(20q), +9, del(13q)
- Molecular genetics: *SETBP1* mutation (~20-30%, exon 4 mutations with D868N most common, associated with -7 and i(17q), *ASXL1* (65%), *SRSF2*, *TET2* (~40%), *KRAS, NRAS, EZH2, ETNK1, CBL, JAK2*, (~10-30%) , *CSF3R* (<1%, T618I most common), *CALR* (rarely or never present)

Juvenile Myelomonocytic Leukemia (JMML)
- Somatic *PTPN11* (20-30%), *KRAS* and *NRAS* (75-80%) mutations
- Clinical *NF1* disease or *NF1* mutation
- Germline *CBL* mutation (10-15%, Y371 common mutation hotspot)

Myeloid/Lymphoid Neoplasms associated with Eosinophilia
- *PDGFRA* rearrangement (often del(4)(q12q12); *FIP1L1-PDGFRA*)
- *PDGFRB* rearrangement (often t(5;12)(q31~33;p12) ;*ETV6-PDGFRB*)
- *FGFR1* rearrangement (various partners)
- t(8;9)(p22;p24.1);*PCM1-JAK2*
- *ETV6-FLT3* fusion

Myeloid Neoplasms with Germline Predisposition
- AML with germline *CEBPA* mutation
- Myeloid neoplasm with germline *DDX41* mutation
- Associated with platelet disorders
  - *RUNX1*, *ANKRD26*, *ETV6* mutation
- Associated with other organ dysfunction
  - *GATA2* mutation
  - *JMML*-type mutations

Langerhans cell histiocytosis, histiocytic sarcoma, Erdheim-Chester disease
- *BRAF* p.V600E mutation in a subset of cases

T-cell Neoplasms

T Lymphoblastic Leukemia/Lymphoma (T-ALL)
- Translocations involving T-cell receptor (TCR); error during TCR gene rearrangements
- *NUP214-ABL1* strictly associated with T-ALL <6% cases
- *MYC* rearrangements (~6% cases)
- *NOTCH1* (70% cases), *CDKN2A/B* (cryptic deletions >70% cases) mutations

Early T-precursor Acute Lymphoblastic Leukemia (ETP ALL)
- *FLT3*, *NRAS*/*KRAS*, *DNMT3A*, *IDH1*/2

Anaplastic Large Cell Lymphoma, *ALK*-negative (ALCL, ALK–)
- Subset have rearrangement at 6p25 (region with *DUSP22* and *IRF4*) - good prognosis
- *TP63* rearrangement - poor prognosis

Anaplastic Large Cell Lymphoma, *ALK*-positive (ALCL, *ALK*+)
- Chromosome translocations involving *ALK* gene at 2p23
- t(2;5)(p23;q35); *ALK-NPM1*
- Other *ALK* rearrangements

T-cell Large Granular Lymphocyte Leukemia (T-LGL)
- *STAT3* mutation
- *STAT5B* mutation

Peripheral T cell lymphoma, NOS (PTCL-NOS)
- *TET2*, *DNMT3A*, *VAV1*
- *GATA3* vs *TBX21* profiles
- Complex cytogenetic abnormalities common; t(5;9)(q33;q32) *ITK-SYK* in follicular variant
- Clonal rearrangements of TRB and TRG, IGH rearrangements in ~30% cases

Angioimmunoblastic T-cell lymphoma (AITL)
- *RHOA, CD28, TET2, DNMT3A, IDH2*

T-cell Prolymphocytic leukemia (T-PLL; with inv(14) or t(X;14))*ATM*, *STAT5B*, *JAK1*, *JAK3*
- Complex karyotypes common with numerical and structural abnormalities including inv(14), t(X;14), i(8)(q10), -11, del11q, -22, -13.
- *TCL1A* (TCL1) rearrangements at 14q32. Multiple submicroscopic abnormalities.

B-cell Neoplasms

B Lymphoblastic Leukemia (B-ALL)
- Good prognosis
  - Hyperdiploid (extra chromosome copies are not random: X, 4, 14, 21 most common)
  - t(12;21)(p13;q22);*ETV6-RUNX1* (typically cryptic fusion)
  - Common in children, ~25% of B-ALL
- Intermediate prognosis- *t(5;14)*(q31;q32); IGH/*IL3*, associated with eosinophilia
- Poor Prognosis
  - t(9;22)(q34;q11.2); *BCR-ABL1*
    - Usually m-BCR (Most pediatric cases have p190; in adults, 50% is p190 and 50% is p210)
  - t(v;11q23); *KMT2A* rearranged
    - Most common leukemia in infants <1 year old; may occur in utero
    - Common translocation partners *AFF1* (4q21) and*MLLT1* (19p13)
  - Hypodiploid
    - Most commonly lost chromosomes include 3, 4, 7, 9, 13, 17, and 20
    - Worse prognosis: near haploid (25-29 chromosomes) and low hypodiploid (33-39 chromosomes)
  - Intrachromosomal amplification of chromosome 21 (iAMP21)
    - Defined by multiple copies of *RUNX1*, not detectable by standard karyotyping
  - *BCR-ABL1* like B-ALL
    - Lacks *BCR-ABL1* translocation
    - 15-20% pediatric ALL
    - *CRLF2* or *EPOR* rearrangement
    - *JAK* mutations
    - *CDKN2A/B* or *IKZF1* deletion/mutation
    - Other translocations involving tyrosine kinases

Follicular Lymphoma (FL)
- t(14;18)(q32;q21) (80-90% cases); IGH/*BCL2*
- Cases without t(14;18) are usually BCL2 negative with increased *CHECK1* expression
- FL cases have ~6 cytogenetic abnormalities (17p and 6q23 worse prognosis)
- Complex karyotype correlates with poorer prognosis
- *BCL6* rearrangements more common in grade 3B tumors

Mantle Cell Lymphoma (MCL)
- t(11;14)(q13;q32); IGH/*CCND1*
- Common secondary abnormalities: loss of 1p, 13q, 17p, gains in 3q. Numerical abnormalities include +3, +12, -8, -9, -X, -Y
- t(8;14)(q24;q32) occurs rarely and has worse prognosis; t(8;14) and *CCND1* rearrangement is called a "double hit" MCL

Burkitt Lymphoma (BL)
- *MYC* rearrangements
  - t(8;14)(q24;q32); IGH/*MYC*
  - t(2;8)(p12;q24); IGK/*MYC*
  - t(8;22)(q24;q11); IGL/*MYC*

Hairy Cell Leukemia (HCL)
- *BRAF* p.V600E (~95% of cases)
- >85% HCL cases demonstrate somatic hypermutation in VH genes
- *MAP2K1* mutations = Hairy Cell Leukemia variant (HCL-v)

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)
- Good Prognosis
  - del(13)(q14) most common cytogenetic abnormality
  - Mutated IGHV
- Intermediate Prognosis
  - Trisomy 12 (a/w *NOTCH1*) (good to intermediate prognosis)
  - del(6q)
- Poor Prognosis
  - 17p13 deletion
  - del(11)(q22-23) (a/w *SF3B1*)

Extranodal Marginal Zone Lymphoma, MALT type
- t(11;18)(q21;q21) – pulmonary and gastric MALT
- t(14;18)(q32;q21) - orbital and salivary gland MALT
- t(3;14)(p14.1;q32) - thyroid, orbital, skin MALT
- t(1;14)(p22;q32)

Lymphoplasmacytic Lymphoma (LPL) and IgM Monoclonal Gammopathy of Unknown Significance (MGUS)
- 6q23 deletion most common cytogenetic finding in LPL
- *MYD88* p.L265P (~90% of cases)
- *CXCR4* mutation (~30% of LPL, ~20% of IgM MGUS)

ALK-positive large B-cell lymphoma
- t(2;17)(p23;q23); *CLTC-ALK*

High-grade B-cell lymphoma
- *MYC* rearrangement with *BCL2* and/or *BCL6* rearrangement

Diffuse large B-cell lymphoma, NOS
- Activated B cell type
  - BCL6 rearrangements/*NOTCH2* mutations
  - *MYD88* & *CD79B* mutations
  - *NOTCH1* mutations
- Germinal center B cell type
  - IGH/*BCL2* & *EZH2* mutations