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; rarely in elderly 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) with t(15;17)(q22;q12); *PML::RARA*
  - Bilobed blasts with granules +/- Auer rods
  - Associated with disseminated intravascular coagulation
  - APL with *PML::RARA* is sensitive to ATRA/arsenic treatment
    - Some APL variants like *ZBTB16::RARA* and *STAT5B::RARA* fusions are resistant to ATRA

- *NPM1* mutation without *FLT3*-ITD

- AML with in-frame bZIP and smbZIP mutated *CEBPA*
  - *FLT3*-ITD mutations occur in 22-33% of cases (poorer prognosis, still better than *FLT3*-ITD without *CEBPA* mutations)

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)
  - Common secondary cytogenetic abnormality, such as +8

- 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
  - Vast majority as sole chromosome abnormality
  - *FLT3*-ITD common

- 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

- t(1;22)(p13.3;q13.1) *RBM15::MRTF1*

- AML with myelodysplasia-related (AML-MR)
  - >20% blasts required by WHO; 10% blasts by ICC
  - *De novo* or history of MDS or MDS/MPN
  - With MDS-associated cytogenetic abnormality (see MDS section)
  - With MDS associated mutations in 8 genes: *ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2*

- 11q23 (non t(9;11), many partners, such as t(4;11) and t(11;19)

- t(9;22) (q34;q11.2); *BCR::ABL1* with P210 or P190, usually with –7, +8, complex karyotype

- *NUP98* rearrangement: 2nd most common driver gene alteration in relapsed pediatric AML, >30 fusion partners

- *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 including del(5q)
- Monosomy 13 or del(13q)*

Intermediate Prognosis
- del(7q)*
- Monosomy 5*
- Trisomy 8, trisomy 19
- del(17p) or i(17)(q10)*
- Any other single or double independent clones

Poor Prognosis
- Monosomy 7*
- inv(3), t(3;3), del(3q), double including –7/7q-, 3 abnormalities*

Very Poor Prognosis
- Complex (>3 abnormalities)*

*MDS defining abnormality in the setting of persistent cytopenia of undetermined origin

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
- bi*TP53* (mutations and/or copy number loss, or cnLOH)

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 (CHIP) — particularly *DNMT3A, TET2, ASXL1, PPM1D* (VAF: ≥2%; ≥4% in X chromosome for male). Mutations 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, i(17)(q10), and+8 or +19

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 out of frame indel mutations (~30% of cases)
- *MPL* W515K/L, S505N/A (~8% of cases)
- Others with poor prognosis: *TET2*, *IDH1*, *IDH2*, *ASXL1*, *SRSF2*, *U2AF1*

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%), *RUNX1*, and *JAK2*

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Other Entities

Chronic Myelomonocytic Leukemia (CMML) (MDS/MPN "bridging" category)

- Cytogenetics: +8, -7, -Y, *PDGFRB* re-arrangement, i(17q)
- Frequent mutations: *TET2* (~50%), *SRSF2* (~30-50%; poor prognosis), *ASXL1* (40-50%, poor prognosis if missense mutations are excluded), *EZH2* (poor prognosis), *RUNX1* (~15%), *KRAS* and *NRAS* (~15%; myeloproliferative phenotype; adverse outcome), *CBL1* (~10-20%), *NF1* (~5-10%), *SETBP1* (~5-10%; poor prognosis), *BCOR* (~5-10%; poor prognosis), *JAK2* (not specific)

Myelodysplastic/myeloproliferative neoplasm with neutrophilia (prior name: Atypical chronic myeloid leukemia; aCML)

- negative for *BCR/ABL1*, *PDGFRA*, *PDGFRB*, *FGFR1*, *JAK2* rearrangement
- Cytogenetics: +8, del(20q), i(17q), abnormalities of chromosomes 13, 14, 17, 19 and 12
- 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)

Myelodysplastic/myeloproliferative neoplasm with *SF3B1* mutation and thrombocytosis (prior name: MDS/MPN with ring sideroblasts and thrombocytosis)

- Molecular mutations: *SF3B1*, *JAK2* (~50%), *CALR*

Juvenile Myelomonocytic Leukemia (JMML)

- Somatic *PTPN11* (35%; poor prognosis), *KRAS* and *NRAS* (~20-25%) mutations (poor prognosis)
- Germline (often) *NF1* (poor prognosis) or somatic *NF1* mutation
- Germline *CBL* mutation (10-15%, Y371 common mutation hotspot; favorable prognosis)
- Secondary mutations: *SETBP1*, *JAK3*, *SH2B3*, *ASXL1*

Myeloid/Lymphoid Neoplasms associated with Eosinophilia and tyrosine kinase gene fusion (MLN-TK)

- *PDGFRA* rearrangement (often del(4)(q12q12); *FIP1L1::PDGFRA*)
- *PDGFRB* rearrangement (often t(5;12)(q31~33;p12) ;*ETV6::PDGFRB*)
- *FGFR1* rearrangement (various partners)
- *JAK2* rearrangement, t(8;9)(p22;p24.1);*PCM1::JAK2*
- *FLT3* rearrangement, such as *ETV6::FLT3* fusion
- *ABL1* rearrangement, such as *ETV6::ABL1* fusion

Myeloid Neoplasms with Germline Predisposition without a pre-existing platelet disorder or organ dysfunction

- AML with germline *CEBPA* mutation
- Myeloid neoplasm with germline *DDX41* mutation
- Myeloid neoplasm with germline *TP53* mutation

Myeloid Neoplasms with Germline Predisposition and pre-existing platelet disorder

- *RUNX1*, *ANKRD26*, *ETV6* mutation

Myeloid Neoplasms with Germline Predisposition and potential organ dysfunction

- Germline *GATA2* mutation
- Bone marrow failure syndrome: Severe congenital neutropenia, Shwachman-Diamond syndrome, Fanconi anemia
- Telomere biology disorders
- RASopathies: Neurofibromatosis type 1, *CBL* syndrome, Noonan syndrome or Noonan syndrome-like disorders
- Down syndrome
- Germline *SAMD9* mutation: MIRAGE syndrome
- Germline *SAMD9L* mutation: *SAMD9L*-related ataxia pancytopenia syndrome
- Biallelic germline *BLM* mutation

Langerhans cell histiocytosis, histiocytic sarcoma, Erdheim-Chester disease

- Clonal *IGH, IGHK,* or *TR* rearrangement
- *BRAF* p.V600E mutation (~25-50%)
- *MAP2K1* (~25%) and *ARAF* mutation

T-cell Neoplasms

T-Lymphoblastic Leukemia/Lymphoma (T-ALL/LBL)

- Clonal rearrangement of T-cell receptor (TR) genes (almost always), IGH gene rearrangement (~20%)
- Translocations involving T-cell receptor (TCR; alpha and delta TR at 14q11.2, beta TR at 7q34, and gamma TR at 7p14.1) with variety of partners, such as *TLX1*, *TLX3* (*TLX1* relatively favorable prognosis)
- *TAL1* translocation, such as *TAL1::STIL* (relatively favorable prognosis)
- t(10;11)(p12.3;q14.2) with *PICALM::MLLT3* fusion (NUP)
- *KMT2A* rearrangement (~8%)
- *NUP214::ABL1* (<6% cases)
- *MYC* rearrangements (~6% cases)
- *NOTCH1* (70% cases), *CDKN2A/B (*cryptic deletions >70% cases) mutations

Early T-precursor Lymphoblastic Leukemia/Lymphoma (ETP-ALL)

- Mutations in *FLT3*, *NRAS/KRAS*, *DNMT3A*, *IDH1/2*, *NOTCH1*, *CDKN1/1*

ALK-negative Anaplastic Large Cell Lymphoma (ALK–ALCL)

- Rearrangement at 6p25 (region with *DUSP22* and *IRF4*; ~30%) - good prognosis
- *TP63* rearrangement (~8%) - poor prognosis
- *JAK1* and/or *STAT3* mutations
- Cytogenetic changes: 1q+, 6p+,8q+, 12q+, 4q-, 6q21-13q-, 17p13.1- (TP53)

ALK-positive Anaplastic Large Cell Lymphoma (*ALK*+ ALCL, long-term overall survival better than ALK–ALCL)

- Clonal rearrangement of T-cell receptor (TR) genes (~90%)
- Variety translocations involving *ALK* gene at 2p23, such as t(2;5)(p23;q35); *NPM1:: ALK*
- Secondary cytogenetic changes: -4, del(11q), del(13q), +7, 17p+, 17q+

T-Large Granular Lymphocyte Leukemia (T-LGL)

- TRG rearrangement in all cases
- *STAT3* mutation
- *STAT5B* mutation

Peripheral T cell lymphoma, NOS (PTCL-NOS)

- Clonal rearrangement of T-cell receptor (TR) genes in most cases
- Complex karyotype
- *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)

- Clonal rearrangement of T-cell receptor (TR) genes (~75-90%)
- Cytogenetic changes: +3, +5, +21, +X, del(6q), 22q+, +19, 11q13+
- *RHOA, TET2, DNMT3A, IDH2, CD28, PLCG1, FYN*

T-cell Prolymphocytic leukemia (T-PLL; aggressive); with inv(14) or t(X;14))*ATM*, *STAT5B*, *JAK1*, *JAK3*

- Complex karyotypes, most common inv(14)(q11.2q32.1) (~80%), t(14;14)(q11;2q32.1) (~10%), or t(X;14) with TRA involvement; other are chromosome 8 abnormalities (~70-80%) such as idic(8)(p11), t(8;8), and 8q+; less commonly -11, del(11q), -22, -13, del(TP53).
- *TCL1A* (TCL1) rearrangements at 14q32. Multiple submicroscopic abnormalities.

B-cell Neoplasms

B Lymphoblastic Leukemia (B-ALL)

Good prognosis

- High Hyperdiploid (usually 50- 66 chromosomes, common ones are +21, +X, +14, and +4 common) (~25% pediatric B-ALL)
- t(12;21)(p13;q22); *ETV6::RUNX1* (typically cryptic fusion) (~25% pediatric B-ALL)
- *ETV6::RUNX1* like B-All: *IGH-DUX4* or *ERG-DUX4* fusion, frequently with introgenic *ERG* deletion, some with *IKZF1* deletion

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; less common in older childhood, then increasingly common in adulthood
  - Common translocation partners *AFF1* (4q21) and *MLLT1* (19p13)
- Hypodiploid
  - Most commonly lost chromosomes include 3, 4, 7, 9, 13, 17, and 20
  - Worse prognosis in near haploid (25-29 chromosomes) and low hypodiploid (33-39 chromosomes) than high hypodiploid (40-43 chromosomes)
- Intrachromosomal amplification of chromosome 21 (iAMP21): multiple copies of *RUNX1* usually found by FISH, may be associated with +x, abnormal 7, del(*RB1*), del(*ETV6*), and/or *CRLF2* rearrangement
- t(1;19) with *TCF3::PBX1*
- t(17;19) with *TCF3::HLF*
- *BCR::ABL1* like B-ALL (no *BCR::ABL1* translocation)
  - 15-20% pediatric ALL
  - *CRLF2, ABL1, ABL2, PDGFRB, JAK2,* or *EPOR* rearrangement
  - *JAK* mutations
  - Other translocations involving tyrosine kinases

Follicular Lymphoma (FL)

- t(14;18)(q32;q21) with IGH::*BCL2* fusion in 80-90% cases; t(14;18) negative cases may have BCL6 (3q27) rearrangement
- IG heavy and light chain gene rearranged, IGV extensive somatic hypermutation
- Additional cytogenetic changes: 1p-, 6q-, 10q-, 17p-,+1, 6p+, +7, +8, 12q+, +X, and 18q+ (deletions of 17p and 6q, as well as worse prognosis)
- *BCL6* rearrangements more common in grade 3B tumors

Mantle Cell Lymphoma (MCL)

- t(11;14)(q13;q32) with IGH::*CCND1* fusion (>95%)
- Common secondary abnormalities: loss of 1p31p13 (~30-50%), 6q23q27 (*TNFAIP3*, ~25-40%), 9p21 (*CDKN2A*; ~20-30%), 11q22q23 (*ATM*; ~20-60%), 13q11q13 (~20-55%), 17p13.1 (*TP53*, ~20-45%); gains in 3q26 (~30-50%), 7p21 (~15-35%), 8q24.2 (*MYC*, 5-25%). Numerical abnormalities include +3, +12, -8, -9, -X, -Y
- Deletion of *TP53* and/or *CDKN2A*, complex karyotype: adverse prognostic factors
- t(8;14)(q24;q32) occurs rarely and has aggressive clinical course; t(8;14) and *CCND1* rearrangement is called a "double hit" MCL
- Mutations: *ATM, CCND1, KMT2A MLL, NOTCH1/2, TP53, CDKN2A, CDKN2C*
- *CCND1*-negative MCL (IHC: absence of SOX11 staining): *CCND2* (~50%), *CCND3* translocation

Hairy Cell Leukemia (HCL)

- Classical HCL (cHCL): *BRAF* p.V600E (~95% of cases)
- HCL variant (HCLv): IGHV4-34 rearrangement (~10-20%), *MAP2K1* mutations, poorer prognosis

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)

- Good Prognosis
  - del(13)(q14) as sole abnormality
  - Mutated IGHV (≥2%)
- Intermediate Prognosis
  - Trisomy 12 (good to intermediate prognosis)
  - Normal karyotype
  - *NOTCH1* and/or *SF3B1* mutation
- Poor Prognosis
  - 17p13 deletion (including *TP53*)
  - del(11)(including *ATM*)
  - *TP53* and/or *BIRC3* mutation
- Mutations in *BIRC3, NOTCH1, SF3B1* more frequently at relapse (fludarabine-refractory CLL)

Extranodal Marginal Zone Lymphoma, MALT type: *MALT1* rearrangements

- t(11;18)(q21;q21)/*API2::MALT1* – pulmonary and gastric MALT
- t(14;18)(q32;q21)/*IGH::MALT1* – liver, skin, ocular adnexa, and salivary gland MALT (~15-20%)
- t(3;14)(p14.1;q32)/*IGH::FOXP1* - thyroid, ocular adnexa, skin MALT
- t(1;14)(p22;q32)/*IGH::BCL10* – stomach, lung, and skin MALT (~1-2%)

Lymphoplasmacytic Lymphoma (LPL) and IgM Monoclonal Gammopathy of Unknown Significance (MGUS)

- *MYD88* p.L265P (~90% of cases)
- *CXCR4* mutation (~30% of LPL, ~20% of IgM MGUS)
- *ARID1A* mutations (~17%)
- Other mutations: *TP53, CD79B, KMT2D, MYBBP1A*

Cytogenetic abnormality: non-specific

ALK-positive large B-cell lymphoma

- t(2;17)(p23;q23); *CLTC::ALK:* most common
- other *ALK* rearrangements

Diffuse large B-cell lymphoma/High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements

- *MYC* rearrangement with *BCL2* and/or *BCL6* rearrangement ("double hit" or "triple hit" lymphoma)

Diffuse large B-cell lymphoma, NOS

- Activated B cell type (ABC)
  - Mutations*: CARD11, MYD88, CD79B*
  - Cytogenetic changes: *BCL6* rearrangements, gains 3q27.3, 11q23q24, and 18q21.3; del(6q21), del(9p21)
- Germinal center type (GCB)
  - Mutations: *EZH2, GNA13*
  - Cytogenetic changes: t(14;18)/IGH::BCL2, gains/ amp 2p16, 8q24; del(1p36), del(10q23)

High-B-cell grade lymphoma with 11q aberration:

- lack *MYC* rearrangement
- with interstitial 11q gain and terminal 11q loss

Large B-cell lymphoma with *IRF4* rearrangement

- lack of *MYC* and *BCL2* rearrangement

Burkitt Lymphoma (BL)

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

additional cytogenetic abnormalities: gains of 1q, 7, 12; losses of 6q, 13q32q34, and 17p