

WHO Revised 4th Edition (2017): Important Revisions/Additions

Part I. Myeloid neoplasms and acute leukemia

[updated 4/5/2019]

-Mastocytosis is no longer considered a subgroup of the MPNs due to its unique clinical and pathologic features, ranging from indolent cutaneous disease to aggressive systemic disease, and is now a separate disease category in the classification.

-CSF3R mutation is strongly associated with chronic neutrophilic leukemia (CNL).

PB WBC $>25 \times 10^9/L$.

Segmented neutrophils plus band forms $>80\%$ of WBCs.

- Criteria for CML, accelerated phase.

Any 1 or more of the following hematologic/cytogenetic criteria or provisional response-to-TKI criteria

- Persistent or increasing WBC ($>10 \times 10^9/L$), unresponsive to therapy
- Persistent or increasing splenomegaly, unresponsive to therapy
- Persistent thrombocytosis ($>1000 \times 10^9/L$), unresponsive to therapy
- Persistent thrombocytopenia ($<100 \times 10^9/L$) unrelated to therapy
- 20% or more basophils in the PB
- 10%-19% blasts in the PB and/or BM
- Additional clonal chromosomal abnormalities in Ph⁺ cells at diagnosis that include “major route” abnormalities (second Ph, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, or abnormalities of 3q26.2
- Any new clonal chromosomal abnormality in Ph⁺ cells that occurs during therapy.
- Hematologic resistance to the first TKI (or failure to achieve a complete hematologic response to the first TKI) or
- Any hematological, cytogenetic, or molecular indications of resistance to 2 sequential TKIs or therapy

- Occurrence of 2 or more mutations in BCR-ABL1 during TKI therapy

-P. Vera: major criteria (1) for elevated Hgb (>16.5 for men and >16.0 for women)

-Morphology for ET vs. pre-PMF:

- ET: normocellular BM, normal M/E ratio, rare meg clusters, large/giant/hyperlobated megs, rare reticulin fibrosis (Gr 1)
- Pre-PMF: hypercellular BM, increased M/E ratio, frequent meg clusters, variable size/bulbous/hyperchromatic/hypolobated megs, frequent reticulin fibrosis (Gr 1)

-(A new subtype) Molecular genetic abnormalities in myeloid/lymphoid neoplasms associated with eosinophilia: PCM1-JAK2 Eosinophilia: t(8;9)(p22;p24.1). Variants include: ETV6-JAK2, BCR-JAK2. May respond to JAK2 inhibitors. Often presents with T-LBL or B-ALL, Bone marrow shows left-shifted erythroid predominance and lymphoid aggregates.

- Refractory anemia with ring-sideroblasts (sideroblasts: at least 5 iron granules over at least 1/3 of nuclear circumference) associated with marked thrombocytosis (RARS-T), used to be a provisional entity within the MDS/MPN unclassifiable group, now termed MDS/MPN with ring sideroblasts and thrombocytosis. Presence of a SF3B1 mutation or, in the absence of SF3B1 mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features. RARS-T may have concurrent mutations JAK2, CALR, or MPL.

-CMML: subtypes

Morphology: Dysplastic (WBC <13k), Proliferative (WBC \geq 13k)

Blast count: CMML-0 (0-4), CMML-1 (5-9), CMML-2 (10-19)

- aCML is associated with SETBP1 and/or ETNK1 mutations in up to a third of cases.
- Juvenile myelomonocytic leukemia (JMML): Approximately 90% of patients carry either somatic or germline mutations of PTPN11, KRAS, NRAS, CBL, or NF1. JMML also has association with monosomy 7 and hyperphosphorylation of STAT5.
- MDS has changed to remove terms such as “refractory anemia” and “refractory cytopenia” and replaces them with “myelodysplastic syndrome” followed by the appropriate modifiers: single vs multilineage dysplasia, ring sideroblasts, excess blasts, or the del(5q) cytogenetic abnormality. There are no changes to childhood MDS; refractory cytopenia of childhood remains as a provisional entity within this category.
- The presence of 1% blasts in the PB, with <5% BM blasts, defines as MDS, unclassifiable (MDS-U). However, because 1% blasts may not be reproducible as a single observation, this finding must now be demonstrated on at least 2 separate occasions in order to diagnose MDS-U according to this criterion.
- There is a major change in the diagnostic criteria for myeloid neoplasms with erythroid predominance (erythroid precursors >50% of all BM cells). In the updated classification, the denominator used for calculating blast percentage in all myeloid neoplasms is all nucleated BM cells, not just the “nonerythroid cells.” The subcategory of acute erythroid leukemia, erythroid/myeloid type (previously defined as a case with >50% BM erythroid precursors and >20% myeloblasts among nonerythroid cells) has been removed from the AML category. Pure erythroid leukemia remains as an AML, NOS subtype and is now the only type of acute erythroid leukemia (>80% erythroid precursors with >30% proerythroblasts).
- Based on recent data showing no adverse effect of 1 chromosomal abnormality in addition to the del(5q), the entity MDS with isolated del(5q) may be diagnosed if there is 1 additional cytogenetic abnormality besides the del(5q), unless that abnormality is monosomy 7 or del(7q). Evaluation for TP53 mutation is recommended in patients with MDS with isolated del(5q) to help identify an adverse prognostic subgroup in this generally favorable prognosis MDS entity. A subset of cases have JAK2, MPL, or SF3B1 mutations.
- Targeted sequencing of a limited number of genes can detect mutations in 80% to 90% of MDS patients; the most commonly mutated genes in MDS are SF3B1, TET2, SRSF2, ASXL1, DNMT3A, RUNX1, U2AF1, TP53, and EZH2. Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of apparently healthy older individuals without MDS, so-called “clonal hematopoiesis of indeterminate potential” (CHIP). Although some patients with CHIP subsequently develop MDS, the natural history of this condition is not yet fully understood; thus, the presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in a patient with unexplained cytopenia.
- If an SF3B1 mutation is identified, a diagnosis of MDS-RS may be made if ring sideroblasts comprise 5-14% of nucleated erythroid cells, whereas at least 15% ring sideroblasts are still required in cases lacking a demonstrable SF3B1 mutation. MDS-RS cases will be subdivided into cases with single lineage dysplasia (MDS-RS-SLD, previously classified as refractory anemia with ring sideroblasts) and cases with multilineage dysplasia (MDS-RS-MLD, previously classified as refractory cytopenia with multilineage dysplasia). If ring-sideroblasts are less than 5%, even with SF3B1 mutation the DX is still MDS-SLD or MDS-MLD.

-A new provisional category of AML with BCR-ABL1 is added to recognize these rare de novo AML cases that may benefit from TKI therapy, Although the diagnostic distinction between de novo AML with BCR-ABL1 and blast transformation of CML may be difficult without adequate clinical information.

-The finding that the improved prognosis associated with AML with mutated CEBPA is associated with biallelic, but not single, mutations of the gene has resulted in a change in that disease definition to require biallelic mutations.

-NPM1 or biallelic CEBPA: these mutations now supersede the presence of multilineage dysplasia in the AML classification (dysplasia is not associated with worse prognosis with these mutations).

-Myeloid neoplasms with germline predisposition: mutations in CEBPA, DDX41, RUNX1, ANKRD26, ETV6, GATA2. Note: underlined mutations are with pre-existing platelet disorder.

-Infants with Noonan syndrome may carry germ-line PTPN11 mutation and develop a JMML-like disorder (may be transient or aggressive)

-Childhood MDS:

Refractory cytopenia of childhood: typically hypocellular BM, dysplasia in 1-3 cell lines (whereas aplastic anemia has no dysplasia)

Childhood MDS EB-1 and EB-2: diagnosed as in adult MDS

- A provisional category of AML with mutated RUNX1 has been added to the classification for cases of de novo AML with this mutation excluding cases that are associated with MDS-related cytogenetic abnormalities, recurrent chrom abnormalities, t-AML, AML with NPM1 or CEBPA. This new provisional disease category appears to represent a biologically distinct group with a possibly worse prognosis than other AML types.

-Both TAM and myeloid leukemia associated with Down syndrome are characterized by GATA1 mutations and mutations of the JAK-STAT pathway.

-Most cases of acute panmyelosis have 20-25% blasts in BM.

-Care must be taken before making a diagnosis of B/myeloid MPAL when low-intensity myeloperoxidase (MPO) is the only myeloid-associated feature. Multiparameter flow cytometry is the method of choice for recognizing MPAL.

-The unique association between low hypodiploid ALL and TP53 mutations is often constitutional.

-B-lymphoblastic leukaemia/lymphoma with t(5;14): a rare disease, < 1% of cases of ALL, patients may present with an asymptomatic eosinophilia, and blasts may be deceptively absent in the peripheral blood. The prognosis is not different from that of other types of ALL.

-“BCR-ABL1-like ALL”: B-ALL with translocations involving tyrosine kinases or cytokine receptors. They account for 10-25% of ALL cases and associated with poor outcome. The cases with translocations involving tyrosine kinase genes involve many different genes including ABL1 (with partners other than BCR), as well as other kinases including CRLF2 (up to 50% of cases), ABL2, PDGFRB, NTRK3, TYK2, CSF1R, and JAK2. Over 30 different partner genes have been described. Some patients, especially those with EBF1-PDGFRB translocations, have shown remarkable responses to TKI therapy, even after failing conventional therapy.

-Indolent T-lymphoblastic proliferation: which was briefly mentioned in the fourth edition classification, is now a more readily recognized non-neoplastic entity that may mimic T-lymphoblastic lymphoma. It typically involves lymphoid tissue of the upper aerodigestive tract but may occur in other locations. Local recurrences are common and systemic dissemination is

rare. Histologic examination of involved lymph nodes shows infiltration and sometimes replacement by proliferations of lymphoblasts that are less cytologically atypical than the usual T-lymphoblastic lymphoma. Although the blasts have an immature thymic phenotype that can be demonstrated by TdT staining in lymph nodes, the phenotype reflects a developmentally normal, non-aberrant phenotype and the proliferations are not clonal. These latter features allow this indolent entity to be distinguished from T-lymphoblastic lymphoma.

-Early T-precursor (ETP) ALL leukemia: has a unique immunophenotypic and genetic makeup indicating only limited early T-cell differentiation, with retention of some myeloid and stem cell characteristics at both the immunophenotypic and genetic level. By definition, blasts in ETP ALL express CD7 but lack CD1a and CD8, and are positive for 1 or more of the myeloid/stemcell markers CD34, CD117, HLA DR, CD13, CD33, CD11b, or CD65. They typically also express CD2 and cytoplasmic CD3 and may express CD4, but these are not part of the definition. CD5 is often negative and when positive is present on <75% of the blast population. Myeloid associated gene mutations, such as FLT3, NRAS/KRAS, DNMT3A, IDH1, and IDH2, are reported at high frequency in ETP ALL, whereas more typical T-ALL-associated mutations such as activating mutations in NOTCH1 or mutations in CDKN1/2 are infrequent. Although initial small series of ETP ALL suggested that outcome was very poor, in the largest series to date, no prognostic significance was found.

-NK-lymphoblastic leukaemia/lymphoma: a provisional entity, may be considered in a case that expresses CD56 along with immature T-associated markers such as CD7 and CD2, and even including cCD3, provided that the case lacks B-cell and myeloid markers, TCR and IG genes are in the germline configuration, and blastic plasmacytoid dendritic cell neoplasm has been excluded.

Part II. Lymphoid neoplasms

CLL/SLL

- Cytopenia or disease-related symptoms are now insufficient to make a diagnosis of CLL with $<5 \times 10^9/L$ PB CLL cells (this should be diagnosed as Monoclonal B-cell lymphocytosis).
- Large/confluent and/or highly proliferative proliferation centers are adverse prognostic indicators.
- Mutations of potential clinical relevance, such as TP53, NOTCH1, SF3B1, ATM, and BIRC3, have been recognized. However, the literature is inconsistent regarding the clinical implications of some of the mutations and combined risk profile, and it will not be recommended at this time
- Proliferation centers (PC) can have cyclin D1 expression in up to about 30% of CLL/SLL, they express MYC protein, and, PCs which are large/confluent and/or have a high proliferative fraction are a significant and independent adverse prognostic indicator.

Monoclonal B-cell lymphocytosis

- Must distinguish low-count from high-count MBL.
- MBL precedes virtually all cases of (CLL/SLL). The updated WHO will retain the current criteria for MBL, but will emphasize that “low-count” MBL, defined as a PB CLL count of $<0.5 \times 10^9/L$, must be distinguished from “high-count” MBL [$0.5-5 \times 10^9/L$] because low count MBL has significant differences from CLL, an extremely limited, if any, chance of progression, and, until new evidence is provided, does not require routine follow-up outside of standard medical care. In contrast, high count MBL requires routine/yearly follow-up, and has very similar phenotypic and genetic/molecular features as Rai stage 0 of CLL.

- The best candidates for tissue-based MBL: lymph nodes with CLL/SLL in which proliferation centers were not observed and patients in whom adenopathy was <1.5 cm based on CT scans
Hairy cell leukemia

- Almost all cases of hairy cell leukemia (HCL) have BRAF V600E mutations.

- HCL without BRAF V600E mutations and half of HCL-variant (HCL-v): use IGHV4-34, have mutations in MAP2K1 which encodes MEK1 (which is downstream of BRAF).

Lymphoplasmacytic lymphoma (LPL)

• MYD88 L265P mutation in vast majority of cases (90%) impacting diagnostic criteria even though finding is not specific for LPL.

• IgM MGUS is more closely related to LPL and other B-cell lymphomas than to myeloma.

-CXCR4 mutations are found in about 30% of LPL.

-IgM MGUS: serum monoclonal IgM < 30 g/L; BM lymphoplasmacytic infiltrate <10%; no anemia/hyperviscosity/lymphadenopathy/hepatosplenomegaly.

-Non IgM MGUS: as previously defined

-Light-chain MGUS: abnormal FLC ratio (<0.26 or >1.65); clonal plasma cells <10%; no CRAB/amyloidosis

-Additional criteria for plasma cell myeloma (only need one): clonal plasma cells in BM \geq 60%; serum FLC ratio \geq 100; >1 bone lesion on MRI

-TEMPI syndrome (telangiectasias, elevated erythropoietin/erythropoiesis, monoclonal gammopathy, perinephric fluid collection, intrapulmonary shunting)

Follicular lymphoma (FL)

• Mutational landscape better understood but clinical impact remains to be determined (no recommended mutations at this time).

In situ follicular neoplasia (ISFN)

• Have a low rate of progression, but are more often associated with prior or synchronous overt lymphomas, thus requiring additional clinical assessment. Flow cytometric studies demonstrate populations of B cells with a FL-type phenotype in about half of all lymph nodes with ISFN.

Pediatric-type FL

-Pediatric FL now known as pediatric-type FL because similar lymphomas may occur in young and rarely in older adults. It is a nodal disease characterized by large expansile highly proliferative follicles that often have prominent blastoid follicular center cells rather than classic centroblasts (or centrocytes). Morphology is similar to FL-Gr 3; however grading should not be done in pediatric-type FL. BCL2 rearrangements must not be present, but there may be some BCL2 protein expression. They also lack BCL6 and MYC rearrangements with ongoing investigations of their genetic/molecular landscape.

- Nearly all cases are localized and may not require treatment other than excision.

-The criteria for pediatric-type FL, however, must be strictly applied to avoid underdiagnosing conventional grade 3 FL, with particular caution required before making this diagnosis in an adult.

Large B-cell lymphoma with IRF4 rearrangement

• New provisional entity to distinguish from pediatric-type FL and other DLBCL.

• Localized disease, often involves cervical lymph nodes or Waldeyer ring. Occurs most commonly in children and young adults, most typically occurs in Waldeyer ring and/or cervical lymph nodes and are low stage. They may have a follicular, follicular and diffuse, or pure diffuse growth pattern resembling FL grade 3B or a DLBCL. Strong IRF4/MUM1 expression is seen

usually with BCL6 and a high proliferative fraction. BCL2 and CD10 are also expressed in more than half of the cases with a minority CD5+. They are most often of germinal center type, particularly based on gene expression profiling (GEP) studies. Most cases have IG/IRF4 rearrangements (tested with FISH) sometimes together with BCL6 rearrangements but they uniformly lack BCL2 rearrangements. This lymphoma is considered to be more aggressive than other pediatric-type FL but patients, at least when treated, have done very well.

Duodenal-type FL

- Having features of a localized overt low-grade FL (typically Gr 1 or 2), is distinct from other GI tract FL, and has many features that overlap with ISFN as well as some features resembling an extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue. Localized process with low risk for dissemination. These patients appear to have an excellent outcome, including some cases managed with a watch-and-wait strategy.

Predominantly diffuse FL with 1p36 deletion

- Accounts for some cases of diffuse FL, lacks BCL2 rearrangement; presents as localized mass, often inguinal.

Mantle cell lymphoma (MCL)

- Two MCL subtypes recognized: one largely with unmutated/minimally mutated IGHV and mostly SOX11+ and the other largely with mutated IGHV and mostly SOX11(-) (indolent leukemic non-nodal MCL with PB, bone marrow (BM), splenic involvement, may become more aggressive with secondary abnormalities, often involving TP53).
- Mutations of potential clinical importance, such as TP53, NOTCH 1/2, recognized in small proportion of cases.
- CCND2 rearrangements in approximately half of cyclin D1(-) MCL cases. These cases have high Cyclin D2 expression (not a specific finding). They need to be diagnosed with positive SOX11.

In situ mantle cell neoplasia (ISM CN)

- New name for in situ MCL, reflecting low clinical risk.
- Characterized by the presence of cyclin D1+ cells, most typically in the inner mantle zones of follicles, in lymphoid tissues that do not otherwise suggest the diagnosis of a MCL, and is often found incidentally, sometimes in association with other lymphomas.

Diffuse large B-cell lymphoma, NOS

- Distinction of GCB vs ABC/non-GC type is required with use of immunohistochemical algorithm acceptable (Hans'), may affect therapy (clinical trials show favorable response of ABC subtype to R-CHOP combined with Lenalidomide).
- Most of DLBCL with concomitant expression of MYC/BCL2 do not carry MYC/BCL2 chromosomal alterations and have been named "double-expressor lymphoma.". Most studies use a cutoff of 40% MYC-expressing cells to define these cases; the cutoff for BCL2 expression 50% is recommended. In several but not all studies, the double-expressor lymphomas have a worse outcome than other DLBCL, NOS but they are not as aggressive as the HGBL, with rearrangements of MYC and BCL2 and/or BCL6.
- Mutational landscape better understood but clinical impact remains to be determined.

EBV+ DLBCL, NOS

- This term replaces EBV+ DLBCL of the elderly because it may occur in younger patients.
- Does not include EBV+ B-cell lymphomas that can be given a more specific diagnosis.

EBV+ mucocutaneous ulcer

- Newly recognized entity associated with iatrogenic immunosuppression or age-related immunosenescence, typically with indolent course. Large B cells with background of numerous T cells (mostly CD8-positive). The most common site is oral cavity including gingiva.

HHV8-associated lymphoproliferative disorders

-HHV8-positive multicentric Castleman disease: generalized lymphadenopathy, splenomegaly, constitutional symptoms. Abnormal follicles, plasmablasts predominantly in mantle zones, interfollicular plasma cell hyperplasia

B-cell antigens +/-, clgM lambda +, IRF4a +, CD138-, polyclonal, HHV8+, EBER-, HIV+/-
Poor prognosis but improved with new therapies

-HHV8-positive DLBCL, NOS: large lymph node, splenic mass, extranodal sites, peripheral blood. Sheets of large plasmablastic cells

B-cell antigens +/-, clgM lambda +, IRF4a +, CD138-, monoclonal, HHV8+, EBER-, HIV+/-
Poor prognosis

-HHV8-positive germinotropic lymphoproliferative disorder: localized or sometimes multifocal lymph node involvement. Retention of architecture with germinal centres containing variable numbers of plasmablasts sometimes replacing follicles

B-cell antigens-, monotypic kappa or lambda+, any heavy chain+, CD138-, CD30 +/-, polyclonal or oligoclonal, HHV8+, EBER+, HIV-

Usually responds to treatment

Burkitt lymphoma

- TCF3 or ID3 mutations in up to ~70% of cases.

Burkitt-like lymphoma with 11q aberration (gain or loss)

- New provisional entity that closely resembles Burkitt lymphoma but lacks MYC rearrangement and has some other distinctive features.

High-grade B-cell lymphoma (HGBL), with MYC and BCL2 and/or BCL6 translocations

- The old “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL” (BCLU)”-> now designated HGBL, with MYC and BCL2 and/or BCL6 rearrangements (“double-/triple-hit” lymphomas).

-A consensus has not yet been reached to provide specific guidelines as to which LBCL should have fluorescence in situ hybridization studies. Some believe that all DLBCL should have genetic studies for the detection of MYC, BCL2, and BCL6 rearrangements, whereas others would limit them, for example, to cases with a GCB phenotype and/or high-grade morphology or to cases with >40% MYC+ cells.

High-grade B-cell lymphoma, NOS

- Includes blastoid-appearing large B-cell lymphomas and cases lacking MYC and BCL2 or BCL6 translocations that would formerly have been called BCLU.

Rare cases of B-lymphoblastic leukaemia/lymphoma with MYC and BCL2 translocations

sometimes transformed from an antecedent or synchronous follicular lymphoma, are not included in double-hit high grade B cell lymphoma category, and should be classified as B lymphoblastic leukaemia/lymphoma with the translocations specified in a comment. Patients with such cases are usually treated like patients with lymphoblastic leukaemia

T-cell large granular lymphocytic leukemia

- New subtypes recognized with clinicopathologic associations.
- STAT3 and STAT5B mutations in a subset, latter associated with more clinically aggressive disease.

Systemic EBV+ T-cell lymphoma of childhood

-Name changed from lymphoproliferative disorder to lymphoma due to its fulminant clinical course and desire to clearly distinguish it from chronic active EBV infection.

-Increased frequency in Asians, and in indigenous populations from Central and South America and Mexico.

-Has a fulminant clinical course usually associated with a hemophagocytic syndrome.

Phenotype: CD2+, CD3+, CD56-, TIA1+. Most cases are secondary to acute primary EBV infection and are CD8+, whereas cases occurring in the setting of severe chronic active EBV are CD4+. Rare cases show both CD4+ and CD8+. EBER is positive.

Hydroa vacciniforme-like lymphoproliferative disorder

-Name changed from lymphoma to lymphoproliferative disorder due to its relationship with chronic active EBV infection and a spectrum in terms of its clinical course.

-Increased frequency in Asians, and in indigenous populations from Central and South America and Mexico.

-The cells have a cytotoxic T-cell phenotype, mostly CD8-positive, with few cases CD4-positive. One third of the cases show expression of CD56. CD30 is often expressed in the infiltrating EBV-positive T cells.

Chronic active EBV infection of T and NK cell type

-Shows a broad range of clinical manifestations from indolent, localized forms (like hydroa vacciniforme-like LPD and severe mosquito bite allergy) to a more systemic form characterized by fever, hepatosplenomegaly, and lymphadenopathy with or without cutaneous manifestations.

-The immunophenotype of the EBV-infected cells varies: T cells in 59% of cases, NK cells in 41%, and both T and NK cells in 4%. The T cells in chronic active EBV infection are predominantly CD4-positive. EBER is positive.

Enteropathy-associated T-cell lymphoma (EATL)

• Diagnosis only to be used for cases formerly known as type I EATL, typically associated with celiac disease, primarily a disease of individuals of northern European origin.

-EATL generally has a polymorphic cellular composition and wide range in cytology.

-Most EATL cases express TCR $\alpha\beta$; negative for CD4, CD8, CD56

Monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL)

• Formerly type II EATL; segregated from type I EATL and given a new name due to its distinctive nature and lack of association with celiac disease.

-Appears increased in incidence in Asians, and Hispanic populations.

-MEITL is monomorphic, and usually positive for CD8, CD56, and MATK

-Gains in chromosome 8q24 involving MYC are seen in a high proportion of cases.

-Many cases of MEITL are derived from $\gamma\delta$ T cells.

Indolent T-cell lymphoproliferative disorder of the GI tract

• New provisional entity with superficial monoclonal intestinal T-cell infiltrate, usually composed of CD3/CD8(+) T cells, with an indolent clinical course, some cases show progression. Their optimal management is not yet determined.

Lymphomatoid papulosis

-Variable loss of pan-T-cell antigens (CD2, CD3, CD5, CD7); CD30-positive; cytotoxic granule associated proteins are expressed in most cases; ALK-negative

-New subtypes described with similar clinical behavior but atypical histologic/immunophenotypic features:

<u>Subtype</u>	<u>Frequency</u>	<u>Phenotype</u>	<u>Morphology</u>
A	>80%	CD4+/CD8-	Many RS-like cells are present together with numerous inflammatory cells
B	<5%	CD4+/CD8-	Small atypical (cerebriform) lymphocytes-Mimic MF
C	~10%	CD4+/CD8-	Large lymphocytes with few inflam cells-Mimic C-ALCL
D	<5%	CD4-/CD8+	Small-intermediate lymphocytes. Mimic C-CD8 pos cytotoxic TCL
E	<5%	CD4-/CD8+	Small-intermediate lymphocytes, necrosis, angiodestructive. Mimic Extranodal NK/TCL
*	<5%	CD4-/CD8+ CD4-/CD8-	Small-intermediate lymphocytes (cerebriform) lymphocytes-Mimic Tumor stage MF

* with chromosome 6p25 rearrangement at IRF4/DUSP22 locus (detected by FISH)

Primary cutaneous $\gamma\delta$ T-cell lymphoma

- Cutaneous $\gamma\delta$ T-cell lymphoma are generally aggressive. Important to exclude other cutaneous T-cell lymphomas/lymphoproliferative disorders that may also be derived from $\gamma\delta$ T cells such as mycosis fungoides or lymphomatoid papulosis. Phenotypes: TCR $\gamma\delta$, CD3+, CD4-, CD8-/+ , Cytotoxic molecules + , CD56+, EBV-. This entity includes previous subcutaneous panniculitis-like TCL with TCR $\gamma\delta$. Note that phenotypes of subcutaneous panniculitis-like TCL are: TCR $\alpha\beta$ +, CD3+, CD4 -, CD8+, Cytotoxic molecules+, CD56-, EBV -

Primary cutaneous acral CD8+ T-cell lymphoma

- New indolent provisional entity, originating in the ear. This needs to be excluded from primary cutaneous CD8+ aggressive TCL.

Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder

- No longer to be diagnosed as an overt lymphoma due to limited clinical risk, localized disease, and similarity to clonal drug reactions.

-The cells have a TFH phenotype.

- Remains a provisional entity.

Peripheral T-cell lymphoma (PTCL), NOS

- Subsets based on phenotype and molecular abnormalities being recognized that may have clinical implications but are mostly not a part of routine practice at this time.

Nodal T-cell lymphomas with T-follicular helper (TFH) phenotype

- An umbrella category created to highlight the spectrum of nodal lymphomas with a TFH phenotype including angioimmunoblastic T-cell lymphoma, follicular T-cell lymphoma, and other nodal PTCL with a TFH phenotype (specific diagnoses to be used due to clinicopathologic differences).

-T follicular helper (TFH) phenotype: the neoplastic cells should express at least 2 or 3 TFH-related antigens, including CD279/PD1, CD10, BCL6, CXCL13, ICOS, SAP, and CCR5.

- Overlapping recurrent molecular/cytogenetic abnormalities recognized that potentially could impact therapy.

ALK(-) anaplastic large-cell lymphoma

- Now a definite entity that includes cytogenetic subsets that appear to have prognostic implications:
 - A subset with 6p25 rearrangements at IRF4/DUSP22 locus (detected by FISH) tends to be relatively monomorphic, usually lack cytotoxic granules, and have been reported to have a superior prognosis, similar to that of ALCL-ALK(+). This 6p25 rearrangements at IRF4/DUSP22 locus is also seen in a subset of lymphomatoid papulosis cases.
 - A small subset of cases with TP63 rearrangements are very aggressive.

Breast implant-associated anaplastic large cell lymphoma

- New provisional entity distinguished from other ALK(-) ALCL; noninvasive disease associated with excellent outcome.
 - Presents as an accumulation of seroma fluid between the implant itself and the surrounding fibrous capsule. Both saline- and silicone-filled implants have been implicated, with a median interval from the time of the implant to the lymphoma of about 10 years. In most cases, the neoplastic cells are confined to the seroma fluid, without invasion of the capsule. In such cases, conservative management is recommended, with removal of the implant and capsule. If there is invasion through the capsule, there is risk of lymph node involvement and systemic spread, warranting systemic chemotherapy.

Nodular lymphocyte-predominant Hodgkin lymphoma

- Variant growth patterns, if present, should be noted in diagnostic report, due to their clinicopathologic associations: (A) Classic B cell rich nodular, (B) Serpiginous nodular, (C) Nodular with many extranodular LP cells, (D) T cell rich nodular, (E) Diffuse, T cell rich (TCRBCL like), (F) Diffuse moth-eaten , B cell rich.
- Cases associated with synchronous or subsequent sites that are indistinguishable from T-cell histiocyte-rich large B-cell lymphoma (THRLBCL) without a nodular component (lacking any follicular dendritic cells) should be designated THRLBCL-like transformation of NLPHL. Progression to a process with features of THRLBCL is associated with a more aggressive clinical course, and requires different management, such that the term NLPHL in this setting may not be sufficient.

Lymphocyte-rich classical Hodgkin lymphoma

- Features recognized that are intermediate between NLPHL and other types of classical Hodgkin lymphoma.

Post-transplant lymphoproliferative disorder (PTLD)

- Categories: (a) Non-destructive: plasmacytic hyperplasia, infectious mononucleosis, florid follicular hyperplasia; (b) Polymorphic; (c) Monomorphic: DLBCL, BL, plasma cell myeloma, plasmacytoma, EBV-positive MZL, PTCL-NOS, hepatosplenic TCL
- Light-chain restriction may be seen focally (subset) in polymorphic PTCL. However if a clear-cut light chain restriction is seen one should consider monomorphic PTLD

Erdheim-Chester disease

- Seen mostly in adults with bone and lung involvement
- Often associated with BRAF mutations
- Should be distinguished from other members of the juvenile xanthogranuloma family

Other histiocytic/dendritic neoplasms

- Clonal relationship to lymphoid neoplasms recognized in some cases.
 - Some of these neoplasms are associated with or preceded by FL, CLL, B- or T-lymphoblastic neoplasms, or PTCL. These cases carry the same TCR or IGHV rearrangements and chromosomal aberrations as the associated lymphoid neoplasms, suggesting a process of trans-differentiation.
 - The BRAF V600E mutation has been reported in the setting of Langerhans cell histiocytosis, histiocytic sarcoma, disseminated juvenile xanthogranuloma, Erdheim-Chester disease, and follicular dendritic cell sarcoma.
 - Indeterminate dendritic cell tumour (also known as indeterminate cell histiocytosis): proliferation of spindle to ovoid cells with phenotypic features similar to those of normal indeterminate cells, the precursor cells of Langerhans cells. The infiltrate is diffuse, consisting of cells resembling Langerhans cells. An accompanying eosinophilic infiltrate is usually not present. The tumor cells are positive for S100 protein and CD1a, negative for Langerin.
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Tests currently available from Genoptix for specific malignancy in the WHO revised 4th Edition

- CML-AP**: abl1 kinase domain mutation (PCR)
 - AML with bcr-abl1**: Bcr/abl1 by FISH or PCR
 - CNL**: CSF3R mutation in Next-Gen Sequencing/MPN Target Profile (a total of 5 genes)
 - MDS-RS**: SF3B1 mutation in Next-Gen Sequencing/AML Molecular Profile (a total of 21 genes)
 - aCML**: SETBP1 mutation in Next-Gen Sequencing/MPN Target Profile (a total of 5 genes)
 - JMML**: PTPN11, KRAS, NRAS, CBL mutations in Next-Gen Sequencing/Myeloid Molecular Profile (a total of 40 genes)
 - AML with RUNX1**: RUNX1 mutation in Next-Gen Sequencing/AML Molecular Profile (a total of 21 genes)
 - MDS with del(5q)**: TP53 mutation in Next-Gen Sequencing/AML Molecular Profile (a total of 21 genes)
 - HCL**: BRAF V600E (PCR); MAP2K1 mutation in Next-Gen Sequencing/NexCourse Complete (a total of 173 genes)
 - LPL**: MYD88 mutation (PCR); CXCR4 mutation in Next-Gen Sequencing/Lymphoid Molecular Profile (a total of 75 genes)
 - BL**: TCF3, ID3 mutations in Next-Gen Sequencing/Lymphoid Molecular Profile (a total of 75 genes)
 - T-LGL**: STAT3, STAT5B mutations in Next-Gen Sequencing/Lymphoid Molecular Profile (a total of 75 genes)
 - BCR/ABL Like ALL**: ALL (Ph-like) FISH Profile
 - Myeloid/lymphoid neoplasm with eosinophilia and PCM1-JAK2**: Eosinophilia FISH profile
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Complete List of Genoptix Tests for FISH / PCR / NGS

FISH PANELS

ALL FISH Profile: TCF3/PBX1 t(1;19), BCR-ABL1-ASS1 t(9;22), CDKN2A (9p21.3), KMT2A (MLL) 11q23.3, ETV6/RUNX1 t(12;21), IGH (14q32.3), TP53

ALL FISH Profile (pediatric): Cen 4, Cen 10(+4/+10), BCR-ABL1-ASS1 t(9;22), KMT2A (MLL) 11q23.3, ETV6/RUNX1 t(12;21), IGH (14q32.3)

ALL (Ph-like) for bcr-abl1-like ALL: PDGFRB (5q32), BCR/ABL1-ASS1 t(9;22), JAK2(9p24.1), EPOR (19p13.2), CRLF2(Xp22.33/Yp11.32)

AML FISH Profile: MECOM 3q26.2, DEK/NUP214 t(6;9), RUNX1T1/RUNX1 t(8;21), KMT2A (MLL) 11q23.3, PML-RARA t(15;17), CBFβ/MYH11 inv(16)

CLL FISH Profile: : IGH/CCND1 t(11;14), ATM(11q22.3), Cen 12(+12), 13q(13q-/-13), TP53(17p13.1)

CML FISH Profile: BCR-ABL/ASS1 t(9;22), +8 (8cen), RARA (17q21)

Myeloma FISH Profile: CKS1B/CDKN2C (1p32.3/1q21.3), 5q(5q-/-5/+5), 13q (13q-/-13), TP53(17p13.1), IGH/FGFR3 t(4;14), IGH/CCND1 t(11;14), IGH/MAF t(14;16), IGH/MAFB t(14;20)

MDS FISH Profile (Standard): 5q(5q-/-5/+5), 7q(7q-/-7), Cen 8(+8), 20q (20q-), KMT2A(MLL) 11q23.3

Eosinophilia FISH Profile: PDBGRα (4q12), PDGFRβ (5q32), FGFR1 (8p11), JAK2 (9p24.1)

MPN FISH Profile: CKS1B/ CDKN2C (1p/1q), 5q(5q-/-5/+5), 7q(7q-/-7), Cen 8 (+8), 20q (20q-), BCR/ABL1 ASS1 t(9;22), 13q(13q-/-13)

B-NHL FISH profile (Low grade B cell): BCL6 (3q27.3), 6q(6q-/-6), MYC (8q24.21), IGH/CCND1 t(11;14), IGH/BCL2 t(14;18), MALT1 (18q21)

B-NHL High Grade FISH profile (High grade B cell): BCL6 (3q27.3), IRF4/DUSP22(6p25.3), MYC (8q24.21), IGH/BCL2 t(14;18)

MOLECULAR TESTS/PROFILES

ABL1 Kinase Domain Mutation: DNA sequence of targeted regions of ABL (exons 4, 6-7) is determined using next-generation sequencing (NGS) technology.

AML Molecular Profile 24 gene mutations by NGS (ASXL1, BCOR, CEBPA, CSF3R, DNMT3A, EZH2, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, PHF6, PTPN11, RAD21, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2)

MPN Target (Molecular) Profile (NGS): CALR, CSF3R, JAK2, MPL and SETBP1

Myeloid Molecular Profile (NGS for 21 genes): ASXL1, BCOR, BRAF, CALR, CEBPA, CBL, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRa, PDGFRb, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1 and ZRSR2

B-Cell Clonality Assessment: to detect clonal B-cell immunoglobulin heavy chain (IGH) gene rearrangement by polymerase chain reaction (PCR) of variable and joining regions on chromosome 14.

T-Cell Clonality Assessment (Gamma): T-cell receptor Gamma gene variable and joining regions on chromosome 7.

Cytogenetics, FISH, and PCR Testing for Hematopathology Samples Protocol

NOTES: for patient already in remission, we will just order tests to cover known positive abnormalities from previous diagnostic bone marrow (translocations, mutations, etc). Only for patients still refractory or with relapse we would order all tests as for new cases to cover any genomic evolution that yield new abnormalities.

1. AML

(a) Sample in green-top tube for

-Cytogenetics

-AML FISH Profile [MECOM 3q26.2, DEK/NUP214 t(6;9), RUNX1T1/RUNX1 t(8;21), KMT2A (MLL) 11q23.3, PML-RARA t(15;17), CBFB/MYH11 inv(16)]

(b) Sample in lavender-top tube for PCR testing for

-Quantitative BCR/ABL1

-FLT3 (ITD/TKD)

-AML Molecular Profile - 24 gene mutations by NGS (ASXL1, BCOR, CEBPA, CSF3R, DNMT3A, EZH2, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, PHF6, PTPN11, RAD21, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, ZRSR2)

-Quantitative PML/RARA for diagnostic and follow-up bone marrows with APL cases

-For AML cases with known t(8;21) or inv(16), typically by FISH from baseline BM, we will send for PCR with f/u BMs. Send a separate purple-top tube to Lab Corp, ordered with a down-time test order slip; alert send-out section so that they can work on the approval process to send to third party lab.

2. ALL

(a) Sample in green-top tube for

-Cytogenetics

-ALL FISH Profile [TCF3/PBX1 t(1;19), BCR-ABL1-ASS1 t(9;22), CDKN2A (9p21.3), KMT2A (MLL) 11q23.3, ETV6/RUNX1 t(12;21), IGH (14q32.3), TP53]

For pediatric: ALL FISH Profile (pediatric) [Cen 4, Cen 10(+4/+10), BCR-ABL1-ASS1 t(9;22), KMT2A (MLL) 11q23.3, ETV6/RUNX1 t(12;21), IGH (14q32.3)]

ALL (Ph-like) for bcr-abl1-like ALL [PDGFRB (5q32), BCR/ABL1-ASS1 t(9;22), JAK2(9p24.1), EPOR (19p13.2), CRLF2(Xp22.33/Yp11.32)]

b) Sample in lavender-top tube for PCR testing for

-Quantitative BCR/ABL1

3. CML

(a) Sample in green-top tube for

-Cytogenetics

-CML FISH profile

(b) Sample in lavender-top tube for PCR testing for
-Quantitative BCR/ABL1 (diagnostic and follow-up bone marrows)

(c) For detection of resistance to Imatinib Mesylate if clinically indicated: Abl1 Kinase Domain Mutation Analysis (sample in purple-top tube for PCR)

4. PV

(a) Sample in green-top tube for
-Cytogenetics

(b) Sample in lavender tube for PCR: JAK2 V617F mutation (if negative reflex to JAK2 Exon 12 mutation)

5. ET, PM, MPN-NOS, MPN/MDS

(a) Sample in green-top tube for
-Cytogenetics
-MPN FISH profile: CKS1B/ CDKN2C (1p/1q), 5q(5q-/-5/+5), 7q(7q-/-7), Cen 8 (+8), 20q (20q-), BCR/ABL1 ASS1 t(9;22), 13q(13q-/-13)

(b) Sample in lavender tube for MPN Target (Molecular) Profile (Next-gen seq): CALR, CSF3R, JAK2, MPL and SETBP1

6. Myeloid and Lymphoid Neoplasms with Eosinophilia

Sample in green-top tube for
-Cytogenetics
-Eosinophilia FISH profile: PDBGR α (4q12), PDGFR β (5q32), FGFR1 (8p11), JAK2 (9p24.1)

7. PLASMA CELL MYELOMA

Sample in green top tube for
-Cytogenetics
-Myeloma FISH Profile (standard): CKS1B/CDKN2C (1p32.3/1q21.3), 5q(5q-/-5/+5), 13q (13q-/-13), TP53(17p13.1), IGH/FGFR3 t(4;14), IGH/CCND1 t(11;14), IGH/MAF t(14;16), IGH/MAFB t(14;20)

8. CLL

(a) Sample in green top tube for
-Cytogenetics
-CLL FISH Profile: IGH/CCND1 t(11;14), ATM(11q22.3), Cen 12(+12), 13q(13q-/-13), TP53(17p13.1)

(b) Sample in lavender-top (baseline bone marrow only) for
-IgVH Hypermutation analysis

9. MDS

Sample in green top tube for

-Cytogenetics

-MDS FISH Profile (standard): 5q(5q-/-5/+5), 7q(7q-/-7), Cen 8(+8), 20q (20q-), KMT2A(MLL) 11q23.3

10. B cell Non-Hodgkin Lymphoma FISH Panel, if needed for diagnosis

(bone marrow aspirate or unstained slides of bone marrow clot)

-B-NHL FISH profile (for low grade B cell): BCL6 (3q27.3), 6q(6q-/-6), MYC (8q24.21), IGH/CCND1 t(11;14), IGH/BCL2 t(14;18), MALT1 (18q21)

-B-NHL High Grade FISH profile (for high grade B cell): BCL6 (3q27.3), IRF4 DUSP22(6p25.3), MYC (8q24.21), IGH/BCL2 t(14;18)

11. Myeloid Molecular Profile, if needed for diagnosis of MDS; not universally adopted

Sample in lavender-top tube for Next-Gen Sequencing testing:

ASXL1, BCOR, BRAF, CALR, CEBPA, CBL, CSF3R, DNMT3A, ETV6, EZH2, GATA2, GNAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRa, PDGFRb, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5b, TET2, TP53, U2AF1, WT1 and ZRSR2

12. LPL

Sample in lavender-top tube for PCR testing: MYD88 (write down test in “Notes” since it is not in menu)

13. HCL

(a) Sample in green top tube for
-Cytogenetics

(b) Sample in lavender-top for
-BRAF by PCR

SOLID TISSUE (lymph node, bone marrow clot)

Note that decalcified bone marrow biopsy is not accepted

FISH sample for solid tissue:

-Unstained slides at 5 micron thickness
-Minimum 2 slides for each probe; add 2 extra slides

PCR sample for solid tissue:

-Unstained slides at 10 micron thickness
-Minimum 2 slides for each probe; add 2 extra slides

EBER sample for solid tissue (technical only, to be read by us):

-3 unstained slides at 5 micron thickness