GUIDELINES FOR DIAGNOSTIC WORKUP OF HEMATOLYMPHOID NEOPLASMS

1. B-CELL NON-HODGKIN LYMPHOMA

1.1 Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is a group of aggressive large B-cell neoplasms. CD10, BCL-6, and MUM-1 expression pattern can be used to distinguish germinal center B-cell (GCB) and activated B-cell (ABC/non-GCB) types. MYC and BCL-2 expression defines “double expressor” phenotype. CD5+ de novo DLBCL has a more aggressive course and may present with a more advanced stage. EBV+ DLBCL is a distinct subtype of DLBCL and EBV as a target may have specific therapeutic implications. Ki-67 assesses proliferation rate and is prognostically relevant. CD30+ DLBCL can be treated with anti-CD30 therapy (brentuximab). Plasmablastic lymphoma is typically EBER+, CD138+, monoclonal kappa or lambda, and CD56 negative. EBER and CD56 are useful to distinguish plasmablastic lymphoma from plasmacytoma. BCL-1 is useful to distinguish pleomorphic mantle cell lymphoma from de novo CD5+ DLBCL. ALK+ DLBCL is very rare and usually has immunoblastic/plasmablastic morphology. FISH for MYC with upfront or reflex BCL-2 and BCL-6 should be performed in all DLBCL to assess for double/triple hit lymphoma.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD20, CD3, CD5, CD10, CD30, BCL-2, BCL-6, MYC, MUM-1, Ki-67, EBER (11)
- CD56, CD138, Kappa, Lambda (for plasmablastic lymphoma), ALK (if plasmablastic ruled out)
- BCL-1 (if CD5+)
- CD23 (if primary mediastinal type)

**Other ancillary studies**
- FISH for MYC, BCL-2, BCL-6

1.2 Burkitt Lymphoma

Burkitt lymphoma (BL) is a rapid growing B-cell neoplasm. Ki-67 proliferation rate is virtually 100% in all BL. BL arises from germinal center B cells that express CD10 and BCL-6. MYC is expressed and is driven by MYC/IG translocation. BCL-2 expression is typically negative. Approximately 30% of sporadic and HIV associated BL is associated with EBV. TdT and CD34 are used to rule out lymphoblastic lymphoma if necessary. FISH for MYC is recommended for all BL.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD20, CD3, CD10, BCL-2, BCL-6, Ki-67, MYC, EBER
- TdT, CD34 (as needed)

**Other ancillary studies**
- FISH for MYC
1.3 Lymphoblastic Lymphoma (B and T cell Types)

Lymphoblastic lymphomas (LBL) are precursor B-cell and T-cell neoplasms. Coexisting leukemic forms are usually present. For tissue diagnosis, flow cytometry provides phenotype which is usually sufficient for the diagnosis along with morphology. In the event of tissue only without flow cytometry, a panel of immunohistochemistry should be performed per discretion of the pathologist. For workup of T-LBL in the mediastinum, absence or minimal cytokeratin staining is useful to distinguish LBL from thymoma.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD34, TdT (as needed);
- Pan-cytokeratin, CD1a, other T-cell markers (for T-LBL as needed);
- CD79a, CD10, other B-cell markers (for B-LBL as needed)

**Other ancillary studies**
- FISH (as needed)

1.4 Follicular Lymphoma

Typical follicular lymphoma (FL) can be readily diagnosed based on morphology and expression of BCL-2. Ki-67 is used to assess proliferation, especially in low grade lesions. High proliferation in low grade FL may be more aggressive than typical low grade lesions. Strong and uniform MUM-1 expression in FL may indicate presence of IRF4 rearrangement (in head and neck location). Typical FL does not require FISH. However, FISH for BCL-2 is recommended in the diffuse variant and in cases with negative BCL-2 by immunohistochemistry. FISH for IRF4 should be performed in MUM1+ head/neck/Waldeyer’s ring cases and FISH for 1p36 should be performed in diffuse variant in inguinal cases.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD20, CD3, BCL-2, Ki-67;
- CD10, BCL-6 (optional);
- CD21 (in diffuse variant);
- MUM-1 (in localized head & neck type and Waldeyer’s ring variants)

**Other ancillary studies**
- FISH for BCL-2 for diffuse variant;
- FISH for IRF4 for MUM-1+, localized head & neck type and Waldeyer’s ring variants;
- FISH for 1p36 for inguinal diffuse variant

1.5 Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is a B-cell neoplasm that expresses BCL-1 (cyclin D1) in the vast majority of the cases. SOX11 is positive in virtually all MCL and is useful to diagnose BCL-1 negative MCL. MCL is typically CD5+ and CD23-. TP53 expression and high Ki-67 proliferation rate indicate worse prognosis.
FISH for $BCL-1$ is only recommended for cases with atypical morphology/phenotype per discretion of the pathologist.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD20, CD3, CD5, CD23, Cyclin D1, TP53, Ki-67;
- Sox-11 (if cyclin D1 negative)

**Other ancillary studies**
- FISH for $BCL-1$ (if Cyclin D1 negative)

1.6 Marginal Zone Lymphoma (Nodal, Splenic, MALT)

Marginal zone lymphoma (MZL) is a group of indolent B-cell lymphomas which include nodal, splenic, and MALT types. MZL is typically CD5- and CD23-. CD43 is expressed in ~20-40% MZL and is useful to confirm the diagnosis. A fraction of MZL (more common in MALT) has plasma cell differentiation with monoclonal plasma cells. Residual germinal centers can be highlighted by CD10+, BCL-6+ and BCL-2-. Ki-67 proliferation rate is usually low. IGH is helpful to establish clonality especially in small biopsies. FISH for MALT1 can be performed in selected cases per discretion of the pathologist.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD20, CD3, CD5, CD10, CD21, CD43, BCL-2, BCL-6, Ki-67;
- CD138, Kappa, Lambda (in nodal and MALT types);
- H.pylori (in gastric type);

**Other ancillary studies**
- FISH for MALT1 (for MALT as needed)
- IGH (for gastric MALT as needed)

1.7 Small Lymphocytic Lymphoma (lymph node)

Small lymphocytic lymphoma (SLL) is CD5+ and CD23+, and has low Ki-67 proliferation rate. LEF-1 is specific for SLL/CLL and can be used in questionable cases. FISH for CLL panel can be performed on selected cases per discretion of the pathologist/oncologist.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD20, CD3, CD5, CD23, Ki-67
- LEF-1 (as needed)

**Other ancillary studies**
- FISH for CLL panel (optional, if not done on blood or bone marrow)
1.8 Lymphoplasmacytic Lymphoma (lymph node)

Nodal Lymphoplasmacytic lymphoma (LPL) usually has expanded interfollicular pattern or diffuse pattern. There are two neoplastic components: B-lymphoid and plasma cells. Lymphocytes are CD5-, CD10-, BCL-6-, and may be CD43+. The plasma cell component expresses monoclonal kappa or lambda. CD10, BCL-6, BCL-2 may identify reactive follicles, which is helpful for the diagnosis. Ki-67 proliferation rate is usually low. MYD88 mutation test is recommended for the initial diagnosis and is useful to distinguish LPL from marginal zone lymphoma with plasma cell differentiation.

Specimen Collection Requirement
H&E slides, touch preps, flow cytometry
cytogenetics (optional)

Immunohistochemistry
CD20, CD3, CD5, CD10, CD21, CD43, BCL-2, BCL-6, CD138, Kappa, Lambda, Ki-67

Other ancillary studies
NGS for MYD88 (for initial diagnosis)

2. T-CELL NON-HODGKIN LYMPHOMA

2.1 Peripheral T-Cell Lymphoma, NOS

Peripheral T-cell lymphoma NOS (PTCL) has frequent pan T-cell antigen loss (variable CD2, CD3, CD5, CD7) and typically expresses CD4. CD30 expression is variable. Strong uniform expression of PD-1 suggests T-zone variant. Some PTCL expresses cytotoxic phenotype (TIA-1+). A small number of PTCL expresses EBER. Ki-67 expression rate is variable and is usually high. TCR is recommended to confirm the clonality.

Specimen Collection Requirement
H&E slides, touch preps, flow cytometry
cytogenetics (optional)

Immunohistochemistry
CD20, CD2, CD3, CD4, CD5, CD7, CD8, CD56, CD30, PD-1, TIA-1, EBER, Ki-67
Granzyme B, Perforin (as needed);
CD10, BCL-6, CD21 (if PD-1+)

Other ancillary studies
TCR

2.2 Anaplastic Large Cell Lymphoma

Anaplastic large cell lymphoma (ALCL) is a CD30+ T-cell neoplasm which may not express T-cell antigens. CD43 is the most commonly expressed T-cell antigen. CD30 expression is uniform and strong. CD15 is always negative. LCA is variable and is more frequently negative. One or several cytotoxic antigens are positive, which are useful to distinguish ALCL from classical Hodgkin lymphoma. ALK is positive in a subset (defines ALK+ type). B-cell antigens (CD20, Pax-5, CD79a) are always negative. ALK expression is a reliable surrogate for ALK rearrangement; therefore FISH for MPN-ALK is usually not indicated. FISH for DUSP22 should be performed in ALK negative cases and DUSP22 rearranged cases have prognosis similar
to ALK+ type. If ALK and DUSP22 are both negative, FISH for TP63 may be performed as per discretion of the pathologist. Positive TP63 rearrangement indicates unfavorable prognosis.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD30, CD2, CD3, CD4, CD5, CD7, CD8, CD43, CD15, CD20, ALK, LCA, Ki-67;
- TIA-1, Granzyme B, Perforin (in ALK negative cases)

**Other ancillary studies**
- TCR
- FISH for DUSP22 (if ALK negative)
- FISH for TP63 (if ALK, DUSP22 negative) - optional

### 2.3 Angioimmunoblastic T-Cell Lymphoma

Angioimmunoblastic T-cell lymphoma (AITL) is a T-cell neoplasm with follicular helper T-cell phenotype. It expresses pan T-cell antigens (CD2, CD3, CD5, CD7) and CD4. One or more of the pan T-cell antigens may be negative. The diagnosis requires the expression of at least 2 (or 3) follicular helper T-cell antigens: CD10, BCL-6, PD-1, CXCR13, ICOS. CD30 expression is variable. EBER is positive in reactive B-immunoblasts. CD21 is useful to highlight expanded follicular dendritic cell meshwork, which is a useful feature in supporting the diagnosis. Ki-67 proliferation rate is variable. Both TCR and IGH are recommended. TCR is rearranged in all cases and IGH is rearranged in ~30%.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry
- Cytogenetics (optional)

**Immunohistochemistry**
- CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD30, CD21, CD10, BCL-6, PD-1, EBER, Ki-67
- CXCR13, ICOS (if <2 FHT markers+)

**Other ancillary studies**
- TCR, IGH

### 2.4 Adult T-Cell Lymphoma/Leukemia

Adult T-cell lymphoma/Leukemia (ATLL) is an HTLV1-associated T-cell neoplasm that expresses strong CD25, pan T-cell antigens (CD2, CD3, CD5, CD7), and CD4 (majority). One or more of the T-cell antigens may be negative. CD30 expression is variable. EBER and cytotoxic markers are negative. Ki-67 proliferation rate is variable. TCR is recommended to confirm clonality.

**Specimen Collection Requirement**
- H&E slides, touch preps, flow cytometry (CD25)
- Cytogenetics (optional)

**Immunohistochemistry**
- CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD30, EBER, Ki-67;
- TIA-1, Granzyme B, Perforin (as needed)
- CD25 (if flow cytometry not performed)
Other ancillary studies
TCR

2.5 Extranodal Peripheral T-Cell Lymphomas (Hepatosplenic, Nasal, Intestinal)

These entities include hepatosplenic T-cell lymphoma (HSTCL), extranodal NK/T-cell lymphoma (ENKTL), enteropathy-associated intestinal T-cell lymphoma (EATL), and monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL). These entities express CD56 (except for EATL) and pan T-cell antigens (with frequent loss of one or more). They usually express TIA-1 and variably express granzyme B and perforin. CD30 expression is variable and is not uniform. Ki-67 proliferation rate is variable. TCR is recommended to confirm clonality.

Specimen Collection Requirement
H&E slides, touch preps, flow cytometry cytogenetics (optional)

Immunohistochemistry
CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD56, CD30, EBER, TIA-1, Granzyme B, Perforin, Ki-67

Other ancillary studies
TCR

3. HODGKIN LYMPHOMA

3.1 Classical Hodgkin Lymphoma

Classical Hodgkin lymphoma (cHL) is CD30+, Pax-5+ (dim), CD15+ (variable intensity), and LCA-. Some cHLs express dim CD20. The background lymphocytes are predominantly T cells. EBER is positive in a subset. Clonality studies are usually not required for the typical cases.

Specimen Collection Requirement
H&E slides, flow cytometry

Immunohistochemistry
CD20, CD3, CD30, CD15, LCA, Pax-5, EBER;
OCT-2, BOB-1, MUM-1, EMA (as needed)

Other ancillary studies
IGH (to rule out primary mediastinal LBCL and gray zone lymphoma as needed)
TCR (to rule out ALCL as needed)

3.2 Nodular Lymphocyte Predominant Hodgkin Lymphoma

Nodular lymphocyte predominant Hodgkin lymphoma (LPHL) is CD20+ (uniform), Pax-5+ (strong), LCA+, CD30-/+ (dim, variable), CD15-. The background small lymphocytes are predominantly B cells. CD57+ T cells are increased in the background. PD-1+ T cells form rosette around LP cells. CD21 highlights follicular dendritic cell meshwork, which is especially useful in the diffuse variant.

Specimen Collection Requirement
H&E slides, flow cytometry
**4. CUTANEOUS LYMPHOMA**

4.1 Cutaneous T-Cell Lymphoma (punch or shave biopsy), other than CD30+ LPD

The majority of cutaneous T-cell lymphoma (CTCL) other than CD30+ LPDs are mycosis fungoides (MF). MF expresses pan T-cell antigens and CD4. One or more pan T-cell antigens may be negative (CD7 most common). CD30 expression is either negative or positive in rare cells. Increased CD30 expression suggests transformation. Ki-67 proliferation rate is low in patch/plaque stage and higher in tumor stage. Other rare types of CTCL include CD8+ type, γδ type, acral type, and small/medium cell type, which express CD8, TCR γδ, CD8, FDC antigens, respectively. TCR is usually necessary to confirm clonality.

**Specimen Collection Requirement**
- H&E slides

**Immunohistochemistry**
- CD20, CD3, CD30, CD4, CD5, CD7, CD8, CD30, Ki-67;
- add PD-1, CD10, BCL-6 if small/medium cell type;
- add TCR delta for γδ type

**Other ancillary studies**
- TCR

4.2 Cutaneous CD30+ Lymphoproliferative Disorder (punch or shave biopsy)

Primary cutaneous CD30+ lymphoproliferative disorder (CD30+ LPD) consists of primary cutaneous anaplastic large cell lymphoma (cALCL) and lymphomatoid papulosis (LYP). CD30 expression is consistent and strong in large cells, which may or may not express T-cell antigens. ALK is negative in large cells. Background lymphocytes are predominantly T cells. TCR is recommended for confirmation of clonality. Up to 50% LYP are TCR clonally rearranged. DUSP22 is rearranged in approximately 25% cALCL.

**Specimen Collection Requirement**
- H&E slides

**Immunohistochemistry**
- CD20, CD2, CD3, CD4, CD5, CD7, CD8, CD30, Ki-67, ALK

**Other ancillary studies**
- TCR
- FISH for DUSP22 (if ALK negative)
- FISH for TP63 (if ALK, DUSP22 negative) - optional

4.3 Cutaneous B-Cell Lymphoma (punch or shave biopsy)
Primary cutaneous B-cell lymphomas consist of cutaneous large B-cell lymphoma, leg type (cLBCL), cutaneous follicular lymphoma (cFL), and cutaneous marginal zone lymphoma (cMZL). cLBCL typically has an “activated B cell” phenotype with expression of MUM-1 and BCL-2, while cFL has an “altered germinal center B cell” phenotype with expression of BCL-6 but could be negative for CD10 and BCL-2. CD21 is useful to highlight follicular dendritic cells in cFL (especially in cases that lack nodular pattern) and in cMZL (to highlight areas with residual follicles). Expression of CD43 and monoclonal plasma cells are helpful for the confirmation of lymphoma. IGH is usually needed to confirm the B-cell clonality.

**Specimen Collection Requirement**
- H&E slides

**Immunohistochemistry**
- CD3, CD10, CD20, CD21, CD30, CD43, BCL-2, MUM-1, BCL-6, Kappa, Lambda, Ki-67

**Other ancillary studies**
- IGH

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## 5. LEUKEMIA AND OTHER BONE MARROW DISEASES

### 5.1 Acute Myeloid Leukemia

Most acute myeloid leukemia (AML) can be diagnosed by morphology and flow cytometry. Cases with discrepant flow cytometry and manual blast count and cases with borderline percentage of blasts require CD34 immunostain. For the initial diagnosis, ancillary studies including FISH, chromosome, FLT3, NGS should be performed. For the follow-up cases, chromosome, targeted FISH (initial FISH positive loci only), FLT3 (initial FLT3 ITD+ cases) are performed. MRD can be performed (send-out) when ordered by clinicians.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD34 (as needed), reticulin

**Other ancillary studies**
- Chromosome, FISH, FLT3, NGS, MRD (flow cytometry or genomic test)

### 5.2 Mixed Phenotype Acute Leukemia

Mixed phenotype acute leukemia (MPAL) consists of mostly mixed myeloid/B-lymphoid and mixed myeloid/T-lymphoid leukemias. The lineages are assigned based on the WHO guidelines. Flow cytometry often is sufficient to determine the lineages. If flow cytometry is ambiguous, immunohistochemistry may be helpful to determine the lineages.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD34, TdT, CD3, CD19, CD79a, CD68, MPO, E cadherin (for lineage assignment as needed);
5.3 Lymphoblastic Leukemia (B and T types)

Vast majority of lymphoblastic leukemia (ALL) can be diagnosed by flow cytometry and morphology. Immunohistochemistry is usually not indicated. FISH and chromosome are performed in all initial diagnosis. Target FISH panel and chromosome are indicated for the follow-up diagnosis. MRD is indicated in follow-up diagnoses and is performed per physician’s order.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD34, TdT (as needed)

**Other ancillary studies**
- Chromosome, FISH, NGS

5.4 Myelodysplastic Syndrome

Myelodysplastic syndrome (MDS) consists of a heterogeneous group of disorders characterized by variable degrees of dysplasia and percentages of blasts. Immunostains are used as needed. CD34 is useful to assess blasts. CD61, factor VIII highlight megakaryocytes. Glycophorin A and E cadherin highlight erythroid precursors. MPO stains for myeloid elements. FISH, chromosome, NGS should be performed on the initial specimens.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD34, CD61 or Factor VIII, Glycophorin A, E cadherin, MPO (as needed);
- Reticulin, iron

**Other ancillary studies**
- Chromosome, FISH, NGS

5.5 Chronic Myeloid Leukemia

Chronic myeloid leukemia (CML) usually does not require immunohistochemistry. In the accelerated phase, CD34 immunostain can be used to assess percentage of blasts. For the initial diagnosis, FISH, chromosome, and rtPCR should be performed. NGS to assess ABL kinase mutation is performed in refractory and relapsed cases.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics
Immunohistochemistry
CD34 (as needed), Reticulin

Other ancillary studies
Chromosome, FISH, rtPCR for BCRABL1; NGS for ABL kinase mutation (as needed)

5.6 Myeloproliferative Neoplasm, other than CML

Myeloproliferative neoplasm (MPN) other than CML consists of polycythemia vera (PV), essential thrombocytopenia (ET), primary myelofibrosis (PMF), and chronic neutrophilic leukemia (CNL). NGS to assess JAK2, CALR, MPL, CSF3R is essential. CD34 immunostain may be used to assess percentage of blasts. Reticulin (in all types) and trichrome (in PMF) are essential to assess fibrosis.

Specimen Collection Requirement
H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

Immunohistochemistry
Reticulin; Trichrome (for PMF); CD34 (as needed); E cadherin, glycophorin A, MPO, CD61 or factor VIII (as needed)

Other ancillary studies
Chromosome, FISH, NGS

5.7 Myelodysplastic/Myeloproliferative Neoplasm

Myelodysplastic/myeloproliferative neoplasm (MDS/MPN) aka overlapping syndromes consists of chronic myelomonocytic leukemia, juvenile chronic myelomonocytic leukemia, atypical chronic myeloid leukemia, MDS/MPN with ring sideroblasts and thrombocytosis, and MDS/MPN, unclassifiable. These entities are diagnosed using a combination of morphology, flow cytometry, cytogenetics, and NGS. CD34 immunostain is useful to assess percentage of blasts.

Specimen Collection Requirement
H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

Immunohistochemistry
CD34 (as needed), Reticulin, iron

Other ancillary studies
Chromosome, FISH, NGS

5.8 Eosinophilic neoplasms

Eosinophilic neoplasms include chronic eosinophilic leukemia (CEL), hypereosinophilic syndrome (HES), and myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement. Increase of blasts, clonal cytogenetic abnormalities, and/or molecular genetic abnormalities are present in CEL. CD34 immunostain is useful to detect increased blasts. FISH for eosinophil panels (PDGFRα, PDGFRβ, FGFR1) is necessary for the diagnosis of the lesions with defined gene rearrangements.
**Specimen Collection Requirement**
H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
CD34 (as needed)

**Other ancillary studies**
Chromosome, FISH for MPN and Eosinophilia panels, NGS

### 5.9 Mast cell neoplasms (bone marrow)
Systemic mastocytosis consists of a spectrum of mast cell neoplasms ranging from indolent lesions to aggressive mast cell leukemia. Mast cells are positive for CD117 and tryptase, and may aberrantly express CD25 and CD2. Nearly all mast cell neoplasms harbor KIT D816V mutation.

**Specimen Collection Requirement**
H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
CD117;
CD2, CD25, tryptase (as needed)

**Other ancillary studies**
Chromosome, FISH, NGS for C-KIT mutation

### 5.10 Hairy Cell Leukemia and Other Splenic B-cell Leukemias
Hairy cell leukemia (HCL) and other splenic B-cell leukemias are diagnosed by flow cytometry and blood/bone marrow morphology. Flow cytometry often underestimates the number of leukemic cells in bone marrow due to associated fibrosis. CD20 immunostain is useful to assess percentage of bone marrow involvement. BRAF immunostain (V600E specific) is useful for confirming HCL and is positive only in HCL and not in other types of splenic B-cell leukemias. NGS is generally not indicated if BRAF immunostain is positive.

**Specimen Collection Requirement**
H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
CD20, BRAF

**Other ancillary studies**
Chromosome, FISH;
NGS (for BRAF V600E as needed)

### 5.11 Chronic lymphocytic Leukemia (blood and bone marrow)
Chronic lymphocytic leukemia (CLL) is usually diagnosed on blood with flow cytometry and blood smear review. Bone marrow biopsy is performed in some patients. Immunohistochemistry is usually not indicated but can be performed as needed (such as to confirm diagnosis and assess amount of bone
marrow involvement). FISH for CLL panel should be performed on all new cases. NGS to assess TP53 mutation can be performed per the request of physician.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD20, CD3, CD5, CD23, LEF-1, BCL-1, TP53 (as needed)

**Other ancillary studies**
- FISH for CLL panel, chromosome;
- NGS (for TP53 per request of physician)

### 5.12 B-Cell Prolymphocytic Leukemia

B-cell prolymphocytic leukemia (B-PL) is diagnosed by flow cytometry, blood morphology and bone marrow morphology. De novo B-PL is typically negative for CD5 while B-PL transformed from CLL usually retains expression of CD5. Mantle cell leukemia mimics B-PL and t(11;14) FISH and/or BCL-1 immunostain is indicated when differential diagnosis of mantle cell leukemia is been considered.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD20, CD3, CD5, CD23, BCL-1

**Other ancillary studies**
- Chromosome, FISH

### 5.13 Lymphoplasmacytic Lymphoma (bone marrow)

Most lymphoplasmacytic lymphoma (LPL) involves bone marrow. The lymphoma has 2 neoplastic components: lymphocytes and plasma cells. The lymphocytes are CD5-, CD10-, and monoclonal. The plasma cells are CD138+ and monoclonal. The lymphocytes and plasma cells should express same immunoglobulin light chain. Nearly all LPL has MYD88 L265P mutation. NGS for MYD88 is recommended for all cases. CXCR4 mutation indicates poor response to ibrutinib.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD20, CD3, CD138, Kappa, Lambda

**Other ancillary studies**
- Chromosome, FISH, NGS for MYD88, CXCR4

### 5.14 Plasma Cell Myeloma

Plasma cell myeloma (PCM) and monoclonal gammopathy of undetermined significance (MGUS) are characterized by infiltration of bone marrow by monoclonal, neoplastic plasma cells. CD138
immunostain is used for quantification of plasma cells in addition to manual count on aspirate smear. Clonality of plasma cells can be assessed by either flow cytometry or in situ hybridization/IHC. Congo red should be performed on all new diagnosis to assess amyloid. FISH and chromosome are indicated for all initial diagnostic samples. Target FISH is indicated for the follow-up samples. MRD can be assess by either flow cytometry or NGS (Clonoseq) as per the physician’s order.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD138, Kappa, Lambda;
- Congo red (initial diagnosis)

**Other ancillary studies**
- Chromosome, FISH;
- MRD (follow-up specimens, Clonoseq or flow cytometry)

### 5.15 T-Cell Prolymphocytic Leukemia

T-cell prolymphocytic leukemia (T-PL) is diagnosed by flow cytometry, blood morphology and bone marrow morphology. TCL-1 expression is both sensitive and specific for T-PL and immunostain of TCL-1 is useful to confirm the diagnosis.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD3, TCL-1

**Other ancillary studies**
- Chromosome, FISH

### 5.16 Large Granular lymphocytic Leukemia, T and NK types

T-cell large granular lymphocytic leukemia (T-LGL) and chronic lymphoproliferative disorder of NK cells (CLPD-NK) are a group of LGL leukemia with indolent clinical behavior. They are diagnosed by flow cytometry, blood morphology and bone marrow morphology. TCR and NGS are indicated in some cases to confirm the diagnosis. T-LGL typically expresses CD57 while CLPD-NK typically expresses CD56. TCR is rearranged in T-LGL and is in germline in CLPD-NK. STAT3 mutation is present in ~30% and STAT5B mutation is present in ~5%.

**Specimen Collection Requirement**
- H&E slides for core biopsy and clot section, Aspirate smears, Touch preparations, Flow cytometry, Cytogenetics, Molecular genetics

**Immunohistochemistry**
- CD3, CD56, CD57 (as needed)

**Other ancillary studies**
- TCR, NGS (for STAT3 mutation), Chromosome, FISH
6. HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS

Histiocytic and dendritic cell neoplasms include Langerhans cell histiocytosis (LCH), follicular dendritic cell neoplasm (FDCN), interdigitating dendritic cell neoplasm (IDCN), histiocytic sarcoma (HS), and Rosai-Dorfman disease (RDD, not a true neoplasm). LCH expresses Langerin, CD1a, S-100, BRAF (subset). FDCN expresses CD21, CD23. IDCN expresses S-100. HS expresses CD68. RDD expresses CD68, S-100. EBER is usually negative in all these lesions.

Specimen Collection Requirement
- H&E slides, touch preps, flow cytometry;
- Cytogenetics (optional)

Immunohistochemistry
- CD68, CD21, CD23, CD1a, S-100, Ki-67, BRAF, EBER, Langerin (as needed)

Other ancillary studies
- NGS (as needed)