FLOW CYTOMETRIC EVALUATION OF MATURE T- AND NK-CELL NEOPLASMS: INTEGRATING MORPHOLOGY, IMMUNOPHENOTYPIC AND CYTOGENETIC / MOLECULAR FINDINGS

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General Considerations

• Th flow cytometric diagnosis of lymphoproliferative disorders relies on two broad approaches:
  • Demonstration of clonality
  • Demonstration of immunophenotypic aberrancies (i.e. differential antigen expression from a normal counterpart)

• In mature B-cell LPDs, demonstration of clonality is accomplished through surface immunoglobulin light chain analysis
  • Often represents the primary method of flow cytometric diagnosis
General Considerations

- In mature T- and NK-cell LPDs, demonstration of clonality employs less-utilized techniques, such as KIR and Vbeta T-cell receptor repertoire analysis
  - These techniques and the required expertise are not readily available in all clinical laboratories

- The primary approach to the flow cytometric diagnosis in T- and NK-cell neoplasms relies on the identification of deviations from normal IP antigen patterns

- The presence of a clone (by Vbeta or PCR analysis) is not synonymous with the presence of a neoplasm
  - Transient clonal T-cell proliferations
General Considerations

• Instrumentation and antibody panels:
  • Custom combinations may be used to fully characterize an abnormal population
  • The complexity of the T-cell immune response renders the task of distinguishing normal from abnormal difficult
  • Typically, the more antibodies in one tube, the easier it becomes to discriminate different populations, by using cluster analysis

• Analytic / gating strategies:
  • Need to be flexible and include review of ungated data
  • Criteria of calling antigen expression (positive or negative) in a population of interest are variable
Flow cytometric evaluation of T and NK-cell neoplasms is hard!
T-Cell Clonality Assessment by FC

T-cell receptor gene rearrangement

Expression of TRBC1 or TRBC2 by TCRαβ T-cells:
- Necessary
- Random
- Mutually exclusive

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TRBC1 Expression In T-Cell Neoplasms

• The anti-TCR antibody clone JOVI-1 is specific for TRBC1 only

• Utility of JOVI-1 (TRBC1) in the flow cytometric identification of T-cell neoplasms
Outline

• Mature T- and NK-cell neoplasms
  • T-cell large granular lymphocytic leukemia
  • Chronic lymphoproliferative disorder of NK cells
  • Aggressive NK-cell leukemia/lymphoma
  • Extranodal NK/T-cell lymphoma, nasal type
  • NK-cell enteropathy
  • Hepatosplenic T-cell lymphoma
  • T-cell prolymphocytic leukemia
  • Mycosis fungoides / Sezary syndrome
  • ALK (+) anaplastic large cell lymphoma
  • Angioimmunoblastic T-cell lymphoma
T-cell Large Granular Lymphocytic Leukemia (T-LGLL)
T-Cell Large Granular Lymphocytic Leukemia

- Defined as persistently increased numbers of LGLs in the PB, without a clearly identified cause

- The absolute LGL count is usually $>2 \times 10^9/L$
  - Cases with lower counts have been described

- Most patients show an indolent clinical course
  - Some require therapy, due to cytopenias

- FC is a powerful diagnostic method, as most cases demonstrate multiple IP aberrancies
T-LGLL - Diagnosis

• CD8(+) >>>> CD4(+) >> CD4(-)/CD8(-)

• Common FC findings in T-LGLL:
  • Positive: TCRαβ, CD16, CD57
  • Negative: CD56; and/or dim CD5 and CD7
  • Additional IP aberrancies: CD2, CD3, TCR Vβ, KIRs

• PCR demonstrates a clonally rearranged TCR gene
  • Confirms a clonal expansion of aberrant T-cells
Normal T-Cell Immunophenotype
Normal T cell immunophenotype

Killer immunoglobulin-like receptors (KIRs)
NK Cell–Associated Receptors

- NK cells have the ability of engaging target cells via inhibitory or activating receptors
  - A similar array of receptors is also found in a subset of memory cytotoxic T-cells
- Receptors that regulate target cell recognition by NK cells
  - Killer cell immunoglobulin-like receptors (KIRs)
  - CD94/NKG2 complexes
- KIRs may be used as potential markers of clonal expansion of NK cells or T cells
KIR and CD94 Expression On NK Cells

- Normal NK cells express several different KIRs
  - Expression is stable and maintained over multiple generations
- Clonal NK cell processes demonstrate restricted KIR expression
  - Similar to light chain restriction in B cells
  - Complete absence of KIRs is also abnormal
- Normal NK cells express both CD94/NKG2A and CD94/NKG2C heterodimers
- Abnormal NK cells show almost exclusively bright CD94/NKG2A
KIR Expression On T Cells

• Normal T cells express several different KIRs
• Clonal T cell processes demonstrate restricted KIR expression
  • Since only a minor subset of cytotoxic T cells express KIRs, the complete absence of KIR expression cannot be used as an indicator of clonality
• Additional studies support the clonal nature of aberrant T cell populations
  • TCR Vβ flow cytometric analysis
  • TCR gene rearrangement RT-PCR
CD8(+) T-LGLL, KIR CD158e(+)

- 45-year-old male with RA and persistent lymphocytosis
CD8(+) T-LGLL, KIR(-), Vβ3(+)

- 72-year-old male with persistent lymphocytosis
CD8(+) T-LGLL, TCR Vβ Panel

• The Vβ panel includes following antibodies: Vb1, Vb2, Vb3, Vb4, Vb5.1, Vb5.2, Vb5.3, Vb7.1, Vb7.2, Vb8, Vb9, Vb11, Vb12, Vb13.1, Vb13.2, Vb12.6, Vb14, Vb16, Vb17, Vb18, Vb20, Vb21.3, Vb22, and Vb23
CD8(+) T-LGLL and Pure Red Cell Aplasia

- 24-year-old female with severe anemia
- Normocellular BM
- Markedly decreased erythropoiesis
  - (M:E ratio = 45:1)
- Parvovirus B19 IHC (-)
T-LGLL – Diagnosis

• Differential diagnosis of T-LGLL:
  • Benign, reactive T-cell expansions
  • Aggressive neoplasms of cytotoxic T cells

• No single laboratory method sufficient to make the diagnosis of T-LGLL

• In general, T-LGLL satisfies 2 of 3 conditions:
  • Immunophenotypically aberrant T cells with NK-cell antigen expression
  • Clonal TCR gene rearrangements
  • Intrasinusoidal bone marrow infiltrates of cytotoxic T lymphocytes
T-LGLL – Molecular Diagnosis

- Mutation analysis for the STAT3-5/NFkB signaling pathway; not part of routine laboratory assessment
- Somatic gain-of-function STAT3 mutations are common in both T-LGLL (up to 75%) and CLPD-NK (up to 50%)
- A subset of T-LGLL expressing CD56 may have a more aggressive behavior associated with STAT5B mutations
- Other reports have found that similar mutations are frequent in CD4(+) T-LGLL, typically found in patients which rarely demonstrate cytopenias, splenomegaly or autoimmune disease, and may be associated with CMV infection or additional malignancy
Chronic Lymphoproliferative Disorder of NK Cells (CLPD-NK)
Chronic Lymphoproliferative Disorder of NK Cells

- Defined as a persistent (>6 months) increase in peripheral blood NK cells, without a clearly identified cause
- The absolute NK cell count is usually $>2 \times 10^9/L$
  - Cases with lower counts have been described
- FC plays a central role in diagnosing CLDP-NK
  - No distinct marker of clonal NK cell expansion
- Morphology:
  - PB shows increased numbers of LGLs
  - BM demonstrates intrasinusoidal infiltrates of NK cells that express cytotoxic molecules (e.g. granzyme B, TIA-1)
PB and BM IHC findings in CLPD-NK

PB

CD3

TIA-1
Normal NK Cell Immunophenotype

- Two distinct functional subsets of NK cells
- CD56(bright +) NK cells (type I)
  - Primarily found in secondary lymphoid tissues
  - CD56(bright +)/CD16(dim +) IP
  - Show limited cytotoxic activity
  - Have little or no KIR expression
- CD56(dim +) NK cells (type II)
  - Primarily found in peripheral blood
  - CD56(dim +)/CD16(bright +) IP
  - Have primarily cytotoxic activity
  - Demonstrate frequent KIR expression
Normal NK Cell Immunophenotype
Aberrant NK Cell Immunophenotype

- Variable CD16 and CD56
  - Similar to normal NK cell subsets

- Dim CD2, CD7, CD8 (may be negative)

- Uniform, bright CD94

- Aberrant KIR expression
  - Uniform expression of a single KIR isoform, with or without the expression of another KIR
  - Absence of all KIR isoforms
CD56(+) CLPD-NK, KIR(-)
CD56(+) CLPD-NK, KIR CD158b(+)

Side scatter
Forward scatter
CD3
CD7
CD2
CD5
CD4
CD56
CD16
CD57
CD45
CD3
CD94
CD158a
CD158b
CD158e
CD56(-) CLPD-NK, KIR CD158e(+)

- Side scatter vs. Forward scatter
- CD3, CD7, CD2, CD5
- CD4, CD56, CD16, CD57, CD11b
- CD8, CD158a, CD158b, CD158e
Aggressive NK-Cell Leukemia/Lymphoma

Extranodal NK/T-Cell Lymphoma, Nasal Type
Aggressive NK-Cell Leukemia/Lymphoma

- Systemic neoplastic proliferation of NK cells
  - Almost always associated with EBV
  - Aggressive clinical course

- Immunophenotypic (and biologic?) overlap with extranodal NK/T-cell lymphoma, nasal type
  - CD2(+), sCD3(-), cCD3(+), CD56(+), TIA-1(+), GranB(+)
  - CD16(+) >> extranodal NK/T-cell lymphoma
  - CD11b(+/-), CD57(-)

- TCR in germline configuration
  - KIR expression useful in establishing clonality
Aggressive NK-Cell Leukemia/Lymphoma

• 82-year-old female with leukocytosis and LAD
• Presented with fever, constitutional symptoms, elevated LDH
• EBER ISH (+)
• Rapid clinical course (DOD 3 weeks after diagnosis)
Aggressive NK-Cell Leukemia/Lymphoma
Extranodal NK/T-Cell Lymphoma, Nasal Type

- Predominantly extranodal lymphoma
  - Almost always associated with EBV
  - NK or cytotoxic T-cell immunophenotype
  - Prominent necrosis and angiodestructive pattern
- Immunophenotypic (and biologic?) overlap with aggressive NK-cell leukemia/lymphoma
  - CD2(+), sCD3(-), cCD3(+), CD56(+), TIA-1(+), GranB(+)
- TCR in germline configuration
  - KIR expression useful in establishing clonality
Extranodal NK/T-Cell Lymphoma, Nasal Type

- 55-year-old Taiwanese female with a sinonasal mass
Extranodal NK/T-Cell Lymphoma, Nasal Type
Extranodal NK/T-Cell Lymphoma, Nasal Type
Extranodal NK/T-cell Lymphoma, Nasal Type

46,XX
NK-Cell Enteropathy
NK-Cell Enteropathy

- Benign NK-cell LPD mimicking lymphoma
  - Unknown etiology (inflammatory vs. autoimmune?)
  - Protracted, indolent clinical course
  - Vague abdominal pain (or asymptomatic)

- Morphology:
  - Medium-to-large cells with pale cytoplasm
  - May show mucosal ulceration
  - No epitheliotropism, necrosis, angiodestruction

- Immunophenotype:
  - Positive: CD2, cCD3, CD56, TIA-1, GranB
  - Negative: sCD3, CD4, CD5, CD8, EBER
NK-Cell Enteropathy

- 59-year-old male; routine colonoscopy
CD56

EBER

TCR βF-1

TCR γ3.20
NK-Cell Enteropathy
NK-Cell Enteropathy

- Differential diagnosis:
  - Extranodal NK/T-cell lymphoma, nasal type
  - Enteropathy-associated T-cell lymphoma
  - Hepatosplenic T-cell lymphoma
  - Peripheral T-cell lymphoma, NOS

- Awareness of and appropriate diagnosis can prevent unnecessary clinical interventions and therapy
Common T And NK-Cell Subsets
## Common T and NK-Cell Subsets

<table>
<thead>
<tr>
<th>Subset</th>
<th>Flow cytometry markers</th>
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</thead>
<tbody>
<tr>
<td>Normal “cytotoxic” NK cells (found primarily in PB)</td>
<td>CD2(+), sCD3(-), CD4(-), CD5(-), CD7(bright +), CD8(partial +), CD16(bright +), CD56(dim +), CD57(variably +), CD94(+), KIR (+)</td>
</tr>
<tr>
<td>Normal “cytokine-secreting” NK cells (found primarily in secondary lymphoid tissues)</td>
<td>CD2(+), sCD3(-), CD4(-), CD5(-), CD7(bright +), CD8(partial +), CD16(dim +), CD56(bright +), CD57(variably +), CD94(+), KIR mostly (-);</td>
</tr>
<tr>
<td>Normal T cells</td>
<td>CD2(+), sCD3(+), CD4(subset +), CD5(+), CD7(+), CD8(subset +)</td>
</tr>
<tr>
<td>Normal γ/δ T cells (comprise &lt;5% of circulating T cells)</td>
<td>CD2(+), sCD3(bright +), CD4(-), CD5(-), CD7(+), CD8(-/dim+), TCR α/β(-), TCR γ/δ (+), KIR (+)</td>
</tr>
<tr>
<td>Normal α/β T cells (comprise &lt;1.5% of circulating T cells)</td>
<td>CD2(+), sCD3(+), CD4(-), CD5(+), CD7(+), CD8(-), TCR α/β (+), TCR γ/δ (-)</td>
</tr>
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Reactive γ/δ T-Cell Expansions

- >95% of PB T-cells express α/β TCR heterodimers
  - <5% (55-120/uL) demonstrate γ/δ TCR expression

- Reactive γ/δ T-cell expansions may be seen in:
  - Infection
  - Autoimmune disease
  - Splenectomy
  - Tumor-elicited immune response

- Normal γ/δ T-cell immunophenotype:
  - CD2(+), CD3(bright +), CD5(dim/-), CD7(+), CD4/CD8(-)
  - CD16 is (+) in ~ 1/3 of cases; CD57 (+); CD56 (-)
Expanded Reactive γ/δ T-Cells

- 47-year-old female with flu-like symptoms and mild lymphocytosis
Hepatosplenic T-Cell Lymphoma (HSTCL)
Hepatosplenic T-Cell Lymphoma

- Extranodal and systemic neoplasm
  - Cytotoxic T-cells demonstrate $\gamma/\delta$ TCR expression
- Epidemiology:
  - Young male patients
  - 20% of cases associated with chronic immunosuppression
- Morphology:
  - Medium-sized lymphocytes
  - Distinct sinusoidal infiltration pattern (BM, liver, spleen)
- Genetics:
  - Isochromosome 7q or trisomy 8
Hepatosplenic T-Cell Lymphoma

- 20-year-old female with pancytopenia
Hepatosplenic T-Cell Lymphoma

Bone marrow aspirate
Hepatosplenic T-Cell Lymphoma

Bone marrow core biopsy, H&E
Hepatosplenic T-Cell Lymphoma

Bone marrow core biopsy, CD3
Hepatosplenic T-Cell Lymphoma

46,XX,del(2)(p21),i(7)(q10),t(10;17)(q22;q35)
T-Cell Prolymphocytic Leukemia (T-PLL)
T-Cell Prolymphocytic Leukemia

- Aggressive mature T-cell neoplasm
  - Extensive PB, BM, LN, liver, spleen, and skin involvement

- Morphology:
  - Medium-sized lymphocytes with prominent nucleoli
  - Small cell variant (25% of cases)

- Immunophenotype:
  - Positive: CD2, CD3, CD7(uniform), CD52
  - CD4(+)/CD8(-) >> CD4(+)/CD8(+)

- Genetics:
  - Inv(14)(q11q32) / t(14;14)(q11;q32) / t(X;14)(q28;q11)
T-Cell Prolymphocytic Leukemia

• 78-year-old female with WBC = 120,000/uL
T-Cell Prolymphocytic Leukemia

47,XX,t(1;12)(p13;q13),+6,add(7)(p15),inv(14)(q11.2q32.1)
Mycosis Fungoides/Sezary Syndrome (MF / SS)
Mycosis Fungoides / Sezary Syndrome

- MF: Epidermotropic, primary CTCL
  - Higher stages show circulating lymphoma cells
- SS: Erythroderma+LAD+circulating lymphoma cells

- Morphology:
  - Small to medium-sized cells with cerebriform nuclei

- Immunophenotype:
  - Positive: CD2, CD3, CD4, CD5
  - Negative: CD7, CD8, CD26

- Genetics:
  - Clonally rearranged T-cell receptor
Mycosis Fungoides, TCR V\(\beta\)20(+)

- 75-year-old male with WBC = 48,000/uL
ALK (+) Anaplastic Large T-Cell Lymphoma (ALCL)
Anaplastic Large T-Cell Lymphoma, ALK(+)

- Frequently involves LNs and extranodal sites
  - BM involvement in 30% of cases
  - Small cell variant may have a leukemic presentation

- Morphology:
  - “Hallmark cells”

- Immunophenotype:
  - Positive: CD30; CD2, CD4, CD5; TIA-1, GranB, EMA, ALK-1
  - Negative: sCD3 (75%), CD7, CD8

- Genetics:
  - NPM/ALK [t(2;5)(p23;q35)]; variant translocations
  - Clonally rearranged TCR
Anaplastic Large T-Cell Lymphoma, ALK(+) 

- 79-year-old male with ANC = 13,000/uL
Anaplastic Large T-Cell Lymphoma, ALK(+)

46,XY,t(2;5)(p23;q35)
Anaplastic Large T-Cell Lymphoma, ALK(+)

• Most ALCLs can be detected and characterized by multiparameter flow cytometry

• Important to employ flexible gating and data analysis strategies
  • Do not rely solely on “lymphocyte gate” analysis on FSC/SSC and CD45/SSC

• Correctly identifying distinct CD30(+) clusters, independent of CD45 expression or light scatter properties
  • Often, ALCL is sCD3(-), CD4(+) (i.e. aberrant)
Angioimmunoblastic T-Cell Lymphoma (AITL)
Angioimmunoblastic T-Cell Lymphoma

- PTCL characterized by systemic disease and typical LN morphology
- Included in the category of T-cell lymphomas of T follicular helper cell (THF) origin
- Diagnosis may be challenging because of the background polymorphous cellular infiltrate
  - Often, reactive T cells outnumber lymphoma cells (by an order of magnitude) by flow cytometry
Angioimmunoblastic T-Cell Lymphoma

- Clinical presentation with hepatosplenomegaly, systemic symptoms, and skin rash; BM involvement is common

- Morphology:
  - Complete LN architectural effacement, with prominent vascular proliferation, and a polymorphic, reactive infiltrate
  - Medium-sized neoplastic T cells with pale cytoplasm
  - Increased FDC meshworks and EBV-positive B immunoblasts

- Immunophenotype:
  - Positive: CD2, CD4, CD5; CD10
  - Negative: sCD3, CD7, CD8
Angioimmunoblastic T-Cell Lymphoma

• 68-year-old male with LAD, skin rash, polyclonal hypergammaglobulinemia and B symptoms
Summary

- T-cell neoplasms are readily diagnosed by FC
  - Immunophenotype can predict specific subtype
- Ancillary techniques may confirm clonality
  - CAVEAT! Clonality ≠ Malignancy
- Morphology is your best friend!
  - Guides interpretation of ancillary studies
Thank you for participating!