Flow Cytometric of Lymphoblastic Leukemias and Acute Leukemias of Ambiguous Lineage

Horatiu Olteanu, MD, PhD
Professor and Medical Director of Flow Cytometry
Mayo Clinic, Rochester, MN
Flow Cytometry in Acute Leukemias

• Roles:
  • Differentiates ALL from AML
  • Distinguishes B-ALL from T-ALL
  • Identifies subtypes of AML: megakaryocytic, monocytic, etc.
  • Treatment and prognostic groups determined partly by immunophenotype
  • Fingerprint for MRD assessment
ALL: Immunophenotypic Features

- **80-85% B-ALL**
  - B-cell antigens such as CD19, CD20, CD22
  - CD10 in 90%
  - Markers of immaturity (CD34 and/or TdT) in most cases

- **15-20% T-ALL**
  - T-cell antigens such as CD2, CD3, CD5, CD7, CD4, and CD8
  - Markers of immaturity such as CD1a, CD34, and TdT

- Aberrant expression of myeloid antigens seen in many cases
B Lymphoblastic Leukemia/Lymphoma
Hematogone Maturation
Kroft SH AJCP 2004; 122: S19-S32
Typical B-ALL Immunophenotype
Typical B-ALL Immunophenotype

- Side Scatter vs. Forward Scatter
- CD22 vs. CD10
- CD34 vs. CD20
- CD19 vs. HLA-DR
- CD38 vs. CD45
- Lambda vs. Kappa
- CD22 vs. TdT
- I.C. CD79a vs. Myeloperoxidase
Case #1
66-year-old F with h/o B-ALL, 3 months s/p alloSCT; pancytopenia
Other results

- **CBC:** WBC=1,700/uL, Hb=8.9 g/dL, Plt=168,000/uL
  - Differential count: 5% segs, 52% lymphocytes, 32% monocytes, 9% eosinophils; 2% basophils

- **Morphology:**
  - BM: 11% blasts; 11% lymphs (including hematogones)

- **Cytogenetics:**
  - 47,XX,+8,t(9;22)(q34;q11.2)[1]/46,XX[19]
  - t(9;22) translocation in 1% of 200 cells analyzed
Diagnosis:

Recurrent B Lymphoblastic Leukemia/Lymphoma (B-ALL) with Hematogone Hyperplasia
**B-Lymphoblasts and Hematogones**

- Hematogones show a reproducible maturation pattern
  - B-lymphoblasts essentially always demonstrate immunophenotype aberrancies
  - Beware of hematogone hyperplasia in the setting of B-ALL

- Flow cytometry and morphology usually provide concordant blast percentages
  - Hemodilution may underestimate blast counts by flow cytometry
Case #2
55-year-old M with leukocytosis

- Presented to PCP with 2-week history of myalgias, night sweats, and 10-15 lbs weight loss
- CBC: Leukocytosis (14,100/uL) and 16% blasts
  - Hgb 15 g/dL, plt 76,000/uL, LDH >2,500 U/L
- PB flow cytometry performed prior to BM biopsy
- BM biopsy: MPO and NSE cytochemical stains (-)
- **Red (78% blasts):** Small cells, CD1a (-), CD2 (-), surface CD3 (-), cytoplasmic CD3 (-), CD4 (-), CD5 (-), CD7 (-), CD8 (-), CD10 (+), CD11b (-), CD13 (-), CD14 (-), CD15 (-), CD16 (-), CD19 (+), CD20 minor subset (+), CD22 dim (+), CD33 (-), CD34 (-), CD36 (-), CD38 (+), CD45 moderate to slightly bright (+), CD45RO (-), CD56 (-), CD64 (-), CD79a dim (+), CD117 (-), HLA-DR (+), MPO (-), TdT (+), and surface immunoglobulin (-).
Cytogenetics

- 47,XY,+i(1)(q10),t(8;14)(q24;q32)[20]

- All 20 metaphase cells analyzed had an extra copy of an abnormal chromosome 1 composed of two copies of 1q resulting in tetrasomy 1q, and what appears to be a balanced translocation between the long arms of chromosomes 8 and 14 (MYC-IGH). No normal cells were observed. FISH performed on the same specimen also revealed an IGH rearrangement in 61.5% of 200 interphase cells analyzed.
Diagnosis:

B-Lymphoblastic Leukemia/Lymphoma with t(8;14)
B-ALL with t(8;14)

• Very rare variant; raises differential diagnosis with Burkitt lymphoma

• Morphology: blasts (immature cells) rather than typical Burkitt morphology

• Immunophenotype:
  • Blasts are positive for Tdt and negative for sIg

• Genetics:
  • May be associated with t(9;22), +21, or complex karyotype
B-ALL with t(9;22)(q34.1;q11.2); BCR-ABL1
B-Lymphoblastic Leukemia/Lymphoma, *BCR-ABL1*-like

- 2016 WHO new provisional entity
- B-ALL with translocations involving TKs or cytokine receptors (e.g. *CRLF2* and *JAK* mutations)
- Gene expression profiles similar to cases of B-ALL with *BCR-ABL1*
- Associated with adverse prognosis
  - May respond to TKI therapy in some cases
MRD Analysis in B-ALL

• MRD-based risk stratification has become standard of care for B-ALL in several ongoing clinical trials

• Negative MRD status (<0.01%) at the end of induction therapy has been proven to be the most reliable indicator of favorable outcome

• Conversely, a high level of MRD early after induction chemotherapy is a poor prognostic factor

• The absence of MRD is being considered as a surrogate therapeutic endpoint for drug approval in clinical trials
MRD Analysis in B-ALL

- COG antibody panel is standardized in North America
  - Tube 1: CD20FITC/CD10PE/CD38PerCPCy5.5/CD19PC7/CD58APC/CD45APCH7
  - Tube 2: CD9FITC/CD13 + 33PE/CD34PerCPCy5.5/CD19PC7/CD10APC/CD45APCH7
  - Tube 3: Syto16*/CD3PerCPCy5.5/CD19PC7/CD45APCH7

- May be performed on PB (day 8) and BM (day 9) post-induction chemotherapy, respectively

- There is broad variation in number/type of antibody combinations and analysis software in different laboratories

- Examples of single-tube panels:
  - CD66c/CD9/CD34/CD19/CD10/CD20/CD38/CD45
  - CD10/CD19/CD20/CD22/CD24/CD34/CD38/CD45/CD58/CD66c
EFS of all patients enrolled on 9900 series therapeutic studies with satisfactory end-induction MRD.
B-ALL MRD (+) (0.24%)
B-ALL MRD (+) (0.02%)
B-ALL MRD (+) (0.03%), s/p CAR-T cells
B-ALL MRD (+) (0.03%), s/p CAR-T cells
T Lymphoblastic Leukemia/Lymphoma
Typical T-ALL Immunophenotype
Typical T-ALL Immunophenotype
Case #3
72-year-old M with pancytopenia

- Presented to ED with 3-week history of fevers, fatigue, night sweats, and 15-20 lbs weight loss
- CBC: Leukopenia (640/uL) and 25% blasts
  - Hgb 9.1 g/dL, plt 123,000/uL, LDH >2,500 U/L
- PB flow cytometry performed prior to BM biopsy
- BM biopsy: MPO and NSE cytochemical stains (-)
Isotype Control

Isotype Control

TdT and Cytoplasmic CD3

MPO
Diagnosis:

Early T-Cell Precursor Lymphoblastic Leukemia
2016 WHO: T-ALL Categories

- Early T-precursor (ETP) ALL
- Has unique IP and genetic profile
  - Blasts express CD7, but lack CD1a and CD8
  - Are positive for myeloid/stem cell antigens: CD34, CD117, HLA-DR, CD13, CD33, CD11b, CD65
  - Typically express CD2, cytoplasmic CD3, and/or CD4 (not required for definition)
  - Frequent FLT3, NRAS/KRAS, DNMT3A, IDH1, and IDH2 mutations
ETP-ALL

Must show definite evidence of T-cell differentiation

PLUS

CD7 positive

CD3 positive (can be either surface or cytoplasmic)
ETP-ALL

Must not show evidence of myeloid lineage differentiation

PLUS

MPO negative

No evidence of monocytic differentiation
ETP-ALL

Must show features of early thymocyte precursors

**CD1a negative**
(less than 5% of total population can be positive)

**CD8 negative**
(less than 5% of total population can be positive)
Note: CD4 is often negative

**CD5 dim/ (-)**
(expressed by <75% blasts)
or
MFI is at least 1 log dimmer than normal T lymphocytes)
ETP-ALL

Must express at least 1 stem cell or myeloid-associated antigen (positive in >10% of blasts)

- CD34 (+) > 10%
- CD117 (+) > 10%
- HLA-DR (+) > 10%
- CD13 (+) > 10%
- CD33 (+) < 10%
Identifying ETP-ALL

• As with any hematolymphoid malignancies, identification of ETP-ALL and typical T-ALL requires knowledge of the immunophenotypic features of the normal cell counterpart, i.e. normal thymocytes.
Normal Thymocyte Maturation
Nearly 100% of neoplastic immature T cells can be detected by examining patterns of expression for CD1a vs. sCD3 and CD4 vs. CD8.

The remaining IP features can then be used to differentiate typical T-ALL from ETP-LL.
Identifying T-ALL and ETP-ALL

Thymocytes

T-ALL

ETP-ALL
Identifying T-ALL and ETP-ALL

Thymocytes

T-ALL

ETP-ALL
Case #4
26-year-old M with diffuse LAD

- Presented with nausea, vomiting, night sweats
  - Diffuse lymphadenopathy and mediastinal mass on CT

- CBC: Mild leukocytosis (12,200/uL) and 25% blasts

- PB flow cytometry performed in 02/2016
• **Red (25% blasts):** Medium to large sized cells with increased SSC (consistent with cytoplasmic vacuoles) that are CD1a (-), CD2 (+), surface CD3 partial dim (+), cytoplasmic CD3 bright (+), CD4 minor subset (+), CD5 minor subset (+), CD7 (+), CD8 (-), CD10 (-), CD11b partial (+), CD13 (-), CD14 (-), CD15 (-), CD16 (-), CD19 (-), CD20 (-), CD22 (-), CD33 variably (+), CD34 (+), CD36 (-), CD38 (+), CD45 moderately (+), CD45RO (-), CD56 (-), CD64 minor subset (+), CD79a (-), CD117 (-), MPO (-), TdT (-), and surface immunoglobulin (-)
Cytogenetics - Diagnosis

- 58-59,XY,+1,+4,+6,+8,del(9)(p21),+10,+18,+19,+19,+20,+20,+22,+22[cp5]/46,XY[26]

- Twenty-six cells were normal, while five cells had a hyperdiploid complement of 58-59 chromosomes in a pattern often observed in hyperdiploid ALL. This pattern included trisomy 4 and trisomy 10 as well as trisomies for 1, 6, 8, 18, tetrasomies for 19, 20 and 22, supported by FISH. In addition deletion 9p (also supported by FISH) was observed; deletion 9p in ALL may be an unfavorable prognostic finding.
Diagnosis:

T-Lymphoblastic Leukemia/Lymphoma
Interval history

- Induction chemotherapy CALGB 10403 protocol
  - BM biopsy from 03/2016: MRD
  - BM biopsy from 05/2016: CR
- Consolidation and maintenance chemotherapy
- Recurrent disease: 05/2017
  - Pancytopenia and 90% blasts
  - PB flow cytometry
• **Red (91% blasts; relapse):** Medium to large sized cells CD1a (-), CD2 variably (+), surface CD3 (-), cytoplasmic CD3 (-), CD4 variably (+), CD5 (-), CD7 (-), CD8 (-), CD10 (-), CD11b (-), CD13 (-), CD14 (-), CD16 (-), CD19 (-), CD20 (-), CD22 (-), CD33 variably (+), CD34 variably (+), CD36 (+), CD38 variably dim (+), CD41 (-), CD45 moderately (+), CD45RO (-), CD56 partial (+), CD61 (-), CD64 (-), CD71 (+), CD79a (-), CD117 (-), HLA-DR partial dim (+), glycophorin A (+), MPO (-), TdT (-), and surface immunoglobulin (-).

vs.

• **Red (25% blasts; diagnosis):** Medium to large sized cells with increased side scatter (consistent with cytoplasmic vacuoles) that are CD1a (-), CD2 (+), surface CD3 partial dim (+), cytoplasmic CD3 bright (+), CD4 minor subset (+), CD5 minor subset (+), CD7 (+), CD8 (-), CD10 (-), CD11b partial (+), CD13 (-), CD14 (-), CD15 (-), CD16 (-), CD19 (-), CD20 (-), CD22 (-), CD33 variably (+), CD34 (+), CD36 (-), CD38 (+), CD45 moderately (+), CD45RO (-), CD56 (-), CD64 minor subset (+), CD79a (-), CD117 (-), HLA-DR (-), MPO (-), TdT (-), and surface immunoglobulin (-).
What is your diagnosis?

• A. T-lymphoblastic leukemia/lymphoma
• B. Acute myeloid leukemia
• C. Acute undifferentiated leukemia
• D. Acute erythroid leukemia
Cytogenetics - Relapse


- All ten cells had a hyperdiploid complement of 52-55 chromosomes in a pattern often observed in hyperdiploid ALL. This pattern included monosomy 2 and trisomies or tetrasomies for 6, 8, 18, 19, 20 and 22. This appears to be a considerably evolved version of a clone that was observed on 02/2016; fewer chromosomes are present (52-55 instead of 58-59), and many more structural abnormalities are present, affecting chromosomes 11, 13, 17, 18, 19, 21.
Diagnosis:

Recurrent T-Lymphoblastic Leukemia/Lymphoma with Evidence of Clonal Evolution
Acute Leukemias of Ambiguous Lineage
Acute Leukemias of Ambiguous Lineage: WHO 2016

- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
- Mixed phenotype acute leukemia with t(v;11q23); *KMT2A* rearranged
- Mixed phenotype acute leukemia, B/myeloid, NOS
- Mixed phenotype acute leukemia, T/myeloid, NOS
- Mixed phenotype acute leukemia, NOS, rare types
- Acute leukemias of ambiguous lineage, NOS
Case #5
42-year-old M with syncopal episode

- Presented to ED after fall at work
  - Small scalp hematoma on back of head
- CBC: Leukocytosis (50,800/uL) and 90% blasts
- PB flow cytometry and BM biopsy was performed
- BM biopsy: MPO and NSE cytochemical stains (-)
• **Red (91% blasts)**: Small to medium sized cells, CD1a (-), CD2 (-), surface CD3 (-), cytoplasmic CD3 (-), CD4 (-), CD5 (-), CD7 partial dim (+), CD8 (-), CD10 (-), CD11b (-), CD13 (-), CD14 (-), CD15 (-), CD16 (-), CD19 (-), CD20 (-), CD22 (-), CD33 (-), CD34 (+), CD36 (-), CD38 variably dim (+), CD45 (-) to dim (+), CD45RO (-), CD56 (-), CD64 (-), CD79a (-), CD117 (+), HLA-DR (+), MPO (-), TdT (-), and surface immunoglobulin (-).
Cytogenetics

- 45,XY, t(5;12)(q13;q24.1),-7, del(12)(p11.2), del(21)(q11.2)[19]/46,XY[1]

- 19 cells each have multiple structural and numerical abnormalities including what appears to be a balanced translocation between the long arms of chromosomes 5 and 12; monosomy 7; and terminal deletions of 12p and 21q. FISH performed on the same specimen also revealed deletion 12p, deletion 21q, and monosomy 7 in 61.5-72.5% of 200 interphase cells analyzed, which is consistent with classical cytogenetic findings.
Diagnosis:
Acute Undifferentiated Leukemia
Acute Leukemias of Ambiguous Lineage

• Contentious topic
  • Somewhat arbitrary requirements for assigning more than one lineage to a single blast population:
    • Myeloid: MPO
    • T cell: Cytoplasmic CD3 (bright, i.e. same intensity as normal T cells)

• Acute undifferentiated leukemia shows no lineage-specific markers
  • Blasts are often positive for HLA-DR, CD34, CD38
  • May be positive for Tdt
Criteria for Lineage Assignment for Mixed Phenotype Acute Leukemia (MPAL)

- **Myeloid lineage**
  - MPO (FC, IHC or cytochemistry) or
  - Monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme)

- **T lineage**
  - Strong cytoplasmic CD3 (with antibodies to CD3 epsilon chain) or Surface CD3

- **B lineage**
  - Strong CD19 with at least one of the following strongly expressed: CD79a, cytoplasmic CD22 or CD10 or
  - Weak CD19 with at least two of the following strongly expressed: CD79a, cytoplasmic CD22 or CD10
MPAL - New Emphases in 2016 WHO

• For MPAL cases, if there are two distinct blast populations, and each individual population meets a definition for either a B, T or myeloid leukemia, it is not necessary that the specific markers be present.

• If ALL or AML is **NOT** MPAL, it is not necessary to meet the more strict MPAL criteria in order to assign lineage.

• Some typical B-ALL cases with homogeneous expression of lymphoid markers on a single blast population may express low-level MPO using IP methods without other evidence of myeloid differentiation. Because the clinical significance of this finding has not yet been established, it is recommended that care be taken before making a diagnosis of B/Myeloid MPAL when low intensity MPO is the only myeloid-associated feature.

• Multi-parameter FC is the preferred method for recognizing MPAL.
AML with t(8;21)(q22;q22.1); \textit{RUNX1-RUNX1T1}
Cross-Lineage Antigen Expression in AML

Partial CD7 and CD56 expression
Cross-Lineage Antigen Expression in AML

Partial CD2 and CD7 expression
MPAL with Two Distinct Blast Populations
Summary

• Routine flow cytometry in ALL requires in-depth knowledge of maturation patterns of normal immature cells (hematogones and T cells)

• Flow cytometry has applications in the differential diagnosis, prognosis, and treatment of ALL

• MRD analysis by flow cytometry is a powerful predictor of outcome in B-ALL, in the clinical trial setting
Thank you for participating!