Case SH2017-0119: A 22-year-old male with a Ph-like mixed phenotype acute leukemia (B/myeloid)

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Clinical history

- A 22-year-old male with a one-month history of worsening dyspnea on exertion and subsequent headaches, oral lesions, and gingival bleeding.

Laboratory findings

- Peripheral blood differential count:
  
  Blasts 41%
  Neutrophils 19%
  Lymphocytes 36%
  Monocytes 3%
  Eosinophils 1%
Core biopsy

≈ 0.5 cm
Core biopsy
Flow cytometry

- CD45-ECD
- SS
- CD19-PE
- CD10-PC5
- MPO-FITC
- CD19-PC5
- CD34-FITC
- CD33-PE
- cCD22-PE
- CD79a-PE
- MPO-FITC
- CD19-PC5
- MPO-FITC
Immunophenotypic summary

Blasts (positive flow cytometry markers)
- CD19 (moderate)
- CD79a (moderate)
- CD10 (dim, very minor subset)
- CD20 (dim, minor subset)
- CD22 (surface/cytoplasmic, dim to negative)
- CD34 (dim, subset)
- CD45 (dim, subset)
- TdT (moderate, subset)
- MPO (dim, subset)

Negative: CD11c, CD13, CD14, CD33, CD38, CD56, CD117, T cell markers
Aspirate smear – Myeloperoxidase cytochemistry

50% of blasts
Core biopsy – Myeloperoxidase immunohistochemistry
Panel differential diagnosis

Mixed phenotype acute leukemia, B/myeloid

vs.

B-ALL (with isolated MPO expression)
The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

Daniel A. Arber,1 Attilio Orazi,2 Robert Hasserjian,3 Jürgen Thiele,4 Michael J. Borowitz,5 Michelle M. Le Beau,6 Clara D. Bloomfield,7 Mario Cazzola,8 and James W. Vardiman9

Table 19. Criteria for lineage assignment for a diagnosis of MPAL

ALL, but only for MPAL. It is also now recognized that some cases of otherwise typical B-ALL with homogeneous expression of lymphoid markers on a single blast population may express low-level myeloperoxidase using immunophenotypic 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 myeloperoxidase (MPO) is the only myeloid-associated feature. Multiparameter flow cytometry is the method of choice for recognizing MPAL, even when there

Weak CD10 with at least 2 of the following strongly expressed: CD7, CD19, cytoplasmic CD22, or CD10
B-ALL with isolated MPO expression shows higher cumulative incidence of relapse

Oberley et al. *American Journal of Clinical Pathology*, Volume 147, Issue 4, 1 April 2017, Pages 374–381
B-ALL with isolated MPO expression shows worse event-free survival
Cytogenetics/Molecular analysis

Karyotype:
46,XY[20] (normal male karyotype)

FISH:
Negative for BCR/ABL1 fusion and KMT2A (MLL) rearrangement.

Molecular genetics:
Negative for JAK2 V617F mutation.
Positive for CDKN2A c.151-6delTinsCCAGGGG mutation.

Cytogenomic array:

327 Kb deletion

Genes
CRLF2 ←
P2RY8 ←
Formation of $P2RY8$-$CRLF2$ fusion in pseudoautosomal region 1 (PAR1)

327 Kb deletion

Resultant fusion
Formation of \textit{P2RY8-CRLF2} fusion in pseudoautosomal region 1 (PAR1) 

FISH \textit{CRLF2} breakapart probe

Region of deletion

Loss of red-orange signal
Historical context of BCR-ABL1-like vs. Ph-like B-ALL

Den Boer et al, The Lancet
February 2009 Vol 10

COALL discovery cohort
DCOG validation cohort

Hierarchical clustering (HC) of 110 gene probe sets identified to predict the major pediatric ALL subtypes (T-cell ALL, ETV6-RUNX1, high-hyperdiploidy, TCF3 or MLL-rearranged, BCR-ABL1). “BCR-ABL1-like”


COG P9906 discovery cohort
St. Jude validation cohort

“Ph-like signature” is based on the prediction analysis of microarrays (PAM) classifier consisting of 257 gene probe sets trained on BCR-ABL1-positive cases.
BCR-ABL1-like cases in pediatric acute lymphoblastic leukemia: a comparison between DCOG/Erasmus MC and COG/St. Jude signatures

Total DCOG/COALL

- HC only: 54
- HC and PAM: 25
- PAM only: 8

COG P9906

- HC only: 18
- HC and PAM: 25
- PAM only: 15
Targetable Kinase-Activating Lesions in Ph-like Acute Lymphoblastic Leukemia


High Frequency and Poor Outcome of Philadelphia Chromosome–Like Acute Lymphoblastic Leukemia in Adults

Testing algorithm for Ph-like ALL in COG trials

(IGJ, SPATS2L, MUC4, CRLF2, CA6, NRXN3, BMPR1B, GPR110, CHN2, SEMA6A, PON2, SLC2A5, S100Z, TP53INP1, IFITM1).

CRLF2 is a frequent rearrangement seen in Ph-like B-ALL

Young Adult (21-39 yrs; n=96)

- CRLF2_JAK mut: 14.6%
- CRLF2_JAK WT: 36.5%
- Other JAK-STAT: 7.3%
- EPORr: 9.4%
- JAK2r: 6.3%
- ABL-class: 10.4%
- Other kinase: 5.2%
- Ras: 5.2%
- None identified: 5.2%
- Unknown: 3.1%
P2RY8-CRLF2 fusion leads to CRLF2 overexpression

CRLF2 acts upstream of JAK-STAT signaling
CRLF2 rearranged cases have frequent concomitant JAK1 and JAK2 mutations
IL-7R transmembrane domain mutations have also been reported in Ph-like B-ALLs

Co-occurrence with CRLF2 rearrangement and SH2B3 deletion has been reported.

Roberts et al., Cancer Cell, 22, 153–166, August 14, 2012
<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
<th>Fusion Partners</th>
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<td>ABL1</td>
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<td>ETV6,11 NUP214,11 RCSD1,11 RANBP2,11 SNX2,19 ZMIZ120</td>
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</table>

* The gene is a previously unreported fusion partner.
† ETV6–NTRK3 has been reported in multiple cancers, including congenital fibrosarcoma25,26 and secretory breast carcinoma,27 but it has not previously been described in acute lymphoblastic leukemia.28,29
There are MANY “actionable” gene fusions in Ph-like B-ALL
Potential methodologies for identifying Ph-like leukemia

Low density array: currently used primarily in the research setting to initially screen for Ph-like ALL

FISH: breakapart probes for known kinase genes would be simple, cost effective but limited in scope

RT-PCR: can screen for or confirm known fusions

Digital molecular barcoding platform

Capture-based RNA sequencing

Next-generation sequencing
Clinical course and response to therapy

- C8811 (Larson) protocol with rituximab
- 'B' arm of hyperCVAD together with ruxolitinib
- Blinatumomab (1 cycle)
- CLOVE chemotherapy (clofarabine, cyclophosphamide, etoposide).
- Inotuzumab (1 cycle)
- At various time points, considered for clinical trials that either closed, where he did not respond, or he was unable to enroll due to either diagnosis or condition (eg infection).

- Persistent disease (34% blasts)
- Persistent disease (85% blasts)
- Persistent disease (96% blasts)
- Persistent disease (70% blasts)
- Persistent disease (50% blasts)
Summary

• Ph-like B-ALL with isolated MPO vs “Ph-like” MPAL
  - Potential for further clarification of diagnostic classification

• Ph-like B-ALL is characterized by kinase rearrangements of which CRLF2 rearrangement is the most prevalent and is often associated with JAK1/2 mutations.
  - Potential for targeted inhibition of JAK/STAT signaling pathways

• Optimal detection of Ph-like B-ALL? Multiple modalities are possible.

• Need for additional clinical trials investigating therapeutic efficacy of kinase inhibitors and optimization of treatment regimens in Ph-like B-lymphoblastic leukemia
  - Consider inclusion of cases of Ph-like MPAL
Final panel diagnosis:

B-lymphoblastic leukemia, *BCR-ABL1*-like

versus

Mixed phenotype acute leukemia, B/myeloid, not otherwise specified