Mixed Phenotype Acute Leukemia with BCL11B Copy Gain: What is the Best Strategy?

Jinjun Cheng[1], Keri Toner[1], and Reuven Schore[1]

[1]Children's National Hospital Center for Cancer and Blood Disorders

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Keri Toner MD[1, 2], Reuven Schore MD[1,2] and Jinjun Cheng MD PhD[1,3,4*]

1. Center for Cancer and Blood Disorders, Children's National Hospital, Washington, DC
2. Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, USA
3. Division of Pathology and Laboratory Medicine, Children's National Hospital, Washington, DC
4. Departments of Pathology and Pediatrics, The George Washington University School of Medicine and Health Sciences, Washington, District of Columbia, USA

***Correspondence to:**

Jinjun Cheng, MD, PhD

Pathology and Laboratory Medicine Division

Children's National Hospital

Room 1629, 111 Michigan Avenue NW

Washington, DC 20010-2970

Telephone: 202-476-4311

Fax: 202-476-4030

Email: jcheng2@childrensnational.org

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List of abbreviations:

Abbreviation	Full term
MPAL	Mixed Phenotype Acute Leukemia
BCL11B	BCL11 Transcription Factor B
FLT3-ITD	FLT3 internal tandem duplication

Abbreviation	Full term
COG	Children Oncology Group
WHO	World Health Organization

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To the Editor:

Mixed-phenotype acute leukemia (MPAL) is a rare, heterogeneous group of leukemia that comprises admixture of myeloid and lymphoid blasts (bilineal) or a single blast population co-expressing lymphoid and myeloid markers (biphenotypic). The accurate diagnosis relies on the immunophenotype of flow cytometric or immunostaining findings.[1-3] European Group of Immunological Characterization of Leukemia (EGIL) was released in 1990s to establish guidelines for the characterization of acute leukemia based on marker expression,[2] and the World Health Organization (WHO) classification also acknowledges lineage-specific antibodies and genetic markers.[2] In brief, the assignment of myeloid lineage depends on the expression of Myeloperoxidase (MPO); the assignment of monocytic differentiation depends on at least 2 of the following: Nonspecific Esterase, CD11c, CD14, CD64 or Lysosome; the assignment of B-lineage depends on CD19 and more than 1 or 2 strong expression of CD79a, cytoplasmic CD22 or CD10; the assignment of T-lineage depends on strong cytoplasmic CD3 (strong is equal to brighter than the normal T cells) or surface CD3 expression.[1]

Recent genetic studies reveal recurrent aberrations in MPAL, which have been integrated in the classification of MPAL with *BCR::ABL1*fusion, *KMT2A* rearrangement, *ZNF384* rearrangement, and*BCL11B* rearrangement.[1] Within these genetic aberrations, *BCL11B* rearrangement is particularly interesting. Inactivating mutations of *BCL11B* have been identified in up to 16% of T-acute lymphoblastic leukemia (T-ALL), indicating its likely role of tumor-suppressor gene for T-ALL.[4,5] However, novel *BCL11B* rearrangements have been identified in MPAL, early T-precursor-ALL, and in poorly differentiated AML with aberrant T-cell antigen expression.[6-10]

The complex phenotype of MPAL creates not just a diagnostic challenge, but challenges in determining and initiating optimal therapy.[11] Lack of prospective trials and varying classification systems leads to inability to define standard therapy. A recent retrospective study by an MPAL cohort from the Children's Oncology Group (COG), suggests that ALL chemotherapy without HSCT may be the preferred initial therapy for pediatric MPAL.[12]Ongoing efforts are underway within COG to prospectively evaluate the use of high risk ALL therapy in MPAL. Here we report a diagnostically challenging T/My MPAL patient with *BCL11B* copy gain.

Case Presentation:

A 9-year-old male presented with lymphadenopathy, leukocytosis with white blood cell count (WBC) 277 $x10^9$/L, Hemoglobin 6.1g/dL, and circulating blasts in peripheral blood. The flow cytometry analysis of peripheral blood confirmed 85% blasts expressing CD34, CD13, CD117, CD15, HLA-DR partial, CD7 and CD2. Most blasts were negative for MPO but equivocally express cytoplasmic CD3 (with an intensity lower than background T cells). In addition, a small population of discrete blast population (less than 5%) were positive for MPO. These flow cytometry findings raised differential diagnoses of MPAL, early T precursor (ETP)-ALL or AML with aberrant T cell antigen expression. A bone marrow biopsy was performed, and the flow cytometry analysis of bone marrow revealed dim cytoplasmic CD3 on blasts with the intensity equal to background T-cells. There was a discrete population of blasts (5-10%) with positive MPO expression. The findings supported a T/My MPAL diagnosis based on the current WHO diagnostic criteria. The immunostains of CD3 and MPO on bone marrow core biopsy also supported the diagnosis. The genetic and cytogenetic karyotype studies on bone marrow aspirate revealed a *BCL11B* copy gain (rearrangement not detected) and FLT3-ITD. The patient was treated on COGAALL1732 protocol and while leukocytosis

responded to initial treatment, on day three of treatment, the patient was found on CT to have a large intraparenchymal hemorrhage and died the following day.

We report a unique case demonstrating the diagnostic and treatment challenge of MPAL with *BCL11B* copy gain. MPAL comprises less than 5% of pediatric acute leukemia.[13] The current diagnostic criteria of MPAL relies on flow cytometry, immunohistochemistry or less frequently by cytochemical stain to determine the blast lineage.[7] However, it is subjective regarding the percentage and intensity of antigen expression when assigning the lineage. For instance, the peripheral blasts in our patient revealed very dim expression of cytoplasmic CD3 and less than 5% MPO+ blasts, which together created a diagnostic challenge.[14]

Because of the rarity of MPAL, there are frequently diagnostic challenges that could lead to delay in treatment initiation as well as a lack of prospective trial data to guide therapy.[11,12,15] *BCL11B* rearrangement is known to associate with a distinct subgroup of acute leukemias with a broadly variable phenotype, including AML, ETP-ALL, and MPAL.[5,6,8,13,16] The functional consequence of*BCL11B* rearrangement is overexpression of*BCL11B* .[8] Our patient had a *BCL11B* copy gain without known rearrangement, which might be an alternative mechanism of *BCL11B* overexpression. Moreover, the leukemias with*BCL11B* aberrations frequently contain FLT3-ITD and other high risk mutations.[8] An ex vivo drug-sensitivity profile study revealed that FLT3 tyrosine kinase inhibitors are effective on these leukemias with *BCL11B* rearrangement and FLT3-ITD,[8] which may provide a novel therapy strategy for MPAL with *BCL11B* rearrangement in the future.

In summary, MPAL is a rare leukemia which presents diagnostic challenges. We report a patient with an identified *BCL11B* copy gain without rearrangement present in T/myeloid MPAL. Identification of this *BCL11B* aberration may contribute to the current molecular knowledge of MPAL, and ideally additional prospective studies of patients with MPAL will more clearly define diagnostic criteria and appropriate therapy.

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Figure Legend:

Figure 1. Flow cytometry and FISH results of T/My mixed phenotype acute leukemia. A: The peripheral blood flow cytometry identified a major blast population expressing equivocal cytoplasmic CD3 (with intensity not reaching to the CD3 intensity of normal T cells), and a small population of MPO+ CD34+ blasts (less than 5%). B: The repeated flow cytometry on bone marrow aspirate revealed cytoplasmic CD3 dim+ (with intensity equal to normal T cells) and cytoplasmic MPO in a sub-population of blasts (5-10%). C: Representative blast on bone marrow aspirate (originally 1000x Gimsa stain). D: The FISH study revealed a copy gain of *BCL11B* in 58 out of 100 cells (green for BCL11B probe; red for TLX3 probe.). CD3 (E, 200x) and MPO (F, 200X) immunostains are positive on a subset of blasts on core biopsy, which also support the diagnosis of T/My MPAL.