Leukemia Research Reports 21 (2024) 100464

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Acute leukemia with *KMT2A* rearrangement: A master of disguise

Sawyer J. Bawek [a,c], Eunice S. Wang [b], Steven D. Green [b,*]

[a] Department of Internal Medicine, University at Buffalo, Buffalo, NY, United States
[b] Leukemia Service, Department of Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States
[c] Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States

ARTICLE INFO

Keywords:
Acute leukemia
KMT2A
Mixed-phenotype acute leukemia
ALL
AML

ABSTRACT

Mixed-phenotype acute leukemia (MPAL) is a rare form of leukemia with ambiguous lineage, and there are challenges in accurately diagnosing this entity according to formal criteria. Here we report a case which was initially diagnosed as "AML" based on atypical peripheral blood flow cytometry that was subsequently determined to be B-ALL with *KMT2A* rearrangement based on marrow results. Although *KMT2A* rearrangements represent a defining genetic abnormality for acute leukemia of ambiguous lineage, this case did not meet the criteria for MPAL based on WHO 2022 criteria. This case highlights the diagnostic challenges of MPAL and the potential limitations of the current classification. We discuss the most appropriate workup and management of these patients and identify areas for future study.

1. Background

There are two main lineages of acute leukemia—Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL), the latter of which includes precursor B-Lymphoblastic (B-ALL) and T-Lymphoblastic (T-ALL) leukemias. Mixed-phenotype acute leukemia (MPAL) is a rare form of acute leukemia with ambiguous lineage and is estimated to represent <3 percent of acute leukemias [1–4].

The diagnosis of MPAL requires ≥ 20 % leukemic blasts in the blood or bone marrow and/or extramedullary leukemia with an ambiguous lineage and cannot be assigned to just one hematopoietic lineage. The World Health Organization (WHO) 2022 classification groups acute leukemia of ambiguous lineage (ALAL) and MPAL under a single category and subdivides them into either ALAL with defining genetic abnormalities or ALAL defined on the basis of immunophenotyping only [5]. Flow cytometry can reveal bilineal or biphenotypic patterns of antigen expression. Acute bilineal leukemia is composed of two or more distinct populations of leukemia cells from two different lineages, whereas biphenotypic leukemia occurs when a single blast cell population co-expresses antigens that are characteristic of both lineages.

Lineage assignment depends on the strength of the association between the antigen and the lineage being assessed, and/or if a coordinated pattern of expression of multiple antigens from the same lineage is demonstrated. For example, in ALL, myeloid-associated antigens such as CD33 and CD13 may be expressed, but their presence does not preclude the diagnosis of ALL nor are they associated with a poor prognosis. Instead, the WHO criteria emphasize certain key lineage markers such as CD19 for B lineage, CD3 for T lineage, and Myeloperoxidase (MPO) for the myeloid lineage (Table 1) [6]. The WHO classification has individual laboratories specify thresholds for positivity. There is currently no consensus cutoff for positive myeloperoxidase expression in acute leukemia, with various groups proposing ranges from 3 to 28 % blasts [5].

Importantly, cases of ALL or AML in which a diagnosis of MPAL is not being considered do not need to meet these stricter MPAL criteria to determine the lineage. Similarly, in cases in which two distinct blast populations can be resolved, it is not necessary that these specific markers be present. Of note, the 2022 International Consensus Classification of myeloid neoplasms did not make any changes to their definition of ALAL, but a working group will report on them separately in the future [8].

Leukemias with multi-lineage protein expression often respond poorly to standard chemotherapy and generally have worse prognoses and outcomes than AML or ALL. Some proposed reasons are that mixed-phenotype blasts can adapt to therapies and switch phenotypes based on selection pressure, and that some MPALs may express high levels of multidrug resistance proteins [9,10].

Due to limited data, particularly in terms of prospective studies, there is no standard of care or consensus of the optimal treatment for MPAL. Philadelphia chromosome-positive (Ph+) MPAL appears to benefit from the incorporation of TKIs [11,12]. For Ph- MPAL, the

* Corresponding author.
E-mail address: steven.green@roswellpark.org (S.D. Green).

https://doi.org/10.1016/j.lrr.2024.100464
Received 18 March 2024; Accepted 14 May 2024
Available online 24 May 2024

Table 1
World Health Organization (WHO) 2022 lineage assignment criteria for mixed-phenotype acute leukemia.

Lineage	Criterion
B-Lineage	
CD19 strong[a]	1 or more also strongly expressed CD10, CD 22, or CD79a[c]
OR,	
CD19 weak[b]	2 or more also strongly expressed CD10, CD22, or CD79a[c]
T-lineage	
CD3 (CYTOPLASMIC OR SURFACE)[d]	Intensity in part exceeds 50 % of mature T-cells level by flow cytometry or, Immunocytochemistry positive with non-zeta chain reagent
Myeloid lineage	
Myeloperoxidase	Intensity in part exceeds 50 % of mature neutrophil level
OR,	
Monocytic differentiation	2 or more expressed: Non-specific esterase, CD11c, CD14, CD64, or lysozyme

Data derived from Khoury et al. [7].
[a] CD19 intensity in part exceeds 50 % of normal B cell progenitor by flow cytometry.
[b] CD19 intensity does not exceed 50 % of normal B cell progenitor by flow cytometry
[c] Provided T lineage not under consideration, otherwise cannot use CD79a.
[d] Using anti-CD3 epsilon chain antibody.

preponderance of data suggests that most patients will benefit from starting with an ALL regimen followed by allogeneic stem cell transplantation in first complete remission (CR), as CR duration may be short [10,13]. For example, a large meta-analysis revealed that MPAL patients were significantly more likely to achieve CR and twice less likely to die with an ALL-like induction therapy compared to an AML-like regimen [14]. Patients <40 years old can be treated with a pediatric-type ALL regimen and those ≥40 years old can be treated with an adult-type ALL regimen. Patients should also have an initial diagnostic lumbar puncture with intrathecal chemotherapy administered. Treatment failure is especially challenging given the even more limited data that exist. Currently, the most common approach is to switch regimens from an ALL-like regimen if used to an AML-like regimen or vice versa [15].

2. Case presentation

A 69-year-old female with a past medical history significant for Diffuse Large B-Cell Lymphoma in complete remission status post 6 cycles of R-CHOP completed two years prior presented to the ED with generalized weakness and bruising. Lab work on presentation was significant for a hemoglobin of 9.2 g/dL and platelets of 14×10^9/L. The WBC count was 5.9 but a peripheral smear revealed 50–70 % blasts consistent with a diagnosis of acute leukemia.

Flow cytometry from the peripheral blood revealed an abnormal blast population expressing CD7 (dim, variable), CD13, CD33, CD19 (dim to negative), CD34 (heterogeneous), CD38, CD45 (dim to negative), CD64 (heterogeneous), and HLA-DR representing 60 % of total cells (Table 2). Cytogenetics revealed t(11;19) (q23;p13.3). Based on these results, the patient was given a diagnosis of 'acute myeloid leukemia with *KMT2A* rearrangement.' A bone marrow biopsy was performed, and she was initiated on treatment with venetoclax and azacitidine.

Two days later, the bone marrow biopsy confirmed acute leukemia with *KMT2A* rearrangement with 91 % blasts in a 95 % hypercellular marrow. However, the blasts expressed B-cell markers CD19 and CD79a (by both flow and IHC), PAX-5 by IHC, CD58 and CD9 (by flow), and were negative for myeloperoxidase. Next-generation sequencing revealed a *DNMT3A* mutation (VAF 38.6 %). The diagnosis was amended to acute leukemia best classified as precursor B-lymphoblastic leukemia (pre-B ALL).

Based on the marrow results, the patient's treatment was then

Table 2
Differences in peripheral blood immunophenotype and marrow at diagnosis and following each treatment.

	Date of bone marrow biopsy	Cytogenetics	Peripheral blasts	Next generation sequencing	Flow cytometry B-cell antigen blasts%	Flow cytometry myeloid cell antigens blasts%	B-cell expression	Myeloid cell expression
Initial Diagnosis Bone Marrow	11/29/2022	46,XX, t(11,19) in 5 cells	91 % (BM)		CD19 = 42 %; CD20 = 0 %; CD22 = 0 %	CD33 = 90 %; CD123 = 100 %;	CD9, CD19, CD34, CD38 CD79a, PAX5, CD58, CD200	CD7,CD13,CD33, CD64, CD117, CD123
Initial Diagnosis Peripheral		46,XX, t(11,19) in 5 cells	68 %	DNMT3S (VAF 38.6 %)	CD19+42 % CD20 = 0 % CD22 = 0 %	CD33 = 90 %	CD19, CD34, PAX5,HLDA-DR (60 %),	CD7, CD13, CD33, C CD38,CD45, CD64
Following ALL Therapy with minihyperCVD	1/5/2023		9 %		CD19 = 98 %, CD20 = 0 % CD22 = 0 %	CD33 = 88 %; CD117 100 %; CD123 = 99 %	CD9,CD19, CD34, CD38, CD58, CD200, HLA-DR	CD7, CD11b, CD13, CD33, CD45, CD64, CD117, CD123
Following Blinatumomab Therapy	2/2/2023	46XXt(11;19) (q23p.13)[5]; 46XX[15]	89 %			CD33 = 100 % CD117 = 98 %; CD123 = 99 %	CD34,CD38,	CD4,CD7,CD13, CD33,CD34,CD36, CD38,CD45,CD64, CD117,CD123
AML Therapy with GO	3/27/2023	46XX[20]	1 %		CD19 = NE%, CD20 = NE%, CD22 = NE%	CD33 = 100 %, CD117 = 100 % CD123 = 47 %	CD34, CD9, CD58	CD7,CD13, CD33, CD36,CD38,CD45, CD64,CD117, CD123
Menin Inhibitor Therapy s/p Cycle 1	6/28/2023		95 %		CD19 = 0 % CD20 = 0 % CD22 = 0 %	CD33 = 98 % CD117 = 23 % CD123 = 81 %	CD9,CD34,CD58, HLA-DR	CD7,CD11b,CD33, CD38,CD45,CD64, CD71,CD117, CD123
Menin Inhibitor Therapy s/p Cycle 2	7/26/2023	No Mitosis	17 %			CD33 = 100 % ; CD117 = 8 %; CD123 = 96 %	CD34,	CD11b,CD13, CD33, CD36,CD38,CD45, CD117, CD123, Lysozyme
Menin Inhibitor Therapy s/p Cylce 4	9/13/2023	46XXt(11;19) (q23;p.13.3) [2] 46XX[18]	6 %			CD33 = 100 %; CD117 = 98 %; CD123 = 100 %	CD34	CD4, CD7,CD13, CD11b,CD33.CD38, CD45, CD64,CD117, Lysozyme

switched to the mini-hyperCVD regimen (cyclophosphamide 150 mg every 12 h days 1–3, vincristine 2 mg IV on days 1 and 8, methotrexate 12 mg IT on day 2, cytarabine 100 mg IT on day 8, dexamethasone 20 mg PO days 1–5). Of note her blasts were negative for CD20 and CD22 and molecular testing did not show clonal rearrangement of the IGH or IGK gene regions.

Because of persistent cytopenias despite the administration of growth factor, a bone marrow biopsy was repeated and revealed a markedly hypocellular marrow (<5 %) with residual acute leukemia (9 % blasts) with similar immunophenotype as the diagnostic specimen.

She received blinatumomab for refractory disease for one cycle with persistence of pancytopenia and new emergence of circulating blasts. Follow-up bone marrow revealed a hypocellular marrow (20 %) with 89 % blasts expressing CD117 and negative for CD34, Pax5, CD19, Tdt, myeloperoxidase, E-cadherin, and CD61. The phenotype differed from the diagnostic specimen with loss of CD19 expression and strong expression of CD33.

She was then treated with gemtuzumab ozogamicin 4.5 mg IV on days 1, 4, and 7 to target CD33-positive blasts. Follow-up bone marrow biopsy revealed a hypocellular marrow (10 %) with no morphological evidence of involvement by acute leukemia. Flow demonstrated measurable residual disease with 0.4 % of abnormal cells expressing CD33 and CD117. She had a prolonged delay in treatment of 4 months due to cytopenias and debility. She then developed circulating blasts in conjunction with a hypocellular marrow (overall 15 %) with 59 % blasts expressing CD33, 117, 123, and negative for CD19 and PAX5.

She recently enrolled in a clinical trial with a novel menin inhibitor and achieved stable disease. After 4 cycles of therapy, repeat marrow shows a hypocellular bone marrow (overall 15 %), with decreased trilineage hematopoiesis, multilineage dyspoiesis, and 6 % blasts. Blasts appear negative for CD34, CD 117, and CD19, and positive for CD33, CD117, and CD123.

3. Discussion

Here we report a case in which an atypical flow cytometric profile was initially labeled as 'AML,' treatment was initiated, and subsequently based on additional testing the patient was given a diagnosis of B-ALL with *KMT2A* rearrangement. *KMT2A* (MLL) rearrangements represent a defining genetic abnormality for ALAL, as well as for both AML and ALL. More than 80 fusion partners with KMT2A have been described, and some rearrangements may be cryptic on conventional karyotyping. Despite a high suspicion for MPAL, our case never met the formal WHO 2022 criteria for MPAL. Specifically, MPO was negative, and there were not ≥2 monocytic differentiation markers present to fulfill the myeloid lineage requirement. Moreover, over the course of treatment, although the immunophenotype changed with loss of B-cell markers, a bilineage MPAL was still never able to be diagnosed.

There is some precedence for this; for example, *ZNF384*-rearranged ALL is known to often have lineage ambiguity with aberrant myeloid antigen expression not rising to the definition of MPAL [13]. Shifts in lineage during disease evolution suggest that ALL with these alterations can retain multi-lineage potential. More rarely, in addition to our report, *KMT2A*-rearranged B-ALL has also been noted to exhibit this behavior following treatment with Blinatumomab [16–18].

Given the known challenges of accurately diagnosing MPAL, including operator-specific flow cytometric analyses, biological heterogeneity, and low disease incidence, it is likely that the classification of this entity will continue to evolve over time due to additional advances in our understanding both of MPAL and the other acute leukemias. For now, as shown in our case, some acute leukemias otherwise considered to be MPAL will continue not to meet the formal criteria and thereby illustrate the potential limitations of the current WHO system. It is also important clinically to be aware that shifts in lineage can occur without reaching MPAL diagnostic thresholds, yet still impact treatment. Ultimately the clinical phenotype supersedes the label applied, particularly in *KMT2A* rearranged ALL with known ambiguous lineage characteristics.

From a practical standpoint, if the flow cytometry diagnosis is unclear, it may be prudent to await the results of comprehensive bone marrow testing including immunohistochemical and/or cytoplasmic testing before initiating treatment. Clinicians and pathologists should provisionally label the disease as a generic 'acute leukemia' until the lineage status can be confirmed to avoid confusion.

Incorporating measurable residual disease (MRD) assessments into cases such as ours in which lineage shifts occur may be particularly challenging and requires further study. Because of the evolution of clonal populations and their immunophenotypes over time and treatment regimens, a wider net of flow cytometry markers may need to be cast to monitor for disease changes.

Finally, after failing approaches directed at both lymphoid and myeloid markers, the patient was enrolled in a clinical trial of a menin inhibitor. Menin inhibitors have demonstrated early evidence of clinical activity in both AML and ALL [19]. Additional research is needed to determine whether targeting specific underlying molecular abnormalities of MPAL/shifting lineage acute leukemias rather than focusing on lineage-specific approaches may be more fruitful than chasing their more malleable immunophenotypes.

Informed consent

Informed written consent was obtained from the patient. Per instructions in the Guide for Authors, written consents must be retained by the author, but copies should not be provided to the journal. If specifically requested please let us know and we will provide a copy.

CRediT authorship contribution statement

Sawyer J. Bawek: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Eunice S. Wang:** Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing. **Steven D. Green:** Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] N.J. Charles, D.F. Boyer, Mixed-phenotype acute leukemia: diagnostic criteria and pitfalls, Arch. Pathol. Lab. Med. 141 (2017) 1462.
[2] L. Yan, N. Ping, M. Zhu, et al., Clinical, immunopheotypic, cytogenetic, and molecular genetic features in 117 adult patients with mixed-phenotype acute leukemia defined by WHO-2008 classification, Haematologica 97 (2012) 1708.
[3] O.K. Weinberg, D.A. Arber, Mixed-phenotype acute leukemia: historical overview and a new definition, Leukemia 24 (2010) 1844.
[4] W. Van Den Ancker, M. Terwijjn, T.M. Westers, et al., Acute leukemias of ambiguous lineage: diagnostic consequences of the WHO2008 classification, Leukemia 24 (2010) 1392.
[5] J.D. Khoury, E. Solary, O. Abla, et al., The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms, Leukemia 36 (2022) 1703–1719, https://doi.org/10.1038/s41375-022-01613-1.
[6] M.J. Borowitz, N.L. Harris, A. Porwit, E. Matutes, Acute leukemias of ambiguous lineage. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, World Health Organization Classification of Tumours, Lyon, France, 2008.
[7] J.D. Khoury, E. Solary, O. Abla, Y. Akkari, R. Alaggio, J.F. Apperley, R. Bejar, E. Berti, L. Busque, J.K.C. Chan, W. Chen, X. Chen, W.J. Chng, J.K. Choi, I. Colmenero, S.E. Coupland, N.C.P. Cross, D. De Jong, M.T. Elghetany, E. Takahashi, A. Hochhaus, The 5th edition of the World Health Organization classification of haematolymphoid tumours: myeloid and histiocytic/dendritic neoplasms, Leukemia 36 (7) (2022) 1703–1719, https://doi.org/10.1038/s41375-022-01613-1.

[8] D.A. Arber, A. Orazi, R. Hasserjian, et al., The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood 127 (20) (2016) 2391–2405.
[9] O. Wolach, R.M. Stone, How I treat mixed-phenotype acute leukemia, Blood 125 (2015) 2477–2485.
[10] O. Hrusak, V. de Haas, J. Stancikova, et al., International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia, Blood 132 (2018) 264–276.
[11] C. Kawajiri, H. Tanaka, S. Hashimoto, Y. Takeda, S. Sakai, T. Takagi, M. Takeuchi, C. Ohwada, E. Sakaida, N. Shimizu, C. Nakaseko, Successful treatment of Philadelphia chromosome-positive mixed phenotype acute leukemia by appropriate alternation of second-generation tyrosine kinase inhibitors according to BCR-ABL1 mutation status, Int. J. Hematol. 99 (4) (2014) 513–518, https://doi.org/10.1007/s12185-014-1531-0. AprEpub 2014 Feb 15PMID: 24532437.
[12] H. Shimizu, A. Yokohama, N. Hatsumi, S. Takada, H. Handa, T. Sakura, Y. Nojima, Philadelphia chromosome-positive mixed phenotype acute leukemia in the imatinib era, Eur. J. Haematol. 93 (4) (2014) 297–301, https://doi.org/10.1111/ejh.12343. OctEpub 2014 May 13PMID: 24750307.
[13] T.B. Alexander, E. Orgel, Mixed phenotype acute leukemia: current approaches to diagnosis and treatment, Curr. Oncol. Rep. 23 (2021) 1–10.
[14] M. Maruffi, R. Sposto, M.J. Oberley, L. Kysh, E Orgel, Therapy for children and adults with mixed phenotype acute leukemia: a systematic review and meta-analysis, Leukemia 32 (7) (2018) 1515–1528, https://doi.org/10.1038/s41375-018-0058-4.
[15] B.S. George, B. Yohannan, A. Gonzalez, A. Rios, Mixed-phenotype acute leukemia: clinical diagnosis and therapeutic strategies, Biomedicines 10 (8) (2022) 1974, https://doi.org/10.3390/biomedicines10081974. Aug 15PMID: 36009521PMCID: PMC9405901.
[16] J. Du, K.M. Chisholm, K. Tsuchiya, K. Leger, B.M. Lee, J.C. Rutledge, C.R. Paschal, C. Summers, M. Xu, Lineage switch in an infant B-lymphoblastic leukemia with t(1;11)(p32;q23); *KMT2A/EPS15*, following blinatumomab therapy, Pediatr. Dev. Pathol. 24 (4) (2021) 378–382.
[17] R.R. He, Z. Nayer, M. Hogan, R.S. Cuevo, K. Woodward, D. Heyer, C.A. Curtis, J. F. Peterson, Immunotherapy- (Blinatumomab-) related lineage switch of *KMT2A/AFF1* rearranged B-lymphoblastic leukemia into acute myeloid leukemia/myeloid sarcoma and subsequently into B/myeloid mixed phenotype acute leukemia, Case Rep. Hematol. 2019 (2019) 7394619, https://doi.org/10.1155/2019/7394619.
[18] A. Rayes, R.L. McMasters, M.M O'Brien, Lineage switch in MLL-rearranged infant leukemia following CD19-directed therapy, Pediatric Blood Cancer 63 (6) (2016) 1113–1115, https://doi.org/10.1002/pbc.25953. JunEpub 2016 Feb 23PMID: 26914337.
[19] G.C. Issa, I. Aldoss, J. DiPersio, et al., The menin inhibitor revumenib in *KMT2A*-rearranged or *NPM1*-mutant leukaemia, Nature 615 (2023) 920–924.