



Expanding diagnostic criteria: Multiorgan T-Cell/myeloid mixed phenotype acute leukemia with t(v;11q23) *KMT2A*-rearrangement successfully treated by allogeneic stem cell transplant

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ABSTRACT

Mixed phenotype acute leukemia (MPAL) consists of a leukemia of two different lineages (myeloid, T, and/or B) co-occurring in the same tissue. *KMT2A*-rearrangement is rare and usually seen in B/myeloid MPAL. We report a unique case of T/myeloid MPAL with a t(v;11q23) *KMT2A*-rearrangement, with acute myeloid leukemia (AML) in the bone marrow but concurrent T-cell acute lymphoblastic leukemia (T-ALL) in lymph node and skin. Genomic interrogation suggests an undifferentiated stem cells with *KMT2A* rearrangement as the founder mutation that acquired additional lineage-specific mutations resulting in AML in the marrow and T-ALL in other sites.

1. Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous hematological malignancies characterized by abnormal stem cell differentiation in the bone marrow [1]. AML is the most common subtype of leukemia and accounts for 1.3% of new acute cancers [2]. Although some patients achieve complete remission after induction, around 50% of patients relapse, highlighting the need for more effective treatment strategies [1].

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 12–15% of all acute lymphoblastic leukemias and is an aggressive uncommon leukemia that affects progenitor T-lymphoid stem cells and causes increased production of T-cell lymphoblasts in the bone marrow and blood [3]. Treatments for T-ALL may use central nervous system-directed therapy and risk-based multiagent chemotherapy. Relapsed disease tend to occur within 2 years of diagnosis and is difficult to treat, with survival rates lower than 25%. Once relapsed, the only treatment measure available is hematopoietic cell transplantation [4].

Mixed Phenotype Leukemia (MPAL) is a subclassification of acute leukemia, where a combined presentation of AML with T-ALL and/or B-ALL is observed. MPALs are rare, composing 2–5% of acute leukemia, with poor prognosis posing a diagnostic and therapeutic challenge [5]. Conventionally, the diagnosis of MPAL requires either a single blast

population with lineage-defining phenotypic expression of multiple lineages (myeloid, B-cell and/or T-cell) (biphenotypic) or two distinct blast populations that each independently satisfy criteria for designation as AML, B-ALL, and/or T-ALL (bilineage) [6]. Given the rarity of MPAL, minimal treatment guidelines exist. In this report, we present a unique patient with bilineal MPAL but with different leukemias in different sites (bone marrow: AML; lymph node and skin: T-ALL) with *KMT2A*-rearrangement. The patient was successfully treated with allogeneic stem cell transplant. This case challenges current diagnostic criteria and offers unique molecular insight into this rare disease.

2. Case presentation

The patient is a 34-year-old female who presented with bilateral neck pain, fatigue, dyspnea, rash, and abdominal pain for several weeks. On admission, CT demonstrated retropharyngeal edema without abscess formation, cervical lymphadenopathy, and a soft tissue mediastinal mass displacing vascular structures. CBC showed WBC of 3.8 K/uL, Hb of 5.9 g/dL, and platelets of 76 K/uL. On 8/13/2020, a bone marrow biopsy was performed, and aspirate smears showed trilineage dysplasia (Fig. 1A and 1B). Flow cytometry showed 45% blasts that were positive for CD11b, CD11c, CD33, CD34, CD45, CD117, HLA-DR., and CD7. The blasts lacked CD3, cyCD3, MPO, and TdT (Fig. 1C). Cytogenetics showed

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47, XX, +5, t(11;19)(q23;p13.1), and add(18)(q21)[20]. FISH showed 88.5% of cells with *MLL* (*KMT2A*) rearrangement. NGS was performed using in-house Trusight Myeloid assay (Illumina, CA) which identified mutations of *PHF6-R257fs*8* and *STAG2-S941fs*6*. A diagnosis of AML with myelodysplasia-related changes was made.

However, the patient also had a concurrent supraclavicular lymph node biopsy performed on 8/14/2020. The biopsy showed diffuse effacement by a population of immature cells with round to irregular nuclear contours and glassy chromatin. IHC studies showed the cells to be positive for CD3, TdT, CD99, and CD1a with co-expression of CD4 and CD8. The neoplastic cells were negative for myeloperoxidase, CD117, and CD34 (Fig. 2A). Flow cytometry detected a distinct T lymphoblastic population that showed expression of cyCD3 and TdT that lacked myeloid markers. NGS was performed using FoundationOne Heme (Cambridge, MA) which showed *KMT2A* (*MLL*) *MLL-ELL* fusion,

CDKN2A/B-p16INK4a loss and *p14ARF* loss, and *STAT5B-T628S*. Right flank skin biopsy from 8/13/20 showed dense peri-adnexal and perivascular infiltrate in the dermis, with no involvement of the overlying epidermis. The atypical cells were intermediate in size with irregular nuclear contours and dispersed chromatin. IHC showed diffuse positivity for CD3 (cytoplasmic pattern), CD1a, CD99, CD4, CD8, and TdT (Fig. 2B). CD34 and CD117 were negative in the neoplastic cells. A diagnosis of T-ALL was made in the lymph node and skin biopsies. Pleural effusion from 8/17/2020 also showed T-lymphoblastic leukemia. As a result, the patient was diagnosed with MPAL with t(v;11q23) *KMT2A*-rearrangement, with bone marrow biopsy consistent with AML, and skin, lymph node, and pleural effusion showing T-ALL.

In August of 2020, she was treated with HyperCVAD. Repeat bone marrow biopsy on 9/22/2020 showed residual disease with 25% of blasts. On 9/30/2020, she was placed on CLAG-M. Bone marrow biopsy

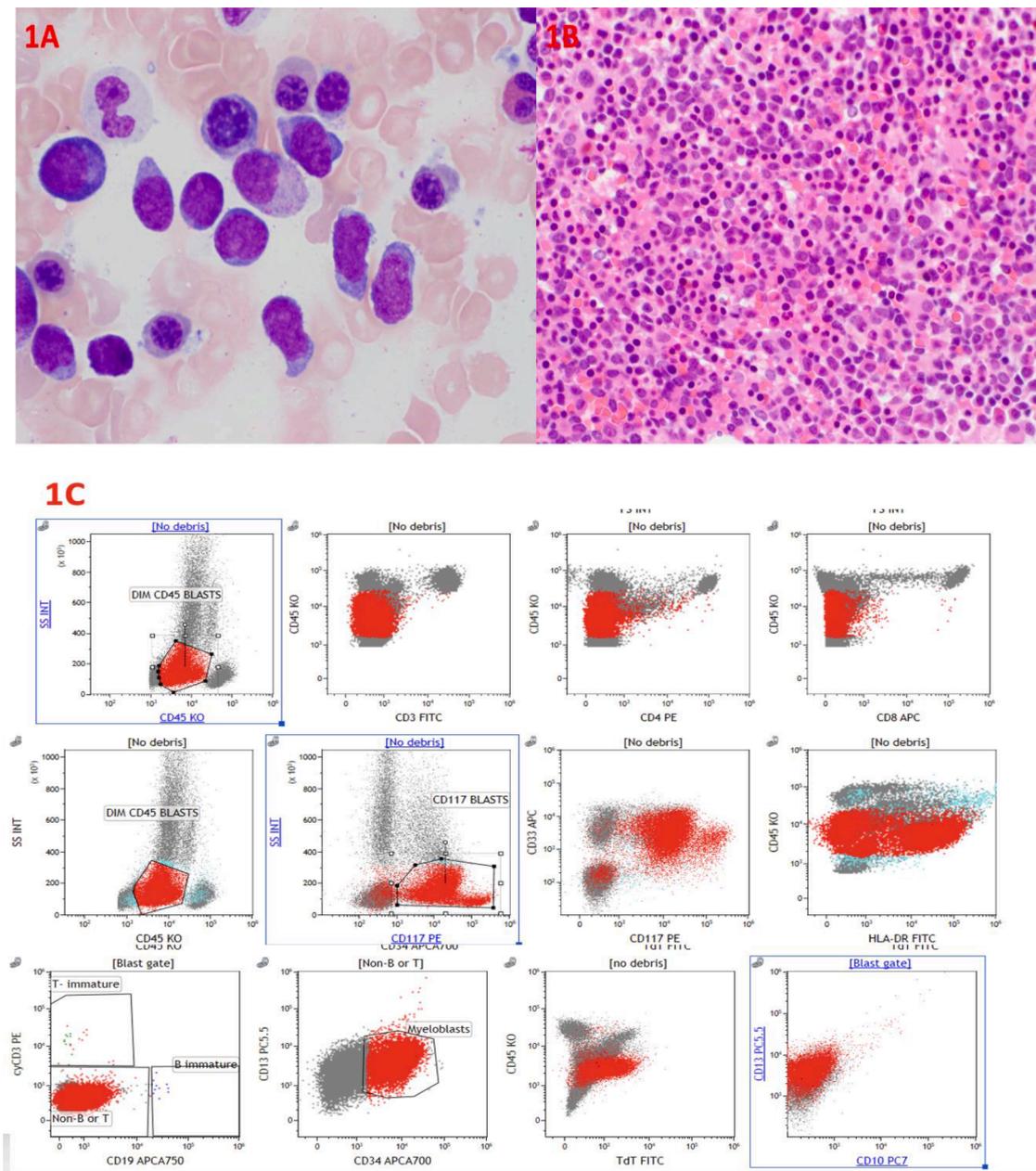
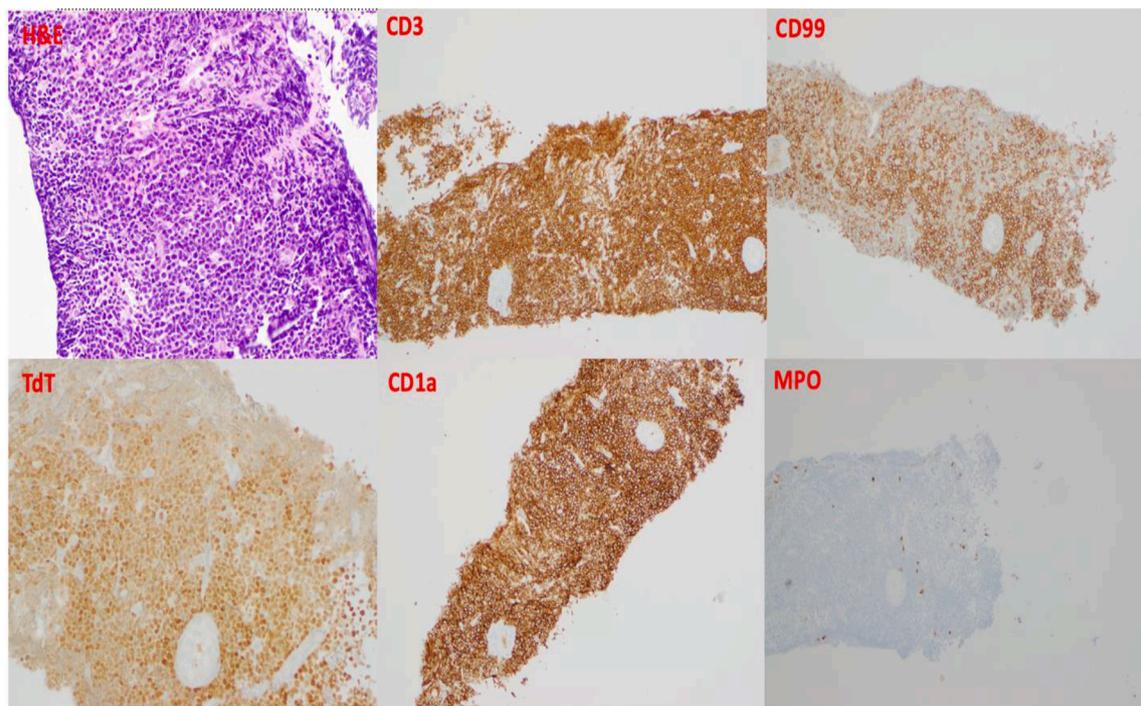


Fig. 1. (A). (1000x) Immature mononuclear cells on AML bone marrow aspirate compatible with blasts. Interestingly some show “hand-mirror” morphology more commonly associated with lymphoblasts while others resemble more conventional myeloblasts. B. Core biopsy showing hypercellularity and diffuse infiltration by blasts. C. Flow cytometric analysis of bone marrow showing AML. The blasts are positive for CD34, CD117, CD13, CD33, HLA-DR, but negative for cytoplasmic CD3, surface CD3, CD4, and CD8.

2A



2B

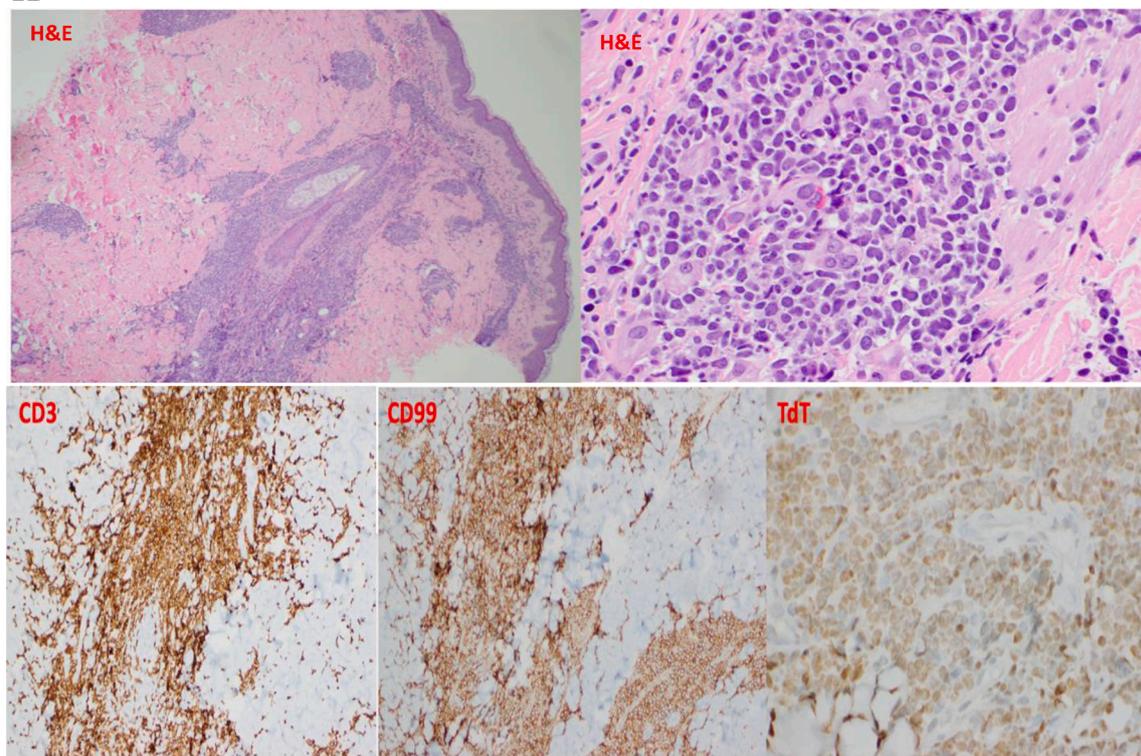


Fig. 2. (A) Supraclavicular lymph node needle core biopsy. Low power H&E section showing diffuse effacement by blasts. They are positive for CD3, CD99, and TdT (nuclear), and CD1a but lack myeloperoxidase (MPO). (B) Skin biopsy showing perivascular and peridnexal infiltrate of blasts that are positive for CD3, CD99, and TdT.

was repeated 10/13/2020 and showed a hypocellular remission marrow with no excess blasts. CT imaging showed resolution of the mediastinal mass but showed the development of bilateral pleural effusions which were negative for malignancy. She received additional consolidation with CLAG-M and pretransplant marrow was negative. She received a matched-unrelated allogeneic stem cell transplant on 2/12/2021 with

conditioning with Flu/Bu(5300). A bone marrow biopsy on 9/2/2021 revealed no morphological evidence of residual acute myeloid leukemia or T-cell lymphoblastic leukemia and the patient remains in complete remission on day +290 post-transplant.

3. Discussion

We hereby present a rare, singular case of MPAL with AML in the bone marrow and simultaneous T-ALL within the skin, lymph nodes, and pleural effusion. There are several unique aspects with regards to this case. First, the existence of two different types of clonally-related leukemias in two different locations has never been described and expands current diagnostic criteria for MPAL. Second, the 11q23/*MLL*-rearrangement is seen in only 8% of MPAL, usually B/myeloid with only two prior cases in T/myeloid MPAL in the literature [7]. Third, *MLL-ELL* fusion has never been described in T/myeloid MPAL. Fourth, we carefully are able to dissect the molecular mutational landscape of the leukemias at different sites. Fifth, we document allogeneic stem cell transplant as an effective strategy in this patient.

MPAL typically presents in two scenarios. The first being a single population of cells with a demonstrated immunophenotype that spans two or more lineages. Myeloid lineage can be established by presence of myeloperoxidase or expression of 2 or more monocytic markers CD11c, CD14, CD64, and lysozyme. T cell lineage is established by the presence of surface or cytoplasmic CD3. B-cell lineage require strong CD19 with at least strong CD79a, cytoplasmic CD22, or CD10. If CD19 is weak then at least two of these additional markers should be strongly expressed. The second diagnostic scenario in MPAL involves two separate populations of blasts with each demonstrating its own singular lineage assignment [8]. However, this type of MPAL typically involves the presence of two distinct (myeloid and lymphoid) blast populations in the same location, namely the bone marrow. The presence of two disparate types of acute leukemia in different organ sites simultaneously, one medullary and extramedullary, is remarkable. Moreover, both tumors shared *MLL* translocation allowing us to infer that they are clonally-related, likely arising from a common stem cell progenitor. We posit that there was an immature stem cell with significant plasticity to allow for bilineal differentiation. The two clones bifurcated by acquiring different mutations and maturing along different lineage pathways. Based on this, we propose to expand the current diagnostic criteria of MPAL to include cases in which there is synchronous presentation of two phenotypically distinct acute leukemia populations (AML plus T-ALL or B-ALL) in different body compartments.

With regards to molecular pathogenesis, we hypothesize that a common, undifferentiated stem cells in different locations (sharing founder *MLL*-rearrangement) acquired additional but distinct driver mutations based on their respective microenvironment, such that those in the bone marrow became myeloid blasts and those in the lymph node and skin differentiated into T lymphoblasts. In the AML component, *STAG2* and *PHF6* mutations were detected. *STAG2* is part of the cohesin complex and has been reported in MDS and secondary AML. *PHF6* mutation, on the other hand, is typically associated with T-ALL and supports the idea of genomic ambiguity within the AML component [9, 10]. On the other hand, the T-ALL showed *CDKN2A/B-p16INK4a* loss, *p14ARF* loss and *STAT5B* p.T628S. Inactivation of the *INK4/ARF* tumor suppressors is a hallmark of T-ALL. Furthermore, *STAT5B* mutations have been implicated as putative drivers of T-ALL [11].

MPAL *KMT2A*-rearranged cases are predominantly seen with B-cell lineage blasts. In the newest edition of the WHO classification of hematopoietic tumors, it was proposed that a *KMT2A* translocation could theoretically lead to a T-cell acute lymphoblastic leukemia, but no such case was reported at that time. Since then, there have been only a two reports of T/myeloid MPAL with *MLL* rearrangement to the best of our knowledge [12, 13]. The *MLL* fusion partner in this case (*ELL*-gene) has never been described.

Prior studies have found treatment with allo-HSCT following chemotherapy offers a survival advantage relative to chemotherapy alone in MPAL (B and T-myeloid) patients [14, 15]. Furthermore, T-myeloid MPAL patients have been found to benefit from allo-HSCT [15]. This particular patient did not respond to initial therapy with HyperCVAD but responded to a more aggressive CLAG regimen followed

by allogeneic hematopoietic stem cell transplant (allo-HSCT), which supports the use of transplantation as a treatment strategy for T/myeloid MPAL patients in remission. Response to treatment data is valuable in these patients given the rarity of disease and their poor prognosis compared to other types of leukemia. Furthermore, it is worthy of mention that the presence of *KMT2A* rearrangement in both clonal lineages in this patient offers a fortuitous biomarker for measuring minimal residual disease post-transplant by either FISH, NGS, or more sensitive allele-specific PCR assay methods [16].

4. Conclusion

This report presents a rare case of T-cell/myeloid MPAL with t(v;11q23) *KMT2A*-rearrangement, which expands current diagnostic algorithms, provides novel molecular insights into this rare disease, and provides data regarding therapeutic approaches to similar patients.

Conflict of Interest

None

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