Case Report

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

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1. Introduction

Immunotherapy targeted at CD19, either antibody-based (blinatumomab) or T-cell mediated (CAR T cells), represents a promising treatment strategy for patients with refractory B-lymphoblastic leukemia (B-ALL). Early phase clinical trials have shown high rates of complete remission in refractory pediatric B-ALL patients after CD19 CAR-T-cell or blinatumomab therapy [1, 2]. However, a rare event, lineage switch from B-ALL to acute myeloid leukemia (AML) can occur following CD19 targeted therapy, most commonly in KMT2A-rearranged B-ALL [3–6]. The KMT2A gene is a critical target of chromosomal rearrangements observed in ALL, AML, mixed phenotype acute leukemia (MPAL), and therapy-related myeloid neoplasms [7]. The presence of KMT2A rearrangement, especially in B-ALL, has long been associated with a higher risk of lineage switch under therapy and is an independent dismal prognostic factor [8]. However, the exact mechanism and management of lineage switch events are unclear. Herein, we report a 40-year-old female with KMT2A/AFF1-rearranged B-ALL that was refractory to conventional chemotherapy. Following administration of blinatumomab, she developed a breast mass proven to be myeloid sarcoma, in addition to bone marrow involvement by AML. Approximately six weeks after cessation of blinatumomab, a repeat bone marrow examination revealed B/myeloid MPAL.
2. Case Description

A 40-year-old female without a past medical history presented with two weeks of easy bruising, fatigue, and muscle aches. A complete blood count revealed leukocytosis (white blood cell count, 71.8 × 10^3/μL; reference, 3.4–9.6 × 10^3/μL), anemia (hemoglobin, 12.6 g/dL; reference, 13.2–16.6 g/dL), and thrombocytopenia (platelet count, 77 × 10^3/μL; reference, 135–317 × 10^3/μL). Peripheral blood smear revealed numerous small-to-intermediate-sized blasts with high nuclear-to-cytoplasmic (N:C) ratio, fine chromatin, and prominent nucleoli. Flow cytometry performed on peripheral blood sample revealed a large population of blasts in the dim CD45 region expressing CD19 (bright), CD34 (dim), and CD15 (dim) and was negative for CD10. A subset of blasts appeared to be positive for myeloperoxidase (MPO). Bone marrow evaluation revealed a hypercellular bone marrow (90%) composed of numerous small-to-intermediate-sized blasts with similar morphology as the blasts are identified in peripheral blood smear (Figure 1, A1 and A2). Flow cytometry of bone marrow aspirate revealed a large population of blasts immunophenotypically identical to the blasts detected in peripheral blood (Figure 1, F). Since it was questionable for MPO positivity in a subset of blasts, immunohistochemical analysis was performed on the bone marrow biopsy specimen. The blasts were strongly positive for PAX5 (Figure 1, A3), CD19, and CD79a; focally positive for CD34; but were completely negative for MPO (data not shown). Taken together, these findings are consistent with a diagnosis of B-ALL.

Chromosome and FISH studies confirmed the presence of KMT2A/AFF1 fusion with additional chromosomal abnormalities (Figure 1, E1; Table 1). Six weeks after discontinuation of blinatumomab, a repeat bone marrow biopsy and aspirate demonstrated a hypercellular marrow (80%) with a dimorphic population of blasts composed of mixed small- and large-sized blasts (Figure 1, D1-D2). Flow cytometry and immunohistochemical studies confirmed the presence of two populations of blasts: (1) B-lymphoblasts phenotypically identical to those in the patient’s initial bone marrow specimen expressing CD19, CD34 (dim), and cytoCD79a and (2) myeloblasts phenotypically similar to those in her second bone marrow specimen expressing CD33 and CD64 (Figure 1, H). The myeloblasts were also positive for myeloperoxidase (data not shown). These findings indicated a diagnosis of B/myeloid MPAL.

We present a case of a 40-year-old female with an initial diagnosis of KMT2A/AFF1 rearranged B-ALL that subsequently switched to more aggressive types of leukemic events and with extramedullary involvement. The unique features of this case include several clonally related, but phenotypically distinct leukemic events (B-ALL, AML, myeloid sarcoma of the breast, and MPAL) that occurred within a six-month period. The transformed AML/myeloid sarcoma and MPAL were associated with administering and cessation of blinatumomab, respectively, and demonstrated additional cytogenetic abnormalities in addition to KMT2A/AFF1 fusion. This case provides evidence that two key factors appear to be involved in this lineage switching event: KMT2A rearrangement and blinatumomab therapy. KMT2A-rearranged acute leukemia represents a heterogeneous group of disease overlapping lymphoid and myeloid with more than 100 different fusion partners identified to date [6]. The presence of KMT2A rearrangement has long been associated with a higher risk of lineage switch under chemotherapy and subsequent failure to treatment even before the emergence of immunotherapies [8, 9]. As a monoclonal antibody with bispecificity for both CD19 on B cells and CD3 on cytotoxic T cells, blinatumomab has shown promising therapeutic results in treating refractory or relapsed B-ALL; however, the risk of inducing lineage switch especially in KMT2A/AFF1 rearranged B-ALL should not be underestimated.

While the exact mechanism of lineage switch remains unclear, several possible mechanisms have been proposed [9–14]. Studies have suggested that inherent lineage plasticity of early progenitor cells and immunotherapeutic...
**Figure 1:** Morphologic, immunohistochemical, flow cytometric, and cytogenetic characteristics of the patient’s leukemia. A1–A3 represent bone marrow evaluation at initial diagnosis. Bone marrow biopsy (A1, H&E, 400x) and aspirate (A2, Wright stain 100x, oil) showing numerous small-sized B-lymphoblasts which are strongly positive for PAX5 (A3). B1–B3 represent biopsy of the breast mass (B1, H&E, 400x) and touch imprint (B2, Wright stain, 100x, oil) showing numerous large-sized blasts with monocytic differentiation, which are patchy positive for lysozyme (B3). C1–C3 represent bone marrow biopsy (C1, H&E, 400x) and aspirate (C2, Wright stain, 100x, oil) showing sheets of myeloblasts which are patchy positive for lysozyme (C3). D1–D3 represent bone marrow evaluation six weeks after cessation of blinatumomab.

**Table 1:** Genetic results obtained at the time of B-ALL diagnosis, transformation to AML following initiation of blinatumomab therapy, and subsequent posttransformation to MPAL upon discontinuation of blinatumomab.

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<td>Conventional chromosome analysis</td>
<td>48, XX, +X, t(4; 11)(q21; q23), +8 [20]/49, idem, +X, i(X) (p10)x2, −8 [2]/46, XX[1]</td>
<td>63−67, XX, +X, +X, +add(X)(q22) x1−2, +1, +2, t(4; 11)(q21; q23), +der(4)(q4; 11), +6, +7, +8, +10, +13, +14, +18, +19, +19, +20, +20, +21, +21, +22, +p10, +p10, 46, XX[10]</td>
<td>48, XX, +X, t(4; 11)(q21; q23), +8 [18]/64−66, idem, +X, +add(X)(q22), +1, +2, +der(4) t(4; 11), +6, +6, +7, +10, +13, +14, +18, +19, +19, +20, +21, +21, +22, +cp2</td>
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<td>FISH</td>
<td>KMT2A rearrangement (93% of 100 interphase nuclei)</td>
<td>Not performed</td>
<td>AFF1/KMT2A fusion (92% of 500 interphase nuclei)</td>
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B-ALL, B-acute lymphoblastic leukemia; AML, acute myeloid leukemia; MPAL, mixed phenotype acute leukemia; FISH, fluorescence in situ hybridization.
pressure-induced lineage reprogramming play important roles [3, 9, 10]. An experimental study demonstrated that cellular microenvironment affects cell fate decisions and lineage interconversions [12]. Other studies hypothesized that immunotherapy-induced cytokine release (notably interleukin 6) may promote myeloid differentiation of a lymphoid clone [1, 3, 13]. Additional studies have postulated that genetic evolutions of leukemic blasts under targeted therapy may contribute to lineage switch [10, 14]. The findings in this case suggest that high biphenotypic potential of KMT2A/AFF1-rearranged B-ALL blasts, blinatumomab-induced blast cell reprogramming, clonal evolution, and cytokine release might have played important roles in this lineage switching event.

In conclusion, the lineage switch events indicate that cautious application of immunotherapy in KMT2A/AFF1-rearranged B-ALL should be advocated in clinical settings. Targeting multiple antigens on leukemia-initiating cells may be a better strategy to reduce the likelihood of lineage switching.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**References**


