LETTER TO THE EDITOR

Blinatumomab-induced lineage switch of B-ALL with t(4:11)(q21;q23) KMT2A/AFF1 into an aggressive AML: pre- and post-switch phenotypic, cytogenetic and molecular analysis

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Lineage switch is a rare phenomenon in which acute leukemia transforms from lymphoid to myeloid lineage, or vice versa. It is typically seen following therapy or at the time of relapse.1 Among the chromosomal aberrations associated with lineage switch, the t(4;11)(q21;q23) rearrangement with KMT2A/AFF1 fusion protein (formerly, MLL/AFF1 or MLL/AF4) is the most common.2 In general, lineage switch disease is often refractory to therapy and portends a poor prognosis.3,4

Blinatumomab is a monoclonal antibody with bispecificity for both CD19 on B cells and CD3 on cytotoxic T cells. Following simultaneous binding to both epitopes, normal and neoplastic B cells are lysed by the host cytotoxic T cells.5 Recent reports have documented lineage switch of acute leukemias following CD19-targeted therapy;6 however, the underlying mechanism and management of these events are unclear. Herein, we present a case of refractory B lymphoblastic leukemia (B-ALL) with t(4;11)(q21;q23) KMT2A/AFF1 transforming to acute myeloid leukemia (AML) shortly following blinatumomab therapy. We studied both molecular and cytogenetic abnormalities at the initial diagnosis of B-ALL and at the time of lineage switch, thereby providing insights into the underlying biology.

CASE HISTORY

A 40-year-old woman presented with vaginal bleeding. Blood work revealed a white blood cell count of 152 × 10^9/l, anemia (hemoglobin, 9.7 g/dl) and thrombocytopenia (61 × 10^9/l). Peripheral blood and bone marrow aspirate smears showed sheets of blasts, comprising 90% of total cellularity (Figure 1c). The morphologic and immunophenotypic features were consistent with B-ALL (Table 1 and Figures 1e, g, and l). A bone marrow biopsy revealed sheets of blasts, comprising 90% of total cellularity (Figure 1c). Flow cytometric studies on peripheral blood demonstrated expression consistent with B-ALL (Table 1 and Figures 1f, h, and j). A FISH study on bone marrow aspirate revealed a white blood cell count of 152 × 10^9/l, anemia (hemoglobin, 9.7 g/dl) and thrombocytopenia (61 × 10^9/l). Peripher-

While the mechanism of lineage switch remains unclear, other cases describing lineage switch after CD19-targeted therapy have postulated that clones from both lymphoid and myeloid lineages are present at diagnosis.6 Following treatment, one clone may be selectively suppressed, thereby unmasking the other and manifesting as a lineage switch.7,9 We re-examined the histograms of the flow cytometric studies and NGS results of the initial B-ALL blasts and failed to identify a separate AML subclone. These results suggest that a phenotypic switch took place after blinatumomab therapy. However, flow cytometry and NGS may not have sufficient sensitivity to identify a minute clone.

Other studies suggest that cell lineage plasticity mediates lineage switch in acute leukemia. In a recent report of pre-B ALL lineage switching to AML, pre-B-cell markers of single cell subclones were characterized before and after CAR-T-cell therapy. The molecular signatures of these clones varied with therapy, suggesting lineage plasticity rather than clonal selection.8 Experimental data in this report also demonstrated that CD19 CAR-T-cell immune pressure against CD19 can result in either relapsed leukemia with CD19 loss or leukemia with complete lineage switch as mechanisms of resistance. Using murine models, the authors
the lineage switch. These data revealed an initial mutation in and an additional mutation in therapy and possibly therapeutic resistance. KMT2A/AFF1-positive leukemia and predict poor response to tumomab in patients with B-ALL and t(4:11)(q21;q23) KMT2A/AFF1 lineage switch or nuclear reprogramming previously.

It is possible that following blinatumomab, the phenotypic switch, suggesting that the original clone harbored bi- at diagnosis and after blinatumomab treatment despite the t(4:11)(q21;q23) rearrangement and similar resistance mechanism may be seen with bi-speci

Distinct morphologic and immunophenotypic features of B-ALL blasts and AML blasts following lineage switch. (a) The lymphoblasts of B-ALL had scant cytoplasm, round nuclei, fine chromatin and inconspicuous nucleoli. (b) The myeloid blasts of AML had abundant cytoplasm, folded nuclei, fine chromatin and occasional prominent nucleoli. (c and d) Bone marrow biopsy revealed packed B-lymphoblasts with scant cytoplasm and myeloid blasts with more abundant cytoplasm, respectively. (e, g, and h) show that the lymphoblasts expressed dim CD45, lower side scatter, CD19, CD34 (partial), and terminal deoxynucleotidyl transferase (TdT). They were negative for myeloperoxidase (MPO). (f, h, and j) show that the myeloid blasts expressed bright CD45, high side scatter and MPO, but are negative for CD19, CD34 and TdT.

Table 1. Differences between B-lymphoblasts and myeloid blasts in flow cytometry, cytogenetics, and next generation sequencing studies at the time of diagnosis of B-ALL and post-transformation to AML 9 days following blinatumomab

| B-ALL at diagnosis | AML at transformation |
|--------------------|----------------------|
| Flow cytometric features of the blasts | Express: CD9, CD15 (partial), CD19, cCD22, CD38, CD45 (dim), cCD79a, HLA-DR, nTdT |
| Cytogenetics | Do not express: CD10, CD3, CD13, CD16, CD33, CD117, CD22, CD7, CD56, CD64, cMPO, CD3, CD20, CD66c |
| FISH for KMT2A-AFF1 fusion | 95.60% |
| Next generation sequencing (NGS) | MPL: c.1653del; p.Lys553Argfs*77 (48%) |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; c', cytoplasmic; FISH, fluorescence in situ hybridization; n', nuclear; MPO, myeloperoxidase. NGS genes included ASXL1, BCL11A, BCR, BRAF, CALR, CEBPA, CSF3R, DNMT3A, ETN6, EZH2, FLT3, GATA1, GATA2, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PHF6, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, TERT, TET2, TP53, U2AF1, WTI and ZRSR2. "FISH was done on a follow-up bone marrow aspirate after patient was initiated on a salvage therapy for AML."
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