Multiple Lineage Switches in KMT2A Rearranged Infant Leukemia, Responsive to Combination Therapy with CPX-351 and Inotuzumab

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Abstract

Rearrangements of the *KMT2A* gene are characteristic of infantile acute lymphoblastic leukemia (ALL) and are associated with increased lineage plasticity and resistance to therapy. Here, we describe the case of a 9-month-old infant with infantile ALL who experienced multiple immunophenotypic switches in her leukemia throughout therapy and ultimately achieved remission with the combination of CPX-351 and Inotuzumab. This case highlights the unique clinical challenges infantile ALL poses on monitoring therapeutic response with current methods of measuring minimal residual disease as well as the challenges in treating infantile B-ALL.

Introduction

Rearrangements of the histone-lysine N-methyltransferase 2A (*KMT2A* -r; formerly mixed lineage leukemia; *MLL*) gene on chromosome 11q23 are the hallmark of infantile acute lymphoblastic leukemia (ALL) and confer particularly poor prognosis.[1] *KMT2A* rearrangements induce transcriptional and epigenetic alterations that confer early blocks in B-cell differentiation resulting in an immature B-cell immunophenotype, increased lineage plasticity, and resistance to therapy.[2–4] Among the >130 partner genes previously identified, the most common *KMT2A* -r is the t(4;11)(q21;q23) that results in the *KMT2A/AFF1* fusion gene and is associated with B-cell acute lymphoblastic leukemia (B-ALL) presenting with a pro-B cell immunophenotype.[5] *KMT2A* -r leukemias have been associated with lineage switching from lymphoid to myeloid immunophenotype at relapse, demonstrating increased lineage plasticity.[6–9]

This report details the case of an infant with *KMT2A* -r ALL who experienced multiple immunophenotype switches during therapy. Her disease was refractory to standard therapy but ultimately went into remission with a combination of myeloid- and lymphoid-directed therapy.

Case

A 9-month-old female presented with hepatosplenomegaly and was found to have hyperleukocytosis (WBC 1100 k/μL), anemia (2.4 g/dL), and thrombocytopenia (27 k/μL). Flow cytometry confirmed the diagnosis of pro-B ALL and cytogenetics revealed a *KMT2A* rearrangement (4;11 translocation). She was initiated on therapy per Children's Oncology Group study AALL15P1 (ClinicalTrials.gov Identifier: NCT02828358, Interfant-06 backbone[10] with addition of azacitidine blocks). Post-induction minimal residual disease (MRD) analysis was positive for residual B-ALL (0.73% by flow cytometry; persistent 4;11 translocation by Fluorescence in situ Hybridization [FISH]). She continued therapy per AALL15P1 protocol with azacitidine block 1, consolidation, azacitidine block 2, and interim maintenance 1, after which she had refractory disease with circulating blasts.

Given persistent disease, CD19 CAR-T therapy was the next intended therapy. T-cells were collected following marrow recovery after interim maintenance 1. While awaiting ex-vivo CAR-T manufacturing, she received venetoclax, vincristine, dexamethasone, and pegaspargase.[11] She tolerated this protocol well, only complicated by neutropenia and pre-septal cellulitis which responded well to antibiotics. End of course bone marrow evaluation with B-cell flow-cytometry MRD analysis was negative. However, cytogenetics revealed persistent *KMT2A*- r in 14% of cells. Flow cytometric analysis demonstrated AML with monocytic differentiation, representing 39% of marrow cells. Given the lack of CD19, Tisagenlecleucel was not infused.

Instead, a myeloid-directed individualized therapy was initiated based on the VENAML study[12], cohort C consisting of venetoclax, cytarabine, and azacitidine. End of course bone marrow evaluation was negative for abnormal myeloid blasts but B-ALL MRD show 12.6% abnormal lymphoblasts, indicating a reversion back to a lymphoid immunophenotype. Given that her disease was positive for CD19 and CD33, she was initiated on therapy consisting of a combination of blinatumomab and gemtuzumab[13]. End of course marrow demonstrated 79% B-cell lymphoblasts that were now CD19 negative by flow cytometry.

She then received another therapeutic approach with myeloid and lymphoid directed components, including both CPX-351 (liposomal daunorubicin and cytarabine) and Inotuzumab. Her course was complicated by neutropenia and Streptococcus mitis bacteremia that did not require intensive care unit support. At the end of this course, the bone marrow biopsy was morphologically normal and flow-based MRD testing was negative on both B-ALL and AML MRD. Furthermore, FISH for *KMT2A* rearrangement was negative for the first time during her treatment. Sequencing for rearrangements of the immunoglobulin receptor (ClonoSeq, Adaptive Biotechnologies) remained positive for low level residual disease (48 residual clones per million nucleated cells). Her CSF was positive for blasts, but cleared with additional intrathecal cytarabine, hydrocortisone, and methotrexate while preparing for transplant.

She then proceeded to an unrelated umbilical cord transplant. Post-transplant course was complicated by sinusoidal obstruction syndrome (SOS), thrombotic microangiopathy (TMA), and multifocal pneumonia resulting in ARDS requiring ECMO support. Her leukemia relapsed on day +37 from transplant, with a conversion back to a myeloid immunophenotype. Cytogenetics redemonstrated her *KMT2A*rearrangement. Ultimately, she died on post-transplant day +57 from multi-organ failure.

Discussion

We present a case of refractory *KMT2A* -r infantile leukemia that underwent multiple lineage switches during therapy, both with and without immunotherapeutic pressure. The underlying mechanism by which*KMT2A* -r leukemia can lineage switch is not fully understood. Recent single cell sequencing studies suggest that some patients with*KMT2A* -r B-ALL harbor subpopulations of blasts that contain myeloid transcriptional profiles, which can become the dominant clone under the selective pressure of therapy.[2,3] It is also described that in mixed phenotype acute leukemia, even distinct phenotypic populations, retain multilineage potential, rather than simple outgrowth of a minor clone.[14] The frequently shifting leukemia immunophenotype presented a challenge for measuring MRD using flow-cytometry based methods. In this case, identifying and sequencing the clonal Ig rearrangements allowed for disease detection throughout therapy despite lineage switching. However, rearrangements of*KMT2A* often occur prior to VDJ recombination and result in oligoclonal IG/TCR rearrangements[15], which limits the broader utility of this approach for tracking MRD in *KMT2A* -r B-ALL. Alternatively, quantitative PCR based approaches for tracking the underlying *KMT2A* rearrangements have been suggested.[16]

KMT2A rearrangements confer poor prognosis in B-ALL[17] and, when combined with detectable disease after consolidation, portend high risk of relapse.[16]Recent genomic analysis suggests that the immature differentiation state of *KMT2A-r* blasts may partially explain this poor therapeutic response through resistance to steroids.[3] Our patient did not achieve remission at any point during initial therapy with standard cytotoxic agents, nor did she achieve meaningful benefit from the addition of the BCL-2 inhibitor venetoclax.

Given the lineage infidelity of her disease, she was treated with combinations of agents to target both the myeloid and lymphoid compartments. Initially, she received therapy containing blinatumomab (bispe-

cific CD19/CD3 monoclonal antibody) and gemtuzumab (anti-CD33 monoclonal antibody conjugated to calicheamicin), which has demonstrated activity in cases of mixed phenotype acute leukemia.[13,18] She did not achieve a clinically meaningful benefit from this combination, despite her blasts expressing both CD19 and CD33. However, the dominant blasts emerging from this therapy lost expression of the CD19 antigen in response to the blinatumomab and her disease did not regain expression of CD19 throughout the rest of her therapy.

The results of Interfant-06 determined that children with high end of induction MRD benefit from an intensive myeloid-directed chemotherapy backbone.[19] These results, in combination with our experiences of frequent lineage switching in this patient, led us to treat her with a novel combination of liposomal daunorubicin + cytarabine (CPX-351) in combination with inotuzumab (anti-CD22 monoclonal antibody conjugated to calicheamicin). This was the only therapy she received that achieved remission, albeit with low level molecular evidence of disease still present.

The growing list of immunotherapeutic approaches provides opportunities to explore different combinations to improve disease control in infants with *KMT2A* -r B-ALL, potentially with improved safety profile in this patient group, who have inherently higher risk of treatment related toxicity. Along these lines, the addition of blinatumomab to the backbone of interfant-06 therapy showed improved survival in a recently published trial of *KMT2A-r* infantile leukemia[20]. The rise of single-cell genomic analysis may eventually lead to better prediction tools to help select which patients with *KMT2A-r* leukemia would respond to a lymphoid, myeloid, or combinatorial directed therapy.

This case highlights the challenges in treating *KMT2A* -r infantile B-ALL. The infidelity of *KMT2A-r* leukemias to either myeloid or lymphoid lineages presents unique challenges with respect to monitoring disease response by flow-based analysis. The inherent treatment resistance and poor prognosis emphasize the need to study novel combinatorial therapeutic strategies.

Conflicts of Interest

Authors have no conflicts of interest to disclose

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Figure 1. Summary of treatment, disease response, and immunophenotype changes. Shown is treatment and resultant diseases status with response evaluation.