EXCEPTIONAL CASE REPORT

blood advances

Spontaneous reversion of a lineage switch following an initial blinatumomab-induced ALL-to-AML switch in *MLL*-rearranged infant ALL

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Key Points

- A case of *MLL*-rearranged leukemia that rapidly adapts to immunological stimuli illustrating the high plasticity of this phenotype.

Introduction

Despite intensified therapy protocols, acute lymphoblastic leukemia (ALL) of infancy remains a difficult-to-treat disease, with a high relapse rate. Only 25% of the patients, treated intensively following the relapse, survive by 3 years. *MLL* rearrangement is detected in the majority (80%) of patients and is known to be the critical driver for clonal expansion.[1,2]

Novel immunotherapies spark hope for an effective treatment strategy in the case of chemoresistant disease. Targeting CD19 has been the fundamental principle for both antibody-based immunotherapy (such as blinatumomab) and T-cell-mediated therapy (using chimeric antigen receptor–engineered T cells [CAR T cells]). However, the efficacy of both therapeutic strategies hinges on the maintained surface expression of CD19 as the target molecule. In a fraction of patients, treated with blinatumomab or CAR T cells, downregulation or loss of CD19 expression is observed as one way to escape from immunological pressure.[3,4] Other reports,[5-11] summarized here (Table 1), describe a lineage switch of the leukemic blasts toward a myeloid phenotype following blinatumomab or CAR T-cell immunotherapy, respectively. Here we report on a child with infant ALL, receiving blinatumomab for early relapse following allogeneic stem cell transplantation (SCT). After only 11 days of treatment, monocytic myeloid blasts displaying an M5 morphology were detected in the peripheral blood and bone marrow, indicating a switch to acute myeloid leukemia (AML). Flow cytometry confirmed a switch of the blast population with an expression of myeloid markers. Most surprisingly, cessation of blinatumomab and a watchful waiting period of 7 days resulted in the spontaneous conversion of leukemia back to the original $CD19^{+}$ lymphoblastic phenotype. The case documents the high plasticity of these early progenitor blasts, stressing the demand for effective therapy combinations that limit tumor escape.

Case description and methods

We report on a 5-month-old girl with pro-B-ALL. An atypical *MLL* rearrangement [KMT2A-AFF4 (insertion 5;11)] was identified. The patient was treated according to the Interfant06 protocol. After 5 cycles of chemotherapy, minimal residual disease markers as assessed by real-time quantitative polymerase chain reaction were negative, and SCT was carried out, using a partially T-cell-depleted 9/10 unrelated donor (T-cell receptor aß-/CD19-depletion). Hematopoietic recovery was uneventful. The child developed grade 2 skin graft-versus-host disease as the maximum degree of graft-versus-host disease, but the course was then complicated by day +50 because of Epstein-Barr virus–associated lymphoproliferative disease involving cervical and parapharyngeal lymph nodes. The girl responded promptly to rituximab treatment. At 16 months of age, 133 days after allogeneic SCT, a relapse of her ALL was diagnosed from her peripheral blood (18 000 leukemic blasts per μL). The blasts exhibited the original lymphoblastoid phenotype with lymphoid markers ($sCD19^{++}$; $NG2^{++}$; $CD22^{+}$). A cytoreductive course with dexamethasone and vincristine resulted in a marked decrease of blast counts (300 leukemic blasts per μL). She was enrolled in the expanded access program for blinatumomab in pediatric and adolescent subjects with relapsed/refractory B-precursor ALL (RIALTO; registered at www.clinicaltrials.gov as #NCT02187354). Blinatumomab (2.5 $\mu g/m^2$ starting dose and gradual

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Table 1. Reported cases of a switch in phenotype following immunotherapy

Reference	Number of patients	Age	Molecular characteristics	Treatment	Time of switch
6	2	1 Infant, 1 adult	*MLL*-rearranged, ins(11;10); (t4;11)	CD19-CAR T cells	D21/D22
7	1	Infant	*MLL*-rearranged t(4;11)	Blinatumomab	D15
5	1	Infant	*MLL*-rearranged	CD19-CAR T cells	Not reported
8	1	8 y	None	Blinatumomab	D21
9	1	15 y	*MLL*-rearranged, t(4;11)	Blinatumomab	D28
11	1	40 y	*MLL*-rearranged, t(4;11)	Blinatumomab	D9
10	2	66 y/73 y	t(9;22)(q34;q11)	Blinatumomab	Third cycle/not reported

increase to 15 $\mu g/m^2$) was initiated and well tolerated. By day 9, monocytosis was observed (maximal leukocytes: 7400/μL). Morphologically, cells appeared in atypical shape with a monocytic, blastoid character (34%). Bone marrow biopsy on day 11 revealed almost complete disappearance of the lymphoblastic cells (5%), but the occurrence of monocytic cells (80%) (Figure 1). Fluorescence-activated cell sorting defined these cells as sCD19low, $CD33^+$ $CD34^-$, $CD14^{++}$, $CD15^{++}$, $CD11b^{++}$, $CD64^+$. Staining with moAb7.1-antibody, which binds to NG2, the presumed product of *MLL* rearrangement, was at least partially positive in this blast population. Thus, a switch to AML was diagnosed (M5 subtype). Cytogenetics showed *MLL* rearrangement in 15 of 20 metaphases, whereas the 5 normal metaphasis were donor derived as evidenced by the Y chromosome of the male stem cell donor. RNA-sequencing analysis identified the KMT2A-AFF4 fusion transcript (5q31;11q23).

Blinatumomab was stopped, but no cytostatic chemotherapy was initiated. Surprisingly, in the following days, the monocytic blasts spontaneously disappeared from the peripheral blood. Bone marrow biopsy by day 9 after cessation of blinatumomab showed ~40% of lymphoblastic blasts and only rare monocytic cells. Fluorescence-activated cell sorting demonstrated a switch back to the original phenotype with a loss of the myeloid markers (Figure 1).

Subsequently, an additional cycle of chemotherapy followed by a stem cell boost was administered (fludarabine, cytarabine, daunoxome). The patient recovered without significant toxicity. Staging showed 8% lymphoblastic blasts in the bone marrow. Clinically, she developed a rapidly growing chloroma in her temporal region requiring palliative irradiation. The clinical condition prevented us from taking a biopsy. In parallel to the irradiation, blinatumomab was reinitiated with intermittent low-dose cytarabine (7 days/3 days). However, while on therapy, additional chloromas developed. Overall conditions worsened significantly, and the best supportive care was provided until the patient's passing.

Results and discussion

CD19-directed immunotherapy, either antibody- or T-cell-based, has shown tremendous clinical success for patients with otherwise refractory $CD19^+$ B-cell leukemia.[12] For infant ALL, high relapse rates and poor outcome of patients with relapsed disease have been reported even with SCT frequently performed in first molecular remission.[1] A lineage switch is a rare event, but more frequent in patients with *MLL* rearrangement. Rossi et al analyzed a cohort of 1482 pediatric leukemia patients and identified 9 patients with a phenotypic switch (incidence 0.6%); 7 of these 9 leukemias showed *MLL* rearrangement.[13] In 2 cases, the switch was observed from AML to ALL, whereas a switch from ALL to AML (M4/M5) was the more common phenomenon. Since 2016, at least 10 patients (including this patient) have been described who responded to CD19-directed immunotherapy with a lineage switch to AML. Four patients were infants, 4 were adults, and 2 were of school age. Except for 2 adults with bcr-abl1$^+$ ALL[10] and 1 child,[8] all of the patients had leukemia with *MLL* rearrangement (Table 1).

Mechanistically, Jacoby et al[5] identified oncogenic drivers, as deletion of PAX5 and Ebf1 recapitulated lineage reprogramming during CD19 CAR pressure in murine models. Notably, phenotypic reprogramming to AML was mostly stable upon several passages in the mouse especially if all B-lineage surface markers had been lost, whereas reexpression of lymphatic markers in tertiary passages was observed if residual B-cell marker expression was maintained.[5]

The case described here is exceptional in that our patient's leukemia spontaneously switched back to the original B-phenotype, once the immunological pressure exerted by blinatumomab was discontinued. The excellent clinical condition at the time prompted us to postpone further therapy and carefully monitor the monocytic blasts. Within days of ending blinatumomab treatment, these blasts disappeared in the periphery, which was confirmed by a repeat bone marrow biopsy only 9 days later. This finding remarkably links immunological pressure and possibly cytokine release to the phenotypic changes within a clonal blast population. Cytokine release following CD19-directed immunotherapy is frequently observed early in the course of treatment, when T cells encounter abundant targets and become fully activated. Clinically, life-threatening cytokine release syndrome after blinatumomab[14] or CAR T cells[12] can be managed by blocking interleukin-6R (IL-6R) signaling using tocilizumab. Gardner et al hypothesized that inflammatory cytokines (notably IL-6) might contribute to the myeloid differentiation of a lymphoid clone.[6] This hypothesis is based on early in vitro work on a human leukemic cell line, showing the capacity to switch to a myeloid phenotype with a specific role of IL-6.[15] Recently, Stevens at al[16] demonstrated a relationship between high IL-6 levels at diagnosis of AML and poor clinical outcome. These results still require confirmation in larger patient cohorts, but intriguingly link IL-6 to chemotherapy resistance and outcome.[16] Our patient only experienced a mild increase of leukocytes and an initially elevated C-reactive protein (53.8 mg/L), both of which decreased after cessation of blinatumomab infusion. However, the study mentioned previously,[16] also could not link IL-6 levels directly to fever episodes or infection.

Thus, this case illustrates a possible short-term course the disease may take naturally and illustrates how rapidly leukemic cells adapt to the

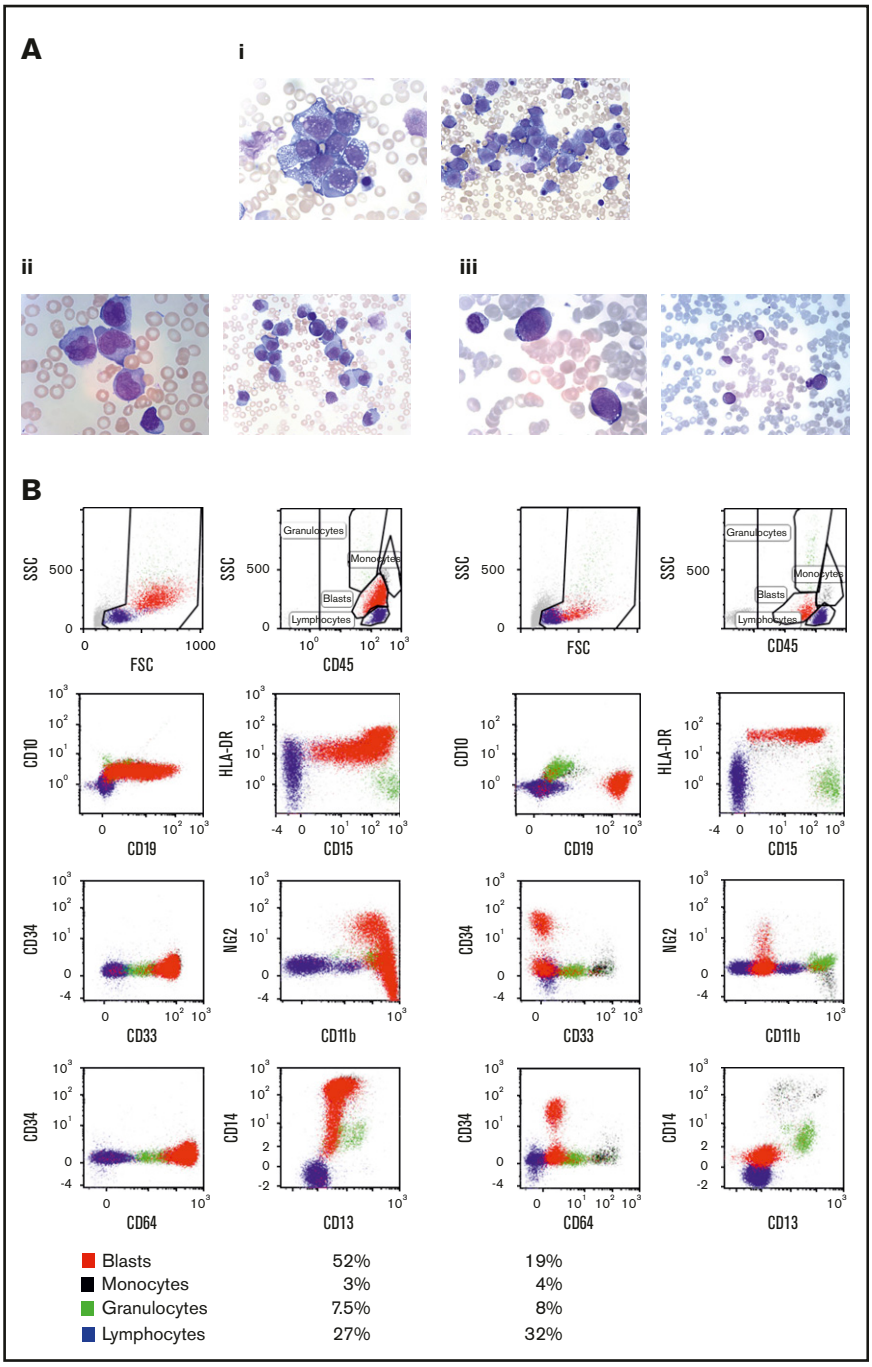

Figure 1. Phenotypic evolution of leukemic blasts after blinatumomab treatment. (A) L1/2 morphology of blasts (bone marrow) at the time of relapse, before blinatumomab (i); myelomonocytic morphology of cells in the bone marrow 11 days after blinatumomab treatment (ii) and 9 days after cessation of blinatumomab (iii). In each image pair, magnification is 1:1000 (left) and 1:500 (right) (Pappenheim staining). (B) Flow cytometry at the 2 respective time points: directly after blinatumomab treatment (left 2 columns) and 9 days later (right 2 columns). Staining shows $CD45^+$ live cells.

microenvironment. In contrast, in all other cases and as far as it is documented, patients were immediately treated with various chemotherapy schedules.

Cytogenetics confirmed the presence of the defining *MLL* rearrangement at the time when primarily monocytic blasts had been present. Also, real-time quantitative polymerase chain reaction for immunoglobulin gene rearrangement at the time of AML diagnosis was highly positive. Thus, the most likely scenario in this patient is that the leukemic clone carrying the *MLL*-rearrangement displayed a myeloid morphology and phenotype, while at the same time carrying the clone-specific VDJ rearrangement.

In summary, *MLL*-rearranged leukemic blasts may show exceptional plasticity in response to their microenvironment, and escape variants need to be carefully monitored especially in the context of advanced immunotherapies. Further studies are required to assess a possible benefit of either intermittent AML-directed chemotherapy (eg, low-dose cytarabine) between shorter courses of blinatumomab therapy or concomitant modulation of the cytokine milieu (eg, by IL-6 blockade).

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Authorship

Contribution: M.W. and P.G.S. decided on critical clinical issues regarding patient care; M.R., M.E., R.S., and D.R. provided critical diagnostic cues and interpretation for this case; M.W. wrote the manuscript; and all authors discussed and approved the manuscript.

Conflict-of-interest disclosure: P.G.S. is a principal investigator of the early access program for blinatumomab in refractory or relapsed pediatric ALL (RIALTO; sponsor: Amgen). The remaining authors declare no competing financial interests.

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