RESEARCH LETTER

blood advances

TO THE EDITOR:

Plasticity of lineage switch in B-ALL allows for successful rechallenge with CD19-directed immunotherapy

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Introduction

CD19-directed immunotherapies have been extremely effective at inducing remission in patients with B-cell acute lymphoblastic leukemia (B-ALL), however, relapse remains a major challenge.[1-3] The rarest but most problematic relapse event after CD19-directed immunotherapies is that of lineage switch (LS), in which neoplastic cells lose B-lymphoid–specific features and acquire a myeloid immunophenotype.[4-8] Optimal salvage strategies for these patients are unknown and the absence of lymphoid targets reduces available immunotherapeutic options. Here, we present 2 cases of LS that are salvaged with myeloid-directed therapy but experience recurrent B-ALL. In both cases, patients were successfully rechallenged with CD19-directed immunotherapy without early recurrence of myeloid disease.

Methods

This is a retrospective case series reporting 2 patients with *KMT2A*-rearranged (*KMT2A*-r) infant B-ALL who experienced LS followed by subsequent B-ALL relapse after hematopoietic cell transplantation (HCT), successfully salvaged with CD19-directed immunotherapy. Each patient received treatment on a clinical trial protocol, with case 1 on PLAT-03 (#NCT03186118) and case 2 on PLAT-05 (#NCT0333691). PLAT-03 was a pilot study that incorporated periodic infusions of CD19-expressing antigen-presenting T cells after the initial infusion of a CD19–chimeric antigen receptor (CAR) T cell with a 4-1BB costimulatory domain (SCRI-CAR19).[9] PLAT-05 is an ongoing phase 1 trial that tests the safety and efficacy of a CD19 × CD22 dual specific CAR T-cell product (SCRI-CAR19x22).[10] Evaluation of leukemic blasts through multiparameter flow cytometry (MFC), fluorescence in situ hybridization, and G-banded karyotyping was performed in Clinical Laboratory Improvement Amendments–certified laboratories. For morphologic complete remissions (CRs), minimal residual disease (MRD) was evaluated by MFC and/or next-generation sequencing (NGS) of the IGH/IGL gene using Clonoseq (Adaptive Biotechnologies, Seattle, WA). MRD status is reported with methodology used (MFC-negative or -positive CR and/or NGS-negative or -positive CR). In addition, for 1 case, an RNA fusion assay using Archer FusionPlex (Seattle Children's Hospital, Seattle, WA) and a targeted DNA-based NGS panel of cancer-related genes was performed via UW-OncoPlex (University of Washington, Seattle, WA).[11] These studies were conducted with approval of the institutional review board of Seattle Children's Hospital and in accordance with the Declaration of Helsinki.

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The full-text version of this article contains a data supplement.

Table 1. Disease characteristics

	Initial B-ALL diagnosis		LS to AML		Relapsed B-ALL	
Case 1						
Blast %	93 B-lymphoblasts		96 abnormal immature monocytes 0.14 B-lymphoblasts (immunophenotype similar to diagnosis)		70 B-lymphoblasts	
Flow cytometry immunophenotype of major blast population	CD19	Positive	CD19	Negative	CD19	Positive
	CD22	Positive	CD22	Negative	CD22	Positive
	CD10	Negative	CD10	Negative	CD10	Negative
	CD15	Partial positive	CD15	Positive	CD15	Negative
	CD34	Negative	CD34	Negative	CD34	Partial positive
	CD38	Positive	CD38	Positive	CD38	Positive
	CD58	Positive (increased)	CD58	Positive	CD58	Positive (increased)
	HLA-DR	Positive	HLA-DR	Positive	HLA-DR	Positive
	CD13	Negative	CD13	Negative	CD13/33	Negative
	CD33	Negative	CD33	Positive	CD33	Negative
	CD11b	Not evaluated	CD11b	Positive	CD11b	Not evaluated
	CD14	Negative	CD14	Partial positive	CD14	Not evaluated
	CD64	Not evaluated	CD64	Positive	CD64	Negative
	CD4	Not evaluated	CD4	Positive (dim)	CD4	Not evaluated
	CD7	Partial positive	CD7	Negative	CD7	Not evaluated
	CD45	Positive (slightly dim)	CD45	Positive	CD45	Positive
	CD123	Not evaluated	CD123	Not evaluated	CD123	Positive
Cytogenetics	Karyotype: 46, XX, t(1;11)(p32;q23)[16]/46,XX[2]		Karyotype: 46, XX, t(1;11)(p32;q23)[5]/47,XX,t(1;11),+8[14]/46,XX[1]		Not performed	
	FISH: positive for a *KMT2A* rearrangement in 81% of nuclei (with loss of 3′ KMT2A signal)		FISH: positive for a *KMT2A* rearrangement in 95% of nuclei (loss of 3′ KMT2A signal)			
ClonoSEQ, %	IGH-SeqA = 60.9 IGH-SeqB = 70.0 IGL-SeqC = 76.2 IGL-SeqD = 60.9 IGH-SeqE = 0.6 IGH-SeqF = 1.8 IGH-SeqG = 0.0013 IGH-SeqH = ND		IGH-SeqA ≥ 99.9 IGH-SeqB ≥ 99.9 IGL-SeqC = 82.2 IGL-SeqD = ND IGH-SeqE = ND IGH-SeqF = ND IGH-SeqG = ND IGH-SeqH = ND		IGH-SeqA = 43.1 IGH-SeqB = 36.2 IGL-SeqC = 85.6 IGL-SeqD = ND IGH-SeqE = 68.4 IGH-SeqF = 67.4 IGH-SeqG = 68.5 IGH-SeqH = 68.5
NGS	RNA fusion panel: positive for *KMT2A-EPS15* fusion		DNA NGS panel: 1. Positive for the 3′ *KMT2A* deletion seen previously		Targeted testing (by DNA NGS): negative for *NRAS* p.G12C	
	DNA NGS panel: positive for 3′ *KMT2A* deletion		2. New findings of trisomy 8, *NRAS* p.G12C, and low-level subclonal KRAS p.G13D.			
Case 2						
Blast %	95.5 B-lymphoblasts		4.5 abnormal immature monocytes		25 B-lymphoblasts	
Flow cytometry immunophenotype of major blast population	CD19	Positive (increased)	CD19	Negative	CD19	Positive (increased)
	CD22	Positive	CD22	Not evaluated	CD22	Positive
	CD10	Negative	CD10	Not evaluated	CD10	Negative
	CD15	Not evaluated	CD15	Positive	CD15	Partial positive
	CD34	Positive	CD34	Negative	CD34	Positive
	CD38	Positive	CD38	Positive	CD38	Positive
	CD58	Positive (increased)	CD58	Not evaluated	CD58	Positive (increased)

AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; LS, lineage switch; ND, not detected; NGS, next-generation sequencing.

Table 1 (continued)

	Initial B-ALL diagnosis		LS to AML		Relapsed B-ALL	
	HLA-DR	Positive	HLA-DR	Positive	HLA-DR	Positive
	CD13	Negative	CD13	Negative	CD13	Negative
	CD33	Negative	CD33	Positive	CD33	Negative
	CD11b	Not evaluated	CD11b	Not evaluated	CD11b	Not evaluated
	CD14	Not evaluated	CD14	Negative	CD14	Not evaluated
	CD64	Negative	CD64	Positive	CD64	Not evaluated
	CD7	Negative	CD7	Negative	CD7	Not evaluated
	CD56	Not evaluated	CD56	Positive	CD56	Negative
	CD45	Positive (dim)	CD45	Positive	CD45	Positive
	CD123	Negative	CD123	Negative	CD123	Not evaluated
Cytogenetics	46, XX, t(4;11)(q21;q23)[20]; FISH: positive for *KMT2A* rearrangement in 100% of nuclei		Karyotype not performed FISH: positive for *KMT2A* rearrangement in 10.5% of nuclei		46, X, t(X;10)(q13;p13), t(4;11)(q21;q23), t(4;15)(p16;q22),t(8;14)(q22;q32) [15]//46,XY[5] FISH: positive for *KMT2A* rearrangement in 30% of nuclei	
ClonoSEQ, %	(performed 2.5 months after diagnosis) IGH-SeqA = 4.0 IGH-SeqB = 3.8 IGH-SeqC = 0.036		IGH-SeqA = 3.6 IGH-SeqB = 0.019 IGH-SeqC = 3.1		IGH-SeqA = 20.9 IGH-SeqB = ND IGH-SeqC = ND	
NGS	Not performed		Not performed		Not performed	

AML, acute myeloid leukemia; FISH, fluorescence in situ hybridization; LS, lineage switch; ND, not detected; NGS, next-generation sequencing.

Results/Discussion

Case 1

A 6-month-old female diagnosed with *KMT2A*-r, CNS2 infant B-ALL had persistent disease after induction chemotherapy on the Children's Oncology Group study AALL15P1 (#NCT02828358). She experienced progressive medullary and central nervous system (CNS) disease during the subsequent consolidation phase of therapy. Salvage therapy was attempted with blinatumomab, but she experienced an LS during the first cycle (Table 1). She was able to achieve an MFC-positive CR after administration of FLA + GO (fludarabine, high-dose cytarabine, and gemtuzumab ozogamicin), with MRD notable for $CD19^+$ B-ALL and no evidence of myeloid disease. She was rechallenged with blinatumomab and achieved MFC-negative but NGS-positive CR.[8] She proceeded with cord blood HCT but relapsed with $CD19^+$ B-ALL by day 28. Blinatumomab was initiated while tapering off immune suppression and she achieved an NGS-negative CR after 1 cycle. After a total of 5 cycles of blinatumomab, she was enrolled on the PLAT-03 trial owing to a CNS relapse of B-ALL. CNS disease was eradicated after infusion of SCRI-CAR19. Twenty-three months after infusion and 6 doses of antigen-presenting T cells, she showed evidence of ongoing persistence of her CAR T cells and remained in an NGS-negative CR (Figure 1A).

Case 2

A 4-month-old female diagnosed with *KMT2A*-r infant B-ALL had persistent MFC-positive MRD and CNS2 status after AALL15P1 induction and modified consolidation therapy. She was enrolled on PLAT-05 and received SCRI-CAR19×22. She achieved an MFC-negative CR with persistent NGS-MRD by day 21 after infusion. In the setting of ongoing CAR T-cell persistence 5 months after infusion, she experienced an LS (Table 1). Salvage therapy with FLA + GO resulted in an MFC-negative CR with persistent NGS-MRD, which was consolidated with a cord blood HCT. Relapse of $CD19^+$ B-ALL was noted 5 months after HCT. She was initiated on blinatumomab and achieved an MFC-negative CR after the first cycle and NGS-negative CR after the second cycle. The patient remained in an NGS-negative CR after 6 courses of blinatumomab and transitioned to maintenance chemotherapy (Figure 1B).

Discussion

We present 2 patients with *KMT2A*-r infant B-ALL with persistent disease after upfront treatment. Owing to dismal outcomes associated with persistent disease, experimental immunotherapies were pursued.[12] Both patients experienced LS after CD19-directed immunotherapy, which was successfully salvaged with intensive myeloid-directed therapy and HCT. In each case, patients experienced recurrent $CD19^+$ B-ALL after clearance of their myeloid disease. Remarkably, both were successfully rechallenged with CD19-directed immunotherapy and have not experienced recurrent disease despite prolonged immunotherapeutic pressure.

LS has been previously reported in patients after cytotoxic chemotherapy and/or HCT,[13,14] but the frequency has increased with the broader use of immunotherapy.[4-8] The incidence of LS ranges anywhere from 1% to 3% after CD19-CAR but is higher in patients with a *KMT2A*-r, including those with infant ALL.[15,16] LS has also been well documented after treatment with blinatumomab.[6,17-19]

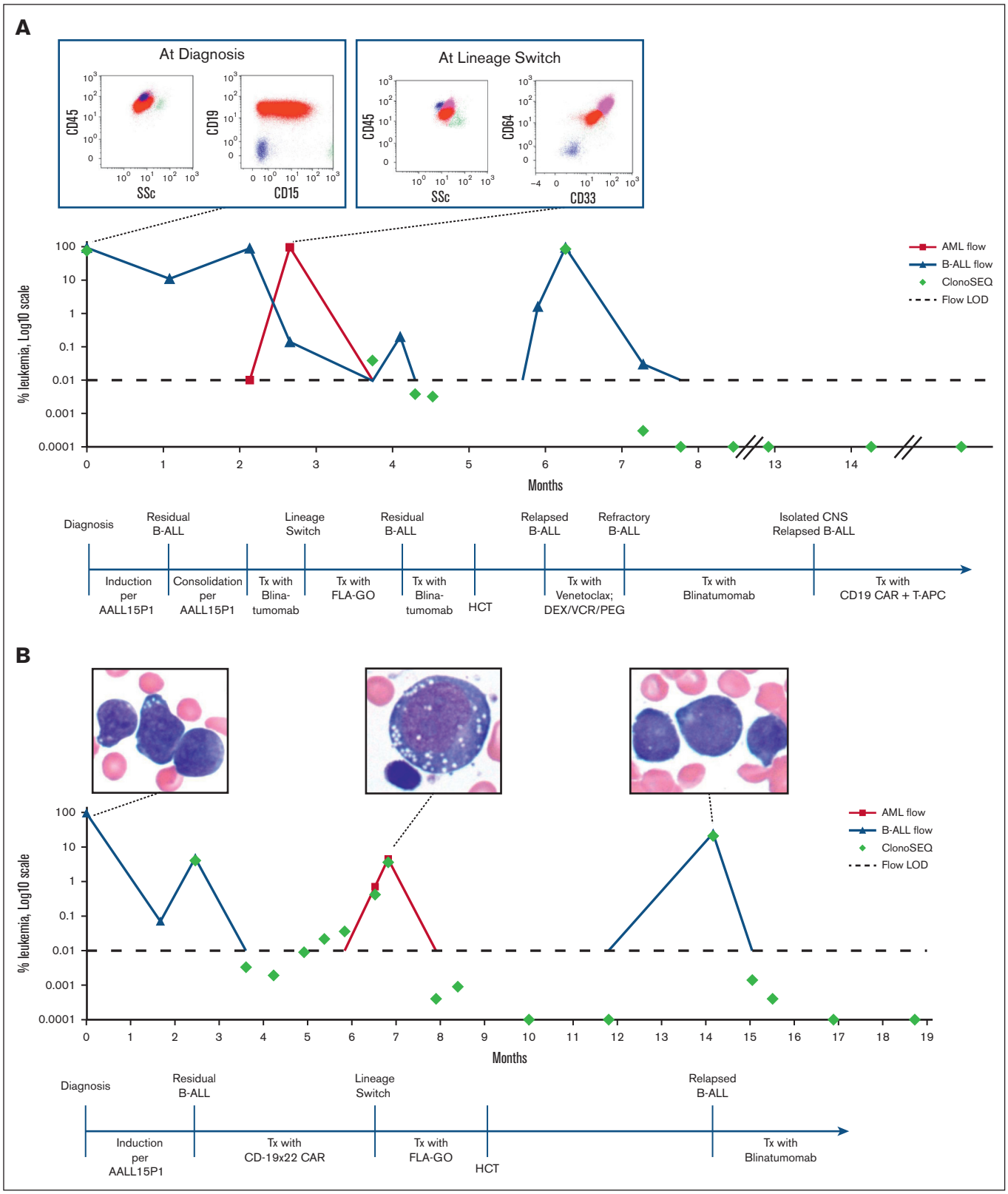

Figure 1. Patient clinical courses. (A) Clinical course of case 1 bone marrow evaluation of leukemic blasts by MFC and by NGS of the IGH/IGL gene through clinical course, with MFC diagrams at diagnosis and at LS. (B) Clinical course of case 2 bone marrow evaluation of leukemic blasts by MFC and by NGS of the IGH/IGL gene through clinical course, with histology images at diagnosis, at LS, and at relapse aftertransplant. AML, acute myeloid leukemia; Blina, blinatumomab; DEX, dexamethasone; LOD, level of detection; PEG, pegaspargase; T-APCs, T-cell antigen-presenting cells; Tx, treatment; SSc, side scatter; VCR, vincristine.

The prognosis in patients with *KMT2A*-r that experience an event after CD19-directed immunotherapy, including LS, is dismal.[15,16] The reason for these poor outcomes is multifactorial and includes the heavy pretreatment, including HCT, patients have often received before CD19-directed immunotherapies. These exposures limit further cytotoxic options because of disease resistance and cumulative toxicity. Furthermore, the phenotypic switch shifts the antigens available for effective immunotherapeutic targeting. Despite this, if patients can tolerate myeloid-directed therapy, there is potential therapeutic benefit with subsequent HCT. In our 2 cases, the LS event occurred after minimal disease–directed therapy, which may have contributed to the higher tolerance and favorable responses to myeloid-directed therapy and subsequent HCT, consistent with previous reports.[4]

We also report on the unique phenomenon of recurrent B-ALL after successful treatment of the LS. Although spontaneous reversion after cessation of blinatumomab has been previously described, we are unaware of any patient being successfully rechallenged with CD19-directed immunotherapy.[6] Our patients' B-ALL recurred after myeloid-directed therapy or HCT and demonstrated sustained responses to CD19-directed immunotherapy without early recurrence of LS. Both of our cases had very short intervals between the initial CD19-directed immunotherapy and LS and, although late LS events have been described, their extended follow-up without LS is encouraging.

The mechanism behind LS is not completely understood and may vary based on disease characteristics. One proposed mechanism involves disease plasticity in which a single genotype results in a fluid phenotype informed by therapeutic pressures. Alternatively, a nonlineage committed progenitor cell harboring the leukemogenic alteration may exist, which can propagate a heterogeneous disease similarly dependent on therapeutic context.[20] Irrespective of the mechanism, it is compelling to speculate that a patient already predisposed to LS is destined to experience recurrent LS with repetitive immunotherapy. Our report challenges this concern, however, whether the responsible clone was eliminated because of intensive myeloid-directed chemotherapy or is more sensitive to the graft-versus-leukemia effect of HCT remains to be elucidated. In addition, it may be continued graft-versus-leukemia surveillance that extinguishes any subsequent LS events that may occur, whereas the ongoing CD19-directed immunotherapy controls the lymphoid component.

This report confirms that LS may be a salvageable entity but requires a combined modality approach. Patients can achieve elimination of their myeloid disease after intensive myeloid-directed therapy coupled with HCT but remain at risk for leukemia relapse. Those who experience relapse restricted to their lymphoid disease could be successfully rechallenged with CD19-directed immunotherapy with durable remissions. Prospective studies exploring manners to prevent and treat LS are needed.

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