Myeloid lineage switch in *KMT2A*-rearranged acute lymphoblastic leukemia treated with lymphoid lineage-directed therapies

Leukemias usually commit to a specific lineage in their development. However, lineage switch (LS), which is the transformation of the original leukemic clone into a different cellular lineage, is associated with a poor survival and usually occurs in leukemias with rearrangements of the *KMT2A* gene (*KMT2Ar*), which already convey a dismal prognosis.[1,2] LS is rare, but its incidence may increase with the use of lymphoid lineage-targeting treatments, such as CD19-directed therapies.[3–7] Herein, we report one of the largest case series of patients with B-cell acute lymphoblastic leukemia (B-ALL) with *KMT2Ar* that had LS to a myeloid phenotype, all after receiving CD19- or CD22-directed therapy. The patients' baseline characteristics and the evolution of their diseases are detailed in Table 1 and Figure 1. Immunophenotype changes between diagnosis and LS are detailed in Figure 2. Detailed information regarding mutations tested in these patients are detailed in the *Online Supplementary Tables S1* and *S2*. This study received ethics approval from the University of Texas MD Anderson Cancer Institutional Review Board (IRB) and was

Table 1. Characteristics of each patient's B-cell acute lymphoblastic leukemia (at diagnosis) and acute myeloid leukemia (after lineage switch).

Patient (sex, age in years)	Acute lymphoblastic leukemia			Acute myeloid leukemia (LS)	
	Cytogenetics and FISH	Mutations	CNS	Cytogenetics and FISH	Mutations
#1 (F, 43)	46,XX,t(4;11)(q21;q23)[12]/ 47,idem,+8[2]/46,XX[6] FISH positive for *KMT2Ar*	*TP53* p.Y163S[A]	-	61~65,XX,-X,+add(1)(p13),-3,der(4)t(4;11)(q21;q23), t(4;11),+del(6)(q15q21),-10,-11,-17,-17,-17,+1~6mar[cp12]/46,XX[8] FISH positive for *KMT2Ar*	*TP53* p.Y163S & p.I251fs[A]
#2 (M, 34)	46,XY,t(4;11)(q21;q23)[11]/ 46,idem,add(18)(p11.2)[8]/ 46,XY[1] FISH positive for *KMT2Ar*	*TP53* p.R282P[A]	-	37~45,XY,add(2)(q33),add(3)(p13), add(3)(p21),t(4;11)(q21;q23), add(9)(p22),-14,add(15)(q24),-16,add(17)(p11.2),-18,i(18)(q10),-19,-21,+1~5mar[cp10]/46,XY[10] FISH positive for *KMT2Ar*	*TP53* p.R282P[A]
#3 (F, 27)	46,XY,t(4;11)(q21;q23) [13]/46,XX[7] FISH positive for *KMT2Ar*	Not assessed	-	53,XX,+der(4)t(4;11)(q21;q23),t(4;11),+6,+7,+8, +13,+19,+20[6]//46,XY[14] FISH positive for *KMT2Ar*	Not assessed
#4 (M, 0)	46,XY,t(4;11)(q21;q23)[15]/46,XY[5] FISH positive for *KMT2Ar*	*CCND3, FLT3, CD28*	+	Cytogenetics not available FISH positive for *KMT2Ar*	*PTPN11* p.D61V[A]
#5 (F, 60)	46,XX,t(11;19)(q23;p13.3)[14]/46,XX[6] FISH positive for *KMT2Ar*	*KRAS* p.G12D[B]	-	46,XX,+i(8)(q10),t(11;19)(q23;p13.3),-16[12]/46,XX,+i(8)(q10),t(11;19),add(14)(p11.2),-16[cp6]/47,XX,+X,t(11;19)[2] FISH not available	Not assessed
#6 (M, 44)	46,XY,t(4;11)(q21;q23) [20] FISH positive for *KMT2Ar*	Not available	-	84~87,XXYY,-1,der(4)t(4;11)(q21;q23),t(4;11),-5,-10,-11,-21[cp20] FISH positive for *KMT2Ar*	*NRAS* p.Q61H[A]

[A]Mutation/s detected using a 81-gene next-generation sequencing panel. [B]Mutation/s detected using a 28-gene next-generation sequencing panel. Panel gene coverage is detailed in the *Online Supplementary Appendix*. LS: lineage switch; CNS: central nervous system involvement; FISH: fluorescence *in situ* hybridization; *KMT2Ar*: *KMT2A* rearrangement; F: female; M: male.

conducted in accordance with the Declaration of Helsinki. Patient 1 was a 43-year-old woman diagnosed with B-ALL with t(4:11)/*KMT2A::AFF1* rearrangement. The patient received treatment with hyper-CVAD (hyper-fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone). She achieved complete remission (CR) but had disease bone marrow relapse after the eighth course. She then received mini-hyper-CVD (mini-hyper-fractionated cyclophosphamide, vincristine, and dexamethasone) with inotuzumab but did not achieve CR. A second salvage therapy consisting of fludarabine, idarubicin, cytarabine, pegylated asparaginase, and blinatumomab elicited CR, but the patient had a relapse with LS after the second cycle of blinatumomab. She then received salvage chemotherapy with venetoclax and later azacytidine plus ipilimumab and nivolumab but had no response.

Patient 2 was a 34-year-old man diagnosed with B-ALL with t(4:11)/*KMT2A::AFF1* rearrangement. He received hyper-CVAD and initially achieved CR with negative measurable residual disease by multiparameter flow cytometry (MRDfc) but had relapse after the fourth course. He then received three courses of dose-reduced hyper-CVD with inotuzumab and achieved CR with negative MRDfc before proceeding to allogeneic stem cell transplantation (allo-SCT). Two months after allo-SCT, the patient had a mixed relapse with 57% lymphoid blasts in the bone marrow and two myeloid sarcomas in the left tonsillar region. He was treated with fludarabine, idarubicin, cytarabine, venetoclax, and blinatumomab and initially had CR with negative MRDfc and a reduction in sarcoma size. However, his disease progressed rapidly; a full LS with full bone marrow involvement by myeloid blasts was accompanied

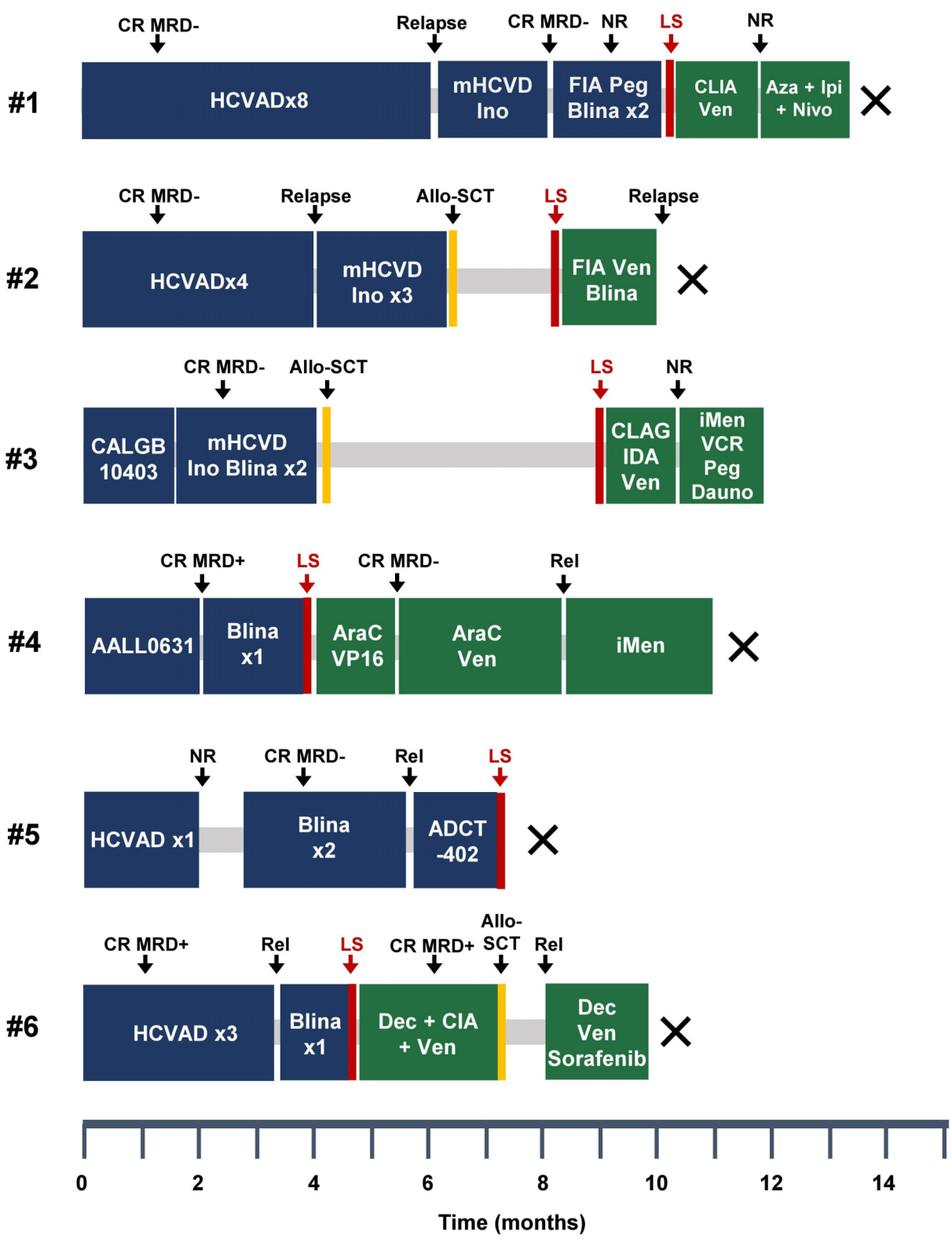

Figure 1. Summary of therapies received. Patient treatments and responses over time. Blue indicates therapies for B-cell acute lymphoblastic leukemia; green indicates therapies for acute myeloid leukemia after lineage switch (LS; red). Allo-SCT: allogeneic stem cell transplantation; AraC: cytarabine; Aza: azacytidine; Blina: blinatumomab; CIA: clofarabine, idarubicin, and cytarabine; CLAG: cladribine, cytarabine, and granulocyte colony-stimulating factor; CLIA: cladribine, idarubicin, and cytarabine; CR: complete remission; Dauno: daunorubicin; Dec: decitabine; FIA: fludarabine, idarubicin, and cytarabine; HCVAD: hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; IDA: idarubicin; iMen: menin inhibitor; Ino: inotuzumab ozogamicin; Ipi: ipilumumab; mHCVD: mini-hyperfractionated cyclophosphamide, vincristine, and dexamethasone; MRDfc: minimal residual disease by flow cytometry; Nivo: nivolumab; NR: no response; Peg: pegylated asparaginase; VCR: vincristine; Ven: venetoclax; VP16: etoposide.

by clinical deterioration, and the patient received no further treatment.

Patient 3 was a 27-year-old woman diagnosed with B-ALL with t(4:11)/*KMT2A::AFF1* rearrangement. The patient initially received induction therapy based on the CALGB 10403 pediatric protocol and then received dose-dense mini-hyper-CVD with inotuzumab and blinatumomab. After three cycles of therapy, she achieved CR with negative MRDfc and proceeded to allo-SCT. Five months after allo-SCT, she had a relapse with LS with multiple myeloid sarcomas without bone marrow involvement. She received FLAG-IDA (fludarabine, cytarabine, idarubicin, and G-CSF) with venetoclax but had no response. The patient is currently enrolled in a clinical trial combining chemotherapy with a menin inhibitor; her sarcomas shrunk after the first cycle of treatment.

Patient 4 was a 2-month-old male diagnosed with B-ALL with t(4:11)/*KMT2A::AFF1* rearrangement. The patient was treated with the AALL0631 pediatric protocol but had persistent MRDfc and received one course of blinatumomab. He then relapsed with LS. He received cytarabine with etoposide and achieved CR. He started maintenance therapy with cytarabine and venetoclax as a bridge to allo-SCT but had relapse with LS. The patient was enrolled in a clinical trial with a menin inhibitor but did not achieve response.

Patient 5 was a 60-year-old woman diagnosed with B-ALL with 46,XX,t(11;19)(q23;p13.3)/*KMT2A::MLLT1* rearrangement. The patient received hyper-CVAD but had progressive disease after the first course and, therefore, received blinatumomab, which elicited CR with negative MRDfc. However, the patient had a relapse and was enrolled in a clinical trial of the CD19-directed antibody–drug conjugate ADCT-402. After one course, the patient had a relapse with LS and received no further therapy.

Patient 6 was a 44-year-old man diagnosed with B-ALL with t(4:11)/*KMT2A::AFF1* rearrangement. The patient received three courses of hyper-CVAD; he initially achieved CR with MRDfc but had a relapse after the last cycle. He then received one cycle of blinatumomab and had a relapse with LS. He was treated with decitabine plus chemotherapy and venetoclax and had CR after two

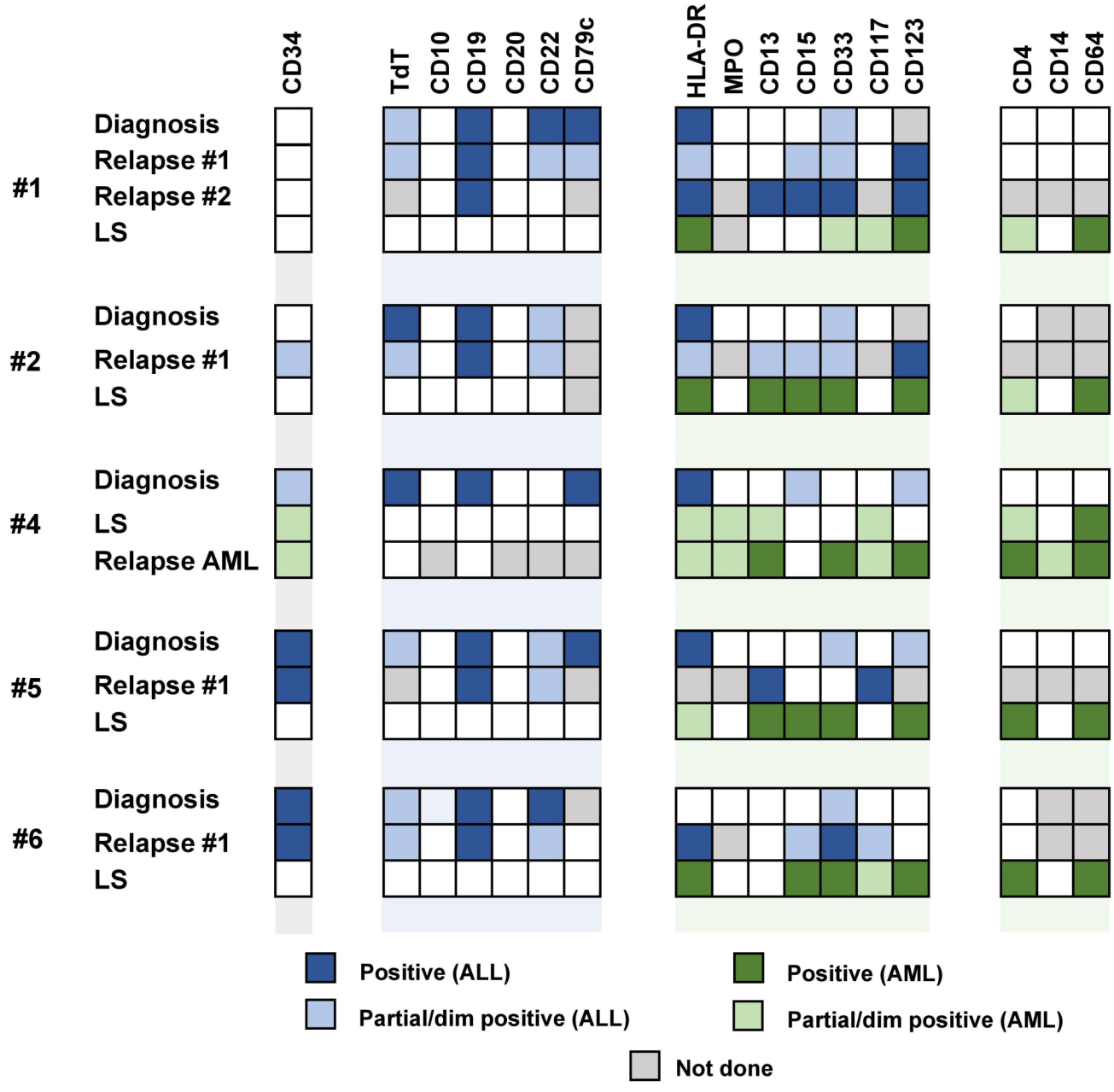

Figure 2. Immunophenotypic changes between diagnosis and lineage switch. Immunophenotypes of the leukemic cells at different time points. LS: lineage switch; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia.

cycles before proceeding to allo-SCT. However, he had a relapse 30 days after allo-SCT. He received decitabine, venetoclax, and sorafenib but did not achieve CR.

LS is rare, occurring in about 6% of all relapsed leukemias in childhood.[2] In our cohort, this phenomenon occurred in 7% of patients with KMT2Ar B-ALL treated in our institution. LS occurs primarily in *KMT2Ar* leukemias, usually in pediatric patients with ALL with t(4;11)/*KMT2A::AFF1* rearrangement,[4–6] this last characteristic present in five of the six patients presented here. Patients with LS have poor prognosis, and current treatments do not have a high rate of success.[4] Therefore, a better understanding of the mechanisms of LS is needed to develop strategies to prevent and treat it.

The specific mechanism of LS in *KMT2Ar* leukemias is unclear. Potential mechanisms include bipotential progenitors, cellular reprograming, de-differentiation, and clonal selection.[8] Tirtakusuma *et al.*,[9] investigating the mechanisms of LS in *KMT2A::AFF1* leukemias, found that LS is related to the rewiring of gene regulatory networks and to changes in chromatin accessibility, with myeloid relapses recurrently associated with *CHD4* gene abnormalities. This suggest that LS is driven and maintained by epigenetic dysregulation. Other studies have shown that the lineage fate of these leukemias is influenced by the bone marrow microenvironment.[10] Of note, four of five patients with available cytogenetics at LS acquired a complex karyotype besides KMT2A rearrangement, two of them with a detectable *TP53* mutation. A biological hypothesis to be validated is that this evident genetic instability that drives these chromosome abnormalities could also favor a phenotype switch under the selective pressure or B-cell-directed therapies.

LS has become a topic of interest among leukemia researchers because it provides a mechanism by which B-ALL cells can escape directed immunotherapies, which requires to switch the highly effective lymphoid-lineage directed therapy to myeloid lineage-directed therapy. Recent studies have shown that in patients with *KMT2Ar* leukemias, LS from B-ALL to AML can occur after treatment with blinatumomab or chimeric antigen receptor T cells.[3,7,11,12] This is important because the increased use of these treatments, which have selective pressure against lymphoid antigens, may increase the rate of LS. In our cohort, all patients received at least one B-cell–directed immunotherapy, against either CD19 or CD22, and these antigens had disappeared by the time of LS. However, whether these therapies can increase the rate of LS is unclear; for example, a recent study in a large cohort of pediatric patients receiving blinatumomab has not detected any LS.[13] It is likely that leukemias presenting with LS have high lineage promiscuity, and B-cell-directed therapies are highly effective at eliminating B-ALL subclones, leaving the myeloid-committed leukemic cells a beneficial environment in which to proliferate. Continuing to monitor LS rates among patients receiving B-cell–directed therapies, especially those who have leukemias with *KMT2Ar*, is crucial.

Regardless of their phenotype, the leukemias reported herein had the genetic hallmarks of *KMT2Ar*, which made them potentially sensitive to targeted therapy with menin inhibitors. These recently developed compounds interfere with the interaction between the KMT2A-rearranged protein and its cofactor, menin, thereby impeding the abnormal expression of the *HOXA* genes responsible for maintaining leukemic cells.[14] The *KMT2A* rearrangement is maintained at the time of LS, therefore there is a rationale for testing the efficacy of menin inhibitors in this situation.[15] In our cohort, two patients with relapsed AML arising from B-ALL received menin inhibitor; as of this writing, one has had a response whereas the other did not respond. It is warranted to further explore the efficacy of menin inhibition in KMT2A rearranged leukemias presenting LS.

In conclusion, LS is a rare but devastating scenario. Studies to determine whether novel directed therapies against B-lineage antigens can increase the incidence of LS in these patients are warranted. Combination therapy of menin inhibitors with targeted therapies could change the treatment paradigm for patients with leukemias with LS.

Authors

Alex Bataller,[1*] Tareq Abuasab,[1*] David McCall,[2] Wei Wang,[3] Branko Cuglievan,[2] Ghayas C. Issa,[1] Elias Jabbour,[1] Nicholas Short,[1] Courtney D. DiNardo,[1] Guilin Tang,[3] Guillermo Garcia-Manero,[1] Hagop M. Kantarjian[1] and Koji Sasaki[1]

[1]Department of Leukemia, [2]Department of Pediatrics and [3]Department of Hematopathology, the University of Texas MD Anderson Cancer Center, Houston, TX, USA

**AB and TA contributed equally as first authors.*

Correspondence:
K. SASAKI - ksasaki1@mdanderson.org

https://doi.org/10.3324/haematol.2023.283705

Received: June 7, 2023.
Accepted: August 23, 2023.
Early view: August 31, 2023.

Disclosures

GCI discloses consultancy from Novartis, Kura Oncology and

Nuprobe; and research funding from Celgene, Kura Oncology, Syndax, Merck, Cullinan and Novartis. EJ discloses research funding from Amgen, Pfizer, Abbvie, Adaptive Biotechnologies, Astex and Ascentage; and consultancy from Amgen, Pfizer, Abbvie, Takeda, Adaptive Biotechnologies, Astex, Ascentage, Genentech, Novartis, BMS, Jazz Pharmaceuticals, Hikma Pharmaceuticals and Incyte. NS discloses consultancy from Takeda Oncology, AstraZeneca, Amgen, Novartis and Pfizer; research funding from Takeda Oncology, Astellas and Stemline Therapeutics; and honoraria from Amgen. CDD discloses membership on an entity's Board of Directors or advisory committees from GenMab, GlaxoSmithKline, Kura and Notable Labs; honoraria from Kura, Astellas, Bluebird Bio, Bristol Myers Squibb, Foghorn, ImmuneOnc, Novartis, Takeda, Gilead and Jazz Pharmaceuticals; is a current holder of stock options in a privately-held company from Notable Labs; consultancy from Abbvie, Servier; and research funding from Servier, Bristol Myers Squibb, Foghorn, ImmuneOnc, LOXO, Astex, Cleave and Forma. GG-M discloses research funding from Astex Pharmaceuticals, Novartis, Abbvie, BMS, Genentech, Aprea Therapeutics, Curis and Gilead Sciences; consultancy from Astex Pharmaceuticals, Acceleron Pharma and BMS; and honoraria from Astex Pharmaceuticals, Acceleron Pharma, Abbvie, Novartis, Gilead Sciences, Curis, Genentech and BMS. HMK discloses research funding from Abbvie, Amgen, Ascentage, BMS, Daiichi Sankyo, Immunogen, Jazz Pharmaceuticals and Novartis; and honoraria from Abbvie, Amgen, Amphista, Ascentage, Astellas, Biologix, Curis, Ipsen Biopharmaceuticals, KAHR Medical, Novartis, Pfizer, Precision Biosciences, Shenzhen Target Rx and Takeda. KS discloses research funding from Novartis; consultancy from Novartis; honoraria from Otsuka Pharmaceuticals; and membership on an entity's Board of Directors or advisory committees from Novartis, Pfizer and Daiichi-Sankyo.

Contributions

KS, HMK, AB and TA conceived and designed the study. AB, TA, DM and BC collected the clinical data. WW and GT provided and interpreted biological data. AB elaborated the figures. GCI, EJ, NS, CDD, GG-M, HMK and KS provided clinical care to the patients. AB and TA wrote the manuscript. All authors contributed intellectually to the study and critically reviewed and edited the manuscript.

Data-sharing statement

The data used for this study is not publicly available in order to protect patient confidentiality. Reasonable requests for de-identified data should be directed to the corresponding author.

References

1. Winters AC, Bernt KM. MLL-rearranged leukemias - an update on science and clinical approaches. Front Pediatr. 2017;5:4.
2. Stass S, Mirro J, Melvin S, Pui CH, Murphy SB, Williams D. Lineage switch in acute leukemia. Blood. 1984;64(3):701-706.
3. Gardner R, Wu D, Cherian S, et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood. 2016;127(20):2406-2410.
4. Takeda R, Yokoyama K, Fukuyama T, et al. Repeated lineage switches in an elderly case of refractory B-cell acute lymphoblastic leukemia with MLL gene amplification: a case report and literature review. Front Oncol. 2022;12:799982.
5. Yost CM, Gaikwad AS, Parsons DW, et al. Aberrant leukemia-associated immunophenotype as potential harbinger of lineage switch in KMT2A-rearranged leukemia: a case series. Leuk Lymphoma. 2020;61(14):3515-3518.
6. Rossi JG, Bernasconi AR, Alonso CN, et al. Lineage switch in childhood acute leukemia: an unusual event with poor outcome. Am J Hematol. 2012;87(9):890-897.
7. Rayes A, McMasters RL, O'Brien MM. Lineage switch in MLL-rearranged infant leukemia following CD19-directed therapy. Pediatr Blood Cancer. 2016;63(6):1113-1115.
8. Dorantes-Acosta E, Pelayo R. Lineage Switching in acute leukemias: a consequence of stem cell plasticity? Bone Marrow Res. 2012;2012:406796.
9. Tirtakusuma R, Szoltysek K, Milne P, et al. Epigenetic regulator genes direct lineage switching in MLL/AF4 leukemia. Blood. 2022;140(17):1875-1890.
10. Hu T, Murdaugh R, Nakada D. Transcriptional and microenvironmental regulation of lineage ambiguity in leukemia. Front Oncol. 2017;7:268.
11. Lamble AJ, Myers RM, Taraseviciute A, et al. KMT2A rearrangements are associated with lineage switch following CD19 targeting CAR T-cell therapy. Blood. 2021;138(Suppl 1):S256.
12. Wölfl M, Rasche M, Eyrich M, Schmid R, Reinhardt D, Schlegel PG. Spontaneous reversion of a lineage switch following an initial blinatumomab-induced ALL-to-AML switch in MLL-rearranged infant ALL. Blood Adv. 2018;2(12):1382-1385.
13. van der Sluis IM, de Lorenzo P, Kotecha RS, et al. Blinatumomab added to chemotherapy in infant lymphoblastic leukemia. N Engl J Med. 2023;388(17):1572-1581.
14. Issa GC, Ravandi F, DiNardo CD, Jabbour E, Kantarjian HM, Andreeff M. Therapeutic implications of menin inhibition in acute leukemias. Leukemia. 2021;35(9):2482-2495.
15. Issa GC, Aldoss I, DiPersio J, et al. The menin inhibitor revumenib in KMT2A-rearranged or NPM1-mutant leukaemia. Nature. 2023;615(7954):920-924.