Clonal evolution from B-cell acute lymphoblastic leukemia with *BCR::ABL1* multilineage involvement to acute myeloid leukemia after multiple anti-CD19 chimeric antigen receptor T-cell therapy

Clonal evolution in leukemia generally arises under the selection pressure of cytotoxic chemotherapy and targeted therapies.[1] Unlike B-cell acute lymphoblastic leukemia (B-ALL) relapse associated with the acquisition of drug resistance mutations in chemotherapy, clonal evolution after CD19-targeted immunotherapies including blinatumomab and anti-CD19 chimeric antigen receptor (anti-CD19 CAR) T-cell therapy, is mainly determined by the immunophenotype of blasts.[1,2] Downregulation of CD19 and selection of the CD19-negative subclones are potential mechanisms of CD19-negative relapse.[3] With the widespread use of cell surface antigen-targeted therapies, lineage switch is being observed with increasing frequency. Recently, a study reported that among patients with lineage switch after T-cell engaging therapies, 48.90% (23/47) patients received CAR T cells and 51.10% (24/47) patients received blinatumomab prior to lineage switch.[4] Here, we present a rare case of a patient with B-ALL with *BCR::ABL1* multilineage involvement who developed immunoglobulin heavy chain (IGH) clonal evolution accompanied by acute myeloid leukemia (AML) phenotypic and morphological changes after receiving of anti-CD19 CAR T-cell consolidation therapy multiple times.

A 56-year-old woman presented with fatigue and excessive sweating in December 2021. Peripheral blood counts revealed elevated white blood cell count (185.32×10^9/L), anemia (hemoglobin, 96 g/L) and thrombocytopenia (14×10^9/L). Bone marrow aspirate smears exhibited intermediate-sized blasts with scant, agranular cytoplasm, delicate chromatin, and inconspicuous nucleoli (Figure 1A). Morphology analysis of bone marrow aspiration indicated that lymphoid blasts comprised 73.50% of the bone marrow mononuclear cells. Immunophenotyping confirmed the diagnosis of B-ALL expressing CD34, CD10, CD19, and cytoplasmic CD79a (cCD79a). Blasts expressed CD13, CD33 and were negative for other myeloid markers (HLA-DR, MPO, etc.) (Table 1; Figure 1B-F). Chromosome analysis revealed 46,XX,t(9;22)(q34;q11)[2]/45,idem,-7[5]/45,idem,-7,20q-[3], and quantitative polymerase chain reaction (PCR) confirmed the *BCR::ABL1* (b2a2/b3a2) fusion. Next-generation sequencing (NGS) of hotspot mutations showed mutations in *RUNX1* with a variant allele frequency of 44.29%, *MYD88* with a variant allele frequency of 50.80%, and no *ABL1* kinase domain mutation was detected (Table 1). Consequently, bone marrow aspiration analysis by morphology, immunophenotyping, cytogenetics, and molecular genetics suggested Philadelphia-positive (Ph^+) B-ALL. After dexamethasone pretreatment and induction therapy with idarubicin (10 mg for 1 day) + vindesine (4 mg for 1 day) combined with imatinib, complete remission (CR) was achieved, confirmed by bone marrow aspiration examination in January 2022. However, flow cytometry (FCM) analysis revealed that the measurable residual disease (MRD) was 18.07% in cerebrospinal fluid (CSF) but negative in bone marrow, indicating isolated central nervous system leukemia (CNSL) in March 2022. The blasts in CSF became negative after two courses of triple intrathecal chemotherapy (methotrexate + cytarabine + dexamethasone) and chemotherapy of high-dose methotrexate (3 g/m^2) with medium-dose cytarabine (2 g/m^2).

Then the patient was recruited into a clinical trial (*clinicaltrials.gov. Identifier: NCT03984968*), incorporating first-time anti-CD19 CAR T-cell therapy, followed by three cycles of anti-CD19 CAR T-cell consolidation combined with $CD19^+$ feeding T cells (FTC, autologous T cells transduced with a CD19 gene expression vector) and tyrosine kinase inhibitors (TKI) to eliminate MRD.[5] The supplementation with $CD19^+$ FTC can restore the anti-CD19 CAR T cells' lost response to residual $CD19^+$ blasts. This research has been approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. At 28 days after the first dose of anti-CD19 CAR T-cell infusion, this patient had a hypocellular marrow with MRD of $<1.4\times10^{-4}$ measured by FCM on a bone marrow aspirate, along with 2263 *BCR::ABL1* copies/10,000 ABL1 copies (Table 1). Therefore, this patient discontinued imatinib and switched to dasatinib maintenance therapy. After three cycles of consolidation therapy, this patient achieved MRD-negative CR and 0.99% *BCR::ABL1* fusion. However, she relapsed 3 months after the last cycle of anti-CD19 CAR T-cell consolidation therapy. Her bone marrow aspirate smears showed the emergence of blasts with more abundant cytoplasm (Figure 1G). Bone marrow aspirate analysis by FCM showed 73.80% of blasts were positive for HLA-DR, CD13, CD33, and CD117, whereas CD19 and other B-lineage antigens were absent (Table 1; Figure 1H-L). Cytogenetic studies of the bone marrow showed the original t(9;22)(q34;q11) (Table 1). Except for the newly emerged AML blasts, there was no evidence of residual B-ALL blasts. NGS revealed persistence of the mutation in *RUNX1* (27.70%) and *MYD88* (48.90%), additional *FAT1* (49.80%), *IKZF1* (25.40%),

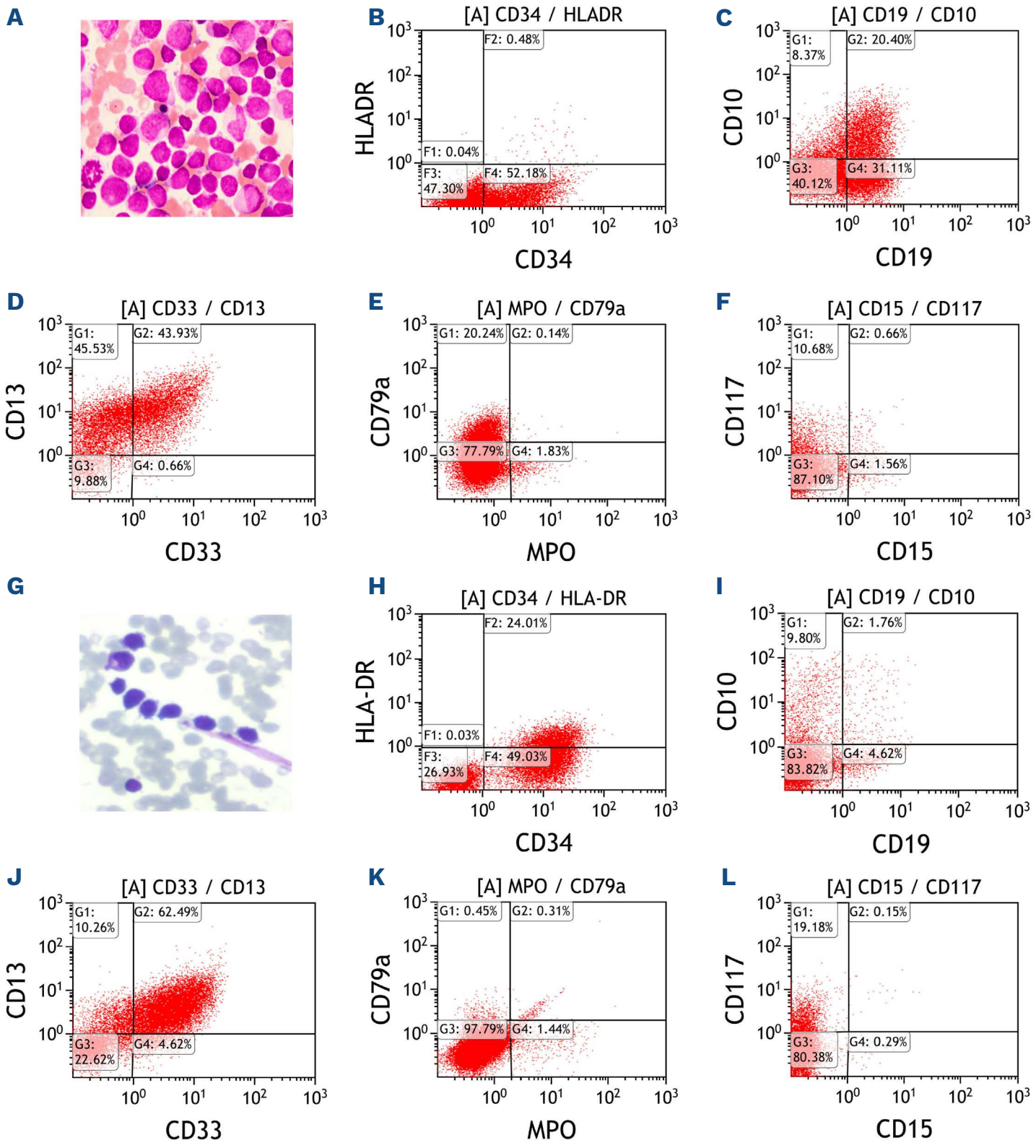

Figure 1. Distinct morphologic and immunophenotypic features of initial B-cell acute lymphoblastic leukemia blasts and acute myeloid leukemia blasts following lineage switch. (A) The lymphoblasts of B-cell acute lymphoblastic leukemia (B-ALL) had scant cytoplasm, round nuclei, fine chromatin, and inconspicuous nucleoli (100X magnification). (B-F) The lymphoblasts (A cell group, SS^{dim} and $CD45^{dim}$) expressed CD19, CD10, cCD79a, CD34, CD13 and CD33. They were negative for HLA-DR and MPO. (G) The myeloid blasts of acute myeloid leukemia (AML) had abundant cytoplasm, folded nuclei, fine chromatin, and occasional prominent nucleoli (100X magnification). (H-L) The myeloid blasts (A cell group, SS^{dim} and $CD45^{dim}$) expressed HLA-DR, CD13, CD33, and CD117, yet they were negative for CD19, CD10 and cCD79a.

and *SETD2* (31.50%). The patient achieved sustained CR after re-induction of dasatinib and venetoclax. Subsequently, the patient intermittently used dasatinib as maintenance therapy and has remained in CR.

Lineage switch is a rare phenomenon characterized by the transformation of acute leukemia from one cell lineage to another, while maintaining the original cytogenetic or molecular aberrations and/or clonal immunoglobulin rearrangement patterns.[2,4,6] In this case, myeloid characteristics were absent at B-ALL diagnosis but became evident upon AML relapse. The patient retained the *BCR::ABL1* fusion and IGH rearrangement upon AML relapse. Furthermore, we observed dynamic changes in IGH subclones. During treatment, the IGH gene may undergo new rearrangements based on the original, disappear entirely with new ones appearing, or alter non-major clone numbers and proportions.[7] In this case, the IGH rearrangement in bone marrow mononuclear cells by NGS revealed the primary clones 1 and 2 at initial diagnosis of B-ALL disappeared after anti-CD19 CAR T-cell treatment, while clones 3 and 4 remained and became the

Table 1. The summary of abnormalities found at the time of B-cell acute lymphoblastic leukemia diagnosis and acute myeloid leukemia relapse.

Characteristics	B-ALL at diagnosis	Pre 1st CAR T-cell infusion	Day 28 post 1st CAR T-cell infusion	AML at relapse
Immunophenotype	$CD34^+$, $CD10^+$, $CD19^+$, $CD13^+$, $CD33^+$, $cCD79a^+$, $CD117^{dim}$, $CD45^{dim}$, $CD7^-$, $HLA\text{-}DR^-$, $CD20^-$, $CD14^-$, $CD2^-$, $CD15^-$, $CD11B^-$, $CD64^-$, $CD56^-$, $CD38^-$, $CD3^-$, $CD4^-$, $CD8^-$, MPO^-	$CD81^+$, $CD123^-$, $CD13^-$, $CD33^-$, $CD22^+$, $CD20^-$, $CD10^+$, $CD19^+$, $CD38^-$, $CD34^+$, $CD45^+$	$CD81^+$, $CD123^-$, $CD13^-$, $CD33^-$, $CD22^-$, $CD20^-$, $CD10^+$, $CD19^+$, $CD38^+$, $CD34^+$, $CD45^+$	$CD7^-$, $CD34^+$, $HLA\text{-}DR^+$, $CD10^-$, $CD20^-$, $CD19^-$, $CD14^-$, $CD13^+$, $CD33^+$, $CD2^-$, $CD117^+$, $CD15^-$, $CD11B^-$, $CD64^-$, $CD56^-$, $CD38^+$, $CD4^-$, $CD8^-$, $CD3^-$, $CD45^\pm$
Cytogenetics	46,XX,t(9;22)(q34;q11)[2]/45,idem,-7[5]/45,idem,-7,20q-[3]	Not done	Not done	46,XX,t(9;22)(q34;q11)[5]/46,XX[5]
MRD by FCM	84.66%	$<9.4\times10^{-5}$	$<1.4\times10^{-4}$	73.80%
BCR::ABL1 by q-PCR	(+); *BCR::ABL1* by q-PCR was performed at diagnosis, but a quantitative analysis was not conducted	(-)	22.63%	103.47%
Mutations	*RUNX1*, *MYD88* mutations positive	(-)	(-)	*RUNX1, FAT1, IKZF1, MYD88, SETD2* mutations positive

B-ALL: B-cell acute lymphoblastic leukemia; CAR: chimeric antgen receptor; AML: acute myeloid leukemia; MRD: measurable residual disease; FCM: flow cytometry; q-PCR: quantitative polymerase chain reaction.

primary clones upon AML relapsed (Figure 2A). In addition, the shared mutational profile between the original B-ALL and the emergent AML (Table 1) supported that this patient suffered relapse with clonally related AML after anti-CD19 CAR T-cell therapy.

Recently, a new subset of B-ALL which appears more closely related to chronic myeloid leukemia (CML) presenting in lymphoid blast phase (LBP) was reported in International Consensus Classification (ICC) of Myeloid Neoplasms and Acute Leukemia.[8] The CML-like disease is characterized by the presence of *BCR::ABL1* fusion in both granulocytes and blasts. *BCR::ABL1* fusion following treatment shows high level positivity whereas both FCM and molecular MRD methods show no or little evidence of MRD contribution to distinguishing this new subset.[8] We reviewed the chromosome specimen at the initial diagnosis of B-ALL. The *BCR::ABL1* fusion was detected not only in B-ALL blasts but also in granulocytes by fluorescence *in situ* hybridization (FISH) (Figure 2B). Besides, quantitative PCR studies for *BCR::ABL1* fusion following 1 month after the first time CAR T-cell therapy showed high level positivity when FCM showed little evidence of MRD (Figure 2C). Therefore, this patient should be diagnosed as B-ALL with *BCR::ABL1* multilineage involvement.

The transition to a myeloid lineage in B-ALL following anti-CD19 CAR T-cell therapy is a rare event, particularly when accompanied by IGH clonal evolution. Previous studies have revealed the high frequency of IGH rearrangement clones in AML.[9,10] We utilized a sensitive NGS method to detect IGH rearrangements and found that clone 3 and 4 could be detected at both initial B-ALL diagnosis and AML relapse. In addition, the *BCR::ABL1* fusion, along with mutations of *RUNX1* and *MYD88*, consistently accompanied AML relapse. Thus, it is highly probable that clones 3 and 4 represent relapsed AML blasts. We assumed that: (1) AML blasts may arise from the original B-ALL, especially from blasts that partially expressed CD13 and CD33 (Figure 2D). Baseline myeloid antigen co-expression was observed in the majority of patients potentially undergoing lineage switch.[4] These B-ALL blasts undergo transcription factor-mediated reprogramming after immunotherapy, leading to a lineage switch towards myeloid cells as a mechanism for immune escape.[2] (2) AML blasts exhibiting IGH rearrangement and *BCR::ABL1* fusion may stem from the minor AML clones at the initial B-ALL diagnosis (Figure 2D). However, given the relatively low abundance of clones 3 and 4 identified at the time of B-ALL diagnosis, the precise phenotypic characteristics of these clones as defined by FCM remain uncertain. The concurrent presence of *BCR::ABL1* fusion and IGH rearrangement in both B-ALL and AML blasts suggests that the aberrant myeloid cells likely originate from progenitor cells harboring *BCR::ABL1* fusion, indicating an aberrant differentiation pathway (Figure 2D). Previous studies have suggested that early genetic mutations can cause progenitor cells to develop abnormally, with these abnormalities remaining stable or changing over time due to treatment and further genetic changes.[11,12]

Clonal evolution may contribute to therapy resistance, and treatment may also accelerate the evolutionary process. The observation that multiple prior cycles of chemotherapy and

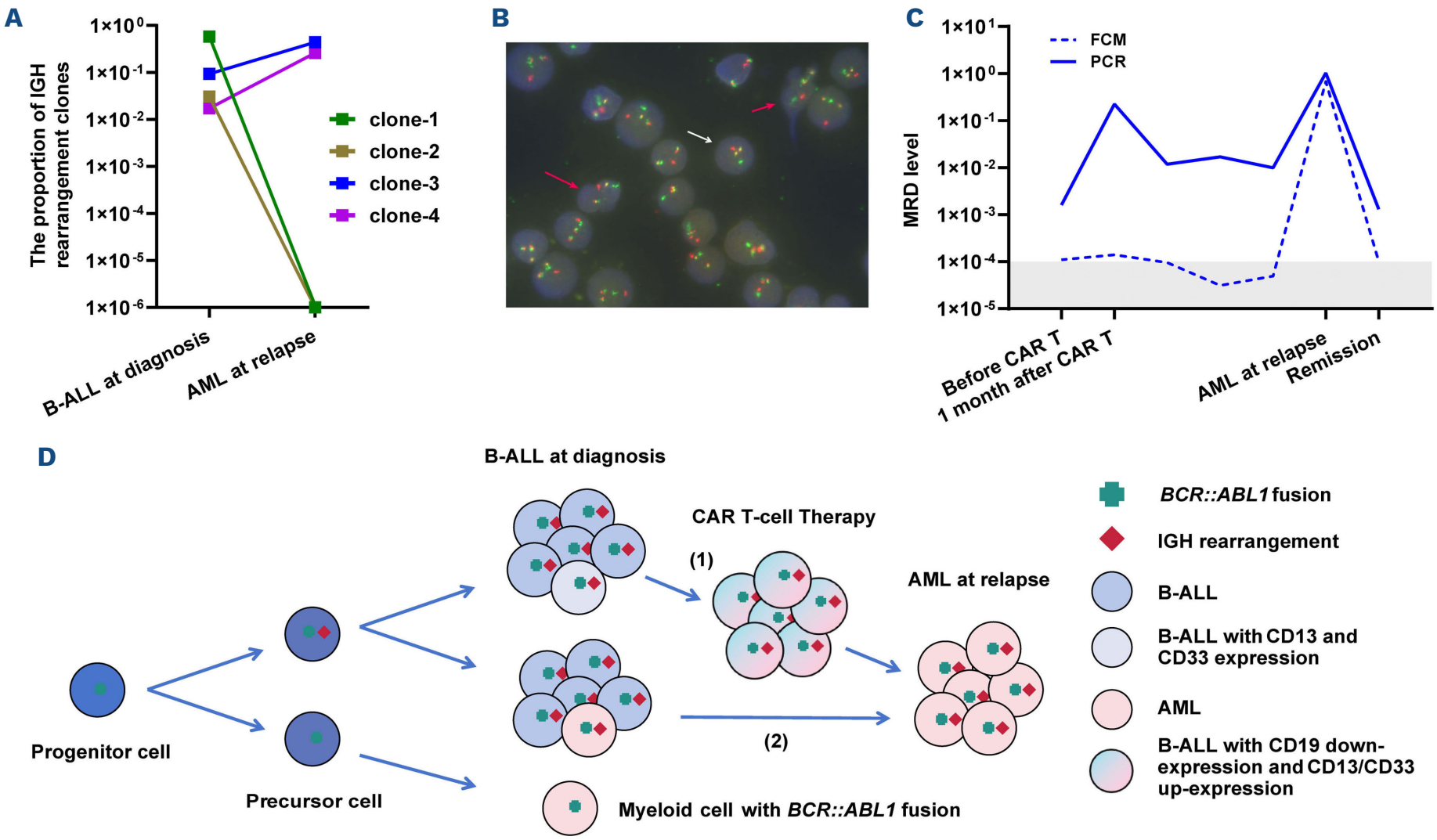

Figure 2. Confirmation that the patient belonged to the subset of Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia (B-ALL) with *BCR::ABL1* multilineage involvement, and model of acute myeloid leukemia relapse from B-ALL after anti-CD19 CAR T-cell therapy. (A) The primary clones 1 and 2 at initial diagnosis of B-ALL disappeared after anti-CD19 chimeric antigen receptor (CAR) T-cell treatment, while clones 3 and 4 expanded and became the primary clones upon acute myeloid leukemia (AML) relapse. (B) Fluorescence *in situ* hybridization detection revealed *BCR::ABL1* fusion not only in B-ALL blasts (white arrow) but also in granulocytes (red arrow). (C) The presence of *BCR::ABL1* fusion detected by polymerase chain reaction (solid line) was inconsistent with the measurable residual disease (MRD) as measured by flowcytometry (dashed line). (D) Models of AML relapse with *BCR::ABL1* fusion and immunoglobulin heavy chain (IGH) rearrangement from B-ALL with *BCR::ABL1* multilineage involvement: (1) B-ALL blasts expressing partial CD13 and CD33 experienced downregulation of CD19 expression, and upregulation of CD13 and CD33, ultimately resulting in AML relapse; (2) The minor AML blasts, undetected at the initial B-ALL diagnosis, expanded after multiple anti-CD19 CAR T-cell treatments that eliminated B-ALL, ultimately leading to AML relapse.

TKI did not induce AML underscores the likely acquisition a myeloid phenotype subsequent to anti-CD19 CAR T-cell therapy. CD19-positive blasts responded well to anti-CD19 CAR T-cell treatment, while residual CD19-negative blasts displayed resistance. Additionally, CD19-negative relapse resulting from lineage switching tends to manifest approximately 1-2 months after anti-CD19 CAR T-cell therapy.[13-15] In this case, AML relapse occurred 11 months after the initial anti-CD19 CAR T-cell infusion, during which the patient received multiple rounds of CAR T-cell therapy. Consequently, AML cell populations at relapse likely evolved from sub-clonal populations present at the B-ALL diagnosis under significant selective pressure.

In summary, this case may partially suggest the potential mechanism of B-ALL with *BCR::ABL1* multilineage involvement and provide a novel mechanism of immune escape in B-ALL. The new subset of Ph^+ B-ALL may have a high-risk of transforming to AML under the pressure of CD19-targeted immunotherapy. In B-ALL with *BCR::ABL1* multilineage involvement, no relevant prognostic feature applicable for therapy tailoring was found so far, multicenter and prospective studies are needed.

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Disclosures

No conflicts of interest to disclose.

Contributions

S-LX and W-JG designed the study and edited manuscript. M-JL, LD and LY collected, analyzed the data, and wrote the manuscript. K-WT, S-MH, C-LW, Y-HH, YZ and H-YC collected the data. W-JG and S-LX read and commented on the manuscript. All authors reviewed the manuscript.

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Data-sharing statement

Please email the corresponding authors to obtain original data.

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