A report on Lineage switch at relapse of CD19 CAR-T therapy for Philadelphia chromosome-positive B-precursor acute lymphoblastic leukemia

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To the Editor: This study reported that a case of Philadelphia chromosome-positive B-acute lymphoblastic leukemia (Ph+ B-ALL) underwent a lineage switch to acute myeloid leukemia (AML) following CD19 chimeric antigen receptor (CAR)-modified T (CAR-T) cells therapy. The study was reviewed and approved by the ethics committee of the first affiliated hospital of Nanjing Medical University (No. 2020-02-109). A 46-year-old woman with a 1-month history of chest pain was admitted to our hospital on January 24, 2014. The results of her laboratory examinations were as follows: (i) White blood cell count 12.0 × 10^9/L; hemoglobin 92 g/L; platelet count 47 × 10^9/L; (ii) Bone marrow (BM) smears showed a massive infiltrate (95.2%) of blast cells; (iii) Immunophenotypic analysis by flow cytometry (FCM) revealed that blast cells accounted for 66.3%, which were positive for CD34, CD10, CD19, CD20, CD22, CD38, and human leukocyte antigen DR; (iv) Karyotype analysis showed no mitotic phase; (v) Fluorescence in situ hybridization demonstrated a positive Philadelphia chromosome (BCR-ABL fusion gene); (vi) BM quantitative real-time polymerase chain reaction (qRT-PCR) detected a positive BCR-ABL p190 transcript (BCR-ABL, 184.1%). She was thus diagnosed with Ph+ B-ALL.

The patient was treated with standard B-ALL induction chemotherapy with rituximab, vincristine, daunorubicin, cyclophosphamide, and prednisone, combined with oral administration of dasatinib (100 mg/day) since January 27, 2014. BM aspiration indicated that complete remission (CR) and the BCR-ABL fusion gene was negative after the first chemotherapy cycle. Consolidation regimen with administering cyclophosphamide, cytarabine, and 6-mercaptopurine were adopted since March 20, 2014. Subsequent six cycles of consolidation therapy were given between April and November 2014. Then, the patient was given a maintenance treatment of oral methotrexate (7.5 mg/week) and 6-mercaptopurine (2.5 mg/day).

Unfortunately, on March 21, 2016, BM and FCM suggested that the patient had a relapse of ALL, and CD19-directed CAR-T therapy was introduced. Then the patient was treated with rituximab, cyclophosphamide, vincristine, and prednisone regimen to reduce tumor burden. BM showed CR on April 7, 2016. The lymphodepleting chemotherapy with FC regimen (fludarabine 37 mg, days 1–3; cyclophosphamide 370 mg, days 1–3) was started from April 8, 2016. A total of 3 × 10^6 engineered CD19-directed CAR-T cells were administered on April 13, 2016. No other immediate infusion-related toxic effect was observed except for the low fever. After anti-infection treatment, the patient’s body temperature quickly dropped to normal and she was discharged half a month later. Within 3 years after CD19-directed CAR-T cells infusion, BM examination performed during that period showed no relapse.

In May 2019, the patient presented at the hospital with fatigue. BM aspirate indicated 88% myeloid progenitors. A normal karyotype was revealed, and a negative BCR-ABL transcript was detected by qRT-PCR. FCM demonstrated that abnormal myeloblasts expressed CD34, CD13, CD33, CD38, CD117, CD15, and human leukocyte antigen DR. Next-generation sequencing showed FLT3-ITD (p.F590delinsLELGSSDNEYF; variant allele fraction 21.99%) mutation, and paired box gene 5 (PAX5) single nucleotide polymorphism (SNP) (p.T264I; variant allele fraction 99.94%). Accordingly, the patient was diagnosed with secondary AML. From May 22, 2019, decitabine in combination with low-dose cytarabine, aclarubicin, and granulocyte colony-stimulating factor (DCAG) was administered to this patient as induction chemotherapy. Subsequent four cycles of DCAG consolidation therapy were...
performed in July, August, October, and November 2019. BM examination showed sustained CR. FCM indicated no minimal residual disease, and neither FLT3-ITD mutation nor PAX5 SNP was detected by next-generation sequencing (the last detection was in November 2019). The patient is now still in the follow-up period.

According to the literature, lineage switch after CAR-T-cell infusion has been described in four cases of B-ALL, including three patients with mixed lineage leukemia (MLL) rearrangement,[1,2] and one pediatric patient with TCF3-ZNF384 fusion.[3] In addition, lineage switch was also reported in murine models bearing E2A-PBX1 leukemia.[4] Table 1 summarizes the characteristics of historically reported cases as well as our patient. It was found that phenotype transformation often occurs in childhood leukemia with MLL rearrangement. The five patients (literature reported and ours) developed lineage switch at 1, 1, 36, 16, and 36 months after CAR-T therapy, respectively. All three patients with MLL rearrangement had severe or mild cytokine release syndrome with high serum concentrations of IL-6 and other cytokines after CAR-T infusion. Regarding the management strategy after lineage transformation, three cases were treated with AML induction regimen, among which one received hematopoietic stem-cell transplantation and one with unclear treatment. However, the prognosis of the reported cases was extremely poor and they died shortly after conversion. After transforming into AML, our patient achieved CR in one course of DCAG regimen chemotherapy treatment. The gene mutation disappeared after four courses of chemotherapy, and the patient is still in CR. Lineage switch is a special type of post-CAR relapses, and several possible mechanisms have been put forward to explain that after CD19-directed treatment, but the exact

### Table 1: Reported cases of myeloid lineage switch following CAR-T therapy in B-ALL.

| References | Age/sex | CRS | Switch time | Initial diagnosis | AML relapse |
|------------|---------|-----|-------------|-------------------|------------|
|            |         |     |             | FCM               | Karyotype  | FISH          | FCM               | Karyotype  | FISH          |
| 1          | 52 y/F  | Severe | 35 d       | Abnormal lymphoblasts expressing CD45, CD19, CD22, CD38, HLA-DR, CD15, CD33, CD13, and TdT | MLL rearranged |             | Abnormal monocytic population expressing, CD13, CD64, HLA-DR, CD15, CD33, CD71, and MPO. No abnormal B cells |             | MLL rearranged |
| 1          | 18 m/F  | Severe | 30 d       | Abnormal lymphoblasts expressing CD19, CD38, CD22, HLA-DR, CD34, and CD45 | MLL rearranged | 46, XX, ins (11;10) (q23; p12) | 46, XX, ins (11;10) (q23; p12) | MLL rearranged |
| 2          | 5 y/M   | Mild  | 3 y        | Expression of CD19, CD20, CD34, variable TdT, and weak variable CD2 | MLL rearranged | 46, XY, del (1) t (1;1) (p36.1q25), t (13;16) (q14; q25) | Positive for CD4, CD7, CD11b, CD13, CD33, CD38, CD56, CD58, CD64, CD71, CD117, partial CD123, and HLA-DR | Not performed | MLL rearranged |
| 3          | 13 m/M  | No    | 16 m       | Positive for CD19, CD20, CD22, CD34, CD38, CD13, CD33, and HLA-DR; negative for CD10, MPO, and TdT | MLL rearranged | 46, XY, del (12) (p13/46, XY) | TCF3-ZNF384 | Positive for CD13, CD33, CD34, CD117, CD123, CD11b, CD38, and CD7; negative for CD19, CD10, CD20, CD24, MPO, TdT, and CD22 | 46, XY | TCF3-ZNF384 |
| Our case   | 46 y/F  | No    | 3 y        | Blast cells accounted for 66.3%, positive for CD34, CD10, CD19, CD20, CD22, CD38, and HLA-DR | BCR-ABL | No mitotic phase | Abnormal myeloblasts expressing CD34, CD113, CD33, CD38, CD117, CD15, HLA-DR | 46, XX | Negative |

CAR-T: Chimeric antigen receptor-modified T cells; B-ALL: B-cell acute lymphoblastic leukemia; CRS: Cytokine release syndrome; FCM: Flow cytometry; FISH: Fluorescence in situ hybridization; AML: Acute myeloid leukemia; MLL: Mixed lineage leukemia; MPO: Myeloperoxidase; HLA-DR: Human leukocyte antigen DR; BCR-ABL: Philadelphia chromosome; d: Days; F: Female; M: Male; m: Months; Switch time: Time from CAR-T-cell infusion to lineage switch; TdT: Terminal deoxynucleotidyl transferase; y: Years; ZNF384: Zinc-finger protein 384.
mechanism remains unclear. Using murine models, Jacoby et al. supported that lineage switch depends on the genetic oncogenic driver. It was indicated that the absence of CD19 antigen epitope alone cannot drive the lineage conversion, whereas the deletion of PAX5 (important B-cell regulatory transcription factors) is associated with the myeloid lineage switch of B-ALL. One of its crucial function is to repress FLT3 (a potential regulator of hematopoietic stem cells) transcription in B-cell progenitors. Besides, PAX5 can up-regulate B-cell-related genes, which is necessary for B-cell commitment and can inhibit myeloid cell proliferation by preventing the response of B cells to myeloid growth factors. In addition, Maeda et al. found that CD 19⁺ B cells and more primitive B-lymphoid progenitors were more likely to be lost, with the increase of IL-6 level. IL-6 can promote uncommitted progenitor cells to express Id1 transcription factor, which can inhibit lymphocyte proliferation and improve BM hematopoiesis. All three patients in Table 1 with MLL rearrangement presented with severe or mild cytokine release syndrome showed high serum concentrations of IL-6 and other cytokines after CAR-T cells infusion, further suggesting that cytokines may play a vital role in lineage switch. Our case described here was special in that FLT3-ITD mutation and PAX5 SNP during transformation was detected, which may be one of the reasons for her transformation.

In the present case, the interval between exposure to multidrug chemotherapy including cyclophosphamide and AML relapse was 5 years and 4 months. Thus, the possibility cannot be ruled out that AML was secondary to chemotherapy drugs. Exogenous factors, such as chemotherapy, or endogenous factors, such as acquired chromosome abnormalities, can change the differentiation process of leukemic cells, resulting in phenotypic transformation during relapse. On the one hand, chemotherapy seems to eliminate the evident leukemia clones at diagnosis, resulting in the amplification of subclones for different phenotypes. On the other hand, drug-induced changes in the original clone may amplify or inhibit the differentiation process, thus, the phenotypic switch is possible.

In conclusion, lineage switch after CD19-directed therapy is a specific mechanism of CAR resistance, with leukemic cells switching from one cell line to another after complete phenotypic alteration, and usually concerns patients with MLL rearrangement. Although there are some hypotheses, the clear mechanism of lineage transformation is still unknown. Further studies are required to design optimal treatment and elucidate the mechanism of lineage switch.

Declarations of interest

None.

References

1. Gardner R, Wu D, Cherian S, Fang M, Hanafi LA, Finney O, 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;127:2406–2410. doi: 10.1182/blood-2015-08-665547.
2. Lucero OM, Parker K, Funk T, Dunlap J, Press R, Gardner RA, et al. Phenotype switch in acute lymphoblastic leukaemia associated with 3 years of persistent CAR T cell-directed-CD19 selective pressure. Br J Haematol 2019;186:333–362. doi: 10.1111/bjh.15812.
3. Oberley MJ, Gaynon PS, Bhownani D, Pulsipher MA, Gardner RA, Hiemenz MC, et al. Myeloid lineage switch following chimeric antigen receptor T-cell therapy in a patient with TCF3-ZNF384 fusion-positive B-lymphoblastic leukemia. Pediatr Blood Cancer 2018;65:e272659. doi: 10.1002/pbc.272659.
4. Jacoby E, Nguyen SM, Fountaine TJ, Welp K, Gryder B, Qin H, et al. CD19 CAR immune pressure induces B-precursor acute lymphoblastic leukemia lineage switch exposing inherent leukemic plasticity. Nat Commun 2016;7:12320. doi: 10.1038/ncomms12320.
5. Maeda K, Malykhin A, Teague-Weber BN, Sun XH, Farris AD, Coggleshall KM. Interleukin-6 aborts lymphopoiesis and elevates production of myeloid cells in systemic lupus erythematosus-prone B6 Sle1icya animals. Blood 2009;113:4534–4540. doi: 10.1182/blood-2008-12-192559.

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