# Myeloid lineage switch following CD7-targeted chimeric antigen receptor T-cell therapy in relapsed/refractory T-cell acute lymphoblastic leukemia

Myeloid lineage switch is a rare phenomenon in relapsed B-cell acute lymphoblastic leukemia B-ALL, predominantly arising in cases harboring the *KMT2A* rearrangement and treated with CD19-targeted immunotherapies including blinatumomab and CD19-targeted chimeric antigen receptor (CAR) T-cell therapy,[1,2] with an incidence of 3% following CD19CAR T-cell therapy in one study.[3] However, myeloid lineage switch has only very rarely been observed in T-cell ALL (T-ALL),[4,5] including one case with *KMT2A* rearrangment. Nevertheless, the extension of antigen-based targeted immunotherapy to T-ALL may increase the prevalence of this phenomenon as a potential escape mechanism.

There has been a growing interest in developing CD7-targeting (CD7) CAR T-cell therapy for relapsed/refractory (r/r) T-ALL as CD7 is expressed in nearly all cases, and early clinical data for CD7CAR T-cell therapy is promising.[6,7] The WU-007 CAR T-cell platform is a novel allogeneic off-the-shelf product manufactured from T cells collected from healthy donors. Allogeneic T cells are genetically edited to delete the CD7 antigen and T-cell receptor $\alpha$ chain (TRAC) to prevent fratricide and graft-*versus*-host disease (GvHD), respectively, and modified cells are subsequently transduced with a CD7CAR.[8] Preclinical data supporting the *in vitro* expansion, antitumor activity and lack of GvHD of WU-007 CAR T cells[8] led to the design of an ongoing international multicenter phase I dose escalation study testing WU-007 in patients with r/r T-ALL (*clinicaltrials gov. Identifier: NCT04984356*), for which our institution is a participating site. Here, we report an unusual case of r/r T-ALL that responded to WU-007 CAR T cells administered at dose level 2 ($300x10^6$ CAR T cells), but subsequently relapsed as an acute myeloid leukemia (AML) (myeloid lineage switch) with no evidence of recurrent T-ALL.

The patient was a 30-year-old male diagnosed with T-ALL with a normal karyotype. *Online Supplementary Figure S1* depicts key time points in this case. He was treated according to the AALL0434 regimen with nelarabine consolidation and achieved complete remission (CR) with negative minimal residual disease (MRD-negative). However, he experienced early hematological relapse during interim maintenance therapy and then received a salvage regimen of nelarabine, cyclophosphamide and etoposide that led to a CR but persistent MRD. A 5-day course of nelarabine was administered as a consolidation therapy while a fully matched unrelated donor was being activated. Unfortunately, he had evidence of frank hematological relapse with increased circulating blasts in the peripheral blood (PB) 4 weeks later. He was enrolled on the CD7CAR T-cell study (*clinicaltrials gov. Identifier: NCT04984356*) and received cytoreduction with dexamethasone while undergoing screening studies. Pre-CAR bone marrow (BM) biopsy showed hypercellular marrow with 68% lymphoblasts (Figure 1A). The immunophenotype of the lymphoblasts (Figures 2A) showed positivity for CD1a, CD2, cytoplasmic CD3 (cCD3), CD4 (minor subset), CD5 (minor subset), CD7, CD33 (dim), CD34, CD38, CD45 (moderate), and HLA-DR (heterogenous). Cytogenetics showed a complex karyotype. Next generation sequencing (NGS) of the BM aspirate revealed mutations in *TP53, PHF6*, and *RUNX1* as well as high RNA expression of *CDK6* and *IL7R* amplification (Table 1). He received lymphodepletion with cyclophosphamide and fludarabine, but white blood cell count and blasts in the PB continued to rise after completing lymphodepletion. WU-007 CAR T cells were infused intravenously. He experienced grade 1 cytokine release syndrome on day 1 following infusion that progressed to grade 2 and resolved with administration of tocilizumab and dexamethasone. Exploratory day 7 BM biopsy showed persistent T-ALL (90%) in a hypercellular marrow; T lymphoblasts expressed cCD3, CD2, CD4 (dim, small subset), CD5 (dim, subset), CD7, CD34, CD45 (dim to moderate) and CD123 (moderate, increased). Cytogenetics showed persistent complex karyotype and fluorescence *in situ* hybridization (FISH) revealed *TP53* deletion (Table 1). On day 10 post CAR T cells, he developed pancytopenia and had increased lactate dehydrogenase (LDH), triglyceride, bilirubin, and ferritin as well as decreased fibrinogen levels. These findings were supportive of macrophage activation syndrome, which was successfully treated with dexamethasone and anakinra.

Day 28 post infusion BM biopsy showed hypocellular marrow with >80% abnormal immature T-cell population. This abnormal T-cell population expressed CD2, cCD3, and CD4, but had lost expression of CD7 by flow cytometry (Figure 2B). Touch imprint showed that atypical cells were mainly blastoid with high nuclear-to-cytoplasmic ratio, multiple prominent nucleoli, and very scant blue and non-granular cytoplasm, some morphologically indistinguishable from the original T lymphoblasts (Figure 1B, C). Flow cytometry for MRD obtained from the marrow identified

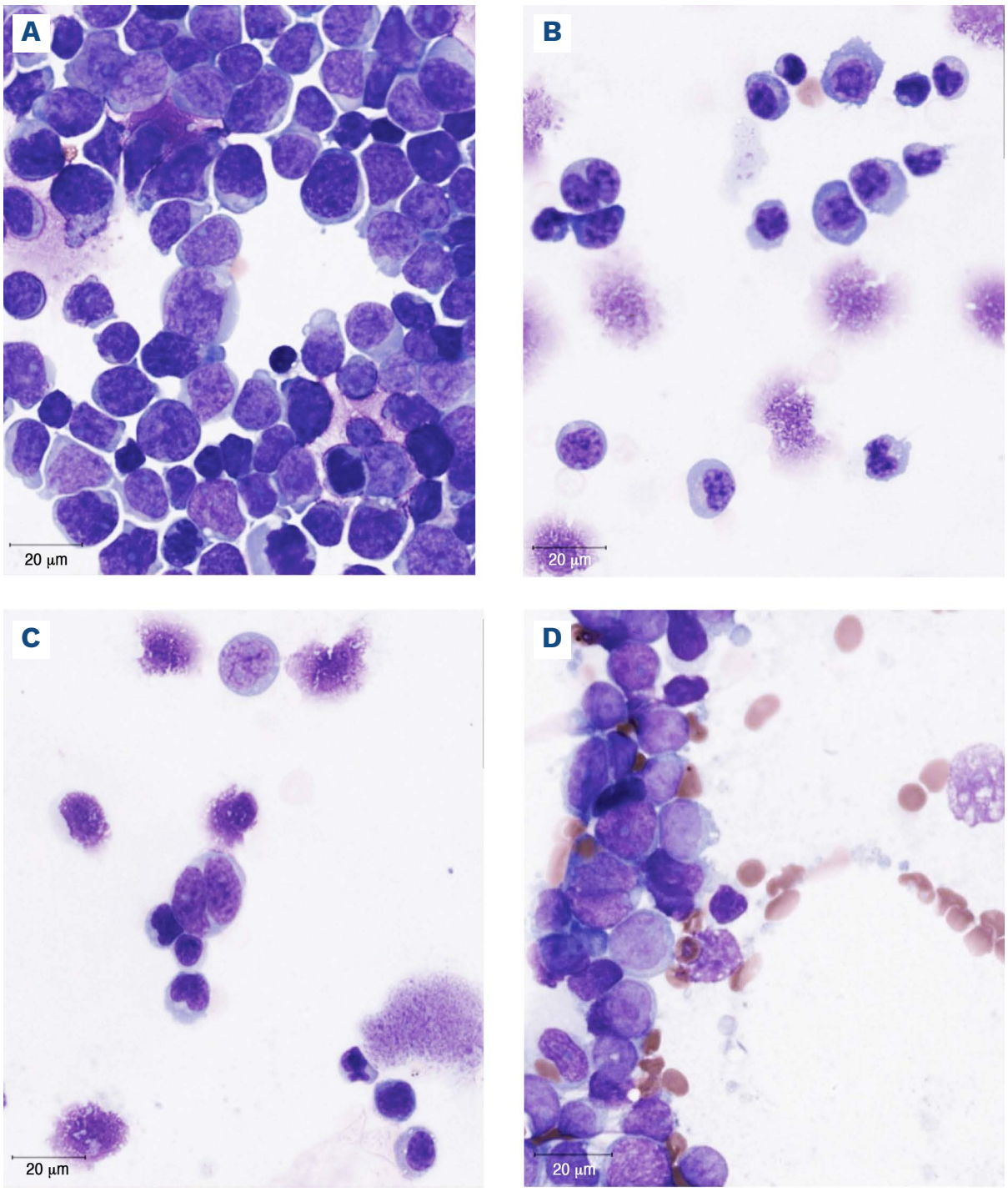


**Figure 1. Cell morphology at different time points.** (A) Morphology of blasts pre-chimeric antigen receptor (CAR) T cells. Large-sized blasts with high nuclear/cytpoplasmic (N/C) ratio, finely dispersed to somewhat coarse chromatin with occasional nuclei, and scant lightly basophilic agranular cytoplasm. (B) Morphology of findings post CAR T cells day 28. Pleomorphic cells with variable size double nucleated and irregular nuclear border and cytoplasmic spike. (C) Morphology of findings post CAR T cells day 28. Large and atypical cells with enlarged nuclei and irregular nuclear contours, predominantly with ample amount of cytoplasm. Cells with bizarre nuclear shapes "Reed-Sternberg-like morphology", depicted in the picture. (D) Morphology of blasts at myeloid linage relapse. Medium-sized blasts with a high N/C ratio, round to irregular nuclear contours, fine chromatin, inconspicuous nucleoli, and scant basophilic cytoplasm.

two T-cell populations, both $CD7^-$. The first population represented 70% of mononuclear cells and had decreased surface CD3 (sCD3), but with normal expression of CD2, cCD3, CD4, CD5, CD38, CD45 and CD48, and absence of CD7, CD16, CD34 and CD56. The second population represented 6% of mononuclear cells and had CD4 predominance (CD4:CD8 ratio of approximately 37) with normal expression of CD2, sCD3, cCD3, CD4, CD5, CD38, CD45 and CD48, and absence of CD7, CD16, CD34 and CD56. These two unusual populations likely represented a combination of CAR T cells and reactive T cells, and there was no definitive immunophenotypic evidence of residual T-cell ALL (Figure 2B). Cytogenetics showed normal karyotype in five metaphases and polymerase chain reaction for *TP53* mutation was negative (Table 1). The patient was deemed to have achieved MRD-negative morphological leukemia-free state but continued to have marked pancytopenia.

During the time in which the patient's donor was activated in preparation for transplant, the patient's LDH levels began to rise, and therefore, we repeated the BM biopsy on day 35 post CAR T-cell infusion. The results of this biopsy showed a hypercellular bone marrow with 80% involvement by myeloid blasts (Figure 1D). Flow cytometry demonstrated an abnormal blast population expressing CD45 (moderate intensity), CD4 (subset), CD13 (weak intensity, subset), CD33 (weak intensity), CD34 (increased), CD64 (weak intensity), CD123 (moderate intensity), HLA-DR and MPO that comprised 82% of total cells analyzed (Figure 2C). The blast population was negative for CD1a, CD2, sCD3, cCD3, CD5, CD7, CD8, CD10, CD14, CD16, cCD22, CD24, CD117, TCR$\alpha/\beta$, TCR$\gamma/\delta$ and TdT (Table 1). NGS analysis demonstrated identical identified mutations in *TP53*, *RUNX1* and *PHF6* in addition to amplification of *PIK3CD* and *BTG1* loss (Table 1). He was treated with salvage of venetoclax and azacitidine, but unfortunately, treatment was complicated by an invasive fungal infection, and he did not respond.

In this case, the r/r T-ALL initially responded to CD7CAR T-cell therapy and the patient achieved MRD-negative morphological leukemia-free state on day 28 post infu-

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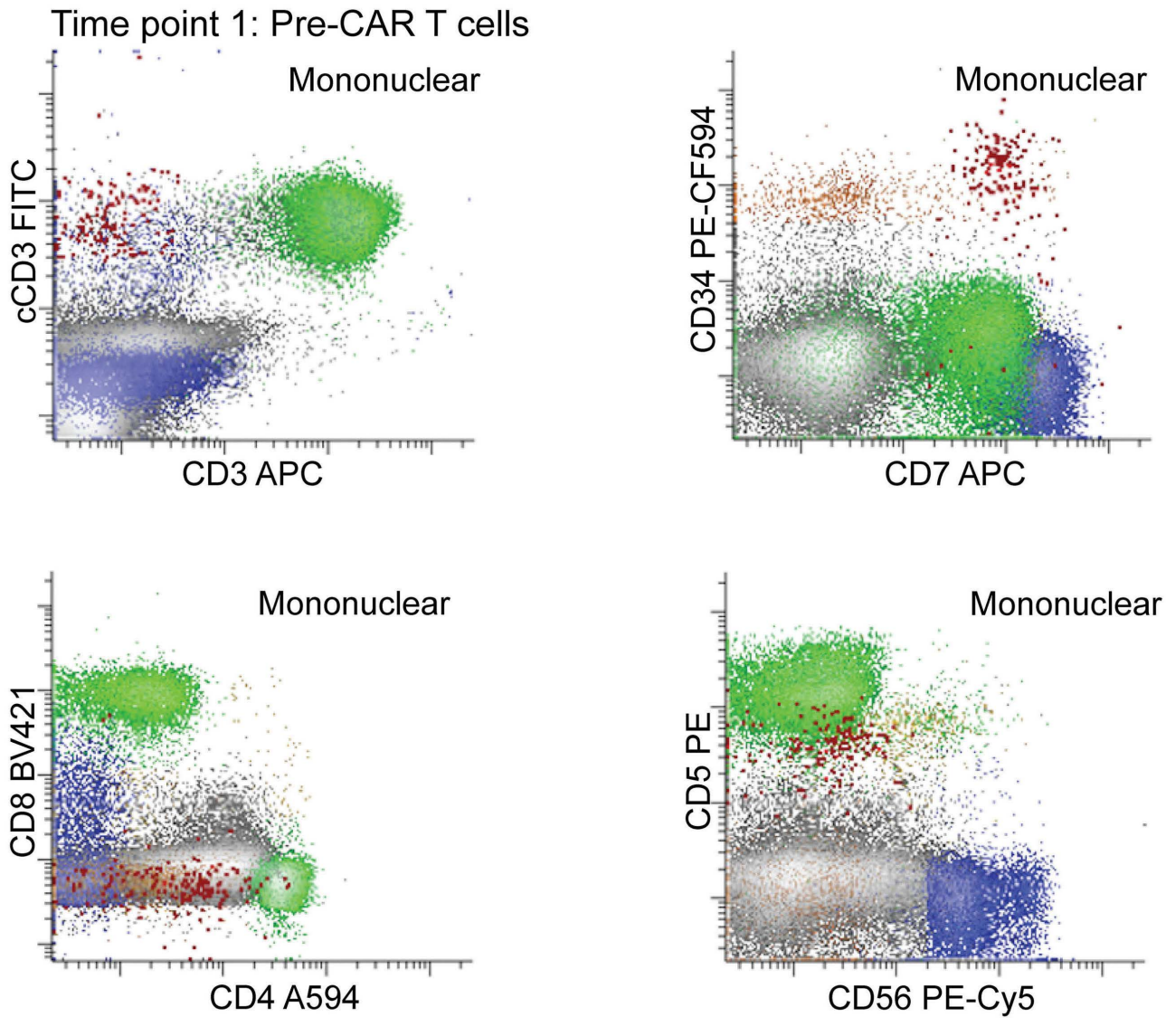


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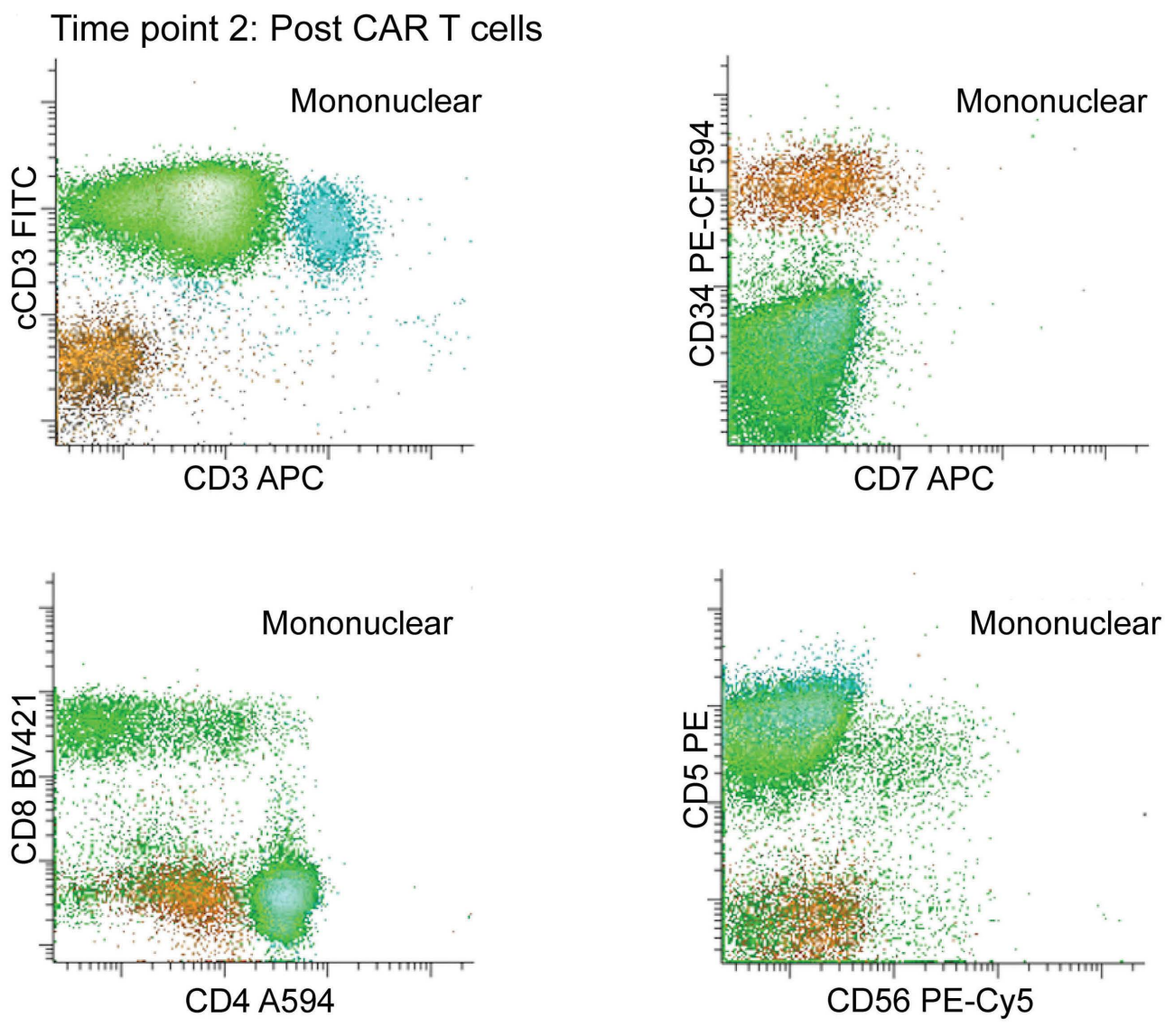


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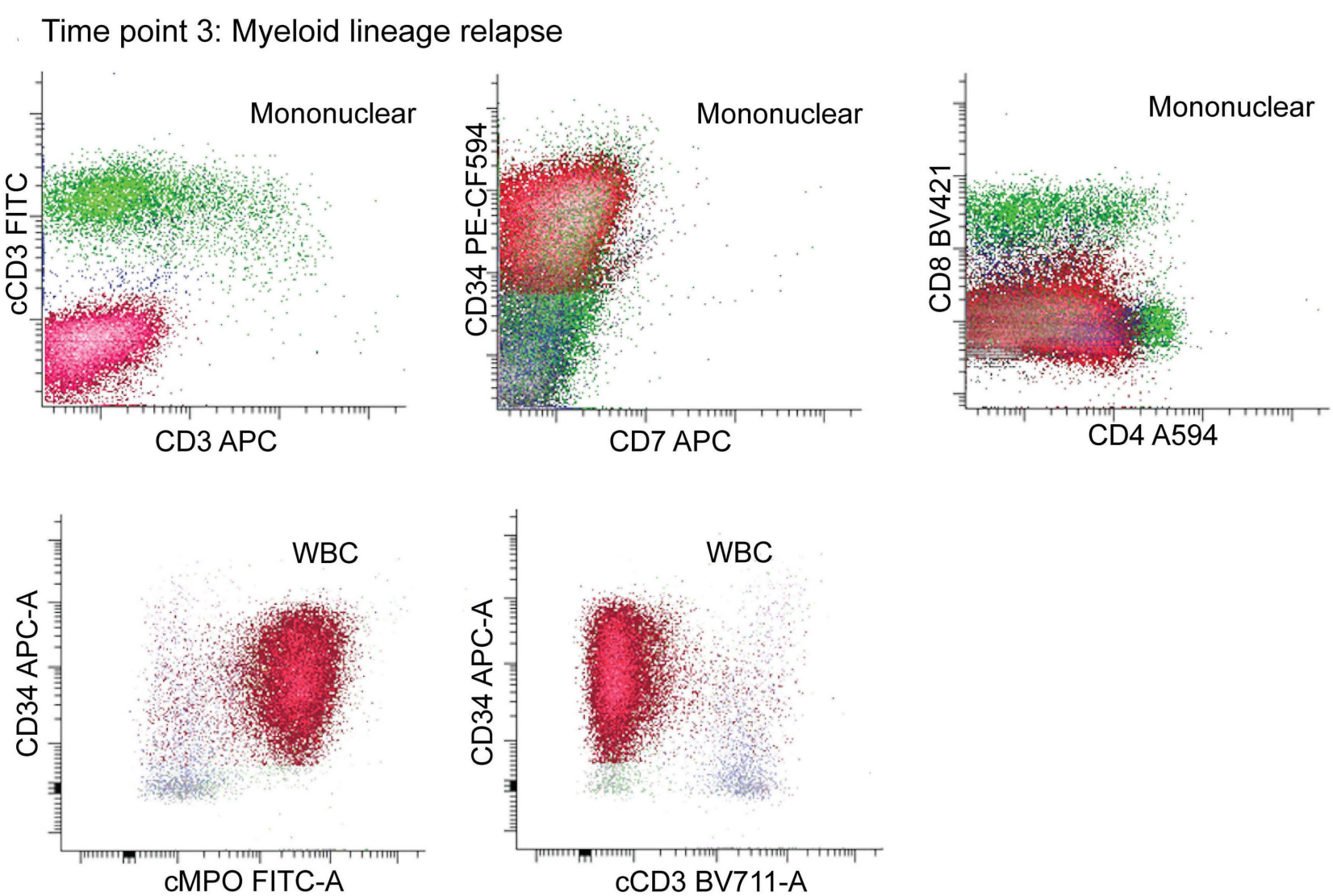


**Figure 2. Phenotype of blast populations over time by flow cytometry.** (A) Pre-CD7 chimeric antigen receptor (pre-CD7CAR) T cells. All plots show mononuclear cells. Abnormal blasts (0.03% of the white blood cells [WBC]) are highlighted in red and express cytoplasmic CD3, CD7, and CD34 without surface CD3. Normal T cells are shown in green and natural killer cells (NK) cells are shown in blue. $CD34^+$ myeloid blast are shown in orange. (B) Post CD7CAR T cells. All plots show mononuclear cells. No $CD7^+/CD34^+$ abnormal blast population is present. T cells are shown in green (surface $CD3^-$, CD4:CD8 ratio of approximately 8:1) and aqua (surface $CD3^+$, almost all $CD4^+$) and lack express of CD7. Essentially no NK cells are present. CD34 blasts are shown in orange, comprise approximately 5% of the WBC and lack expression of all T-cell antigens. At the time of initial evaluation, these were favored to represent early regeneration. (C) Myeloid lineage relapse. The specimen consists largely of $CD34^+$ blasts (red, approximately 75% of the WBC) which express cMPO without cCD3 or CD7. T cells (green) still include a major subset lacking surface CD3 and have a CD4:CD8 ratio of approximately 1:0.6). NK cells remain rare.

sion, but the disease relapsed shortly thereafter and manifested as AML. The shared mutational profile between the original T-ALL and the emergent AML post CD7CAR T-cell therapy supported the notion that the AML originated from the same T-ALL clone as a lineage switch. While CAR T cells effectively eliminated $CD7^+$ T-ALL clones, relapse with lineage switch to myeloid occurred, potentially as an escape mechanism under the pressure of targeting CD7. We postulate that the lineage switch could be the result of either a pre-existing small population of myeloid clones at the time of treatment initiation or due to transformation of early leukemic clones that were less committed to the lymphoid lineage. Indeed, a recent report provides evidence of a significant incidence of myeloid mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) in NGS performed on both B- and T-ALL samples.[9] The allogeneic nature of WU-007 CD7CAR T cells raises concern that the development of AML in this case could originate from donor cells as donor-derived leukemia. However, the similarity of the mutational profile for both leukemias makes this scenario unlikely.

While myeloid lineage switch has been reported in patients with B-ALL with *KMT2A* rearrangement treated with CD19-targeted immunotherapies,[1,2] the occurrence in T-ALL is very rare with only a small number of reported cases.[4,5] Whereas the loss of CD7 antigen upon relapse following CD7CAR T-cell therapy was observed in other studies utilizing different CAR T-cell platforms, there have been no reports on myeloid lineage switch with CD7CAR T-cell therapy.[6,7] As innovative immunotherapeutics are being extended to T-ALL, the phenomenon of lineage switch may become more prevalent and necessitate additional studies to comprehend which patients are at risk and to introduce novel approaches to avert these outcomes.

Our case highlights the potential activity of CD7CAR T-cell

therapy with the WU-007 platform, with the study currently in progress and the dose of WU-007 being escalated per the study design. Nonetheless, our experience exemplifies some challenges and a potential morphologic pitfall in differentiating residual T-ALL from modified CAR product and reactive T cells post treatment as we encountered on day 28 post infusion. The cumulative findings such as extended immunophenotype and molecular results supported disease response in this case before lineage switch.

**Table 1.** Leukemia phenotype, cytogenetics and molecular findings at various time points.

| | Immunophenotype | Cytogenetics/FISH | Mutations and gene expression |
|---|---|---|---|
| Diagnosis | $CD2^+$, $sCD3^-$, $cCD3^+$, $CD4^-$, $dimCD5^+$, $CD7^+$, $CD8^-$, $CD10^-$, $CD11b^-$, $CD13^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD20^-$, $CD23^-$, $CD30^-$, $CD33^-$, $CD34^+$, $CD38^+$, $CD56^-$, $CD64^-$, $CD117^-$, HLA-$DR^+$ (24%), $MPO^-$, $TdT^+$, $CD123^+$ | 46,XY; FISH showed tetrasomy of chrom 6 and 21 in 8.5%, and 1-2 extra signals/copies on chrom 8, 9, 11, and 22 in 5-13.5% | ND |
| 1st relapse | $CD1a^-$, $CD2^+$ (partial dim), $sCD3^-$, $CD4^-$, $CD5^+$ (predominantly), $CD7^+$, $CD8^-$, $CD10^-$, $CD19^-$, $CD33^+$, $CD34^+$, $CD38^+$, $CD45^+$ (dim) | 85-90<4n>XXYY,add(6)(p23)x2, add(8)(p21)x12, der(11)t(11;14)(p13;q11.2)x2, -2,del(17)(p11.2)x12,+58mar, inc[cp4]; FISH showed 17p deletion | ND |
| Pre CAR T- cell infusion | $CD1a^+$, $CD2^+$, $sCD3^-$, $cCD3^+$, $CD4^+$ (minor subset), $CD5^+$ (minor subset), $CD7^+$, $CD8^-$, $CD33^+$ (dim), $CD34^+$, $CD38^+$, $CD45^+$ (moderate), HLA-DR (heterogenous), $TdT^-$. (65% of total analyzed cells). On concurrent peripheral blood flow CD123 dim, partial expression is seen on 71% of total analyzed cells | 45,XY,add(8)(p?21),add(9)(p?21), der(11)t(11;14)(p1?3;q11.2), -4,del(16)(q22),del(17)(p11.2)[4]; FISH studies 17p deletion | *TP53, RUNX1* and *PHF6* mutations; *CDK6* elevated expression and *IL7R* amplification |
| Day 7 post CAR T-cell infusion | $CD1a^-$, $CD2^+$, $sCD3^-$, $cCD3^+$, $CD4^+$ (dim, small subset), $CD5^+$ (dim, subset), $CD7^+$, $CD8^-$, $CD15^-$, $CD34^+$, $CD45^+$, $CD56^-$, $CD64^-$, $CD123^+$ (95% of total analyzed cells) | 75~94<4n>,XY,-X,-Y,add(1)(q44), 4,-4,-5,-5,add(6)(p23)x2,add(8)(p?21)x2,+9,add(9)(p?21)x2, der(11)t(11;14)(p1?3;q11.2)x2, -12,-12,-14,-14,+del(16)(q22), +17,del(17)(p11.2)x2, -22,+1~5mar[cp16] | ND |
| Day 28 post CAR T-cell infusion | No definite abnormal T-lymphoid blast population. T cells present showed phenotype of: $CD1a^-$, $CD2^+$, $cCD3^+$, $CD4^+$, $CD7^-$, $CD8^-$, $CD45^+$, $TdT^-$ (86.6% of total analyzed cells) | 46,XY [5] | *TP53* mutation was negative by PCR |
| Day 35 post CAR T-cell infusion | $CD1a^-$, $CD2^-$, $sCD3^-$, $cCD3^-$, $CD4^+$ (minor subset), $CD5^-$, $CD7^-$, $TdT^-$, $CD34^+$, $MPO^+$ (70% of the total analyzed cells) | 45,XY,add(8)(p?21),add(9)(p?21), t(11;14)(p1?3;q11.2), del(17)(p11.2),-19[16] | *TP53, RUNX1*, and *PHF6* mutations; *BTG1* loss and *PIK3CD* amplification; PCR for *TP53* mutation was positive at 50% |
| Day 38 post CAR T-cell infusion | $CD1a^-$, $CD2^-$, $sCD3^-$, $cCD3^-$, $CD4^+$ (subset), $CD5^-$, $CD7^-$, $CD8^-$, $CD10^-$, $CD13^+$, $CD14^-$, $CD16^-$, cCD22-, $CD24^-$, $CD33^+$ (weakly), $CD34^+$, $CD117^-$, $CD123^+$, $TdT^-$, HLA-$DR^+$, $MPO^+$ | ND | ND |

FISH: fluorescence *in situ* hybridization; ND: not done; PCR: polymerase chain reaction; chrom: chromosome.

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**Contributions**

IA and MA designed the study, collected data, performed analyses and wrote the paper. IA, PT, DB, JM, JYS, SC, RP, YK, KG, KJ, JD, SJF, HW and MA collected and analyzed data. PT, DS, JYS, SC, RP, YK, NM, MJ, HW and MA reviewed bone marrow morphology, cytogenetics and molecular findings. IA and JKM treated the study patient. MCC wrote the paper. All authors reviewed the final draft of the paper.

**Data-sharing statement**

For original data, please contact ialdoss@coh.org

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