ORIGINAL ARTICLE

Sequential CD7 CAR T-Cell Therapy and Allogeneic HSCT without GVHD Prophylaxis

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ABSTRACT

BACKGROUND

Patients with relapsed or refractory hematologic cancers have a poor prognosis. Chimeric antigen receptor (CAR) T-cell therapy as a bridge to allogeneic hematopoietic stem-cell transplantation (HSCT) has the potential for long-term tumor elimination. However, pre-HSCT myeloablation and graft-versus-host disease (GVHD) prophylaxis agents have toxic effects and could eradicate residual CAR T cells and compromise antitumor effects. Whether the integration of CAR T-cell therapy and allogeneic HSCT can preserve CAR T-cell function and improve tumor control is unclear.

METHODS

We tested a novel "all-in-one" strategy consisting of sequential CD7 CAR T-cell therapy and haploidentical HSCT in 10 patients with relapsed or refractory CD7-positive leukemia or lymphoma. After CAR T-cell therapy led to complete remission with incomplete hematologic recovery, patients received haploidentical HSCT without pharmacologic myeloablation or GVHD prophylaxis drugs. Toxic effects and efficacy were closely monitored.

RESULTS

After CAR T-cell therapy, all 10 patients had complete remission with incomplete hematologic recovery and grade 4 pancytopenia. After haploidentical HSCT, 1 patient died on day 13 of septic shock and encephalitis, 8 patients had full donor chimerism, and 1 patient had autologous hematopoiesis. Three patients had grade 2 HSCT-associated acute GVHD. The median follow-up was 15.1 months (range, 3.1 to 24.0) after CAR T-cell therapy. Six patients remained in minimal residual disease–negative complete remission, 2 had a relapse of CD7-negative leukemia, and 1 died of septic shock at 3.7 months. The estimated 1-year overall survival was 68% (95% confidence interval [CI], 43 to 100), and the estimated 1-year disease-free survival was 54% (95% CI, 29 to 100).

CONCLUSIONS

Our findings suggest that sequential CD7 CAR T-cell therapy and haploidentical HSCT is safe and effective, with remission and serious but reversible adverse events. This strategy offers a feasible approach for patients with CD7-positive tumors who are ineligible for conventional allogeneic HSCT. (Funded by the National Natural Science Foundation of China and the Key Project of Science and Technology Department of Zhejiang Province; ClinicalTrials.gov numbers, NCT04599556 and NCT04538599.)

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N Engl J Med 2024;390:1467-80.
DOI: 10.1056/NEJMoa2313812

Patients with relapsed or refractory hematologic cancers have limited therapeutic options and a poor prognosis, with a 5-year overall survival of less than 20%.[1-3] Although allogeneic hematopoietic stem-cell transplantation (HSCT) provides a pivotal strategy to treat aggressive hematologic cancers, its application is impeded by complications including graft-versus-host disease (GVHD), conditioning-associated toxic effects, and severe immunosuppression after long-lasting anti-GVHD treatment.[4] In addition, patients who have severe physiological issues or are in poor health are often ineligible for allogeneic HSCT, and those without prerequisite complete-remission status can receive only salvage allogeneic HSCT, which is associated with an increased incidence of relapse.[5] Additional therapeutic options are needed.

Chimeric antigen receptor (CAR) T-cell therapy has been an exciting breakthrough against hematologic cancers.[6] We and others have shown the clinical safety and efficacy of allogeneic CD7 CAR T cells that target CD7-positive cancers, including T-cell acute lymphoblastic leukemia (ALL), T-cell lymphoblastic lymphoma, and some acute myeloid leukemias (AMLs) with CD7 expression.[7-10] Allogeneic HSCT has been evaluated in multiple clinical studies as consolidation treatment after CAR T-cell therapy to maintain long-term tumor elimination and reduce the risk of relapse.[11-13] Nevertheless, such "bridging" approaches incorporate pre-HSCT conditioning chemotherapy and GVHD prophylaxis agents, which eliminate residual CAR T cells and pose risks of severe toxic effects,[14] thereby potentially compromising therapeutic outcomes.

We endeavored to address these challenges by developing a novel "all-in-one" strategy of sequential CD7 CAR T-cell therapy and haploidentical HSCT without pharmacologic myeloablation or GVHD prophylaxis (Fig. 1A). This case series describes 10 patients with relapsed or refractory CD7-positive cancers treated by this strategy.

Methods

Patients and Study Design

Ten patients with relapsed or refractory CD7-positive cancers were enrolled in one of two prospective clinical studies from November 2021 through September 2023. Nine patients were enrolled in a clinical study of donor-derived CD7 CAR T cells (ClinicalTrials.gov number, NCT04599556) (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org); because of limitations in the manufacturing of donor-derived CAR T cells due to the coronavirus disease 2019 pandemic, one patient was consequently enrolled in a compassionate-use program that provided access to universal CD7 CAR T cells after the end of the clinical study (NCT04538599). Patients who had complete remission with incomplete hematologic recovery and severe bone marrow hypocellularity or pancytopenia after CD7 CAR T-cell therapy, had detectable CD7 CAR T cells, and did not have a history of allogeneic HSCT were deemed to be eligible for the "all-in-one" strategy (Fig. 1B).

The design and manufacture of CAR T cells are described in the Supplementary Methods section and Fig. S2, as previously reported.[7,10] An intensified lymphodepleting regimen consisting of fludarabine (30 mg per square meter of body-surface area), cyclophosphamide (300 mg per square meter), and etoposide (100 mg) was administered for 5 consecutive days to all the patients, followed by infusion of CD7 CAR T cells at a dose of 2×10^6 cells per kilogram of body weight (haploidentical CD7 CAR T cells, nine patients) or 5×10^6 cells per kilogram (universal CD7 CAR T cells, one patient) on day 0. Allogeneic HSCT was used as a salvage therapy for pancytopenia after CAR T-cell therapy in Patient 1 and as prophylactic therapy in the remaining patients. No additional pharmacologic pre-HSCT conditioning regimens or GVHD prophylaxis drugs were used, with reliance instead on the immunosuppressive effects of CD7 CAR T cells and lymphodepletion before CAR T-cell therapy. For the nine patients receiving haploidentical CD7 CAR T cells, hematopoietic stem and progenitor cells (HSPCs) were obtained from the same donor; for the patient receiving universal CAR T cells, HSPCs were obtained from a new haploidentical donor.

The protocol was approved by the ethics committee of the First Affiliated Hospital, Zhejiang University School of Medicine, and is available at NEJM.org. All the patients provided written informed consent in accordance with the principles outlined in the Declaration of Helsinki.

ASSESSMENTS

The evaluation of response was conducted after CAR T-cell therapy (before haploidentical HSCT) and after HSCT, on the basis of corresponding National Comprehensive Cancer Network guidelines for each disease.[15,16] Minimal residual disease (MRD) negativity was defined as no evidence of leukemia cells in the bone marrow–based detection that used multiparameter flow cytometry (sensitivity, 1 in 10^4 cells). Overall survival was measured from the day of CAR T-cell infusion (day 0) until the date of death or last follow-up, and disease-free survival was calculated from the day of CAR T-cell infusion (day 0) until the date of disease progression, death, or last follow-up.

STATISTICAL ANALYSIS

Overall and disease-free survival were estimated with the use of the Kaplan–Meier method with two-sided 95% confidence intervals. Additional statistical analyses are indicated in figure legends. All analyses were conducted with the use of SPSS software (version 26) and R software (version 4.0.2).

RESULTS

PATIENT CHARACTERISTICS

A total of 10 patients were enrolled, including 7 with AML, 2 with T-cell ALL, and 1 with T-cell lymphoblastic lymphoma (grade IVA). The characteristics of the patients at enrollment are summarized in Table 1 and Table S1. The median age of the patients at enrollment was 56.5 years (range, 13.7 to 72.5). All the patients had been heavily pretreated, with a median of 9.5 courses of therapy (range, 4 to 15) (Table S2). Bone marrow involvement was identified in all the patients, with a median percentage of blasts of 36.0% (range, 2 to 87). Two patients had extramedullary disease. The median percentage of CD7 expression on blast cells was 93.0% (range, 80.7 to 97.7).

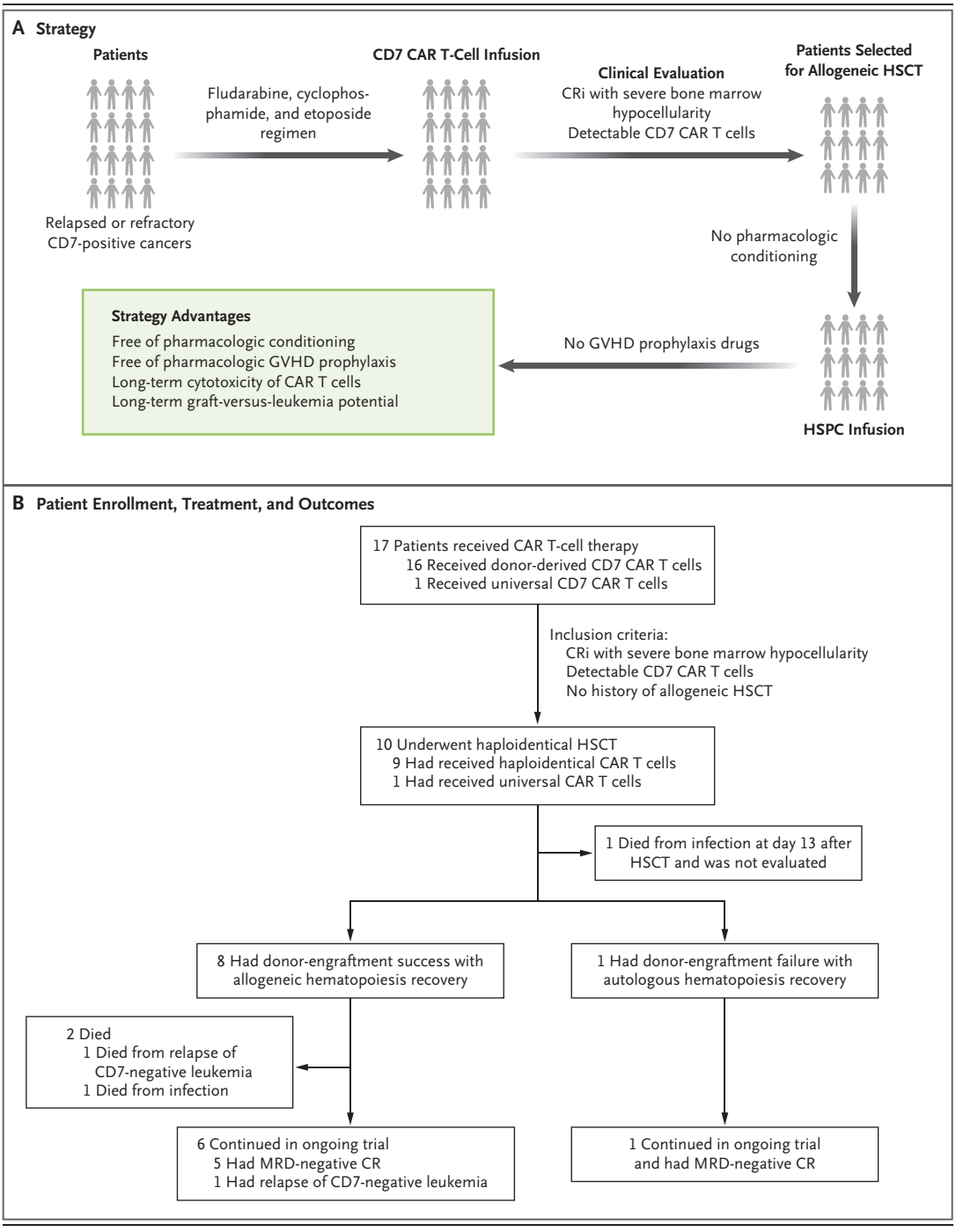
The median time from diagnosis to CAR T-cell infusion was 13.1 months (range, 4.6 to 33.7), and the characteristics of CAR T cells are provided in Table S3. The median time from CAR T-cell infusion to HSPC infusion was 19 days (range, 15 to 89). The first patient (Patient 1) had persistent grade 4 pancytopenia for 3 months after CAR T-cell infusion, complicated with candida sepsis followed by *Enterococcus faecalis* infection. A salvage haploidentical HSPC infusion was thus performed. The subsequent nine patients with similar conditions promptly proceeded to undergo haploidentical HSCT within 1 month after CAR T-cell infusion (Tables S4 and S5 and Supplementary Methods). The median doses of infused mononuclear cells, CD34+ HSPCs, and CD3+ T cells were 9.2×10^8 per kilogram (range, 4.8 to 20.3), 4.8×10^6 per kilogram (range, 3.5 to 8.4), and 5.5×10^8 per kilogram (range, 2.9 to 13.7), respectively.

SAFETY PROFILES

All adverse events that were considered by the investigators to be related to CAR T-cell therapy are documented in Table S6. Cytokine release syndrome occurred in nine patients (five with grade 1 and four with grade 2), with a median time to onset of 1 day (range, 1 to 5) and a median duration of 9.5 days (range, 8 to 13). All episodes of cytokine release syndrome were successfully controlled (Table S7). No cases of immune effector cell–associated neurotoxicity syndrome occurred. All the patients had grade 4 pancytopenia without signs of abatement after medical intervention, and bone marrow assessments suggested severe hypocellularity after CAR T-cell infusion (Tables S8 and S9 and Fig. S3).

GVHD occurred in four patients (one with GVHD related to CAR T-cell therapy and three with HSCT-related GVHD) (Table S10). Patient 2 had grade 2 skin GVHD on day 7 after CAR T-cell infusion, which was completely resolved on day 11 after glucocorticoid and antipruritic treatment. Patients 1, 4, and 6 had short-term grade 2 acute GVHD after haploidentical HSCT. No chronic GVHD occurred.

Five patients had bacterial or fungal infections of any grade. Patient 4 had multidrug-resistant bloodstream infection at 3.3 months followed by intracranial infection, and the patient died of septic shock at 3.7 months. Patient 8 died at 13 days after HSCT of septic shock due to *Staphylococcus haemolyticus* infection and encephalitis due to human herpesvirus 6 infection. Infections in other patients were successfully treated with antibiotics. Reactivation of Epstein–Barr virus (EBV) and cytomegalovirus (CMV) was detected in nine patients (not in Patient 10) (Fig. S4).



EFFICACY

Pancytopenia was successfully relieved after allogeneic HSCT. At 1 month after HSCT, seven of nine evaluable patients (78%) had full donor chimerism (Table S11). In Patient 3, mixed donor chimerism was maintained within the first 3 months (quantified at 88.35%), and full donor chimerism was present at 6 months after HSCT. For patients with full donor chimerism, successful hematopoietic recovery within 1 month after

Figure 1 (facing page). An "All-in-One" Strategy of Sequential Allogeneic CD7 CAR T-Cell Therapy and Haploidentical HSCT.

Panel A is the schematic diagram of this "all-in-one" strategy. Patients received CD7 chimeric antigen receptor (CAR) T-cell therapy and had complete remission (CR) with incomplete hematologic recovery (CRi) and then proceeded to undergo haploidentical hematopoietic stem-cell transplantation (HSCT) without a pharmacologic myeloablative conditioning regimen; no graft-versus-host disease (GVHD) prophylaxis drugs were administered. This innovative strategy enables the preservation of CAR T cells together with the establishment of full donor chimerism, which consequently resulted in prolonged leukemia-free remission. HSPC denotes hematopoietic stem and progenitor cell. Panel B shows patient enrollment, treatment, and outcomes. A total of 10 patients were selected from the clinical study of allogeneic CD7 CAR T cells, with inclusion criteria of CRi after CAR T therapy, detectable CAR T cells, and no history of allogeneic HSCT. Eight patients (80%) had full donor chimerism. At the last follow-up, 6 patients had ongoing CR (5 with full donor chimerism and 1 with autologous hematologic recovery), and 1 was alive with a relapse of CD7-negative leukemia. One patient died from a relapse of CD7-negative leukemia, and 2 died from infections. MRD denotes minimal residual disease.

HSCT was observed, with a median time to neutrophil engraftment of 11.5 days (range, 8 to 17) and a median time to platelet engraftment of 12 days (range, 8 to 29). Patient 5 had autologous hematopoietic recovery at 1 month after HSCT, possibly due to the delayed infusion and compromised expansion of CAR T cells. Recovery of immune cells was also observed (Fig. S5 and Table S12).

All the patients had complete remission with incomplete hematologic recovery after CAR T-cell therapy, including nine with MRD-negative incomplete hematologic recovery and one (Patient 6) with MRD-positive incomplete hematologic recovery. Post-HSCT efficacy was evaluated on day 28 after allogeneic HSCT in nine patients, and all had MRD-negative complete remission. Positron-emission tomography–computed tomography showed the complete regression of extramedullary lesions in Patient 1 at 3 months and Patient 10 at 1 month after HSCT. The median duration of follow-up among the survivors was 15.1 months (range, 3.1 to 24.0). Two patients with AML had a relapse of CD7-negative leukemia in bone marrow, occurring at 5.6 months (in Patient 6) and 4.3 months (in Patient 7) after CAR T-cell therapy. Patient 7 died of disease progression at 4.8 months after CAR T-cell therapy. As of the data-cutoff date (November 8, 2023), six patients remained in MRD-negative complete remission without any treatment (Fig. 2A). The estimated overall and disease-free survival at 1 year was 68% (95% confidence interval [CI], 43 to 100) and 54% (95% CI, 29 to 100), respectively (Fig. 2B).

EXPANSION AND PERSISTENCE OF CAR T CELLS

Robust in vivo expansion of CAR T cells was detected in all the patients. The median time to maximum CAR T-cell expansion as assessed by quantitative real-time polymerase-chain-reaction (PCR) assay was 16 days (range, 7 to 20), and the median level of maximum expansion was 2.9×10^5 copies per microgram of DNA (range, 0.1 to 6.9) (Fig. 2C). The median time to maximum CAR T-cell expansion as assessed by flow cytometry was 11.5 days (range, 8 to 18), and the median level of maximum expansion was 316.5 cells per microliter (range, 155.4 to 6501.9) (Fig. 2C). Of the six patients remaining in MRD-negative complete remission at the data-cutoff date, five with donor engraftment had detectable CAR T cells at the last assessment. The patient with autologous hematopoietic recovery had no detectable CAR T cells at 3 months after CAR T-cell infusion. In two patients with a relapse of CD7-negative leukemia, CAR T cells were undetectable by flow cytometry at relapse yet remained detectable by quantitative real-time PCR.

T-CELL DYNAMICS AND CHARACTERISTICS

CD7+ T cells in the peripheral blood were eradicated at a median time of 8.5 days (range, 5 to 13), accompanied by the expansion of CD7– CD3+ T cells (Fig. 3A). Among nine evaluable patients, CD7+ T and natural killer cells remained undetectable in eight patients until the last follow-up, except for Patient 5 with autologous hematopoiesis recovery. In the two patients with a relapse of CD7-negative leukemia, all normal T cells also remained CD7-negative. ATAC (assay for transposase-accessible chromatin) sequencing on CD7– T cells revealed a reduction of chromatin accessibility at the CD7

Table 1. Baseline Characteristics and Clinical Outcomes.*

Characteristic	Value (N=10)
Median age (range) — yr	56.5 (13.7–72.5)
Sex — no. (%)	
Female	6 (60)
Male	4 (40)
Primary disease — no. (%)	
Acute myeloid leukemia	7 (70)
T-cell acute lymphoblastic leukemia	2 (20)
T-cell lymphoblastic leukemia	1 (10)
Median no. of previous therapies (range)	9.5 (4–15)
Median percentage of blasts in bone marrow (range)	36.0 (2–87)
Median CD7 expression on blast cells (range) — %	93 (80.7–97.7)
Extramedullary involvement — no. (%)	2 (20)
ECOG performance-status score — no. (%)†	
0	4 (40)
1	5 (50)
2	1 (10)
CAR T-cell products and doses — no. (%)	
Donor-derived: 2×10^6/kg	9 (90)
Universal: 5×10^6/kg	1 (10)
Cytokine release syndrome — no. (%)	
None	1 (10)
Grade I	5 (50)
Grade II	4 (40)
Immune effector cell–associated neurotoxicity syndrome — no. (%)	0
Clinical efficacy after CAR-T cell therapy — no. (%)‡	
MRD-negative complete remission with incomplete hematologic recovery	9 (90)
MRD-positive complete remission with incomplete hematologic recovery	1 (10)
Median interval from CAR T-cell infusion to HSPC infusion (range) — days	19 (15–89)
Donor relationship — no. (%)	
Children	8 (80)
Brother	2 (20)
Median donor age (range) — yr	33.9 (17.2–42.4)
HLA match of HSCT graft — no. (%)	
5 of 10 alleles matched	7 (70)
6 of 10 alleles matched	3 (30)
Donor–recipient ABO match — no. (%)	
Matched	6 (60)
Mismatched	4 (40)
Donor sex — no. (%)	
Female	1 (10)
Male	9 (90)

Table 1. (Continued.)

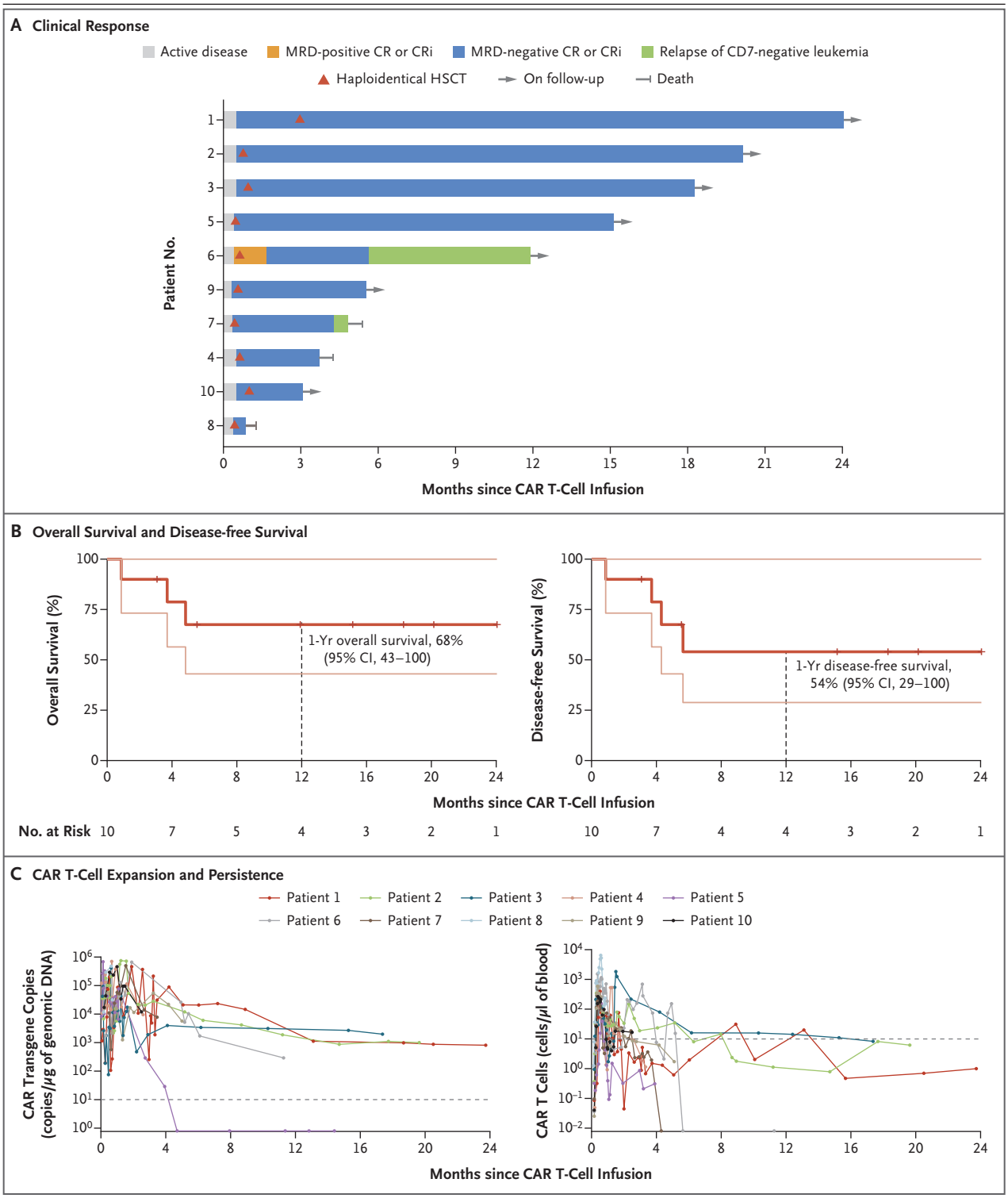
Characteristic	Value (N=10)
Graft composition	
Median no. of mononuclear cells (range) — $\times 10^{-8}$/kg	9.2 (4.8–20.3)
Median no. of CD34+ HSPCs (range) — $\times 10^{-6}$/kg	4.8 (3.5–8.4)
Median no. of CD3+ T cells (range) — $\times 10^{-8}$/kg	5.5 (2.9–13.7)
Median post-HSCT engraftment (range) — days	
Neutrophils	11.5 (8–17)
Platelets	12 (8–29)
GVHD after HSCT — no. (%)	
Acute	
Grade I or II	3 (30)
Grade III or IV	0
Chronic§	0
Median follow-up time among survivors (range) — mo after CAR T-cell infusion	15.1 (3.1–24.0)

* CAR denotes chimeric antigen receptor, GVHD graft-versus-host disease, HSCT hematopoietic stem-cell transplantation, HSPCs hematopoietic stem and progenitor cells, and MRD minimal residual disease.
† Eastern Cooperative Oncology Group (ECOG) performance-status scores range from 0 to 5, with higher scores indicating greater disability.
‡ Complete remission with incomplete hematologic recovery is defined as complete remission in the context of a neutrophil count of less than 1×10^9 per liter, a platelet count of less than 100×10^9 per liter, or both without evidence of extramedullary disease.
§ Six patients who survived at least 100 days were evaluated for chronic GVHD.

locus (Fig. S6). These results suggested that the persisting CAR T cells are able to eliminate CD7+ cells and that surviving T cells have suppressed expression of CD7 as a consequence of the selective pressure.

To further clarify the low incidence of GVHD in the absence of pharmacologic GVHD prophylaxis, we performed a one-way mixed-lymphocyte reaction (MLR) assay on T cells from three patients with more than 18 months of follow-up. T cells from the three patients showed reduced proliferation after stimulation with allogeneic cells as compared with T cells from corresponding donors (Fig. 3B and Fig. S7), which indicates that donor-derived CD7– normal T cells after HSCT indeed had lower alloreactivity. Next, we analyzed the dynamics of the T-cell receptor (TCR) repertoire in these three patients at serial time points (1, 3, 6, and 12 months after HSCT) and their corresponding donors. We observed a post-HSCT increase in clonality, together with a decrease of diversity of the TCRβ complementary determination region 3 repertoires over time, which indicates clonal expansion (Fig. S8 and Table S13). Similar trends of TCR dynamics were also observed in other patients. In Patients 1, 2, and 3, clonal expansion was further supported by the top-50 prevalent TCR clones at 12 months (total frequency, >70%), which were of low frequency in donor T cells (total frequency, <25%) (Fig. 3C).

The function of recovered CD7– T cells was evaluated by ex vivo stimulation with phorbol myristate acetate and ionomycin, CD3 and CD28 beads, or CMV and EBV peptide pools, which showed subtle differences between the T cells obtained from the patients and those obtained from the corresponding donors (Fig. S9). The autologous T cells obtained from Patient 5 also showed an activation potential that was similar to that for donor T cells (Fig. S10). Collectively, these data suggest that the long-lasting CD7 CAR T cells can eradicate donor-derived CD7+ T cells and may contribute to GVHD prophylaxis. On the other hand, the recovered T cells still maintained their capability for activation, and their TCR dynamics indicated the potential for a graft-versus-leukemia effect.



RELAPSE OF CD7-NEGATIVE LEUKEMIA

Flow cytometric analyses confirmed a relapse of CD7-negative leukemia in two patients (Fig. 4A). Whole-exome sequencing revealed no mutations or genetic alterations of the CD7 locus in relapsed leukemia cells in either patient (Fig. 4B). Single-cell RNA sequencing analyses of tumors from both patients confirmed the reduction of

Figure 2 (facing page). Clinical Response, Long-Term Survival Outcomes, and In Vivo Expansion and Persistence of CD7 CAR T Cells.

Panel A shows a swimmer plot illustrating the clinical responses and follow-up of individual patients, as indicated with different colors in the swimmer lanes. Each bar represents one patient. Panel B shows Kaplan–Meier curves of overall survival (left panel) and disease-free survival (right panel). The thick red curve indicates the estimated value, and the thin red curves indicate 95% confidence intervals. Panel C shows the kinetics of CAR T cells in the peripheral blood at serial time points, measured by quantitative real-time polymerase-chain-reaction assay of the CAR transgene (left panel) and flow cytometry (right panel). Different colors indicate different patients.

CD7 expression in relapsed tumor cells (Fig. S11). CD7 was also not expressed on normal T cells at relapse (Fig. 4A). The down-regulation was associated with a reduction of chromosome accessibility on the CD7 locus. In Patient 6, myeloid clusters 4, 5, and 8 — all expressing low levels of CD7 as compared with other myeloid cell clusters — were enriched for relapsed leukemia cells. Similarly, in Patient 7, myeloid clusters 5 and 8 expressed relatively low levels of CD7 and were enriched for relapsed leukemia cells. In both patients, baseline leukemia cells had heterogeneous CD7 expression and consisted of cells from both CD7-high and CD7-low clusters (Fig. S11). The relapsed leukemia cells showed deregulation of pathways involving hematopoiesis and cytokine and chemokine signaling. We thus infer that the relapse was due not to genetic mutations of CD7 but to expansion of preexisting CD7– blasts or transcriptional suppression of CD7 expression.

DISCUSSION

We report that sequential allogeneic CD7 CAR T-cell therapy and haploidentical HSCT, without pharmacologic conditioning or GVHD prophylaxis, can be successfully performed in a challenging cohort of patients with relapsed or refractory CD7-positive cancers. Although CD7 is expressed in only a subset of AMLs, its expression is associated with a poor prognosis.[17,18] As of the data-cutoff date, 6 of 10 patients (60%) remained in MRD-negative complete remission. This innovative procedure exploited allogeneic CD7 CAR T-cell therapy (including lymphodepletion before CAR T-cell infusion) to create a specific condition for successful allogeneic HSPC engraftment, GVHD control, and concomitant persistence of CAR T cells, thus opening a new strategic approach for allogeneic HSCT.

Allogeneic CAR T cells, from haploidentical donors ("donor-derived") or third-party persons ("universal"), are an important approach to increase accessibility of cellular therapy.[19,20] Allogeneic CD7 CAR T cells have been clinically evaluated, but the general outcome is still not satisfactory.[13] Allogeneic HSCT has been considered as consolidation treatment after CD7 CAR T-cell therapy. A recent retrospective study analyzed data from 12 patients with relapsed or refractory T-cell ALL or T-cell lymphoblastic lymphoma (median age, 14 years) who received donor CD7 CAR T-cell therapy as a bridge to conventional allogeneic HSCT; 1-year progression-free survival was 57%, and acute or chronic GVHD developed in 6 patients.[11] Zhang et al. also reported favorable outcomes in young patients (median age, 19 years) who proceeded to undergo allogeneic HSCT after CD7 CAR T-cell therapy (1-year progression-free survival, 67.2%, vs. 15.0% without allogeneic HSCT).[12] Our patient cohort predominantly comprised older persons (median age, 56.5 years) with comparatively worse underlying medical conditions at enrollment, and the long-term efficacy and safety were similar to those in younger persons with conventional HSCT. Furthermore, 6 patients had previous exposure to etoposide, which indicates that etoposide as as lymphodepleting agent might have had only a minor direct effect on their tumor cells. Together, our strategy offers a feasible approach to these patients who were previously deemed to be ineligible for allogeneic HSCT.

One key feature of this study is the avoidance of pharmacologic myeloablation before HSCT by exploiting the effects of the lymphodepleting regimen required for the engraftment of CAR T cells. This approach allows for the long-term persistence of CAR T cells after HSCT, as evidenced by the eradication of CD7+ cells and the persistence of detectable CAR T cells in all the patients with ongoing complete remission. Eight of nine evaluable patients had full donor chimerism after HSCT, which indicates that even without pharmacologic myeloablation, CD7 CAR T-cell

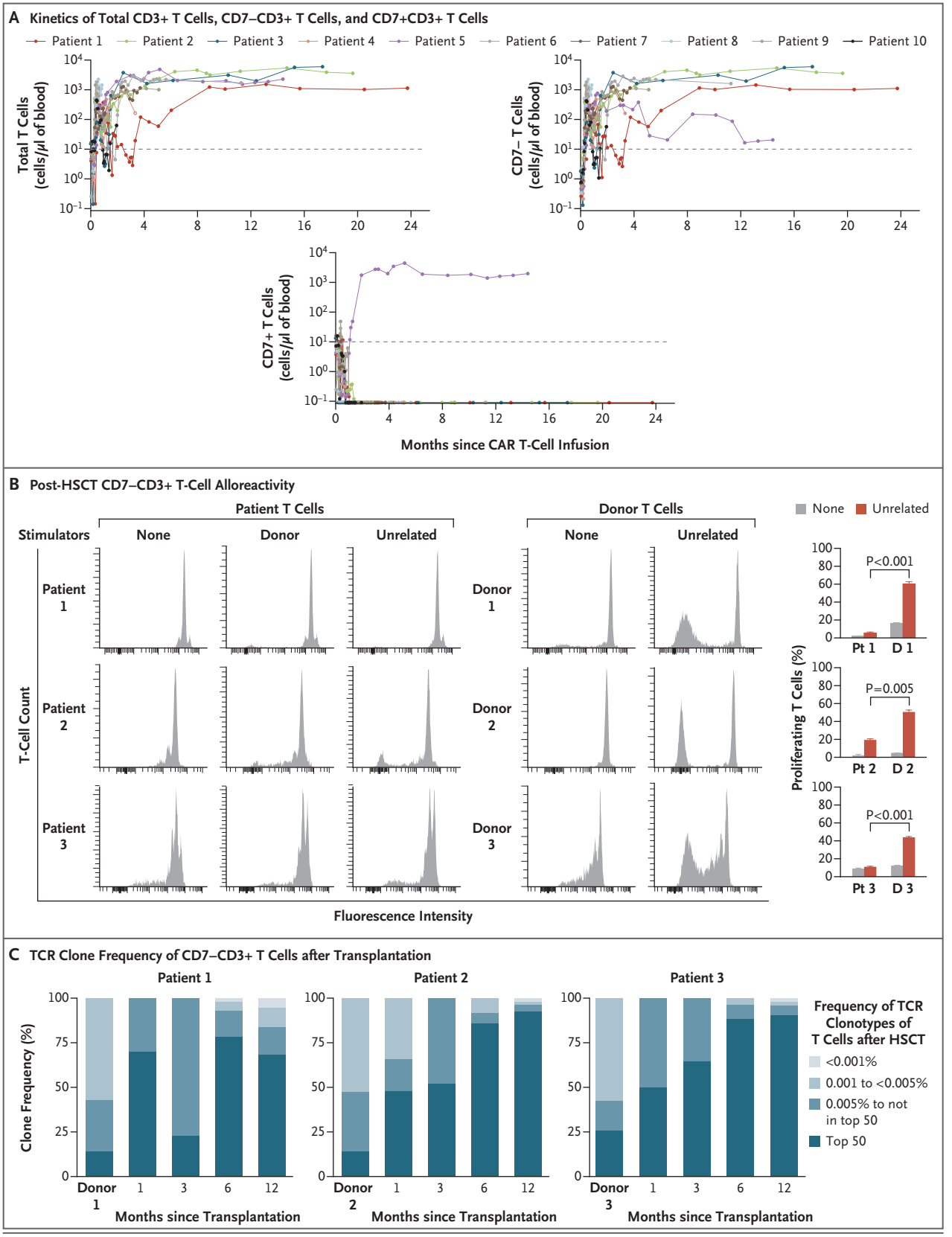


Figure 3 (facing page). Characteristics of CD7– T Cells after HSCT.

Panel A shows the kinetics of total CD3+ T cells, CD3+CD7+ T cells, and CD3+CD7– T cells in the peripheral blood of all treated patients, as measured by flow cytometry. Cells had been gated for negative CAR expression. Different colors indicate different patients. Panel B shows the results of a one-way mixed-lymphocyte reaction assay that analyzed the alloreactivity of T cells from three patients with more than 18 months of follow-up (Patients 1, 2, and 3). Peripheral-blood mononuclear cells (PBMCs) from each patient after HSCT (left panels) and their corresponding HSCT donors (middle panels) were labeled with carboxyfluorescein succinimidyl ester (CFSE) and stimulated by irradiated PBMCs from the HSCT donors or unrelated persons. (The "None" columns in Panel B refer to unstimulated cells from patients and donors.) At 7 days after stimulation, CD3+ T cells were gated and analyzed by flow cytometry, with the reduced CFSE fluorescence intensity indicating cell proliferation. Percentages of proliferating (CFSE-low) T cells in response to allogeneic stimulation were respectively summarized (right panels, three replicates). D denotes Donor, and Pt Patient. Panel C shows T-cell receptor (TCR) clonotypes of T cells from Patients 1, 2, and 3 at indicated time points after HSCT. TCR clones were categorized according to their frequency at 12 months after HSCT to track their frequencies in PBMC samples obtained at 1, 3, and 12 months after HSCT, as well as in infused donors' PBMCs. The composition of TCR clones is indicated by the color key.

treatment can create an environment conducive for allogeneic HSPC engraftment and function. These phenomena might be the consequence of the particularly severe pancytopenia and bone marrow aplasia after CD7 CAR T-cell therapy, which could be due to the allogeneic activity of donor-derived CAR T cells, lymphodepletion that is relatively stronger than that for autologous CAR T-cell therapy, and cytokine release syndrome–related cytokines that can also contribute to cytopenia.[21,22] In contrast, the successful engraftment of donor HSPCs may result from their autologous nature with the CAR T cells, as well as the diminished effect of cytokine release syndrome. We were intrigued to find clues of TCR clonal expansion over time in post-HSCT CD7– T cells, which has been associated with a graft-versus-leukemia effect.[23] The cytotoxic effect of persisting CAR T cells, together with the graft-versus-leukemia potential of donor-derived T cells, may contribute to the potent antitumor efficacy that was seen in this challenging cohort of patients.

GVHD prophylaxis is crucial for the management of post-HSCT toxic effects, but long-lasting immunosuppressive agents can increase the risk of immunodeficiency. In our study, one surprising observation was the absence of severe GVHD even without pharmacologic GVHD prophylaxis. HSCT-associated low-grade acute GVHD occurred in only three patients, and no chronic GVHD was observed. The incidence of GVHD in our study was lower than or similar to that reported (30 to 60%) for conventional allogeneic HSCT with GVHD prophylaxis.[24-26] The persistent CD7 CAR T cells and the subsequent elimination of donor-derived alloreactive CD7+ T cells may contribute to the prevention of GVHD, an idea that was further supported by the MLR assays showing the diminished alloreactivity of donor-derived CD7– T cells. It is important that these CD7– T cells still preserved the reactivity against viral antigens, which suggests the maintenance of some immune-protective function. However, CMV and EBV reactivation was detected, and post-transplantation lymphoproliferative disorder developed in one patient. These results warrant future mechanistic study of the biologic effects of CD7 on T-cell function.

Antigen escape is a prominent cause of disease relapse after CAR T-cell therapy. In this study, two patients had a relapse of CD7-negative leukemia after having had complete remission with full donor chimerism. One of these patients was evaluated as being MRD-positive before HSCT, which is associated with disease relapse.[5] In both patients, no genetic alterations on CD7 were detected in relapsed tumor cells. Instead, we found that relapsed leukemia cells were enriched for specific clusters of myeloid cells with low CD7 expression and had a generally low chromosome accessibility on the CD7 locus. These results together suggest that because of the heterogeneity of AML, the expansion of preexisting CD7-low or CD7– blasts may account for the relapse. We plan to further explore the mechanisms that underlie tumor relapse after this specific treatment strategy.

Although only one patient received universal CAR T cells, the results here indicate that both donor-derived and universal CAR T cells can be efficacious in this "all-in-one" strategy. Given its nature as an exploratory study, a limitation of this investigation is the varied intervals from CAR T-cell infusion to haploidentical HSCT

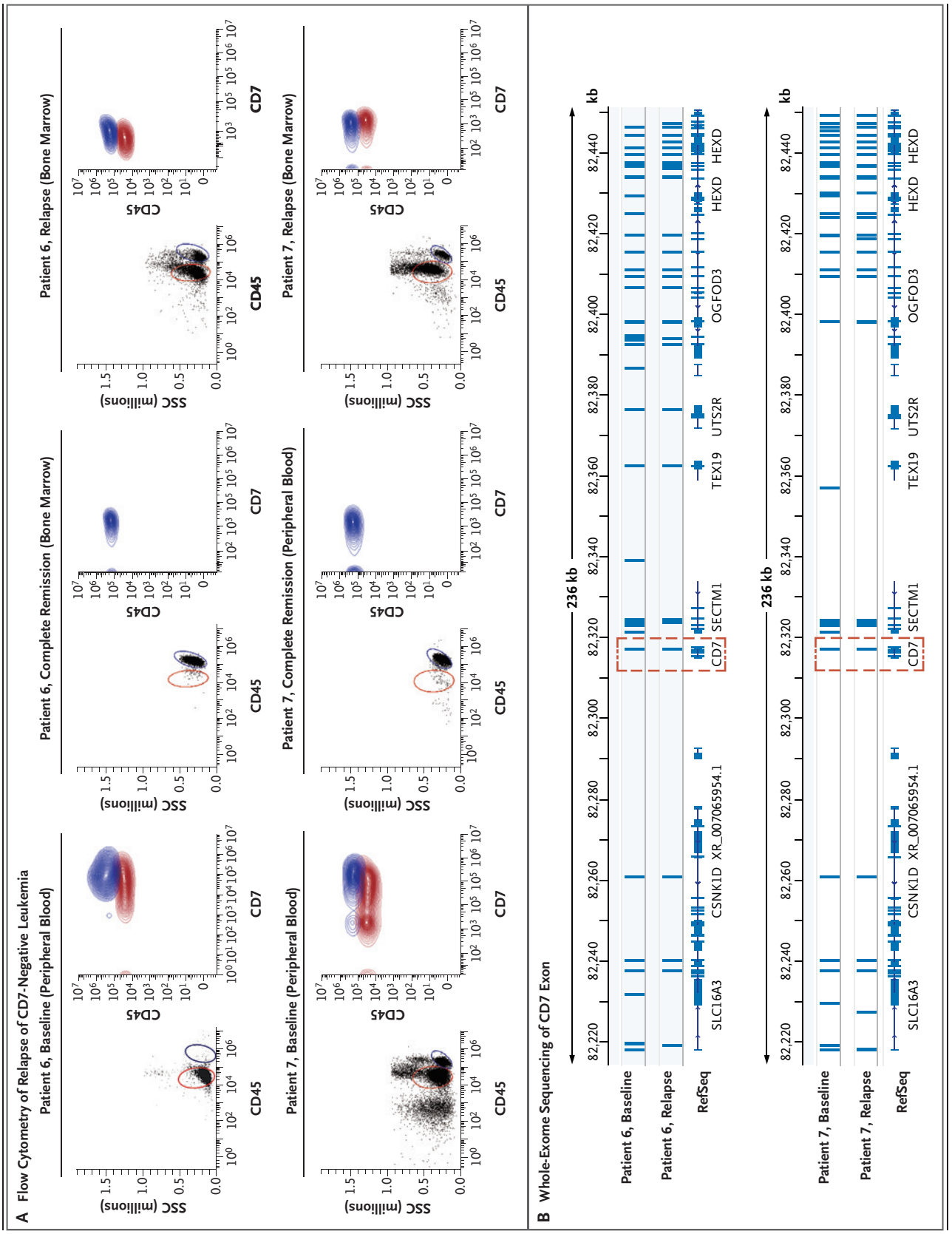


Figure 4 (facing page). Relapse of CD7-Negative Leukemia in Two Patients.

Panel A shows flow cytometry plots of CD7 expression on blasts (red) and normal T cells (blue) in the peripheral blood and bone marrow of Patients 6 and 7 at baseline, complete remission, and relapse. Contour plots show CD7 expression on blasts (red) and normal T cells (blue) that were merged into the same plot. The values on the x and y axes (except for side-scatter [SSC] values) denote the relative intensity of fluorescence signal or scattered-light signal. In Panel B, whole-exome sequencing revealed no additional genetic mutations, deletions, or structural variants in the CD7 exon region in leukemia cells obtained from Patients 6 and 7 between baseline and relapse. The human hg38 RefSeq genome was used for gene annotations. The results of single-cell RNA sequencing analyses of tumors from both patients are shown in Figure S11.

across patients. Meanwhile, although the median HSPC doses in this study align with our previous reports of conventional allogeneic HSCT,[27-29] the most effective HSPC dose may still need further elucidation. Because we were evaluating a new therapeutic strategy, the findings of this study still need to be extended and confirmed in a larger and more homogeneous cohort of patients.

Collectively, our integrated strategy maximized antileukemic efficacy from both persisting CAR T cells and graft-versus-leukemia potential, providing a feasible approach for patients with relapsed or refractory CD7-positive cancers who are ineligible for conventional allogeneic HSCT. A phase 1 clinical trial of sequential allogeneic CD7 CAR T-cell therapy and haploidentical HSCT in a larger cohort is ongoing (ClinicalTrials.gov number, NCT05827835).

Supported in part by grants from the National Natural Science Foundation of China (82270234 and 82130003), the Key Project of Science and Technology Department of Zhejiang Province (2021C03010 and 2023C03060), and the Key Research and Development Program of Zhejiang Province (2024SSYS0023, 2024SSYS0024, and 2024SSYS0025).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

Data will be made available on request (huanghe@zju.edu.cn).

We thank the patients enrolled in this study and their families, as well as the physicians, nurses, and staff of the flow cytometry core facility at the First Affiliated Hospital, Zhejiang University School of Medicine, who provided clinical care and research support, and other staff members who coordinated the clinical study.

APPENDIX

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