ORIGINAL ARTICLE

Base-Edited CAR7 T Cells for Relapsed T-Cell Acute Lymphoblastic Leukemia

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ABSTRACT

BACKGROUND

Cytidine deamination that is guided by clustered regularly interspaced short palindromic repeats (CRISPR) can mediate a highly precise conversion of one nucleotide into another — specifically, cytosine to thymine — without generating breaks in DNA. Thus, genes can be base-edited and rendered inactive without inducing translocations and other chromosomal aberrations. The use of this technique in patients with relapsed childhood T-cell leukemia is being investigated.

METHODS

We used base editing to generate universal, off-the-shelf chimeric antigen receptor (CAR) T cells. Healthy volunteer donor T cells were transduced with the use of a lentivirus to express a CAR with specificity for CD7 (CAR7), a protein that is expressed in T-cell acute lymphoblastic leukemia (ALL). We then used base editing to inactivate three genes encoding CD52 and CD7 receptors and the β chain of the $\alpha\beta$ T-cell receptor to evade lymphodepleting serotherapy, CAR7 T-cell fratricide, and graft-versus-host disease, respectively. We investigated the safety of these edited cells in three children with relapsed leukemia.

RESULTS

The first patient, a 13-year-old girl who had relapsed T-cell ALL after allogeneic stem-cell transplantation, had molecular remission within 28 days after infusion of a single dose of base-edited CAR7 (BE-CAR7). She then received a reduced-intensity (nonmyeloablative) allogeneic stem-cell transplant from her original donor, with successful immunologic reconstitution and ongoing leukemic remission. BE-CAR7 cells from the same bank showed potent activity in two other patients, and although fatal fungal complications developed in one patient, the other patient underwent allogeneic stem-cell transplantation while in remission. Serious adverse events included cytokine release syndrome, multilineage cytopenia, and opportunistic infections.

CONCLUSIONS

The interim results of this phase 1 study support further investigation of base-edited T cells for patients with relapsed leukemia and indicate the anticipated risks of immunotherapy-related complications. (Funded by the Medical Research Council and others; ISRCTN number, ISRCTN15323014.)

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*A list of members of the Base-Edited CAR T Group is provided in the Supplementary Appendix, available at NEJM.org.

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The use of chimeric antigen receptor (CAR) T cells for the treatment of B-cell cancers has been approved by regulatory authorities. However, the treatment of T-cell cancers has been challenging because the targeting of T-cell antigens can trigger CAR T-cell fratricide (in which CAR T cells target one another because each expresses the protein that it is engineered to target) and have damaging effects on the broader T-cell population. Genome editing to disrupt T-cell antigens, such as CD7, can prevent fratricide, and disrupting endogenous T-cell receptors, such as TCRαβ, can prevent graft-versus-host disease (GVHD).[1] However, the use of nuclease reagents to achieve multiple-target editing by means of the creation of multiple, simultaneous, double-stranded breaks in DNA can trigger translocations and other chromosomal aberrations.[2]

In contrast, base editing by means of cytidine deamination guided by clustered regularly interspaced short palindromic repeats (CRISPR) mediates highly precise C→U→T conversion,[3] which can be directed to create premature stop codons

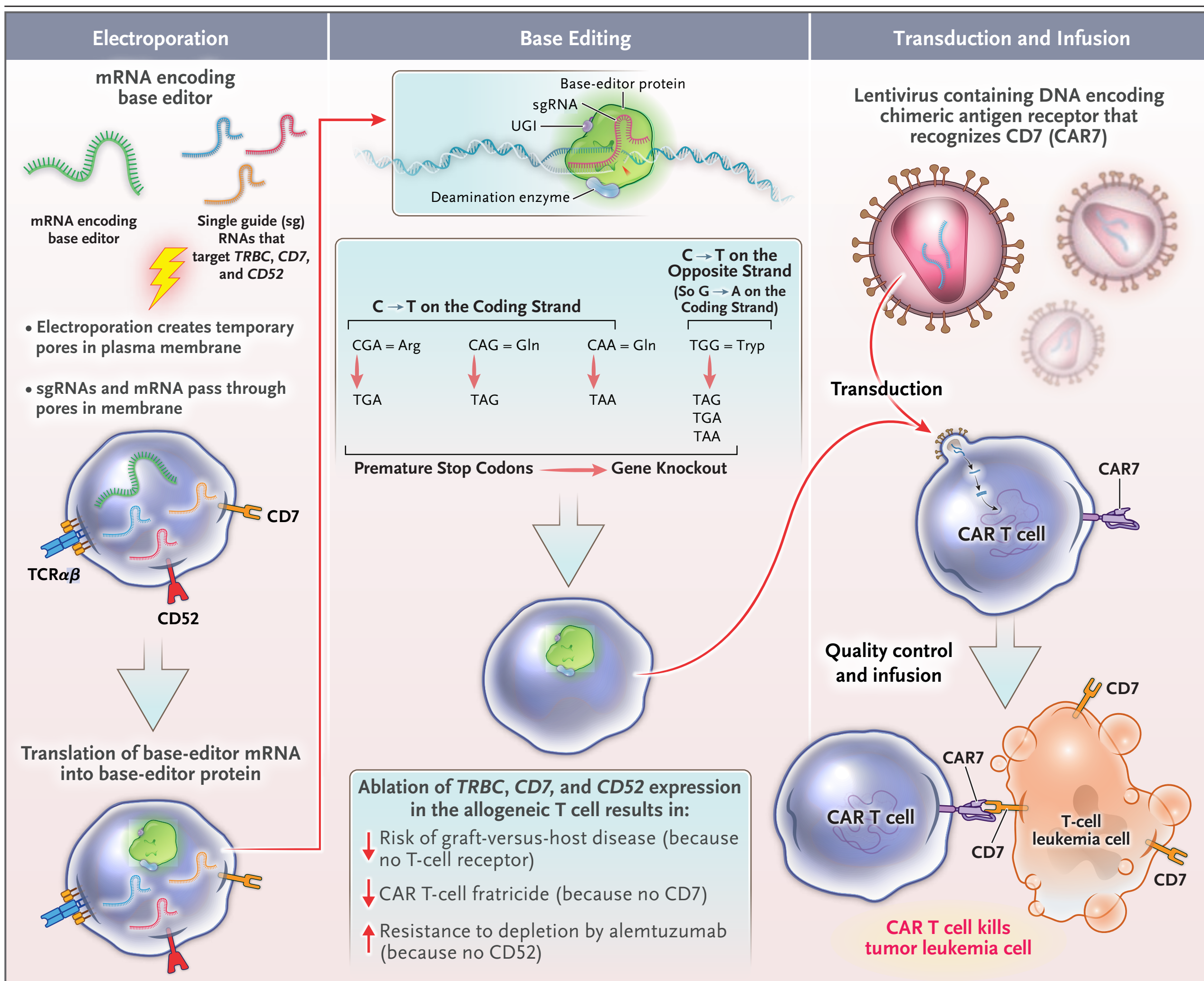

Figure 1. Base-Editing Donor T Cells to Target T-Cell Leukemia.

Base editing by cytidine deamination offers the possibility of highly targeted C→T conversion and the introduction of stop codons or removal of splice sites to disrupt gene expression without causing double-strand DNA breaks. BE-CAR7 T cells were generated from healthy donor peripheral-blood lymphocytes by electroporation of three sgRNAs against *TRBC*, *CD7*, and *CD52* in combination with mRNA encoding codon-optimized BE3. This process allowed expression of CAR7 after lentiviral transduction without fratricide as well as specific targeting of CD7+ leukemia cells.

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or disrupt splice sites without the induction of double-stranded DNA breaks.[4] In this study, we electroporated three single-guide RNAs (sgRNA) targeting *TRBC1* and *TRBC2*, *CD7*, and *CD52* messenger RNAs (mRNA), along with a codon-optimized cytidine base editor (coBE) mRNA, to edit the DNA of healthy donor T cells (Fig. 1). We then transduced these cells with a lentivirus vector encoding a CAR that recognizes CD7, a T-cell antigen, thereby generating base-edited CAR7 (BE-CAR7) T-cell banks (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org) for use in a phase I feasibility and safety study involving pediatric patients with relapsed or refractory T-cell acute lymphoblastic leukemia (ALL). Here, we describe the results from the procedure in the first three study participants.

METHODS

PREPARATION OF BE-CAR7 T CELLS

We obtained peripheral blood mononuclear cells from healthy volunteers with the use of steady-state apheresis. Using a Prodigy device (Fig. S2)[5] that enabled a largely automated process, we activated T cells with Transact (anti-CD3 and CD28 antibody), and the next day we electroporated them with coBE mRNA and sgRNAs targeting *TRBC1* and *TRBC2* (encoding alternate forms of the T-cell receptor β chain; one sgRNA targeted both genes), *CD52*, and *CD7*. The coBE mRNA encodes the base-editor protein: a catalytically impaired Cas9 nickase fused to a rat-derived single-stranded DNA deaminase and a uracil glycosylase.

Mechanistically, hybridization of guide RNA to genomic DNA with Cas9 binding results in the displacement of a short stretch of DNA (called the protospacer adjacent motif, or PAM) and exposes any distal cytosine within a 5-bp window (positions 4 through 8), which renders them vulnerable to deamination (by the deaminating part of the fusion protein) and favors the conversion of cytosine to uracil. Uracil is read by DNA polymerases as a thymine, which results in the incorporation of adenine in the opposing, complementary strand of DNA. The targeted codon is thus rendered a stop codon, which inactivates the gene.[3] The cells were then transduced with a lentiviral vector containing *CAR7* cDNA. Details of our preparation of four full-scale batches of BE-CAR7 cells (two for research and development and two according to good manufacturing practice) are described here and in the Supplementary Appendix.

PATIENTS AND THERAPY

This study began at Great Ormond Street Hospital, London, on April 14, 2022. The protocol specifies a study population of 10 children in the United Kingdom between 6 months and 16 years of age with relapsed or refractory CD7+ T-cell cancer that is quantifiable in bone marrow (>10^{-4} as measured with the use of flow cytometry or polymerase-chain-reaction [PCR] assay). Exclusion criteria are active, uncontrolled infections, preexisting GVHD, or the detection of anti-HLA antibodies against BE-CAR7 cells (see the protocol, available at NEJM.org).

Patients in the study received lymphodepletion with fludarabine (150 mg per square meter of body-surface area), cyclophosphamide (120 mg per kilogram of body weight), and alemtuzumab (1 mg per kilogram) followed by infusion of 0.2×10^6 to 2.0×10^6 BE-CAR7 T cells per kilogram (with a maximum of 5×10^4 per kilogram of TCR$\alpha\beta$+ T cells, to limit the risk of GVHD). Patients with disease in molecular remission at day 28 underwent allogeneic stem-cell transplantation, at which point any persisting BE-CAR7 cells were depleted by the conditioning regimen used before the transplant.

OVERSIGHT

The study is being conducted with the approval of the United Kingdom Health Research Authority, with review by an independent data and safety monitoring committee conducted after every three patients undergo treatment. Written informed consent was provided by the parents of the patients, with additional written participant assent provided by Patients 1 and 3. Ethics approval was awarded by the West London Ethics Committee, and volunteer donor harvests were arranged by the Nolan Registry.

RESULTS

BASE-EDITED CELLS

After base editing and one round of exposure to a lentiviral vector, 59 to 62% of cells expressed CAR7 (Table S1). The remaining TCR$\alpha\beta$+ T cells were depleted by automated magnetic-bead pro-

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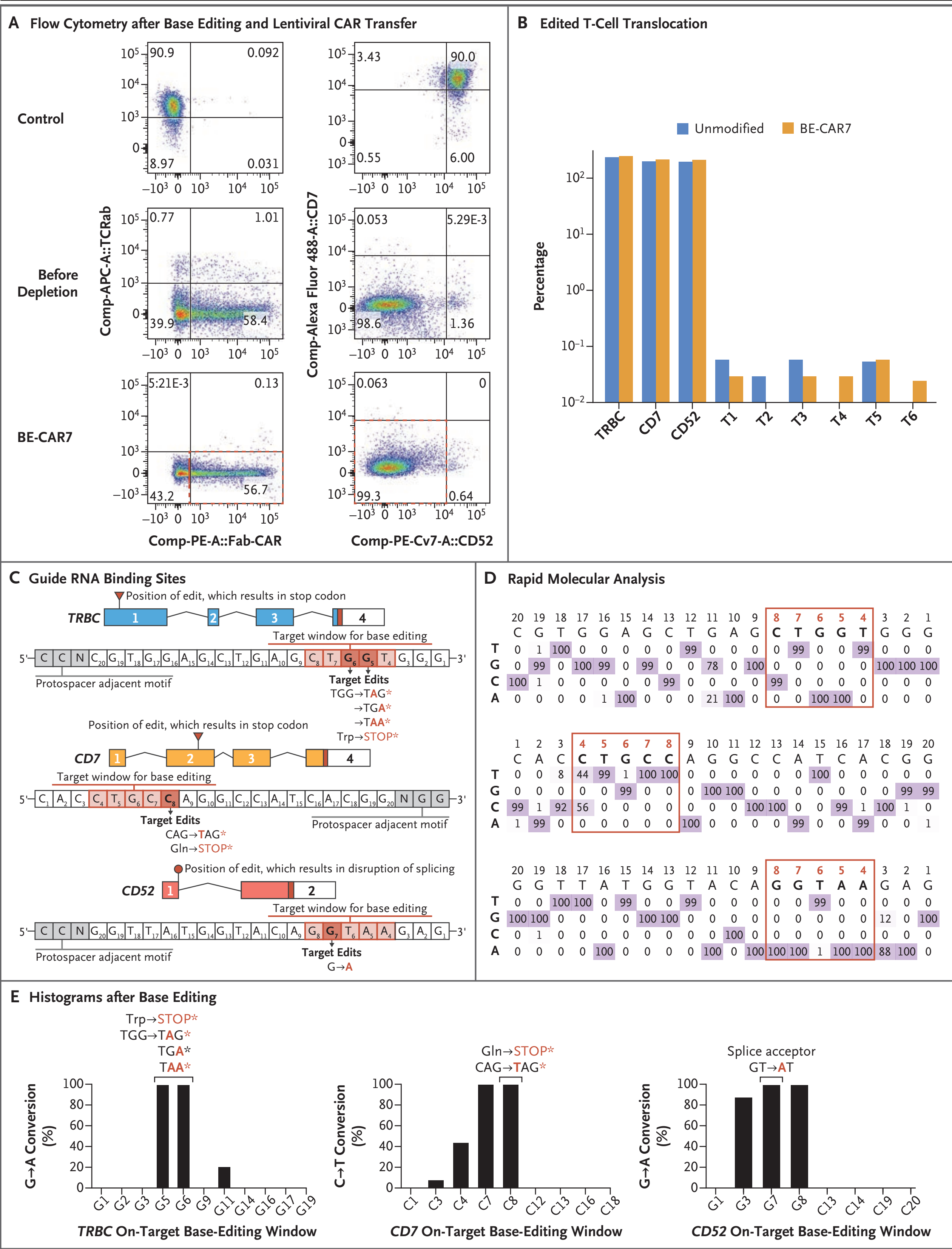

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Figure 2 (facing page). Production of Base-Edited CAR7 T Cells.

Panel A shows flow cytometry after base editing and lentiviral transduction with high-level disruption of TCR, CD7, and CD52. A final processing step to remove residual TCR$\alpha\beta$ T cells was included to ensure no patient received TCR$\alpha\beta$ T cells in amounts more than 5×10^{-4} per kilogram of body weight, which would risk causing graft-verus-host disease. Panel B shows the frequency of translocations previously identified for Cas9 edited T cells by droplet digital PCR for six predictable translocations, which were similar to background levels in unmodified controls (<0.1%). The detection of both alleles of each respective target site was used to validate PCR primers and manifested as a value of 200%. Panel C is a schematic diagram of guide RNA binding sites at TRBC, CD7, and CD52 loci and the expected window of activity for coBE3. Panel D shows rapid molecular analysis by direct sequencing and edit deconvolution by inference of traces in R (EDITR) analysis–confirmed C→T conversions within the expected 5-BP window of coBE3 for all three sgRNAs. In Panel E, histograms show the position of cytosine residues and proportion modified after base editing.

cessing; when the cells were analyzed with the use of flow cytometry at the end of production, they showed residual TCR$\alpha\beta$ expression of 0.1 to 1.9%, and CAR7-expressing cells were devoid of surface expression of CD7 (>99% of the cells) and CD52 (>92% of the cells) (Fig. 2A and Fig. S3). Quality-control testing also included microbiologic screening (the results of which were negative), droplet digital PCR for vector copy number (range, 2.35 to 3.60), and the exclusion of replication-competent lentivirus (Table S1). We used droplet digital PCR to test for the presence of six possible predictable chromosomal translocations that could theoretically be generated by the creation of double-stranded breaks induced by CRISPR–Cas9 editing (Fig. S4) using the six different sgRNA combinations and found the presence of such translocations to be no higher than in unmodified control cells (<0.1%) (Fig. 2B).

Molecular analysis confirmed editing (62 to 100%) of *TRBC*, *CD7*, and *CD52* at the targeted cytosine positions within the expected editing windows (Fig. 2C through 2E; note that editing can be initiated on either strand, so what appears to be a G→A edit on the coding strand is in fact a C→U→T edit that was initiated on the opposite strand). Genome sequencing had indicated no off-target activity at 20 sites predicted (by computer analysis) to be the most likely to be affected (Fig. S5). BE3 protein was not detectable by Western blot at the end of production of the BE-CAR7 T cells (Fig. S6). Functional potency was confirmed for each batch in vitro as compared with two cell lines (Fig. S7) and in abbreviated human–murine chimera experiments (Fig. S8).[6]

PATIENT 1

A 12-year-old girl with T-cell ALL with chromosomal translocation t(10;11) and deletion of chromosome 9p had received induction chemotherapy that had produced a poor response, but after multiple lines of additional treatment, including nelarabine, she had morphologic remission, albeit with detectable minimal residual disease (disease frequency, 7×10^{-4}). There was no central nervous system or extramedullary disease, and the patient underwent a transplantation of HLA-matched unrelated donor stem-cells after receipt of conditioning with total-body irradiation of 12 Gy, etoposide at a dose of 60 mg per kilogram, and antithymocyte globulin (Thymoglobulin) at a dose of 7.5 mg per kilogram. Minimal residual disease was still detectable 4 weeks after stem-cell transplantation, and at 4 months there was evidence of morphologic relapse with CD7 expression on 100% of blasts (Fig. S9).

The patient was 13 years of age at the time she received the study treatment. Before she underwent lymphodepletion, 9% blasts were detectable in bone marrow (Fig. 3A). On examination of her bone marrow immediately before infusion of 50×10^{6} BE-CAR7 cells (0.7×10^{6} per kilogram CAR7 T cells and $<5\times10^{4}$ per kilogram TCR$\alpha\beta$ T cells), we observed 0.3% blasts.

There were no immediate infusion-related events, but a fever developed on day 2, followed by hypotension that led to the administration of fluid boluses without additional intervention for grade 2 cytokine release syndrome (grades range from 1 to 4, with higher grades indicating more serious symptoms). The levels of ferritin (3499 ug per liter) and C-reactive protein (71 mg per liter) were elevated, but we detected no rise in cytokines, including interleukin-6 (Table S2). Histologic analysis on day 6 of a biopsy specimen of a transient, maculopapular rash yielded no evidence of inflammation or GVHD. Systemic cytokine release syndrome resolved by day

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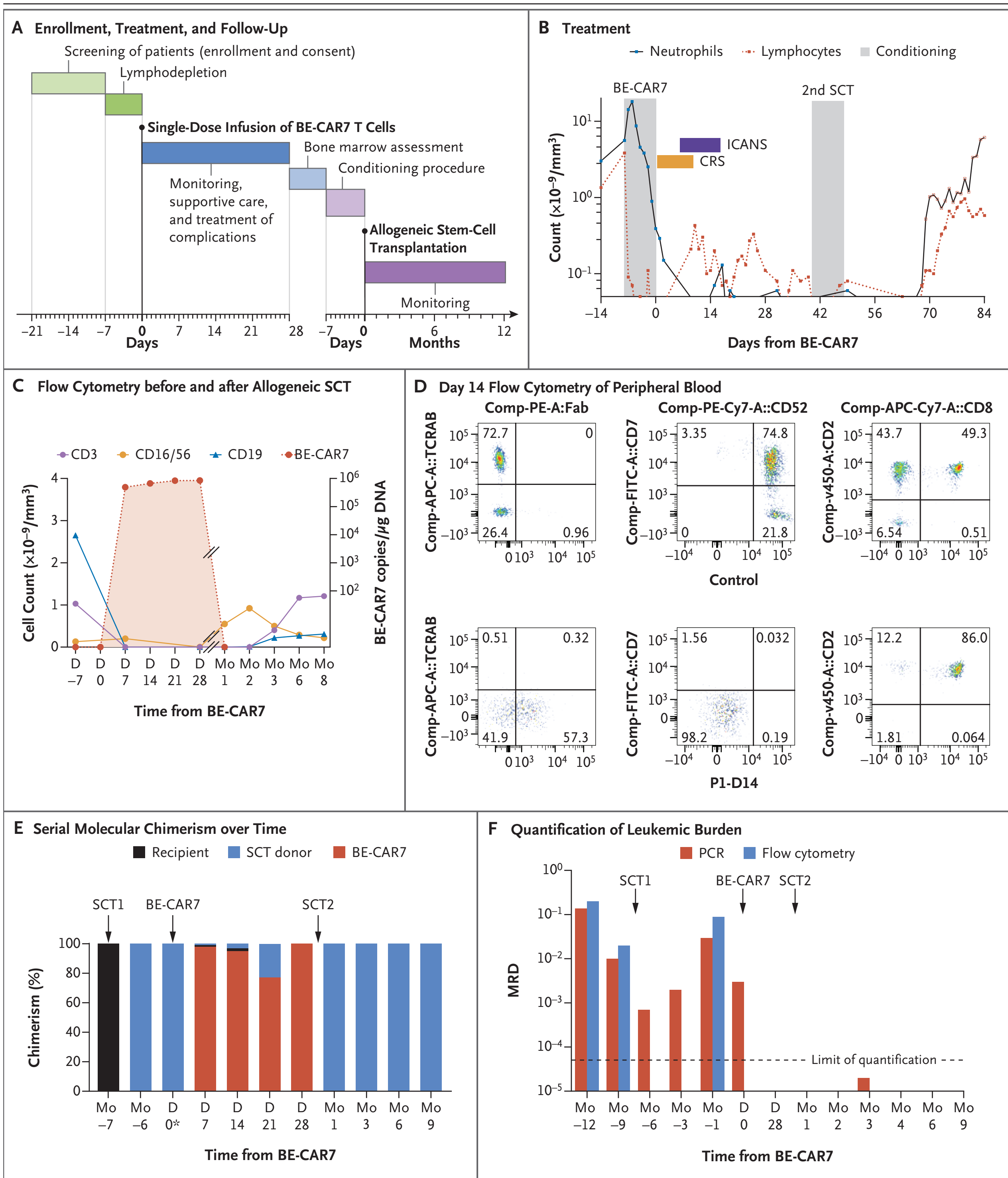

8, but emotional lability, memory loss, and confusion were consistent with grade 1 immune effector cell–associated neurotoxicity syndrome (ICANS; grades ranging from 1 to 4, with higher grades indicating more impairment). Results of magnetic resonance imaging of the brain and electroencephalography were normal, and neurotoxicity resolved spontaneously by day 12 (Fig. 3B).

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Figure 3 (facing page). Treatment and Outcomes, Patient 1.
As shown in Panel A, screening of the patients (enrollment and informed consent) was conducted approximately 3 weeks before the scheduled infusion of BE-CAR7 (day 0). Lymphodepletion was conducted with the use of fludarabine at a dose of 150 mg per square meter of body area, cyclophosphamide at a dose of 120 mg per kilogram of body weight, and alemtuzumab at a dose of 1.0 mg per kilogram administered over 7 days, followed by a single-dose infusion of BE-CAR7 T cells (CAR7 dose range, 0.2×10^6 to 2.0×10^6 per kilogram) with TCR$\alpha\beta$ T cells administered at a maximum dose of 5×10^4 per kilogram. The 28 days after infusion included monitoring, supportive care, and treatment of complications. Assessment of bone marrow on day 28 included quantification of minimal residual disease (MRD) with the use of flow cytometry and PCR; patients with MRD less than 10^{-4} were eligible to proceed to allogeneic stem-cell transplantation (SCT). The conditioning procedure for allogeneic SCT also removed any persisting BE-CAR7 cells, providing a time-limited exposure to the base-edited cells. Panel B shows the treatment course of Patient 1, from mapping of lymphocyte and neutrophil cytopenia after lymphodepletion (shading) and infusion of BE-CAR7 (day 1) through day 28 assessments and further chemotherapy (shading) to reconstitution after a second allogeneic SCT procedure on day 49. Transient cytokine release syndrome (CRS) in the first week and mild neurotoxicity (ICANS) in the second week were documented. Panel C shows flow cytometry analysis of peripheral blood lymphocyte subsets from Patient 1 during lymphopenia (T cells, CD3; B cells, CD19: NK cells, CD16/CD56), which confirmed cellular immune recovery from 1 month (M1) to 8 months (M8) after a second allogeneic SCT. Sensitive droplet digital PCR quantification of lentiviral copies allowed tracking of BE-CAR7 T cells until day 28, after which chemotherapy conditioning for allogeneic SCT removed any remaining cells. Panel D shows flow cytometry of peripheral blood from Patient 1 on day 14, which detected CAR+TCR$\alpha\beta$− BE-CAR7 T cells in the circulation, mostly CD8+ and exhibiting a CD7−/CD52− phenotype. Panel E shows serial molecular chimerism by variable-number tandem repeat (VNTR) that quantified the proportion of recipient cells from Patient 1, allogeneic SCT donor cells, and BE-CAR7 cells over time in peripheral blood and bone marrow (asterisk). Between days 7 and 28, BE-CAR7 cells comprised the majority of circulating mononuclear cells. After conditioning and a second allogeneic SCT, full donor-derived chimerism was observed. As shown in Panel F, the leukemic burden was quantified with the use of flow cytometry and PCR from the time of diagnosis. The dashed line indicates the limit of quantification. Seven months before BE-CAR7, Patient 1 had undergone a first allogeneic SCT, when MRD was negative on flow cytometry but detectable by quantitative PCR. One month before BE-CAR7, blasts were readily detected by flow cytometry and confirmed CD7+. After BE-CAR infusion, the disease had remained undetected by flow cytometry on serial bone marrow samples, and the patient remained in molecular remission at the most recent assessment 9 months after infusion.

The patient had cytopenia from day 0 onward and was dependent on blood and platelet support (Fig. S10).

We detected BE-CAR7 in peripheral blood using lentiviral-specific primers during a period in which the patient had very low absolute lymphocyte subset counts (Fig. 3C). On day 14, flow cytometry captured the presence of CAR T cells, which were predominately CD8+ and TCR$\alpha\beta$−/CD7−/CD52− (Fig. 3D). By day 27, lymphopenia persisted and CAR+TCR$\alpha\beta$− still made up the majority of circulating lymphocytes (>77%), with a small proportion (<9%) of CAR+TCR$\alpha\beta$+ cells also detected (Fig. S11). We used single-cell RNA (scRNA) sequencing to analyze cells that were captured by flow sorting and found evidence of lentivirus-mediated CAR7 expression without CD7 or CD52 expression (Fig. S11).

Molecular chimerism analysis also confirmed the presence of BE-CAR7 T cells, which accounted for the majority of circulating mononuclear cells during the 28 days after infusion (Fig. 3E). Infectious complications during this period included cytomegalovirus (CMV) reactivation (starting on day 6), which was treated with foscarnet, ganciclovir, and letermovir, and episodes of *Escherichia coli* bacteremia (on days 27 and 39) that necessitated intravenous antibiotics and replacement of the central catheter.

On day 27, the patient's bone marrow was hypocellular and in morphologic remission with undetectable minimal residual disease (Fig. 3F). Table S3 lists all the adverse events observed from the start of lymphodepletion to 28 days after BE-CAR7 infusion. Minimal-intensity conditioning for a second stem-cell transplantation from the original donor began on day 43 and comprised fludarabine (160 mg per square meter), cyclophosphamide (120 mg per kilogram), total body irradiation (2 Gy), and antithymocyte globulin (Grafalon, 15 mg per kilogram). Peripheral-blood stem cells at a CD34 cell dose of 9.57×10^6 per kilogram and a CD3 cell dose of 2.73×10^8 per kilogram were infused on day 49 with GVHD prophylaxis consisting of cyclosporine and methotrexate.

After transplantation, mucositis, BK-virus–related hemorrhagic cystitis, enterococcus bacteremia, and pericardial effusion (which occurred during engraftment) developed. We documented neutrophil recovery (which was supported by the administration of granulocyte colony-stimulat-

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ing factor) and full donor chimerism 1 month after transplantation; there were no detectable BE-CAR7 cells. The patient's bone marrow was in morphologic remission with no minimal residual disease, and the patient was discharged 52 days after stem-cell transplantation. Subsequent suspected GVHD grade 2 (grades range from 1 to 4, with higher grades indicating more serious disease) of the skin and gastrointestinal system was treated with a short course of glucocorticoid therapy, and a reactivation of CMV was treated with ganciclovir. Epstein–Barr virus viremia was monitored without intervention. Analysis of bone marrow at 9 months after stem-cell transplantation showed normal morphologic characteristics and confirmed ongoing molecular remission (Fig. 3F). Immunoglobulin G levels and absolute lymphocyte subset numbers were within normal ranges.

PATIENT 2

A 13-year-old boy had received a diagnosis of cortical T-cell ALL 3 years before enrollment in the study and had relapsed while receiving maintenance treatment. Despite receiving reinduction therapy and two blocks of high-intensity chemotherapy, he had refractory disease that manifested as persistent cytopenia with more than 80% blasts in bone marrow, all of which were expressing CD7. He underwent lymphodepletion and received 1×10^6 BE-CAR7 T cells per kilogram (Fig. 4A). Fever with elevated levels of interleukin-6 and ferritin (indicating cytokine release syndrome) developed within 3 days after BE-CAR7 infusion and was treated with tocilizumab (in three doses of 8 mg per kilogram), methylprednisolone (1 mg per kilogram), and transient inotrope support (for <24 hours) before it resolved by day 9. A generalized maculopapular rash with no specific pathologic histologic features on biopsy also resolved, and intravenous antifungal therapy was administered for *Candida krusei* fungemia.

Investigations for suspected ICANS (grade 1) from day 16 onward included normal cerebrospinal fluid (CSF) analysis and cranial imaging, which incidentally revealed maxillary *Aspergillus fumigatus* infection. Bone-marrow assessments on day 19 and day 25 revealed hypocellular marrow in morphologic and flow remission, but with evidence (obtained by means of PCR testing) of minimal residual disease. *A. niger* was repeatedly cultured from a pleural effusion on the right side, and there was associated lung consolidation and hypoxic respiratory deterioration, with notable re-elevation of interleukin-6 and ferritin, despite treatment for a second phase of cytokine release syndrome (Fig. 4B, Table S2) with further anti-interleukin-6 biologics, glucocorticoids, and anakinra.

There was ongoing neutropenia and lymphopenia throughout the investigational period, with BE-CAR7 T cells detected by PCR at serial time points in blood, marrow, and pleural fluid (Fig. 4C). Progressive lung complications from overlapping *A. niger* infection led to palliation and death on day 33.

PATIENT 3

A 15-year-old boy had initially presented in 2016 with mixed-phenotype acute leukemia and had undergone a first allogeneic stem-cell transplantation from a matched unrelated donor after myeloablative conditioning with total body irradiation. After discovery of minimal residual disease in the bone marrow, he had received two donor lymphocyte infusions, but the disease relapsed.

In 2017, he had received a second allogeneic stem-cell transplantation, from an umbilical-cord donor, after a reduced-toxicity conditioning regimen. After relapse in the bone marrow, the disease switched from mixed-phenotype acute leukemia to CD7+ T-cell ALL, and associated central nervous system disease was treated with intrathecal therapy. After informed consent was obtained, lymphodepletion was administered and BE-CAR7 T cells were infused at a dose of 0.9×10^6 per kilogram (Fig. 4D). Transient grade 2 cytokine release syndrome was associated with a transient rash and elevated levels of interleukin and ferritin (Table S2), and two doses of tocilizumab were administered.

Despite the presence of T-cell ALL in the central nervous system, there was no evidence of acute neurologic toxic effects. A recurrent maculopapular rash with nonspecific histologic changes was treated empirically with a 3-day course of methylprednisolone (1 mg per kilogram) to prevent GVHD. The patient had continuing lymphopenia, and viral reactivations, including reactivation of CMV, were treated with antiviral therapy. BE-CAR7 T cells were detected in blood and marrow by flow cytometry and chimerism

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signals at serial time points (Fig. 4E). Assessments of the bone marrow and CSF at day 28 revealed complete morphologic and molecular remission, enabling progression to a consolidative matched, unrelated donor allogeneic stem-cell transplantation (Fig. 4F).

DISCUSSION

The majority of children with T-cell ALL can be treated with standardized chemotherapy regimens, but those with induction failure or with elevated minimal residual disease after consolidation generally proceed to allogeneic stem-cell transplantation.[7] For patients who relapse after allogeneic stem-cell transplantation, the prognosis is poor, with less than 15% long-term survival.[8] In this context, cellular immunotherapy could potentially lead to deep leukemic clearance, but CAR T strategies against T-cell cancers have been challenging, partly because of the fratricide effects between T cells and the serious attendant risks of protracted T-cell lymphopenia. Fratricide can be avoided during manufacturing in situations in which an antigen is down-regulated after activation of CAR T cells[9,10] or when subsets of antigen-negative T cells can be harnessed.[11] Alternatively, preemptive cell-engineering steps have included the expression of inhibitory proteins to sequester target antigens such as CD7[12,13] and the disruption of *CD7* expression by genome editing.[14] The latter has also been combined with simultaneous disruption of T-cell-receptor gene expression[6,15,16] and a molecule critical to the synthesis of major histocompatibility complex class II in an attempt to generate universal CAR T cells.[17]

A variety of genome-editing platforms have reached clinical-phase applications for therapeutic T-cell engineering. Protein-guided zinc-finger nucleases, homing endonucleases, and transcription activator–like effector nucleases (TALENs) have been investigated for ex vivo T-cell modification, as have RNA-guided systems based on CRISPR–Cas9. Editing with nucleases, whether to knock out genes by DNA breakage and nonhomologous end-joining repair, or to target transgene insertion by template-mediated homology-directed repair, is associated with chromosomal aberrations.[1] A trial of TALEN-edited CAR19 T cells was recently halted while translocation events involving chromosome 14q (the site of the T-cell receptor alpha constant [TRAC] locus) were investigated.[18] Vector-related genotoxicity (damage to a patient's DNA caused by viral vectors) has not been a major issue in T-cell therapies, although complications due to malignant transformation appear to have arisen in a trial of transposon-modified CAR19 T cells.[19] In this study, we used CRISPR-guided cytidine deamination to introduce premature stop codons or modify critical splice sites[3,4] in combination with coBE3.

We previously reported a strategy for screening for possible guide-dependent off-target activity on DNA (due to guide RNA binding at partially matched DNA sites) and for promiscuous, guide-independent effects on DNA or RNA.[6] Limitations of screening investigations on the basis of sequencing of computer-predicted sites of ectopic editing are well recognized, and uncertainties remain regarding the significance of molecular changes detected by more comprehensive, nonbiased approaches. Quality control karyotyping of BE-CAR7 T cells showed normal karyotypes on metaphase spreads, and droplet digital PCR assays to detect possible translocations had negative results. In addition, our strategy of a time-limited in vivo application of engineered cells for this study provided mitigation against concerns of risks from unexpected genotoxicity that might arise after long-term engraftment. A treatment period of 28 days was sufficient to elicit antileukemic responses and secure deep remission in two of the three patients, an outcome that mirrored results from allogeneic CAR in patients with B-cell ALL, in whom complete remissions occurred within a similar time frame.[20-22]

This early report includes informative data covering an investigational period from lymphodepletion to day 28 after infusion for three initial patients, all of whom were otherwise facing palliation for refractory leukemia. The first patient had a relapse after an initial matched unrelated donor stem-cell transplantation, which took place when she was in morphologic remission but with minimal residual disease (detected by PCR) and which was preceded by full-intensity myeloablative conditioning and total-body irradiation. After the first stem-cell transplantation, residual disease was detected and a second transplant procedure was considered futile without additional intervention to eradicate the residual disease.

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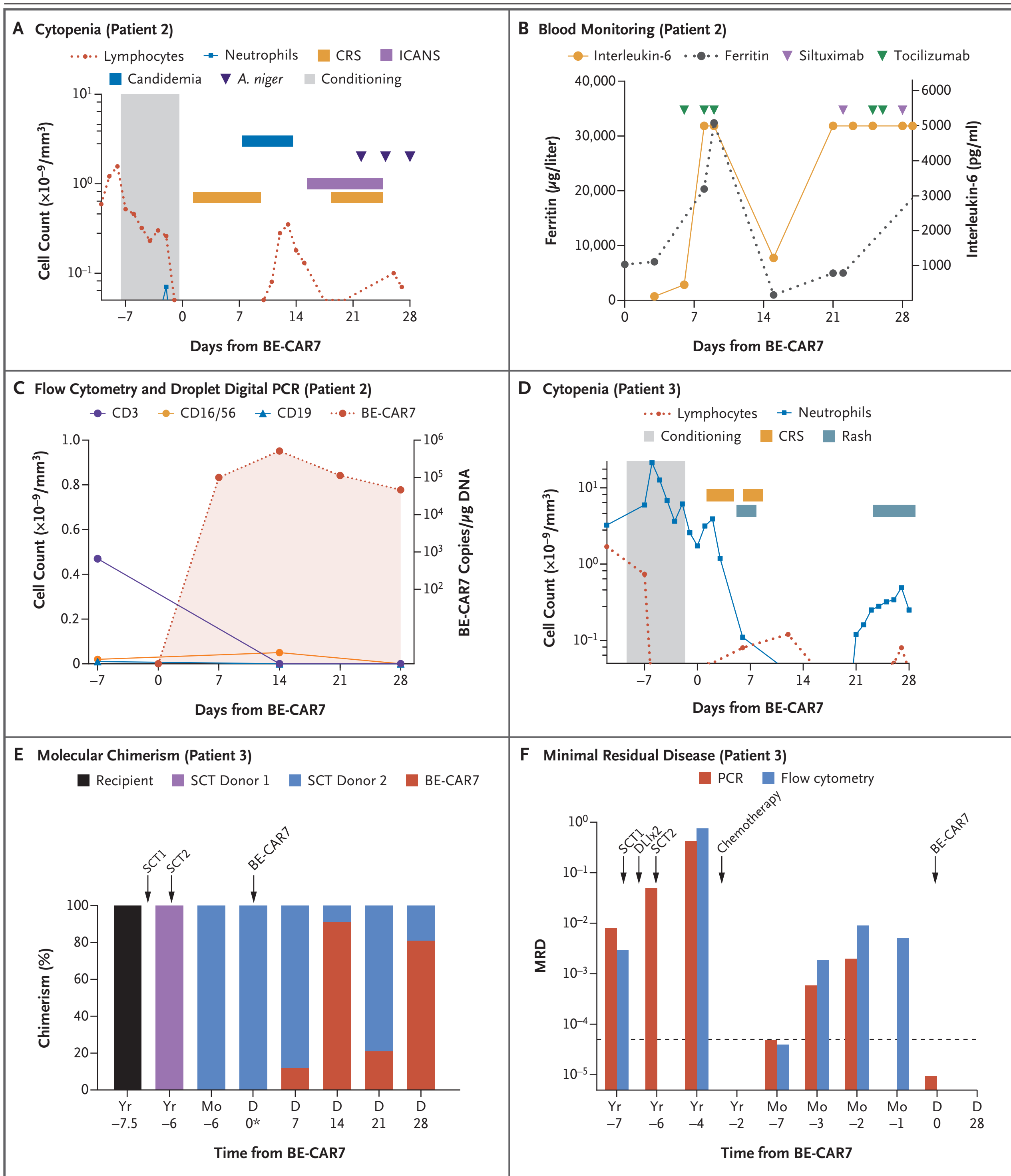

In the second patient, allogeneic stem-cell transplantation had likewise been ruled out, given the refractory, poorly responsive disease and the high blast count. The third patient had relapsed after two allogeneic stem-cell transplantations from different donors and had developed central nervous system involvement, which led to intrathecal chemotherapy. In each case,

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Figure 4 (facing page). Treatment and Outcomes, Patients 2 and 3.

As shown in Panel A, shading indicates lymphocyte and neutrophil cytopenia in Patient 2 before and after lymphodepletion. Infusion of BE-CAR7 (day 0) through day 28 was associated with candida sepsis and culture of *Aspergillus niger* in the lungs. Cytokine-release syndrome (CRS) initially responded to therapy but a second phase, with associated ICANS grade 1, overlapped with progressive fungal complications and respiratory deterioration. As shown in Panel B, monitoring of blood samples obtained from Patient 2 led to the detection of elevated ferritin and interleukin-6 (IL6), which initially responded to tocilizumab and glucocorticoid therapy. However, subsequent levels remained elevated (above the quantifiable range) despite further treatment with tocilizumab and siltuximab. Despite lymphopenia, the presence of circulating BE-CAR7 T cells was documented by flow cytometry and droplet digital PCR for lentiviral copies, as shown in Panel C, up to day 28. In Patient 3, Panel D shows lymphocyte and neutrophil cytopenia after lymphodepletion (shading) and infusion of BE-CAR7 (day 0) through day 28 with transient CRS (grade 2) and rash with nonspecific histologic changes seen on biopsy that resolved after administration of a short course of glucocorticoids. Panel E shows molecular chimerism by VNTR in peripheral blood and bone marrow (asterisk) and was used to monitor cells from Patient 3 (recipient) and two previous stem-cell transplant donors (SCT1 and SCT2) and BE-CAR7 mononuclear cells. BE-CAR7 cells were detectable in the circulation of Patient 3 despite lymphopenia and comprised the majority of circulating mononuclear cells by day 28. As shown in Panel F, Patient 3 had undergone SCT1 more than 7 years earlier for mixed-phenotype acute leukemia, followed by two donor lymphocyte infusions for minimal residual disease (MRD) which were monitored by flow cytometry and PCR. He underwent a second transplant (SCT2) from a matched, unrelated donor umbilical-cord blood graft, and after further chemotherapy (Chemo) sustained remission for more than 2 years. His most recent relapse had been in bone marrow and the central nervous system and consisted of CD7+ T-cell ALL; 28 days after BE-CAR7 infusion, there was morphologic and molecular remission with no evidence of CNS disease.

the application of a universal cell-based intervention was designed to eradicate residual disease ahead of allogeneic stem-cell transplantation.

In each of the three patients, the presence of preexisting anti-HLA antibodies was excluded and lymphodepletion by means of alemtuzumab, augmented-dose fludarabine, and cyclophosphamide was administered. A similar regimen has proven successful for TALEN-edited[20,21] and CRISPR-edited[22] allogeneic universal CAR19 T cells. In each of the patients, fever, rash, and other manifestations of cytokine release syndrome developed within the first week of treatment. Levels of interleukin-6 and ferritin were elevated in Patient 2 and Patient 3. Patient 2 was considered at particular risk for cytokine release syndrome given his high disease burden; indeed, biphasic cytokine release syndrome developed, with overlapping sepsis and fungal lung disease leading to treatment in intensive care. All three patients had multilineage cytopenia, which has been noted in other CAR7 studies,[13] a condition that was probably reflective of anti-CD7 activity against multilineage hematopoietic precursors that probably exacerbated infectious complications, including invasive fungal disease in Patient 2. Cellular immune deficiency after serotherapy and BE-CAR7 lymphodepleting activity was also associated with viral reactivations, including that of CMV, which were managed with antiviral medications. Otherwise, side effects and complications were similar to those associated with other CAR T-cell therapies[23] and were managed with the use of established transplant and cell-therapy protocols.

We report a proof-of-concept study that supports base editing as a therapeutic approach. Our data are consistent with the antileukemic effects of allogeneic CAR T cells, effects that are sufficient for securing remission and deep clearance of T-cell ALL. However, the strategy is not without risk: the immunosuppressive and cytopenic effects of the trial protocol are substantial, and immune-cell manipulation carries risks. In this study, subsequent allogeneic transplantation ensured donor-derived immune reconstitution and also limited the persistence of engineered cells. Additional safety measures could include the incorporation of a drug-inducible safety switch (or elimination system) to enable the controlled removal of infused cells once the useful therapeutic effects are derived or in the event of serious toxic effects.

This phase 1 study aims to recruit 10 children in the United Kingdom for an initial cohort, which includes the patients described here. Similar studies in the United States are in preparation,[24] as well as a related approach using anti-CD33 CAR T cells[25] for deep conditioning ahead of allogeneic stem-cell transplantation for relapsed acute myeloid leukemia in Europe.

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