Universal base-edited CAR7 T Cells for T-Cell Acute Lymphoblastic Leukemia (R1b)

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Abstract

Background

CD7 is an attractive target for CAR T-cell therapy in relapsed/refractory T-cell acute lymphoblastic leukemia (r/r T-ALL). We previously reported supportive 'first-in-human' experiences of base-edited CAR7 (BE-CAR7) T cells with triple C>T deamination-mediated knockouts of TCRαβ, CD52 and CD7.

Methods

Nine children <16-years with r/r T-ALL received BE-CAR7 T cells after lymphodepletion with fludarabine, cyclophosphamide and alemtuzumab in a Phase-1 setting (ISRCTN15323014). Two adults were treated under compassionate access arrangements. Participants achieving remission by D28 after BE-CAR7 T-cell infusion proceeded to allogeneic hematopoietic stem cell transplantation. Primary objectives related to safety and secondary objectives to duration of remission, disease-free and overall survival.

Results

Lymphodepletion and BE-CAR7 infusions were tolerable and circulating CAR7 T cells were detected in all patients. Complications included grade 1-4 cytokine release syndrome, transient skin rashes, multilineage cytopenia and opportunistic infections. All patients exhibited complete morphological remission with incomplete count recovery at D28. Nine patients (82%) achieved deep remissions (by flow and/or PCR) that allowed them to proceed to stem cell transplant, while two patients with quantifiable MRD in bone marrow received palliation. Transplant eliminated remaining BE-CAR7 T cells and supported donor-derived, multi-lineage reconstitution. Viral reactivations were frequent and three patients experienced significant virus-related morbidities post-transplant. Overall, 7/11 (63%) patients dosed are in ongoing remission 3-36 months after transplant, and CD7 negative leukemic escape was documented in 2 patients.

Conclusions

Universal BE-CAR7 T can induce leukemic remission for patients with r/r T-ALL allowing successful allogeneic hematopoietic stem cell transplant.

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Introduction

Patients with T-cell acute lymphoblastic leukemia (T-ALL) managed with standard chemotherapy protocols can anticipate good outcomes, but in case of induction failure or detectable minimal residual disease (MRD) after consolidation, allogeneic hematopoietic stem cell transplant (allo-SCT) is generally advised.[1] The risk of relapse after allo-SCT is higher, when pre-transplant MRD $>10^{-3}$, and deep remission with disease levels $<10^{-4}$ is generally required for transplant eligibility. Survival in patients relapsing after allo-SCT is poor with <15% long-term survival.[2] A number of CAR-T strategies targeting suitable antigens in T-ALL have been proposed,[3-7] and CD7 has emerged as one of the most compelling targets because, even while it is present on normal lymphocytes and hematopoietic precursors, expression is consistently high and stable on T-ALL blast populations. Fratricide effects between cells engineered to express anti-CD7 CARs have been addressed through the use of $CD7^{neg}$ T cell subsets,[8] or by co-expressing protein expression blockers (PEBL) to sequester and prevent CD7 expression at the cell surface.[9] Clinical phase testing using such approaches is underway both in the autologous and matched allogeneic setting.[10-17] Alternatively, genome editing had been applied to disrupt CD7 expression,[18] and has been combined with simultaneous disruption of T-cell receptor gene expression for the production of 'universal' donor CAR T-cells devoid of TCRαβ. [19-23] Recently, the application of base-editing (CRISPR-guided cytidine deamination) to introduce premature stop codons or modify critical splice sites for TCRαβ, CD7 and additional multiplexed knockout effects has been combined with lentiviral transduction of T cells with an anti-CD7 CAR **(Figure 1).**[20,24,25] Advantages of base editing over nuclease editing include minimizing karyotype aberrations or chromosomal translocations as previously reported for TALEN,[26,27] and CRISPR-Cas9 nuclease-mediated products.[28] We previously reported first-in-human experience of BE-CAR7 T cells infused to secure deep molecular remissions of refractory T-

ALL ahead of allo-SCT. Compelling outcomes in 2 of the first 3 children dosed are updated in this report of the completed phase 1 pediatric cohort, alongside additional experience from two 'compassionate-use' applications in adults with refractory T-ALL.

Methods

Base-Edited CAR7 T cells

Design, manufacture, characterization and release of BE-CAR7 T-cell banks was described previously.[24] All dosing used vials generated from the same registry donor (Anthony Nolan Stem Cell Registry, London) and were generated in a single campaign (GMP3) where 59% of CD45+ cells expressed CAR7, with 3.6 vector copies per cell (VCN) and residual TCRαβ expression of 0.1%, and cells were >99% $CD7^-$ and >98% $CD52^-$ **(Table S1**). Vials were cryopreserved in 10, 20 or 50 million cells aliquots of $CD45^+$ mononuclear cells.

Treatment schedule

A Phase 1 study (ISRCTN15323014) considered children aged between 6 months and 16 years with relapsed/refractory CD7+ T-ALL that was quantifiable in bone marrow ($>10^{-4}$ by flow cytometry or PCR). Two additional adult patients were treated under Specials license arrangements and managed using the same protocol. Exclusion criteria included progressive disease, uncontrolled infections, pre-existing graft versus host disease (GVHD) or presence of anti-HLA antibodies against BE-CAR7 batches **(Table S2)**. Patients received lymphodepletion with fludarabine (150 mg/sqm), cyclophosphamide (120 mg/kg < 16years; 1500mg/m2 >16 years) and alemtuzumab (1 mg/kg), followed by infusion within an allowed range of $0.2\text{-}2.0\text{x}10^6$ BE-CAR7 T cells/kg (with a maximum $5\text{x}10^4$/kg TCRαβ+ T cells to limit the risk of GVHD). Patients in MRD remission (by flow or PCR) by day 28 proceeded to allo-SCT, at which point any persisting BE-CAR7 T cells were depleted by conditioning regimens used ahead of transplant **(Figure 1B)**.

The study protocol, data sharing arrangements and statistical analysis plan are provided via the supplement at NEJM.org.

Results

Patient characteristics

Study recruitment started on 01/04/2022 and dosing was scheduled to complete on 31/05/2025. During this period 11 children (aged 5-15 years) were enrolled and screened, of whom 9 met eligibility criteria and were dosed by 31/05/2025 (Figure 1C). One child was excluded due to disease progression despite debulking, and the other deferred for further disease assessments. In addition, two females aged 38 and 28 years (Sp1, Sp2) were treated under specials license arrangements for compassionate access **(Table 1, Table S3)**. All dosed patients (6 females, 5 males) had been categorized as refractory T-ALL with quantifiable leukemia-associated immunophenotype (LAIP) assessed by flow cytometry as CD7 >99% in bone marrow **(Table S4**). All had previously received multiple lines of standard therapies and P001, P002 and Sp2 had undergone previous matched unrelated donor (MUD) transplantation(s), which had included conditioning with total body irradiation (12Gy TBI) **(Table S3).** At last relapse, P002 had documented CNS disease, and Sp2 had previous CNS and ocular involvement. Lymphodepletion was successfully completed for all patients **(Figure S1)** and infusions were well tolerated with children receiving 30-50x10^6 cells in total, and adults 100x10^6 total $CD45^+$ cells, comprising between 0.7-1.0 x10^6 $CAR7^+$ cells/kg, from the BE-CAR7(GMP3) bank [24]

Complications and toxicities

Between 1-5 days after BE-CAR7 T-cell infusion, nine patients (81%) developed grade 1-2 cytokine release syndrome (CRS) **(Figure 2A)** which was managed under standard institutional procedures, including biomarker tracking of interleukin-6 and ferritin **(Figures 2B, S2)**. Two patients (19%) experienced grade 3-4 CRS**;** P003 was described previously,[24] with high disease burden (80% bone marrow blasts) and disseminated fungal infections which likely contributed to inflammatory complications with elevated markers including ferritin and IL-6 despite anti-cytokine therapies and glucocorticoids**.** P004 also had a high marrow burden (50% bone marrow blasts) ahead of lymphodepletion and developed grade 2 CRS within one day of infusion which responded to tocilizumab but recurred at grade 3 from D7 with elevated ferritin and IL-6 requiring interventions with further tocilizumab, anakinra and transient inotrope support before resolving by D11 **(Figure S2)**. Concurrent with resolving CRS, all patients except P008 developed erythematous maculo-papular skin rashes between 6-10 days after BE-CAR7 T-cell infusion, which were managed with topical or systemic glucocorticoids where required and resolved by D21 in all patients **(Figure S3).** Where undertaken, skin biopsy changes were reported as non-specific without significant lymphocytic infiltration. Grade 1 immune effector cell-associated neurotoxicity syndrome (ICANs) was documented in three patients (P001, P003, P008) **(Figure 2A, Table 1)**.
As anticipated, multilineage cytopenia**,** with neutropenia **(Figure 2C),** lymphopenia **(Figure 2D),** and thrombocytopenia developed from lymphodepletion through to D28 and beyond to allo-SCT **(Figure S1)**. **Table 2** summarizes all adverse events (AEs) and serious adverse events (SAEs) recorded from the start of lymphodepletion to 28 days after BE-CAR7 T-cell infusion.

BE-CAR7 T-cell expansion and elimination

Multiparameter tracking of BE-CAR7 T cells in the circulation and bone marrow comprised, flow cytometry **(Figure S4)**, ddPCR for vector copy number (VCN) **(Figure 2E)**, and molecular chimerism signatures **(Figure S5)**. Despite lymphopenia with low absolute T cell counts, ddPCR mapped rising vector copies in DNA isolated from peripheral blood mononuclear cells (MNCs) within 7-14 days, and this was corroborated by chimerism (variable number tandem repeat signatures) analysis Flow cytometry captured the presence of circulating $CAR7^{+}TCR^{-}CD52^{-}CD7^{-}$ T cells in serial blood samples and in D28 marrow samples. Conditioning for transplant ensured elimination of BE-CAR7 T cells and absence of lentiviral ddPCR signal and switch to full donor chimerism was documented in all patients proceeding to transplant **(Figure 2ES5)**.

Anti-leukemic activity

Disease assessments were undertaken before the start of lymphodepletion with flow cytometric assessments to confirm >99% CD7 expression on bone marrow blasts and to quantify disease burden **(Table S4).** Nine of eleven patients had molecular markers suitable for PCR based quantification of MRD. Bone marrow assessments indicated that all patients had exhibited anti-leukemic responses with reductions in marrow disease resulting in morphological and flow remissions in the context of incomplete count recoveries and multilineage cytopenia. All patients in flow and/or PCR remission progressed to transplant with MRD $<10^{-4}$ in all except one child, P010 who continued to allo-SCT with MRD $<10^{-3}$ **(Table 1, Figure 2E, S8).** Transplant conditioning was reduced intensity (RIC) in patients who had a prior first transplant having received 12Gy TBI. In these cases, low dose 2Gy TBI and anti-thymocyte globulin (ATG) was included to ensure removal of BE-CAR7, building on previous strategies in the allo-CAR19 setting. Standard conditioning regimens for first transplants included 4-12Gy TBI and ATG, and all patients have sustained complete

remissions post -transplant except P008 who experienced CD7 negative relapse around 3 months after SCT **(Table 1)**.

P003 and P004 were found to have MRD $>10^{-3}$ by PCR and were not eligible for allo-SCT. Manifestations of extramedullary disease (EMD) involving the left orbit had first declared during the period of CRS experienced by P004, and localised swelling was investigated by magnetic resonance imaging and samples obtained by open biopsy **(Figure S9).** Flow cytometry detected the presence of CD7 negative leukemia, with similar blast populations also identified in D28 bone marrow and presence of disease corroborated by PCR based MRD quantification. Interestingly, presence of BE-CAR7 T cells was confirmed in both bone marrow and peripheral blood, but not in the orbital biopsy sample **(Figure S10)**. Next generation sequencing (NGS) did not detect evidence of escape mutations in exons 1-4 of CD7, and additional investigations will be required to elucidate underlying mechanisms of CD7 loss.

Viral complications and post-transplant immune reconstitution

Routine twice weekly surveillance for common viral infections was instituted due to predicted risk of reactivations after lymphodepletion and BE-CAR7. All patients had documented viraemia with adenovirus, Epstein Barr virus (EBV), cytomegalovirus (CMV) and human herpes virus (HHV)-6 detected frequently in blood and BK virus detected in urine (**Table 1**). Antiviral drugs were given where appropriate (cidofovir/brincidofovir for adenovirus, and ganciclovir/foscarnet for CMV) until T-cell immune recovery and cell-mediated clearance (**Figure 3A, S6**). In pediatric patients (P005, P008) and Sp2, adenovirus and BK reactivations contributed to compromised cardiorespiratory and renal function after transplant necessitating significant support. Sp2 required extended ventilatory assistance and ongoing dialysis following hemorrhagic cystitis, bladder perforation and renal compromise.

Post transplant, P001 experienced late (Month 11 after allo-SCT) reactivation of EBV associated with pericardial effusion which resolved after a short course of systemic glucocorticoids. Kinetics of neutrophil **(Figure 3B)** and lymphocyte **(Figure 3C)** recovery after SCT were broadly as anticipated with no evidence of possible BE-CAR7 activity on engraftment or reconstitution. All surviving patients achieved donor-derived engraftment within a month and stable lymphocyte recovery by around 2 months after allo-SCT (Mean 1.06, range 0.46-1.46 $x10^9$/L). Recovery of CD4 T-cell counts above 250/µl was considered the threshold for T-cell recovery sufficient to initiate withdrawal of prophylaxis for opportunistic infections **(Figure S11)**. Overall survival for all patients dosed ahead of the cut-off date 31/05/2025 was 73%, with 63% disease-free survival after accounting for one patient (P008) with $CD7^{neg}$ post-transplant relapse **(Figures 43D, 3E)**. Longer term studies have been initiated to track outcomes and provide safety monitoring for patients beyond transplant with the first patient now 36 months disease-free (**Figure 3F)**.

Discussion

We previously described how C>T base-editing was applied to generate BE-CAR7 T cells with triple knockouts for experimental therapy against T-ALL in three children enrolled into a first-in-class clinical trial.[24] Two of those patients, both of whom had relapsed after previous allo-SCT, are in ongoing, long-term, remissions. The initial experience provided compelling evidence of the potential medical applications of base editing. We now report primary safety objectives and efficacy data for the pediatric study cohort and additional data from two adult subjects treated using the same protocol under compassionate arrangements. The study design and strategy aimed to maximize the likelihood of successful outcomes even in a Phase-1 setting, including stringent criteria stipulating complete CD7 coverage across the entire blast population for inclusion, and exclusion of patients with pre-existing anti-HLA antibodies directed against the product. While two different donor banks had been established

before the study initiated, the trial so far has only drawn on a single donor for all the patients dosed to date, and this may have contributed to the remarkable consistency of responses encountered across the cohort. In most cases, patients developed fever followed by a generalized rash within a week of infusion, with elevations of non-specific indicators of inflammation such as ferritin, as well as specific biomarkers of CAR activity such as IL6. Interventions for CRS and ICANS, when required, followed standard institutional procedures for managing predictable CAR toxicities and prioritized anti-cytokine biologics to manage complications without potentially impeding anti-leukemic activity. Timing and resolution of skin rashes suggested an immunological basis and could have been mediated by residual TCR$\alpha\beta^{+}$ allo-reactive T cells or tracking of $CD7^{neg}$ T cells to the skin, but no evidence of lymphocytic infiltration in biopsy samples argues against direct mediation by the adoptively transferred effector cells. Most episodes resolved as patients defervesced. Cutaneous manifestations after autologous CAR therapies are estimated to arise in up to 5% of commercial products, but much higher incidences have been reported in some studies.[29] We recognized the importance of debulking leukemia ahead of CAR7 infusion to reduce the risk of serious CRS or neurotoxicity, while noting that similar issues were not encountered when using autologous CAR7 products, perhaps reflecting more variable cell fitness and potency in that setting.[30] Allogeneic cells also have advantages in mitigating against the possibility of blast transduction by lentiviral vectors during CAR T-cell manufacture which could result in CAR7 expression and CD7 antigen masking, as reported for CAR19 vectors targeting $CD19^{+}$ B-ALL.[31] Finally, autologous T cells could harbor underlying genetic predispositions that are of heightened concern for transformation risk following a small number of secondary cancers associated with approved CAR T products.[32]

CAR7 products can be generated without gene editing by transducing T-cell subsets that are naturally CD7 negative or have masked or sequestrated CD7 after expression of

CAR7. Studies have reported morphological remissions of T-ALL using such an approach, but progression-free survival was better in patients who underwent subsequent transplant.[10,11] Another strategy has employed anti-CD7 protein expression blockers (PEBL) for targeted capture and restriction of CD7 expression to address fratricide effects.[12,13] One such study recently reported MRD negativity in 16/17 subjects[30], and similar CAR7 T cells manufactured from matched stem-cell donors delivered complete remissions in 18/20 patients, with seven then proceeding to stem-cell transplantation.[14,15] In the HLA mismatched setting, haploidentical parental donors have been used for the generation of allogeneic CAR7 T cells ahead of transplant from the same donor.[16] Entirely non-matched 'universal' allogeneic donor strategies have exploited additional genome editing steps using CRISPR/Cas9 to disrupt endogenous TCRαβ and CD7 expression to achieve complete remissions.[21,33,22,23,34] All these approaches documented cytopenia or marrow aplasia effects in clinical settings, likely to be multifactorial due to chemotherapy, cytokine release and direct anti-CD7 activity against myeloid precursors, T cells and NK cells.[35] [36] Such effects were exploited in an "all-in-one" strategy where 10 patients who achieved complete remission (CR) and experienced pancytopenia after CAR7 were swiftly transplanted without additional conditioning or GVHD prophylaxis from haploidentical donors resulting in 68% survival at 12 months.[37] Inevitably, protracted lymphopenia and neutropenia clearly increase risks for infectious complications including with opportunistic pathogens and viral reactivations. Complications were previously noted when using similarly augmented lymphodepletion incorporating alemtuzumab in the setting allogeneic genome edited CAR T cells for B cell malignancies.[38] With additional CAR7 effects contributing additional immunosuppressive effects, we documented frequent reactivation of latent viruses such as adenovirus, BK and HHV6 as well as fungus related complications. While intense surveillance and prophylaxis offered mitigations, and the study was designed to reduce risk

through transplant and donor derived reconstitution, virus related complications extending into the post-transplant period caused significant morbidity in two children and an adult. Nonetheless, our experience suggests that CAR7 anti-leukemic effects peak within two weeks, and once CRS has resolved, it is feasible to move rapidly to transplant to secure prompt donor derived reconstitution to address infectious complications. The conditioning strategies for transplant are also critical and were based on standard-of-care approaches for T-ALL, which routinely include 12Gy TBI, except in small infants. Otherwise, reduced intensity regimens were used to promote rapid engraftment and recovery rather than provide additional antileukemic effects. We also exploited T-cell replete umbilical cord blood (UCB) transplants, which can be tailored to support accelerated T-cell recovery from naïve T cells carried in the graft. Patients with longer follow up have documented recovery of T-cell compartments without significant GVHD. Infrequently, $CD7^{neg}$ escape phenomena have been documented in clinical studies, and in handful of cases, underlying mechanisms have been elucidated including frameshift and missense mutations.[15,30]We verified integrity of exonic CD7 sequences by NGS in a child with low level $CD7^{neg}$ disease in bone marrow after BE-CAR7 and in emergent extramedullary disease in an orbit. Further investigations will be required to interrogate epigenetic factors involving DNA methylation and histone acetylation of CD7 that may have downregulated CD7 in primary T-ALL[39,40] and to determine if pre-existing $CD7^{neg}$ leukemic clones were present below detection thresholds before therapy, or arose spontaneously and selectively expanded under pressure from BE-CAR7.

Finally, next generation universal CAR iterations are in development using advanced base editors, with compact architecture and improved editing fidelity.[41] Additional multiplexed base editing to promote immunological stealth by disruption of HLA class I and II disruption may provide an alternative to intense lymphodepletion, serotherapy and attendant risks of viral reactivation.[42] The strategy will also be further refined to minimize the

period of CAR7 exposure and to foster a seamless passage into allo-transplant as soon as effects of cytokine release resolve. In the meantime, this early phase experience supports further investigations of BE-CAR7 for the treatment of r/r T-ALL in both children and adults through extended trial cohorts. Among the open questions are the molecular basis of the CD7- relapses, whether alemtuzumab could be eliminated from the preparative regimen to reduce viral reactivation without diminishing antitumor effects, and would BE-CAR7 cells generated from other donors be comparably effective.

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

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References

1. Teachey DT, O'Connor D. How I treat newly diagnosed T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma in children. Blood 2020;135(3):159-166. DOI: 10.1182/blood.2019001557.
2. Kuhlen M, Willasch AM, Dalle JH, et al. Outcome of relapse after allogeneic HSCT in children with ALL enrolled in the ALL-SCT 2003/2007 trial. British journal of haematology 2018;180(1):82-89. DOI: 10.1111/bjh.14965.
3. Mamonkin M, Rouce RH, Tashiro H, Brenner MK. A T-cell-directed chimeric antigen receptor for the selective treatment of T-cell malignancies. Blood 2015;126(8):983-92. DOI: 10.1182/blood-2015-02-629527.
4. Rasaiyaah J, Georgiadis C, Preece R, Mock U, Qasim W. TCRalphabeta/CD3 disruption enables CD3-specific antileukemic T cell immunotherapy. JCI Insight 2018;3(13). DOI: 10.1172/jci.insight.99442.
5. Sanchez-Martinez D, Baroni ML, Gutierrez-Aguera F, et al. Fratricide-resistant CD1a-specific CAR T cells for the treatment of cortical T-cell acute lymphoblastic leukemia. Blood 2019;133(21):2291-2304. DOI: 10.1182/blood-2018-10-882944.
6. Maciocia PM, Wawrzyniecka PA, Maciocia NC, et al. Anti-CCR9 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia. Blood 2022;140(1):25-37. DOI: 10.1182/blood.2021013648.
7. Xiang J, Devenport JM, Carter AJ, et al. An "off-the-shelf" CD2 universal CAR-T therapy for T-cell malignancies. Leukemia 2023;37(12):2448-2456. DOI: 10.1038/s41375-023-02039-z.
8. Watanabe N, Mo F, Zheng R, et al. Feasibility and preclinical efficacy of CD7-unedited CD7 CAR T cells for T cell malignancies. Molecular therapy : the journal of the American Society of Gene Therapy 2023;31(1):24-34. DOI: 10.1016/j.ymthe.2022.09.003.
9. Kamiya T, Wong D, Png YT, Campana D. A novel method to generate T-cell receptor-deficient chimeric antigen receptor T cells. Blood Adv 2018;2(5):517-528. DOI: 10.1182/bloodadvances.2017012823.
10. Zhao L, Li C, Zuo S, et al. Autologous CD7 CAR-T cells generated without T cell pre-selection in pediatric patients with relapsed/refractory T-ALL: A phase I trial. Molecular therapy : the journal of the American Society of Gene Therapy 2024. DOI: 10.1016/j.ymthe.2024.09.006.
11. Cao XY, Zhang JP, Lu Y, et al. A safety and efficacy study of allogeneic haematopoietic stem cell transplantation for refractory and relapsed T-cell acute lymphoblastic leukaemia/lymphoblastic lymphoma patients who achieved complete remission after autologous CD7 chimeric antigen receptor T-cell therapy. British journal of haematology 2024;204(6):2351-2364. DOI: 10.1111/bjh.19445.
12. Xiao J, Liu Y, Shen Y, et al. Clinical Outcomes of CD7 CAR-T Cell Therapy in Relapsed or Refractory T-Cell Acute Lymphoblastic Leukemia and Lymphoblastic Lymphoma Patients. Hematol Oncol 2025;43(3):e70081. DOI: 10.1002/hon.70081.
13. Li Z, Zheng Q, Yang K, et al. CD7 CART Therapy Bridging Allo-HSCT Remarkably Improves Long-Term DFS in Refractory/Relapsed T-ALL/LBL. Transplant Cell Ther 2025;31(2):73 e1-73 e11. DOI: 10.1016/j.jtct.2024.11.009.
14. Pan J, Tan Y, Wang G, et al. Donor-Derived CD7 Chimeric Antigen Receptor T Cells for T-Cell Acute Lymphoblastic Leukemia: First-in-Human, Phase I Trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2021:JCO2100389. DOI: 10.1200/JCO.21.00389.

15. Tan Y, Shan L, Zhao L, et al. Long-term follow-up of donor-derived CD7 CAR T-cell therapy in patients with T-cell acute lymphoblastic leukemia. J Hematol Oncol 2023;16(1):34. DOI: 10.1186/s13045-023-01427-3.
16. Song Y, Liu Z, Wang Q, Gao K, Wu T. Case report: Two pediatric cases of long-term leukemia-free survival with relapsed acute T-lymphoblastic leukemia treated with donor CD7 CAR-T cells bridging to haploidentical stem cell transplantation. Front Immunol 2024;15:1333037. DOI: 10.3389/fimmu.2024.1333037.
17. Pan J, Zhao L, Zhang Y, et al. Donor-derived CD7 CAR T cells for pediatric and adult relapsed/refractory T-ALL/LBL: a phase 2 trial. Blood 2025. DOI: 10.1182/blood.2025029299.
18. Gomes-Silva D, Srinivasan M, Sharma S, et al. CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. Blood 2017. DOI: 10.1182/blood-2017-01-761320.
19. Cooper ML, Choi J, Staser K, et al. An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies. Leukemia 2018;32(9):1970-1983. DOI: 10.1038/s41375-018-0065-5.
20. Georgiadis C, Rasaiyaah J, Gkazi SA, et al. Base-edited CAR T cells for combinational therapy against T cell malignancies. Leukemia 2021. DOI: 10.1038/s41375-021-01282-6.
21. Li S, Wang X, Yuan Z, et al. Eradication of T-ALL Cells by CD7-targeted Universal CAR-T Cells and Initial Test of Ruxolitinib-based CRS Management. Clinical cancer research : an official journal of the American Association for Cancer Research 2021;27(5):1242-1246. DOI: 10.1158/1078-0432.CCR-20-1271.
22. Hu Y, Zhou Y, Zhang M, et al. Genetically modified CD7-targeting allogeneic CAR-T cell therapy with enhanced efficacy for relapsed/refractory CD7-positive hematological malignancies: a phase I clinical study. Cell Research 2022;32(11):995-1007. DOI: 10.1038/s41422-022-00721-y.
23. Ghobadi A, Aldoss I, Maude SL, et al. Phase 1/2 Trial of Anti-CD7 Allogeneic WU-CART-007 in patients with Relapsed/Refractory T-cell Malignancies. Blood 2025. DOI: 10.1182/blood.2025028387.
24. Chiesa R, Georgiadis C, Syed F, et al. Base-Edited CAR7 T Cells for Relapsed T-Cell Acute Lymphoblastic Leukemia. The New England journal of medicine 2023;389(10):899-910. DOI: 10.1056/NEJMoa2300709.
25. Diorio C, Murray R, Naniong M, et al. Cytosine base editing enables quadruple-edited allogeneic CART cells for T-ALL. Blood 2022;140(6):619-629. DOI: 10.1182/blood.2022015825.
26. Qasim W, Zhan H, Samarasinghe S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Science translational medicine 2017;9(374). DOI: 10.1126/scitranslmed.aaj2013.
27. Benjamin R, Graham C, Yallop D, et al. Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. Lancet 2020;396(10266):1885-1894. DOI: 10.1016/S0140-6736(20)32334-5.
28. Ottaviano G, Georgiadis C, Gkazi S, et al. Phase 1 clinical trial of CRISPR-engineered CAR19 universal T cells for treatment of children with refractory B cell leukemia. Science translational medicine 2022;14(668). DOI: 10.1126/scitranslmed.abq3010.
29. Nusbaum KB, Dulmage B, Choi JN, Jaglowski SM, Korman AM. Cutaneous manifestations of chimeric antigen receptor T-cell therapy: An introduction for

dermatologists. Journal of the American Academy of Dermatology 2022;87(3):597-604. DOI: https://doi.org/10.1016/j.jaad.2021.07.017.
30. Oh BLZ, Shimasaki N, Coustan-Smith E, et al. Fratricide-resistant CD7-CAR T cells in T-ALL. Nat Med 2024;30(12):3687-3696. DOI: 10.1038/s41591-024-03228-8.
31. Ruella M, Xu J, Barrett DM, et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. Nat Med 2018. DOI: 10.1038/s41591-018-0201-9.
32. Ottaviano G, Qasim W. Current landscape of vector safety and genotoxicity after hematopoietic stem or immune cell gene therapy. Leukemia 2025;39(6):1325-1333. DOI: 10.1038/s41375-025-02585-8.
33. Li S, Wang X, Liu L, et al. CD7 targeted “off-the-shelf” CAR-T demonstrates robust in vivo expansion and high efficacy in the treatment of patients with relapsed and refractory T cell malignancies. Leukemia 2023;37(11):2176-2186. DOI: 10.1038/s41375-023-02018-4.
34. Ghobadi A, Aldoss I, Maude S, et al. Anti-CD7 allogeneic WU-CART-007 in patients with relapsed/refractory T-cell acute lymphoblastic leukemia/lymphoma: a phase 1/2 trial. Res Sq 2024. DOI: 10.21203/rs.3.rs-4676375/v1.
35. Kriegsmann K, Loffler H, Eckstein V, et al. CD7 is expressed on a subset of normal CD34-positive myeloid precursors. Eur J Haematol 2018;101(3):318-325. DOI: 10.1111/ejh.13100.
36. Milush JM, Long BR, Snyder-Cappione JE, et al. Functionally distinct subsets of human NK cells and monocyte/DC-like cells identified by coexpression of CD56, CD7, and CD4. Blood 2009;114(23):4823-31. DOI: 10.1182/blood-2009-04-216374.
37. Hu Y, Zhang M, Yang T, et al. Sequential CD7 CAR T-Cell Therapy and Allogeneic HSCT without GVHD Prophylaxis. The New England journal of medicine 2024;390(16):1467-1480. DOI: 10.1056/NEJMoa2313812.
38. Guardo D, Mishra AK, Rashed H, et al. Long term outcomes of genome edited 'universal' CAR19 T-cells for relapsed/refractory B-ALL at a single pediatric centre. Blood Advances 2025. DOI: 10.1182/bloodadvances.2025016366.
39. Rogers SL, Zhao Y, Jiang X, Eaves CJ, Mager DL, Rouhi A. Expression of the leukemic prognostic marker CD7 is linked to epigenetic modifications in chronic myeloid leukemia. Mol Cancer 2010;9:41. DOI: 10.1186/1476-4598-9-41.
40. Rohrs S, Scherr M, Romani J, Zaborski M, Drexler HG, Quentmeier H. CD7 in acute myeloid leukemia: correlation with loss of wild-type CEBPA, consequence of epigenetic regulation. J Hematol Oncol 2010;3:15. DOI: 10.1186/1756-8722-3-15.
41. Neugebauer ME, Hsu A, Arbab M, et al. Evolution of an adenine base editor into a small, efficient cytosine base editor with low off-target activity. Nature biotechnology 2022. DOI: 10.1038/s41587-022-01533-6.
42. Georgiadis C, Preece R, Qasim W. Clinical development of allogeneic chimeric antigen receptor alphabeta-T cells. Molecular therapy : the journal of the American Society of Gene Therapy 2025. DOI: 10.1016/j.ymthe.2025.03.040.

Figures

Figure 1. **A)** Base editing by CRISPR-guided cytidine deamination was used for multiplexed editing ahead of lentiviral transduction with CAR7 of T cells collected from a healthy volunteer donor using a largely automated process on a CliniMacs Prodigy device. Precise C>T conversions introduced premature stop codons or disrupted splice sites at high efficiency following off-device electroporation. Removal of cell surface CD7 prevented fratricide and CD52 negative cells evaded alemtuzumab. Following expansion, residual TCRαβ T cells were depleted by magnetic bead selection to prevent graft versus host disease (GVHD). Finally, cells were cryopreserved in dose-banded aliquots and submitted for quality control and release assessments. **B)** Subjects were consented and screened around two weeks ahead of lymphodepletion which comprised fludarabine, cyclophosphamide, and alemtuzumab. BE-CAR7 infusion was undertaken on D0 and patients were managed in hospital until bone marrow assessments on D28. Subjects in remission (target MRD $<10^{-4}$) proceeded to allogeneic stem-cell transplantation (allo-SCT) followed a conditioning step to remove BE-CAR7 T cells and clear bone marrow niches. Trial monitoring continued post-transplant for 12 months before enrolment into a long term follow up study. **C)** Consort diagram of patients enrolled, screened and dosed with BE-CAR7 T cells. NHS eligible paediatric patients in the UK and Eire were considered at national leukemia panels. Referrals were eligible for UK National Health Service (NHS) care, including under S2 reciprocal care arrangements with Europe. Two additional adults were dosed under specials licence arrangements for compassionate use and were managed under the same protocol. LD, lymphodepletion; SCT, stem cell transplant; TRM, transplant related mortality; DRM, disease related mortality

Figure 2. A) Major toxicities encountered for all subjects during the investigational period from BE-CAR infusion to day 28, with cytokine release syndrome (CRS), rash and immune cell associated neurotoxicity (ICANS). CRS ranging from grade 1 to grade 3 in all subjects with only one subject experiencing grade 4 CRS. All patients except one developed transient rash, either resolving spontaneously or requiring steroids. **B)** Serum IL-6 as a biomarker of CRS became elevated within 7-14 days after BE-CAR7 T cells. **C)** Neutropenia followed lymphodepletion and in most cases continued beyond D28. **D)** Lymphopenia following chemotherapy and BE-CAR7 T cells was expected and continued beyond D28 in all patients. Red-lines represent means of individuals samples collected at the timepoints marked.

E)Quantification of vector copy number by digital droplet PCR (ddPCR) confirmed BE-CAR7 T cells were circulating even during lymphopenia until allo-SCT, after which no further signals were detected. F) All dosed patients exhibited morphological complete remissions and all but three achieved minimal residual disease (MRD) remissions $<10^{-4}$ by flow and/or PCR.

Figure 3. Viral reactivations were frequent during the investigational and transplant lymphopenic periods. **A)** Adenovirus reactivations were detected in blood from around D14 after BE-CAR7 and were managed with antiviral therapy until post-transplant reconstitution, with protracted complications in P005, P008 and Sp2. **B)** Kinetics neutrophil and **C)** lymphocyte recovery were as expected following allo-SCT, with no evidence of persistence or impact from BE-CAR7 T cells. **D)** Overall survival (OS) for all dosed subjects and **E)** cumulative incidence of leukemia progression or relapse over time. F) Swimmer plot summary for all dosed patients and their status during BE-CAR7 therapy and following transplant (Triangle) with the first subject now 36 months disease-free.

Table 1. Patient characteristics and summary of study interventions and outcomes.

MPAL Mixed phenotype acute leukemia; ETP: early T-cell precursor leukemia; N-ETP: Near ETP; Y Yes; N No; F Fludarabine; C Cyclophosphamide; A: Alemtuzumab; G Grade; ADV Adenovirus; BK BK virus; HHV6 Human Herpes virus-6; Neg: Negative; HC Hypocellular marrow; ANA Assay not available; N/A Not applicable; MUD Matched unrelated donor; MMUD Mismatched unrelated donor; UCB Umbilical cord blood; T Treosulfan; E Etoposide; M Melphalan; Rtx Rituximab; CR Complete remission; DRM Disease related mortality; TRM Transplant related mortality

Pt	P001	P002	P003	P004	P005	P007	P008	P009	P010	Sp1	Sp2
Diagnosis	T-ALL	MPAL	T-ALL	T-ALL	ETP	ETP	N-ETP	T-ALL	T-ALL	T-ALL	T-ALL
Previous SCT	Y	Y	N	N	N	N	N	N	N	N	Y
Pre-LD (LAIP)	9%	0.51%	86%	53%	3%	0.18%	15%	0.5%	0.02%	63%	8.4%
Lymphodepletion mg/kg/*mg/m2	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C120 A1	F150 * C1500* A1	F150 * C1500* A1
Cell Dose x10^6/kg	0.7	0.9	1.0	0.8	0.8	0.8	0.7	0.9	0.8	0.8	0.9
CRS	G2	G2	G4	G3	G1	G2	G2	G1	G2	G2	G2
ICANS	G1	N	G1	N	N	N	G1	N	N	N	N
Viral reactivations	BK CMV EBV HHV6	ADV BK CMV EBV HHV6	BK	ADV HHV6	ADV BK	ADV BK EBV HHV6	ADV BK HHV6	ADV HHV6	ADV RSV	ADV BK CMV EBV	ADV BK EBV
Day 28 BM flow	HC	HC	Neg	HC	Neg	Neg	Neg	HC	Neg	HC	Neg
Day 28 BM PCR	$<10^{-4}$	$<10^{-4}$	$>10^{-2}$	$>10^{-2}$	ANA	ANA	$<10^{-4}$	$<10^{-4}$	$4x10^{-4}$	ANA	$<10^{-4}$
Allo-SCT Donor	10/10 MUD	10/10 MUD	N/A	N/A	9/10 MMUD	9/10 UCB	8/10 UCB	10/10 UCB	Haplo	10/10 MUD	10/10 MUD
Chemotherapy mg/kg/ *mg/m2	F160* C120	F120* C120	N/A	N/A	E60	F150* T42000*	E60	E60	E60 Rtx200*	C120	F150* M140*
ATG	Y	Y	N/A	N/A	Y	N	Y	N	Y	N	Y
Radiotherapy	2 Gy	2 Gy	N/A	N/A	12 Gy	4 Gy	12 Gy	8 Gy	12 Gy	13.2 Gy	N/A
Outcome	CR	CR	DRM	CD7neg DRM	TRM	CR	CD7neg Relapse	CR	CR	CR	CR

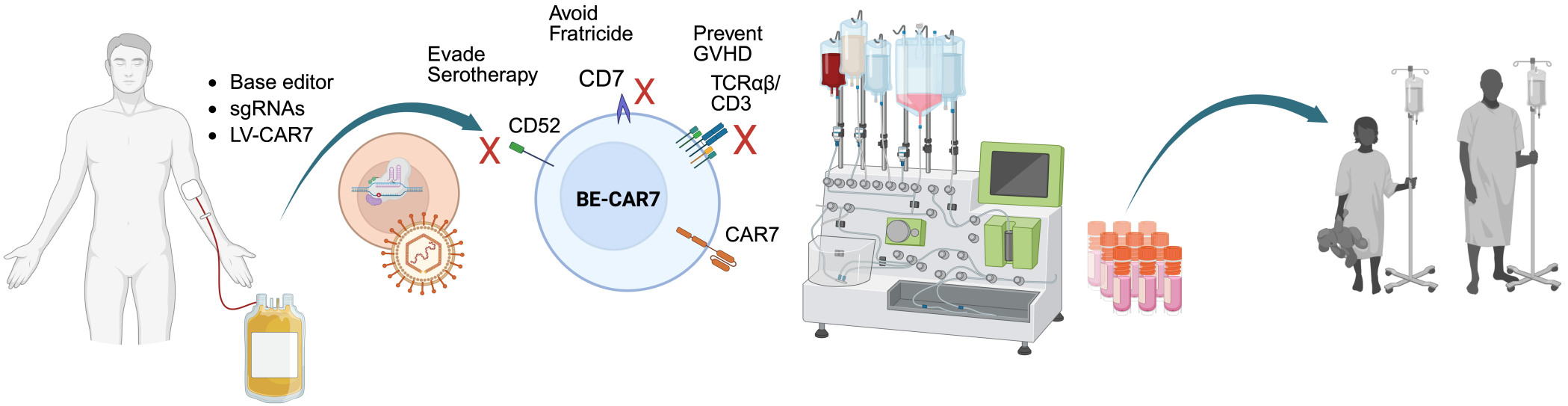
Table 2: Summary of adverse events

Summary of adverse events during investigational period from the start of lymphodepletion to Day 28 following BE-CAR7 infusion. Complications arsing during subsequent allo-SCT were considered unrelated to the investigational product in the absence of BE-CAR7 persistence after conditioining. CRS Cytokine Release Syndrome; G Maximum grade; ICANS Immune Effector Cell Associated Neurotoxicity Syndrome; ICAHT Immune cell associated haemotoxicity; Asp Aspergillosis; *radiological suspicion; Pl.Efn Pleural effusion; Pancr Pancreatitis.

Patients	**P001**	**P002**	**P003**	**P004**	**P005**	**P007**	**P008**	**P009**	**P010**	**Sp1**	**Sp2**
CRS	G2	G2	G4	G3	G1	G2	G2	G1	G2	G2	G2
ICANS	G1		G1				G1				
Skin rash	G1	G3	G1	G2	G2	G2		G2	G2	G1	G2
ICAHT	G4	G4	G4	G4	G4	G4	G4	G4	G4	G4	G4
Viraemia	G3	G2	G1	G1	G2	G2		G2	G2	G3	G1
Bacteriamia	G4						G3			G3	G3
Aspergillus			G5	*G3							
Liver Function Derangement		G1	G3	G1		G2	G3		G2		
Renal/Elec Derangement	G1	G1	G3	G1		G2	G3		G3		
Coagulopathy	G1		G3	G1			G4		G1	G2	
Other AEs >G3			Pl.Efn G4		Pancr G3						

Figure 1A

Allogeneic T cells



Healthy volunteer donor **Automated manufacture** **Multiple recipients**

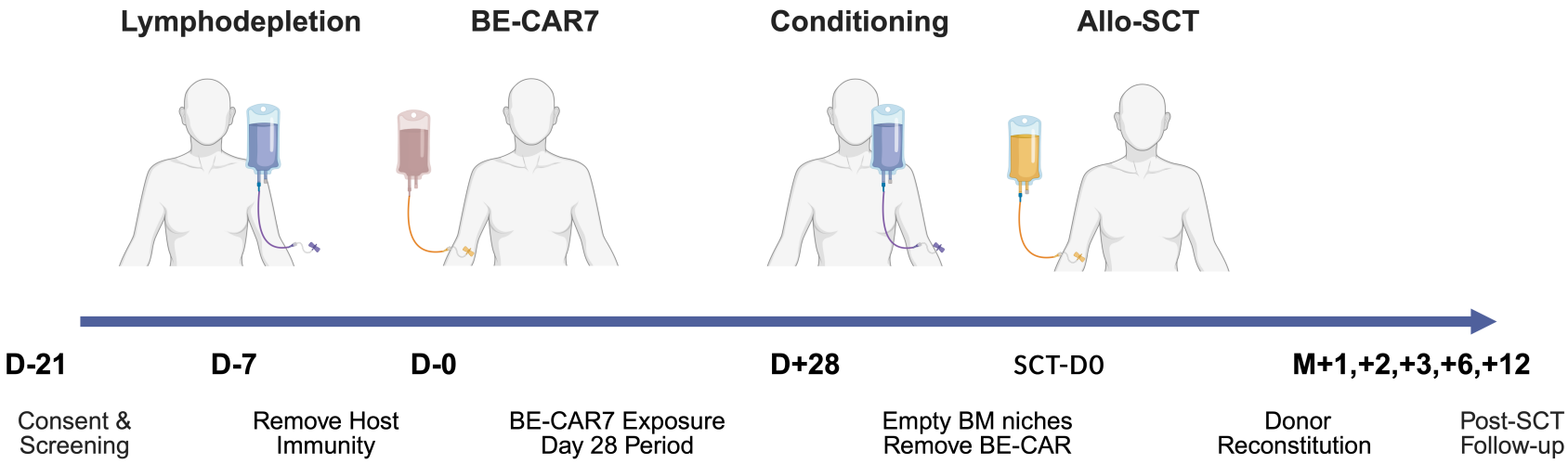

Lymphodepletion
BE-CAR7
Conditioning
Allo-SCT
D-21
D-7
D-0
D+28
SCT-D0
M+1,+2,+3,+6,+12
Consent & Screening
Remove Host Immunity
BE-CAR7 Exposure Day 28 Period
Empty BM niches Remove BE-CAR
Donor Reconstitution
Post-SCT Follow-up

Figure 1C **Flow diagram of patients enrolled, screened and dosed with BE-CAR7 T cell**

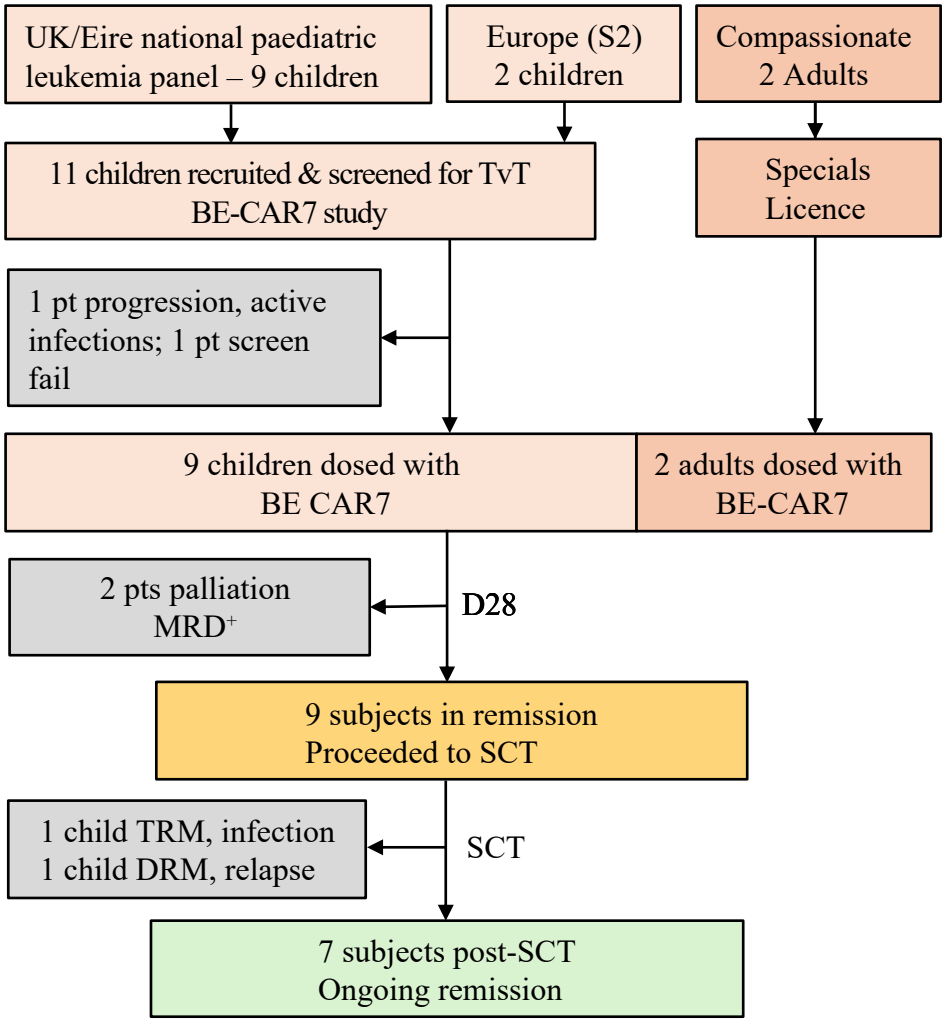


Figure 2

Figure 2A. Cytokine release, rash and neurotoxicity

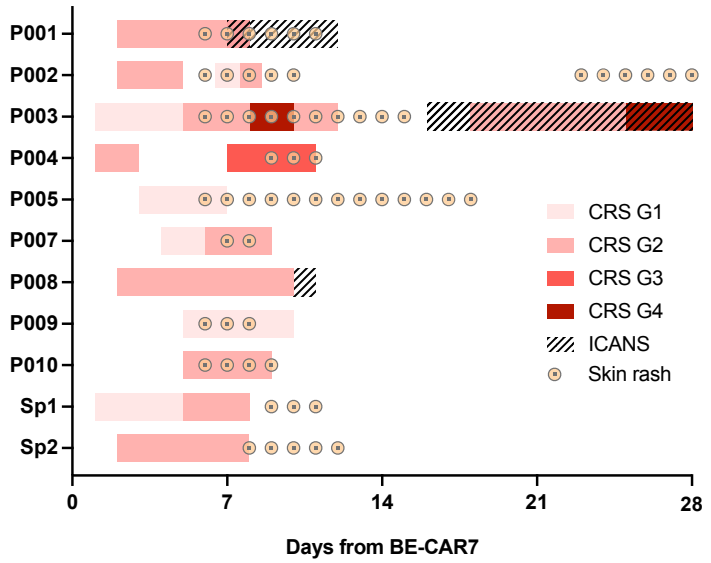


Figure 2B. IL-6 levels after BE-CAR7

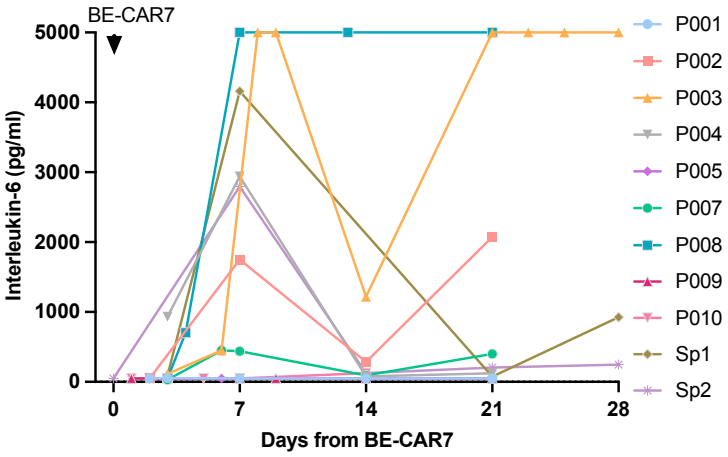


Figure 2C. Neutrophil counts after BE-CAR7

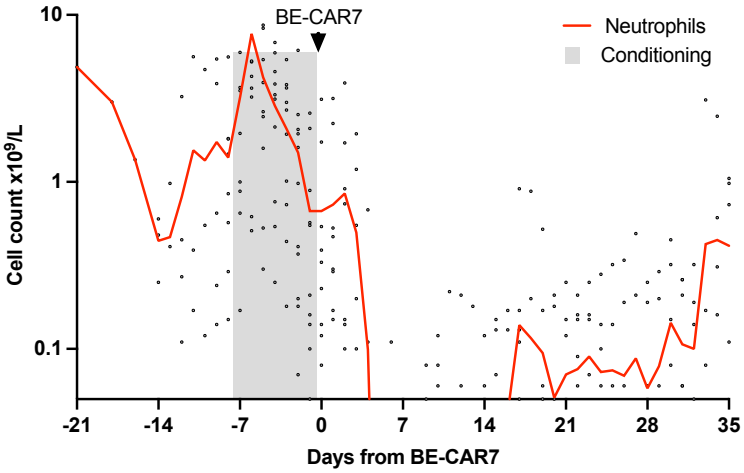


Figure 2D. Lymphocyte counts after BE-CAR7

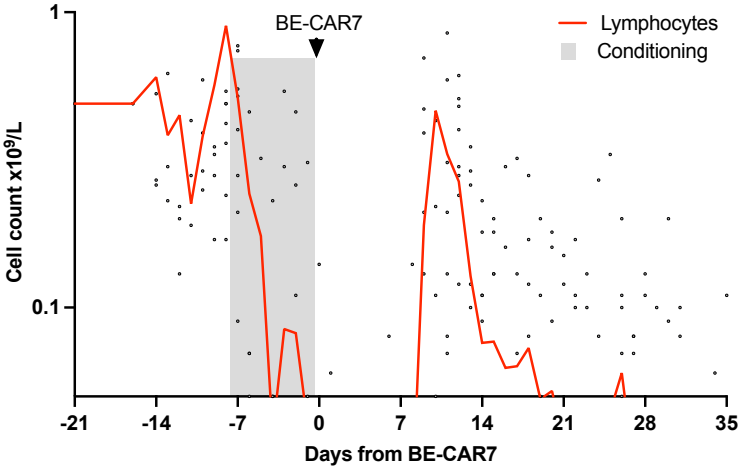


Figure 2E. BE-CAR7 tracking by vector copies

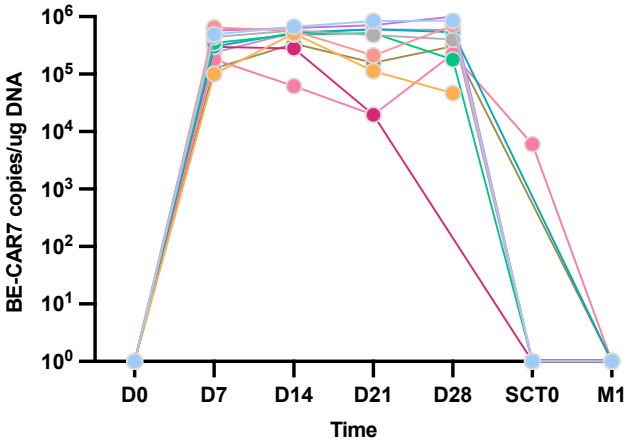


Figure 2F. MRD before and after BE-CAR7

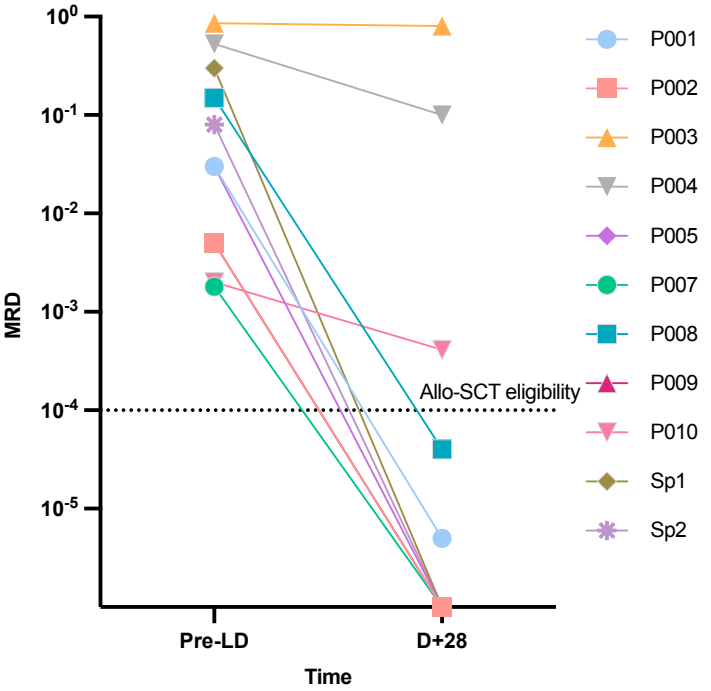


Figure 3

Figure 3A. Adenoviraemia after BE-CAR7

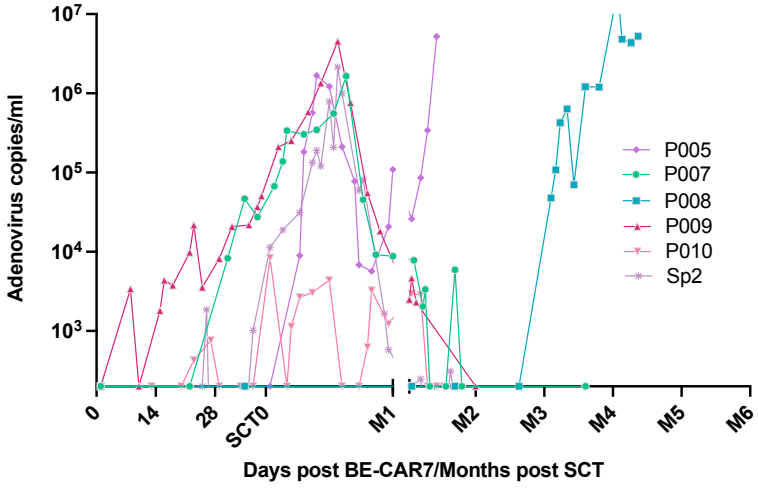


Figure 3B. Neutrophil engraftment after SCT

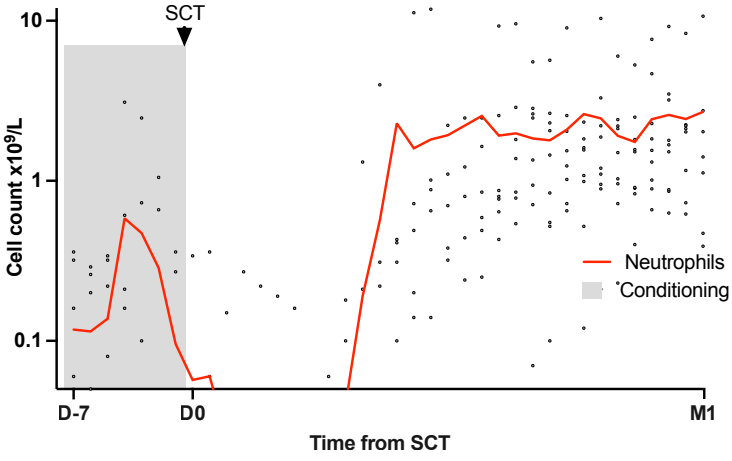


Figure 3C. Lymphocyte count post SCT

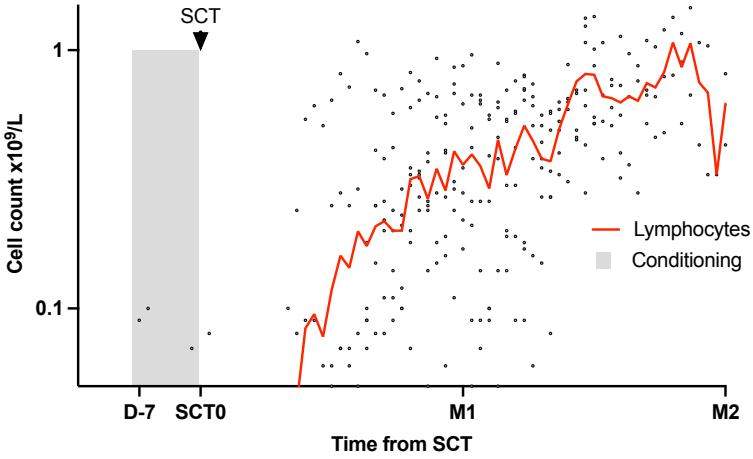


Figure 3D. Overall Survival after BE-CAR7

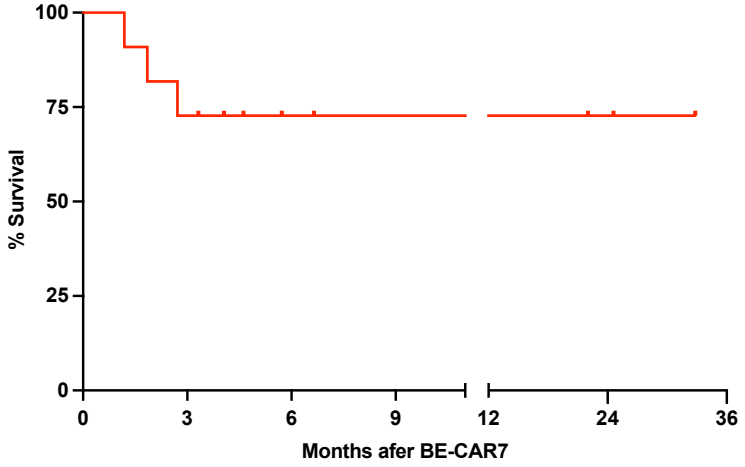


Figure 3E. Cumulative incidence of r/r T-ALL

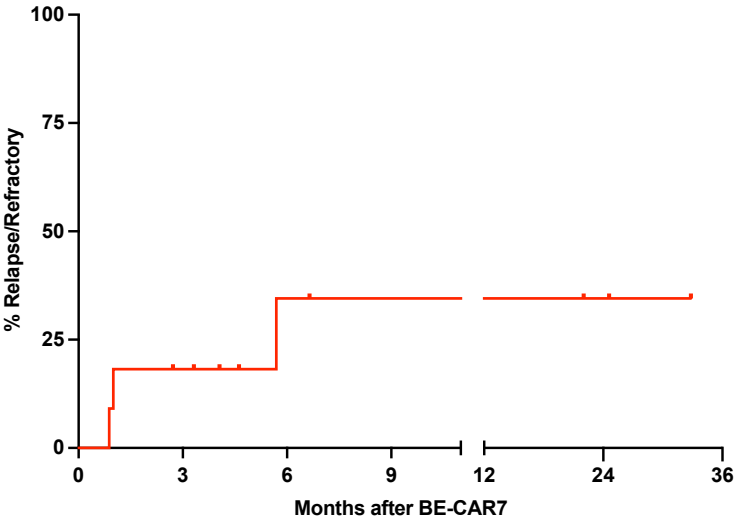


Figure 3F. Swimmer plot summary after BE-CAR7