Outcome of donor-derived TAA T cell therapy in patients with high-risk or relapsed acute leukemia post allogeneic BMT

Tracking no: ADV-2021-006831R1

Hannah Kinoshita (Childrens National Medical Center, United States) Kenneth Cooke (Johns Hopkins University School of Medicine, United States) Melanie Grant (Emory University School of Medicine, United States) Maja Stanojevic (Children's National Hospital, United States) Conrad Russell Cruz (Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, United States) Michael Keller (Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, United States) Maria Fortiz (Childrens National Medical Center, United States) Fahmida Hoq (CNMC, United States) Haili Lang (Children's National Health System, United States) A. John Barrett (George Washington University, United States) Hua Liang (The George Washington University, United States) Jay Tanna (Childrens National Medical Center, United States) Nan Zhang (Children's National Medical Center, United States) Anushree Datar (Childrens National Medical Center, United States) Kenneth Fulton (Childrens National Medical Center, United States) Divyesh Kukadiya (Childrens National Medical Center, United States) Anqing Zhang (Division of Biostatistics and Study Methodology, The George Washington University, United States) Kirsten Williams (Emory University, United States) Hema Dave (Children's National Hospital, United States) Jeffrey Dome (Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, United States) David Jacobsnh (Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, United States) Patrick Hanley (Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, United States) Richard Jones (Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, United States) Catherine Bollard (Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, United States)

Abstract:
Patients with hematologic malignancies relapsing after allogeneic blood or marrow transplantation (BMT) have limited response to conventional salvage therapies with an expected 1-year overall survival (OS) of <20%. We evaluated the safety and clinical outcomes following administration of a novel T-cell therapeutic targeting three tumor associated antigens (TAA-T) in patients with acute leukemia who relapsed or were at high-risk of relapse after allogeneic BMT. Lymphocytes obtained from the BMT donor were manufactured to target TAAs; WT1, PRAME and survivin, which are over-expressed and immunogenic in most hematologic malignancies. Patients received TAA-T infusions at doses of 0.5-4x10^7/m2. Twenty-three BMT recipients with relapsed/refractory (n=11) and/or high-risk (n=12) acute myeloid leukemia (n=20) and acute lymphoblastic leukemia (n=3) were infused post-transplant. No patient developed cytokine-release syndrome or neurotoxicity, and only one patient developed grade III GVHD. Of the patients who relapsed post-BMT and received bridging therapy, the majority (n=9/11) achieved complete hematologic remission before receiving TAA-T. Lymphocytes obtained from the BMT donor were manufactured to target TAAs; WT1, PRAME and survivin, which are over-expressed and immunogenic in most hematologic malignancies. Patients received TAA-T infusions at doses of 0.5-4x10^7/m2. Twenty-three BMT recipients with relapsed/refractory (n=11) and/or high-risk (n=12) acute myeloid leukemia (n=20) and acute lymphoblastic leukemia (n=3) were infused post-transplant. No patient developed cytokine-release syndrome or neurotoxicity, and only one patient developed grade III GVHD. Of the patients who relapsed post-BMT and received bridging therapy, the majority (n=9/11) achieved complete hematologic remission before receiving TAA-T. Relapsed patients exhibited a 1-year OS of 36% and 1-year leukemia-free survival of 27.3% post-TAA-T. The poorest prognosis patients (relapsed <6 months after transplant) exhibited a 1-year OS of 42.8% post-relapse (n=7). Median survival was not reached for high-risk patients who received pre-emptive TAA-T post-transplant (n=12). Although as a Phase-I study concomitant anti-leukemic therapy was allowed, TAA-T were safe and well-tolerated and sustained remissions in high-risk and relapsed patients were observed. Moreover, adoptively transferred TAA-T detected by T-cell receptor V-beta (TCRVb) sequencing persisted up to at least 1 year post-infusion. (ClinicalTrials.gov numbers, NCT002203902)

Conflict of interest: COI declared – see note
**COI notes:** CMB has stock or ownership in Cabaletta Bio, Catamaran Bio and Neximmune. CMB also has equity interest in Mana Therapeutics which subsequently licensed the technology used in this study. As Sponsor of the study she helped design the clinical trial but was not responsible for final decision making regarding data collection, interpretation of outcome data, or decision to publish. PH and CRC also have stock or ownership in Mana Therapeutics and serve on the board of directors (PH) or scientific advisory board (CRC) and were not responsible for clinical trial design, data collection, interpretation of outcome data or decision to publish. PJH is also on the scientific advisory board of Cellevolve and an advisor for Maxcyte. The remaining authors declare no competing financial interests.

**Preprint server:** No;

**Author contributions and disclosures:** This paper was primarily written by HK, KRC, AJB, RJ and CMB. The study was developed and designed by Richard J. Jones (RJJ) Kirsten M. Williams (KMW) and Catherine M. Bollard (CMB). The CNH principal investigators (PI) on the clinical trial were JD, DJ and KMW, and the JHU site PI was KRC. The IND was held by CMB. Programmatic oversight and quality assurance was provided by PH, MF, KF, DK. DJ, JD, KRC, KMW, AJB, RJJ cared for the transplantation patients enrolled on the trial and were responsible for data integrity and capture. Cells were generated and evaluated by PH, AS, AD, JT, NZ. Regulatory oversight by FH. Data analysis was accomplished by HK, MG, CRC, HL, MS, KMW. Statistical studies were performed by HL, AZ and HK. All authors reviewed the manuscript, made the decision to submit for publication, vouching for the accuracy and completeness of the data reported and fidelity to the protocol.

**Non-author contributions and disclosures:** No;

**Agreement to Share Publication-Related Data and Data Sharing Statement:** Data and protocols may be accessed by contacting the corresponding author. Individual participant data will not be shared.

**Clinical trial registration information (if any):** ClinicalTrials.gov numbers, NCT002203902
Outcome of Donor-derived TAA-T cell therapy in Patients with High-risk or Relapsed Acute Leukemia Post Allogeneic BMT

Short Title: Donor-derived TAA-T cells after allogeneic BMT

**Hannah Kinoshita1-3, **Kenneth R. Cooke4, Melanie Grant6, Maja Stanojevic1, C. Russell Cruz1,5,6, Michael Keller1,5, Maria Fernanda Fortiz1, Fahmida Hoq1, Haili Lang1, A. John Barrett6, Hua Liang7, Jay Tanna1, Nan Zhang1, Abeer Shibli1, Anushree Datar1, Kenneth Fulton1, Divyesh Kukadiya1, Anqing Zhang5, Kirsten M. Williams8, Hema Dave1,3,5, Jeffrey S. Dome1,3,5, David Jacobsohn1,2,5, Patrick J. Hanley1,5, ***Richard J. Jones4, ***Catherine M. Bollard1,2,5,6

1Center for Cancer and Immunology Research, Children’s National Research Institute, Children’s National Hospital, Washington, DC

2Division of Blood and Marrow Transplantation, Children’s National Hospital, Washington DC

3Division of Oncology, Children’s National Hospital, Washington DC

4Department of Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Johns Hopkins University School of Medicine, Baltimore, MD

5Department of Pediatrics, The George Washington University School of Medicine and Health Sciences, Washington DC

6Stem cell Transplantation and Cell Therapy Program, George Washington Cancer Center, Washington, DC

7Department of Statistics, The George Washington University, Washington DC

8Department of Pediatric Hematology/Oncology, Aflac Cancer & Blood Disorders Center, Children’s Healthcare of Atlanta and Emory University School of Medicine

9Department of Pediatrics, Emory University School of Medicine

** Co-first authors; **Co-last authors; *Co-Corresponding Authors:

Catherine M. Bollard, MBChBS, MD
Children’s National Health System
The George Washington University
111 Michigan Ave, NW, Washington, DC 20010
Ph: 202-476-4776, Fax: 202-476-2280
Email: cbollard@childrensnational.org

Richard J. Jones, MD
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
University School of Medicine
401 N Broadway, Baltimore, MD 21231
Email: rjjones@jhmi.edu

** Co-first authors; **Co-last authors; *Co-Corresponding Authors:
ABSTRACT

Patients with hematologic malignancies relapsing after allogeneic blood or marrow transplantation (BMT) have limited response to conventional salvage therapies with an expected 1-year overall survival (OS) of <20%. We evaluated the safety and clinical outcomes following administration of a novel T-cell therapeutic targeting three tumor associated antigens (TAA-T) in patients with acute leukemia who relapsed or were at high-risk of relapse after allogeneic BMT. Lymphocytes obtained from the BMT donor were manufactured to target TAAs; WT1, PRAME and survivin, which are over-expressed and immunogenic in most hematologic malignancies. Patients received TAA-T infusions at doses of 0.5-4x10^7/m^2. Twenty-three BMT recipients with relapsed/refractory (n=11) and/or high-risk (n=12) acute myeloid leukemia (n=20) and acute
lymphoblastic leukemia (n=3) were infused post-transplant. No patient developed cytokine-release syndrome or neurotoxicity, and only one patient developed grade III GVHD. Of the patients who relapsed post-BMT and received bridging therapy, the majority (n=9/11) achieved complete hematologic remission before receiving TAA-T. Relapsed patients exhibited a 1-year OS of 36% and 1-year leukemia-free survival of 27.3% post-TAA-T. The poorest prognosis patients (relapsed <6 months after transplant) exhibited a 1-year OS of 42.8% post-relapse (n=7). Median survival was not reached for high-risk patients who received pre-emptive TAA-T post-transplant (n=12). Although as a Phase-I study concomitant anti-leukemic therapy was allowed, TAA-T were safe and well-tolerated and sustained remissions in high-risk and relapsed patients were observed. Moreover, adoptively transferred TAA-T detected by T-cell receptor V-beta (TCRVb) sequencing persisted up to at least 1 year post-infusion. (ClinicalTrials.gov number NCT02203903)

INTRODUCTION

Relapse is the most frequent cause of death after allogeneic blood or marrow transplantation (BMT) for high-risk, hematologic malignancies. The chance of relapse post-BMT for patients with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) ranges from 4 to 80% with a <10% chance of remaining alive and disease-free at 1-year if relapse occurs within 6 months of BMT. Pediatric patients may fare better than adults, but 1-year overall survival (OS) following relapse within 6 months of BMT still remains limited at 20-25%. Current strategies to treat post-BMT relapse, such as chemotherapy and donor lymphocyte infusions (DLI) to enhance the immunologic graft-versus-leukemia (GVL) effect, have low efficacy and are associated with graft-versus-host disease (GVHD). Although second allo-transplants to reinstate GVL effects are occasionally effective in some patients who enter a remission, they are not effective in patients who relapse early after BMT. New immunotherapeutic strategies, notably transgenic chimeric antigen receptor (CAR) T-cells, have shown promise in
patients with ALL,\textsuperscript{11,12} but can have significant toxicities, and have shown minimal efficacy in AML to date. Furthermore, the shared expression of CAR-targeted AML surface antigens on normal myeloid precursors, can incur myelotoxicity.\textsuperscript{13}

An alternative strategy to boost the GVL effect is to infuse donor-derived T-cells targeting tumor-associated antigen (TAA) peptides as circulating TAA-specific T-cells are associated with maintenance of remission post-BMT.\textsuperscript{14} In this Phase-I clinical trial, a novel, multi-targeted, T-cell therapeutic was administered in an attempt to decrease the likelihood of tumor evasion, while broadening treatment options for BMT recipients with relapsed or high-risk acute leukemia regardless of HLA type. The TAAs, WT1, survivin, and PRAME, were selected as targets since they are widely overexpressed in relapsed and refractory hematopoietic malignancies.\textsuperscript{15,16,17} Here we report on the safety, tolerability, persistence and clinical effects of this novel TAA-T treatment as an approach to prolong survival of patients with high-risk or relapsed leukemia after BMT.

METHODS

\textit{Study design.}

A prospective, open-label, pilot clinical trial (NCT02203903; IND16135) was conducted at Children’s National and Johns Hopkins Hospitals as a dose-escalation study with doses ranging from $0.5 \times 10^7/m^2$ (Dose Level 1) to $4.0 \times 10^7/m^2$ (Dose Level 4) with up to 7 infusions per patient depending on availability of the product. This study was approved by the Institutional Review Board at each institution and was conducted in accordance with the principals of the Declaration of Helsinki and Good Clinical Practice guidelines. See \textit{Supplemental Methods} for full details including eligibility. Steroids exceeding 0.5mg/kg/day prednisone equivalent at the time of TAA-T, or investigational T-cell suppressive therapies in the prior 28 days were exclusion criteria.
Patients with relapsed disease could receive cytotoxic therapies prior to and after TAA-T as deemed necessary.

**TAA-T generation**

TAA-T products were manufactured under Good Manufacturing Practice (GMP) conditions from peripheral blood mononuclear cells (PBMCs) obtained from corresponding BMT donors as previously described see Supplemental Methods. Briefly, donor-derived dendritic cells were pulsed with TAA peptide libraries and co-cultured with donor-derived T-cells in the presence of cytokines (IL-7, IL-15, IL-12, IL-6) and re-stimulated in the presence of IL-2. TAA-T products were validated for identity, phenotype, sterility, lack of alloreactivity, and cryopreserved prior to administration.

**TAA-T immunophenotyping and functional analysis**

TAA-T products were analyzed with monoclonal antibodies against: CD3, CD4, CD8, CD127, CD14, CD19, CD25, CD16, CD56, TCRγδ, CD95, CD45RO, CD28, CCR7, CD62L.

TAA-T product-specific activity against WT1, survivin, PRAME pepmixes (JP Peptide Technology, Berlin, Germany) was evaluated as previously described using anti-IFNγ Enzyme-Linked Immunospot (ELISpot). Assays were read by Zellnet consulting (Fort Lee, NJ). TAA-T functionality was also assessed by flow cytometric detection of intracellular IFN-γ and tumor necrosis factor-α (TNF-α) in response to WT1, PRAME or survivin peptides, as described in Supplementary Methods section and as published. Cells were acquired on a Cytoflex S (Beckman Coulter). Gating strategies are shown in Supplemental Figure 1-4. Data analyses were performed using FlowJo version 10.5.

**Cytotoxicity assay**
The cytolytic function of the TAA-T product was evaluated against the HLA-A*02 AML cell line (THP-1) as described in the Supplementary Methods section. Briefly, Cell Trace™ Violet-labeled THP-1 tumor cells were co-cultured with TAA-T or non-specific T-cells (PBMC). Co-cultures were labeled with monoclonal antibodies against: CD3, CD45RO, CD33 and the cytotoxicity of TAA-T products versus non-specific T-cells was assessed by lysis of CD33+Violet+ cells. The number of tumor cells remaining was reported as a proportion of the number of tumor cells plated. Tumor cells alone provided background viability. Cells were acquired on a Cytoflex S (Beckman Coulter). Data analysis was performed using FlowJo version 10.5.

Post-infusion monitoring

Patients were monitored for immediate infusion-related toxicities for one hour after each TAA-T infusion. Patients were subsequently evaluated weekly for 6 weeks for GVHD and other toxicities using the CTCAE version 4.03.

Clinical response definitions

Clinical responses after TAA-T were categorized as: continued complete remission (CCR; continued absence of morphologic or extramedullary disease); complete response (CR; absence of morphologic or extramedullary disease that is either minimal residual disease (MRD) positive or negative); partial response (PR; >10% decrease in leukemia disease without meeting CR criteria); mixed response (MR; decreased evidence of disease in some sites but not all); progressive disease (PD; worsening of all areas of disease); or stable disease (SD; changes insufficient to qualify for CR, PR, MR, or PD).

Immunosequencing
Samples of DNA isolated from the TAA-T product and recipient research blood (0.5x10^6/sample) were sent to Adaptive Biotechnologies (Seattle WA) for TCRVbeta sequencing on the Immunoseq platform with analysis and compilation of sequence results.

**Statistical analysis**

Overall survival (OS) and leukemia-free survival (LFS) were calculated from the time of TAA-T infusion to death from any cause, except when otherwise indicated. Survival curves were estimated by Kaplan-Meier and described as 1-year OS, LFS or as median survival for groups with follow-up less than 1-year. Curves were compared using the log-rank test. Paired Wilcoxon test was used to compare mean antigen responses with a cut-off p-value for significance of <0.05. No adjustments were made for multiple comparisons.

**Data availability**

For original data and protocols, please contact cbollard@childrensnational.org. Individual participant data will not be shared.

**RESULTS**

**Patient characteristics**

Twenty-three patients with relapsed/refractory (n=11) or high-risk (n=12) AML (n=20) and ALL (n=3) were infused with a donor-derived TAA-T-cell product post-BMT. The median age at time of first TAA-T infusion was 33 years (range 1-74), with five pediatric patients (<18 years). Eleven patients were female, 12 male. The donor was HLA-matched for 11 patients and haploidentical-related in 12. All TAA-T products were manufactured from the patient's BMT donor.
In the group with relapsed/refractory disease post-transplant (n=11), the median time from first transplant to relapse was 5 months (range: 56-310 days) (Table 1). Notably, 9 of these patients were MRD+ (n=2), had hematologic relapse (n=5) or extramedullary relapse (n=2) within 6 months after transplant, emphasizing the poor prognosis of the patients in this study. All relapsed patients with hematologic and extramedullary relapse received at least one other therapy prior to TAA-T infusion, including one or more courses of chemotherapy (n=9), second transplant (n=2), DLI (n=2), interferonα (n=1) and/or tyrosine kinase inhibitors (TKI) (n=2).

Patient P8 had persistent MRD with bcr/abl positivity in the marrow following transplant and treatment with dasatinib and was considered refractory. Two patients (P9, P10) received their first TAA-T infusion after a second BMT. Patient P9 received a second BMT for relapse 10 months following his first transplant and achieved second complete remission (CR2) prior to the first TAA-T infusion. Patient P10 developed MRD 180 days after her first BMT. She became MRD+ again 271 days after a second transplant. MRD was reduced to 0.01% with DLI and interferonα prior to receiving the first TAA-T infusion. Ultimately, most patients (9/11, 81.8%) achieved hematologic and extramedullary CR prior to TAA-T-cell infusion, while 3 additional patients remained MRD+ (P1, P8, P10).

Twelve patients remaining in remission after BMT received TAA-T as pre-emptive therapy to prevent relapse for high-risk features including Philadelphia chromosome positivity (ALL), FLT3 positivity and mixed lineage leukemia (MLL) rearrangement (AML). Co-administration of chemotherapy and TKIs along with TAA-T was permitted throughout the study (See Table 2). These 12 patients remained in CCR and were MRD negative at the time of TAA-T infusion (Table 2).

*Polyclonal effector and effector memory TAA-T recognizing WT1, PRAME and Survivin were expanded from healthy donors for clinical use*
The median time from donor collection to product manufacture and release testing of the TAA-T products (n=23) was 29 days (range: 20-41). The 23 patients treated received 1-4 infusions each, with a total of 29 infusions administered.

The phenotype of TAA-T products ultimately infused are shown in Figure 1a-c with variable compositions of CD8+ T-cells (median:43.86%; range:8.56–69.53%), CD4+ T-cells (19.51%; 2.15–53.9%) and CD3-CD16+CD56+ cells (4.57%; 0.05–57.9%; Figure 1a). B-cells and monocytes accounted for <2% (0.11%; 0-1.08%) of all final products (data not shown). Evaluable products (n=11) were predominantly comprised of effector memory T-cell (TEM;CD3+CD4/8+CD45RO+CCR7-CD62L-) populations with a median of 68.75% of CD8+ (range:13.08-87.97%) and 49.72% of CD4+ cells (range:10.56–79.1%). Central memory T-cell (TCM;CD3+CD4/8+CD45RO+CCR7+CD62L+) populations, shown to be important for long-term persistence of adoptively transferred T-cells in vivo,22 comprised a median of 3.8% of CD8+ (range:1.13-19.23%) and 6.75% of CD4+ cells (range:1.69-16.52%). CD8+ effector T-cell (TEFF;CD3+CD8+CD45RO-CCR7-CD62L-) comprised a median of 2% (range:0.2-8.6%) while minimal numbers (<1%) of CD4+ TEFF populations were detected in the infused products (Figure 1b,c). Finally, the TAA-T products showed broad TCR diversity, consistent with virus-specific T-cell products derived from naïve donors (examples shown in Figure 1d).23,24,25

**TAA-T were antigen-specific, polyfunctional and cytolytic against leukemia blasts in vitro**

Antigen specificity of all TAA-T products (n=23) was determined by anti-IFNγ ELISpot assay prior to initial infusion and was repeated on evaluable products prior to subsequent infusions (n=25; Figure 2a). All but one product demonstrated response to the SEB positive control. There was insufficient sample to repeat the analysis to evaluate for failure of the assay. Median (and range) antigen-specific responses (IFNγ production per 1x10^5 cells) were evaluated for WT1 (16 spot forming units (SFU); 0-450 SFU), PRAME (39 SFU; 0-523), survivin (10 SFU;0-114) and all 3 peptides combined (TAA; 44 SFU;0-582). The median negative control
(actin) was 12 SFU (range: 0-144) and the median positive control (SEB) was 732 SFU (range: 0-too many to count). Mean antigen responses (IFNγ production per 1x10^5 cells) were statistically significantly different from actin (negative control) for WT1 (p=0.0469), PRAME (p=0.0001) and TAA (p<0.0001) but not for survivin (p=0.7028). A positive antigen response in each product was classified as an IFNγ production greater than the upper 95% confidence interval (CI) of the median IFNγ production to actin, the negative control. These results similarly identify PRAME as the target antigen with the most consistently positive response across products. This data is shown in the supplemental results.

Antigen specificity was also evaluated using intracellular cytokine staining (ICS) for TNFα and IFNγ by flow cytometry for evaluable products (n=7;Figures 2b-d, Supplemental results).

Finally, the specific cytolytic function of products generated for HLA-A*02+ patients with AML was evaluated using a co-culture assay against the HLA-A*02+ AML cell line THP-1. The in vitro cytolytic activity of the TAA-T product against Violet+ CD33+ THP-1 cells was superior to that of the pseudo DLI product (PBMCs; Figure 2e). These results were reproduced in three other A*02+ donor-derived TAA-T products evaluated (Figure 2f).

**TAA-T administration following allogeneic BMT was safe**

After confirming eligibility, TAA-T were infused as outpatients, with no immediate infusion-related toxicities or adverse events. One patient withdrew from the study on day 23 post-infusion with bacterial sepsis and died from complications unrelated to TAA-T, leaving the recipients of 22 products evaluable for toxicity monitoring over a 45-day period (Table 1, 2, Supplemental Table 1). No patients developed cytokine release syndrome (CRS) or neurotoxicity attributable to TAA-T. Infection and alterations in renal function occurring during the 45-day safety monitoring period were not deemed attributable to TAA-T. Two patients developed elevated c-reactive
protein (CRP) and one developed leukopenia post-infusion, likely related to TAA-T. Two patients developed dose-limiting liver toxicity with alterations in hepatic enzymes and bilirubin that were possibly attributable to TAA-T infusion and fully resolved with treatment.

Ten patients were diagnosed with acute and/or chronic GVHD post-transplant prior to TAA-T. However, only four patients developed GVHD of any grade after TAA-T infusion with only one patient (P9) developing severe (grade 3) GVHD (stage 3 liver, stage 2 skin), which was diagnosed four weeks post-TAA-T (4.0 x10^7/m^2; DL4) and was associated with a concurrent Haemophilus influenzae B (HIB) and Varicella zoster (VZV) infection. This patient's liver biopsy was indeterminant for the underlying cause of the liver toxicity, favoring drug injury versus GVHD. However, this was characterized as a dose-limiting, grade 4 liver toxicity possibly related to the TAA-T. Systemic steroids, tacrolimus and extracorporeal photopheresis in addition to antiviral treatment successfully controlled the liver toxicity and he remains in remission >1-year post-TAA-T. Three other patients developed grades 1 and 2 skin GVHD post-TAA-T (at DLs 1, 3 and 4), all were steroid-responsive. One patient with grade 2 skin GVHD developed a dose-limiting toxicity with grade 4 transaminitis possibly attributed to the TAA-T.

**Disease response post-TAA-T infusion (all patients)**

Seventeen of 23 patients (73.9%) with acute leukemia were in continued complete hematologic (morphologic) remission in the first 45 days following TAA-T. The median duration of survival post-infusion for all patients was 644 days at the time of submission. The 1-year OS for all patients with AML (n=20) was 66.4% (95% CI, 39.84-83.33) post-TAA-T.

**Disease outcomes for patients who relapsed after first BMT**

Nine of 11 patients who relapsed post-BMT were in complete morphologic remission at the time of TAA-T infusion. One patient had detectable disease after achieving a PR in response to bridging chemotherapy, and one patient had disease progression at the time of TAA-T. The 1-
year OS after TAA-T for patients who relapsed after transplant (n=11) was 36.4% (95% CI, 11.2-62.7). Median survival was 255 days (including P1 who died from sepsis and was not evaluable for response at week 6) with 3 long-term survivors [B-ALL (n=1), AML (n=2)] at 812-1160 days post-infusion (Figure 3a). LFS 1-year post-TAA-T infusion was 27.3%, and median LFS was 64 days.

Patients characterized as “responders” (defined as CCR 3 months after TAA-T) had a median LFS of 839 days vs 42 days in the “non-responder” (defined as relapse or PD in the first 3 months after TAA-T) group (p=0.003; Figure 3b). The median OS in the responders was 1150 days compared to 150 days in the non-responder group (p=0.003; Figure 3c). Notably, in the poorest prognosis group (patients with hematologic or extramedullary relapse ≤6 months post-BMT who did not undergo a second transplant prior to TAA-T (n=7)), the median survival was 159 days with a 1-year OS of 25% after infusion (95% CI, 3.7-55.8) and 1-year OS of 42.8% from time of relapse (Figure 3a,d).

Immunoflorescence staining to evaluate expression of targeted antigens (WT1, PRAME and survivin) on the blast populations from evaluable patients showed varying degrees of positivity. No inferences could be made with respect to the correlation of immunofluorescent antigen positivity and disease response because of the small sample size (Figure 3e, Supplemental Figure 5). Individual courses of representative patients who had relapsed after first BMT and had demonstrable responses with in vivo T-cell expansion after TAA-T are shown in Figure 3f,g.

**Disease outcomes for patients at high-risk for relapse post-BMT**

The median survival in patients defined as high-risk for relapse and treated with TAA-T as preemptive therapy (n=12) was not yet reached at the time of submission with 9/12 patients
remaining alive. Eight of the nine (88.9%) evaluable patients were alive at 1-year and of those, five (62.5%) remained alive and in remission at the time of submission (Figure 4a). Nine patients were in persistent remission (no relapse within 6 months of TAA-T) with two patients relapsing at 275 and 700 days, respectively. Patient P19 who relapsed at 275 days subsequently underwent a second transplant from a second donor and ultimately died of disease progression 644 days post-initial TAA-T (Figure 4a). Three patients with AML relapsed within 6 months of TAA-T (Figure 4b), two of these patients died at 228 and 728 days, respectively post-infusion. The third patient remains alive 126 days post-TAA-T (Figure 4c). None of the patients treated preemptively had “early” relapse (defined as relapse within 6 months after 1st transplant). This cohort experienced a median LFS of 792 days, and median OS of 917 days after first transplant (Supplemental figure 7a,b).

**T-cell receptor clonotype expansion and persistence**

T-cell receptor sequencing was performed on available TAA-T products (n=11), and on recipient peripheral blood samples obtained pre-infusion versus post-infusion (Range: 28-365 days post-infusion). Evaluation of unique T-cell receptor clonotypes (present in the product but not the recipient prior to infusion) at post-infusion timepoints demonstrated expansion of unique TCR clonotypes in patients receiving TAA-T after relapse (Figure 3f,g, Supplemental figure 6a-d) and for high-risk disease (Supplemental figure 6e,f). The unique T-cell clones were shown to persist up to 6 months post-infusion in the relapsed group and up to 1-year in the pre-emptively treated group.

**DISCUSSION**

This study found that administration of donor-derived TAA-T targeting WT1, PRAME and survivin was safe and feasible for patients with high-risk leukemia after allogeneic BMT. Eleven patients had relapsed prior to TAA-T infusion, nine of whom relapsed within 6 months of transplant or had residual/refractory disease. The addition of TAA-T to treat or preempt post-
transplant relapse in this subset of patients was accompanied by few treatment-related serious adverse events and a 1-year OS of 36% and LFS of 27.3% in the relapsed group. Moreover, in patients who relapsed <6 months post-BMT and subsequently received TAA-T, their 1-year OS from time of relapse after first transplant was 42.8%. Treatment failure and death is due in part to an insufficient GVL effect, dysregulation of immune function and from toxicity of chemotherapy. McIver et al found that patients who relapse <6 months after allogeneic BMT had a <5% probability of survival irrespective of whether second BMT or less intensive management was chosen, with a 0% OS at 1-year after relapse in the patients who underwent a second transplant. Patients who had late relapse (>6 months post 1st transplant) and subsequently underwent a second transplant, DLI with or without chemotherapy or chemotherapy alone had a 1-year OS of 33% after relapse, similar to other studies also showing later relapse associated with improved outcomes.

Our cohort of patients with “early” relapse (defined as relapsing within 6 months of transplant) who did not undergo a second transplant prior to TAA-T infusion fared better with less relapse and appreciably longer survival: 1-year OS from time of relapse after first transplant 42.8% (Figure 3d) compared to 0%. These outcomes were more comparable to those with late relapse in the McIver cohort, although in the time since that publication, development of other novel therapies used the in the management of this cohort likely also contribute to the improved outcomes observed. Hence, these results are comparable or superior to similar cohorts of AML and ALL patients treated with alternative therapies, suggesting that donor-derived TAA-T are at least as effective as chemotherapy and DLI, or second transplant, for the treatment of post-BMT relapsed leukemia but with appreciably less toxicity, particularly with respect to GVHD.

Donor-derived TAA-T infusions provide an opportunity to expand a subset of cytotoxic T-cells specific to tumor antigen targets, while greatly reducing the risk of GVHD as compared to DLI. Extensive clinical experience with T-cells expanded ex vivo with peptide antigens specific for the treatment of viral infections post-BMT indicate that such T-cells lack alloreactivity
and seldom, if ever, cause GVHD. The safety profile of the TAA-T product described herein is in line with these observations and also contrasts with the frequent occurrence of CRS and neurotoxicity which complicates CD19-CAR-T used for relapsed ALL.

A recent study evaluated the effect of a different tumor-associated antigen-specific T-cell product for patients with AML refractory to salvage chemotherapy with active disease at the time of infusion. Two of 6 patients had partial or complete response to cell therapy alone with three patients treated with demethylating agents and T-cells in the adjuvant setting. In contrast, the majority of the relapsed patients in our study achieved CR with salvage chemotherapy prior to TAA-T. Several (n=4) were treated with decitabine or azacitadine as bridging therapy, which has been shown to increase expression of some TAAs including PRAME and potentiate in vitro killing of AML blasts by PRAME-specific T-cells. Therefore, further studies comparing patients receiving TAA-T therapy in combination with hypomethylating agents to those receiving TAA-T infusions with alternative or no salvage therapy are warranted to assess any synergistic effect these agents may have.

Key issues in designing and expanding effective anti-leukemic T-cells is the selection of appropriate TAAs ubiquitously present on malignant cells and, ideally, not expressed on healthy human tissues. We chose to expand T-cells against multiple TAAs (WT1, PRAME and survivin) to target most malignancies through at least one expressed antigen. Of the available samples, the recipient’s malignancy expressed at least one of these TAAs by immunofluorescence (Figure 3e). However, preliminary data from this Phase I study is not sufficient to analyze a potential correlation between immunofluorescent antigen positivity and disease response to TAA-T. Antigen specificity of the TAA-T products as evaluated by ELISpot demonstrated robust positivity for PRAME and TAA but not consistently for WT1 and survivin across all products. Similarly, CD8+ T-cell responses evaluated by ICS were variable across all patient products, with WT1 and PRAME trending above that of the negative control, although not statistically
significant. This represents an opportunity for increasing the potency of the product for future studies by ensuring equal targeting of all antigens in this single product. Targeting multiple TAAAs, reduces the risk of tumor escape through down-regulation of one antigen but restricting to a maximum of TAAAs also reduces the risk of antigenic competition observed with increased antigen targeting.34 Response to TAA-T infusions may also correlate directly with the number of target antigens expressed on the recipients’ tumor cells, with the poorest responses anticipated in patients not expressing any of the three TAA targets. This hypothesis remains to be definitively tested in larger, later Phase trials.

Growing experience with T-cell infusions to treat malignancy indicates that anti-tumor efficacy is enhanced by lymphodepletion and by persistence of the infused cells.37,38 We did not deliberately use prescribed lymphodepletion before TAA-T infusion, but the therapeutic effect of our TAA-T product may have been enhanced by lymphopenia from “bridging” chemotherapy or prior transplantation. Moreover, characterization of TCR sequencing post-infusion may predict benefit of TAA-T. Patients with disease response following TAA-T infusion had evidence of both persistence and expansion of TCR clonotypes that were present in the product but not present prior to infusion. However, given the small sample size and correlative nature of the data, we are not able to make any conclusions of causality. In future studies, identification and tracking of target antigen-specific unique clonotypes from the T cell product and demonstration of cytotoxicity of the product against individual patient samples could provide further support of this hypothesis.

Finally, two patients were treated concomitantly with the TKI dasatinib for Philadelphia chromosome positive ALL. This therapy was continued before and after TAA-T infusion and therefore may have suppressed antigen expression and activation of the T-cell product.35,36 One of these patients had refractory disease and died early in the follow-up time period. The second patient had prolonged survival and remains alive at the time of submission.
Rapid progress has been made in the manufacturing of highly effective leukemia-specific T-cells. Notably, CD19-CAR-T have achieved spectacular results in B-cell malignancies with apparent cures of ALL relapsing post-BMT.\textsuperscript{11,12} However, they have appreciable post-infusion toxicity and tumor escape can occur from downregulation of CD19.\textsuperscript{39,40} In the absence of suitable myeloid surface antigens, CAR-T therapy for AML remains more challenging and still under early stages of investigation. Our non-genetically engineered TAA-T product is therefore of particular relevance for patients with high-risk AML.

In conclusion, this Phase-I study demonstrates the safety and tolerability of donor-derived TAA-T-cell infusions following allogeneic BMT for the treatment of patients with high-risk/relapsed/refractory hematologic malignancies. Further studies are needed to determine long-term clinical disease outcomes and to optimize the dose and timing of TAA-T infusions post-transplant, particularly for pre-emptively treated patients. However, these data provide robust evidence that donor-derived TAA-T infusion is a well-tolerated therapy. Later phase studies are warranted to further evaluate the effect of TAA-T on prolonging remission in patients with high-risk hematologic malignancies post-BMT.
AUTHOR CONTRIBUTIONS

This paper was primarily written by HK, KRC, AJB, RJ and CMB. The study was developed and designed by Richard J. Jones (RJJ) Kirsten M. Williams (KMW) and Catherine M. Bollard (CMB). The CNH principal investigators (PI) on the clinical trial were JD, DJ and KMW, and the JHU site PI was KRC. The IND was held by CMB. Programmatic oversight and quality assurance was provided by FH, MF, KF, DK. DJ, JD, KRC, KMW, A JB, RJJ cared for the transplantation patients enrolled on the trial and were responsible for data integrity and capture. Cells were generated and evaluated by PH, AS, AD, JT, NZ. Regulatory oversight by FH. Data analysis was accomplished by HK, MG, CRC, HL, MS, KMW. Statistical studies were performed by HL, AZ and HK. All authors reviewed the manuscript, made the decision to submit for publication, vouching for the accuracy and completeness of the data reported and fidelity to the protocol.

DECLARATION OF INTEREST

CMB has stock or ownership in Cabaletta Bio, Catamaran Bio and Neximmune. CMB also has equity interest in Mana Therapeutics which subsequently licensed the technology used in this study. As Sponsor of the study she helped design the clinical trial but was not responsible for final decision making regarding data collection, interpretation of outcome data, or decision to publish. PH and CRC also have stock or ownership in Mana Therapeutics and serve on the board of directors (PH) or scientific advisory board (CRC) and were not responsible for clinical trial design, data collection, interpretation of outcome data or decision to publish. PJH is also on the scientific advisory board of Cellevolve and an advisor for Maxcyte. The remaining authors declare no competing financial interests.

ACKNOWLEDGEMENTS

This work was supported by NIH grant P01-CA-015396 (RJ/CMB/KC) and a Leukemia and Lymphoma Society SCOR 7018-04 (CB/KW/PH), a Hyundai Hope on Wheels grant (CB/KW),
Ben’s Run Foundation (CB/KW), Rising Tides Foundation (KW/CB) and a Ruth L. Kirschstein National Research Service Award Institutional Research Training Grant awarded to the Children’s Research Institute Hematology Training Program by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health, 5T32HL110841-08 (HK). This project was supported by Award Number UL1TR001876 from the NIH National Center for Advancing Translational Sciences. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Center for Advancing Translational Sciences or the National Institutes of Health. The funding sources had no role in trial design, data collection, interpretation of data, or decision to publish. We would also like to acknowledge the work of Maria Martin Manso for her role in T-cell product manufacturing and Sean Gillen for study coordination in the early stages of this trial.
REFERENCES

1. Barrett J, Battiwalla M. Relapse after allogeneic stem cell transplantation. Expert Rev Hematol 2010; 3(4):429-441

2. Bejanyan N, Weisdorf D, Logan B, Wang H, Devine S, Lima M, Bunjes D, Zhang M. Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: A center for international blood and marrow transplant research study. Biology of blood and marrow transplantation 2015; 21(3):454-459.

3. McIver Z, Yin F, Hughes T, Battiwalla M, Ito S, Koklanaris E, Haggerty J, Hensel N, Barrett J. Second hematopoietic stem cell transplantation for leukemia relapsing after myeloablative T cell depleted transplants does not prolong survival. Bone Marrow Transplant 2013; 48(9):1192-1197.

4. Menon NN, Jenkins LM, Cui H, Jenkins C, Anwer F, Yeager AM, Katsanis E. Factors associated with improved outcomes after second allogeneic hematopoietic cell transplantation for relapsed pediatric leukemia. Ann Hematol. 2016 Mar;95(4):637-44. doi: 10.1007/s00277-016-2599-9. Epub 2016 Jan 20. PMID: 26787415.

5. Eapen M, Giralt SA, Horowitz MM, Klein JP, Wagner JE, Zhang MJ, Tallman MS, Marks DI, Camitta BM, Champlin RE, Ringdén O, Bredeson CN, Martino R, Gale RP, Cairo MS, Litzow MR, deLima M. Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. Bone Marrow Transplant. 2004 Oct;34(8):721-7. doi: 10.1038/sj.bmt.1704645. PMID: 15322568.

6. Naik S, Martinez C, Leung K, Sasa G, Nguyen N, Wu M, Gottschalk S, Brenner M, Heslop H, Krance R. Outcomes after Second Hematopoietic Stem Cell Transplantations in Pediatric Patients with Relapsed Hematological Malignancies. Biol Blood Marrow Transplant. 2015; 21(7):1266-72.

7. Orti G, Barba P, Fox L, Salamero O, Bosch F, Valcarcel D. Donor lymphocyte infusions in AML and MDS: Enhancing the graft-versus-leukemia effect. Experimental Hematology. 2017. 48; 1-11.
8. Rautenberg C, G entire sentence is cut off, please provide the full sentence.

9. Imus PH, Blackford AL, Bettinotti M, Iglehart B, Dietrich A, Tucker N, Symons H, Cooke KR, Luznik L, Fuchs EJ, Brodsky RA, Matsui WH, Huff CA, Gladstone D, Ambinder RF, Borrello IM, Swinnen LJ, Jones RJ, Bolaños-Meade J. Major Histocompatibility Mismatch and Donor Choice for Second Allogeneic Bone Marrow Transplantation. Biol Blood Marrow Transplant. 2017 Nov;23(11):1887-1894. doi: 10.1016/j.bbmt.2017.07.014. Epub 2017 Jul 25. PMID: 28754545; PMCID: PMC5881910.

10. Orti G, Sanz J, Bermudez A, Caballero D, Martinez C, Sierra J, Cabrera Marin JR, Espigado I, Solano C, Ferrà C, García-Noblejas A, Jimenez S, Sampol A, Yañez L, García-Gutiérrez V, Pascual MJ, Jurado M, Moraleda JM, Valcarcel D, Sanz MA, Carreras E, Duarte RF. Outcome of Second Allogeneic Hematopoietic Cell Transplantation after Relapse of Myeloid Malignancies following Allogeneic Hematopoietic Cell Transplantation: A Retrospective Cohort on Behalf of the Grupo Español de Trasplante Hematopoyetico. Biol Blood Marrow Transplant. 2016 Mar;22(3):584-8. doi: 10.1016/j.bbmt.2015.11.012. Epub 2015 Nov 26. PMID: 26631751.

11. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 2013; 5: 177

12. Lee DW, Kochenderfer JN, Stetler-Stevenson M, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. Lancet 2015; 385: 517–528.

13. Tasian SK. Acute myeloid leukemia chimeric antigen receptor T-cell immunotherapy: how far up the road have we traveled?. Ther Adv Hematol. 2018;9(6):135-148.
14. Rezvani K, Yong A, Savani BN, Mielke S, Keyvanfar K, Price D, Douek D, Barrett AJ. WT1-specific T lymphocytes participate in the elimination of acute lymphoblastic leukemia (ALL) following allogeneic stem cell transplantation. Blood, 2007;110:1924-3

15. Candoni A, Tiribelli M, Toffoletti E, Cilloni D, Chiavessio A, Michelutti A, Simeone E, Pipan C, Saglio G, Fanin R. Quantitative assessment of WT1 gene expression after allogeneic stem cell transplantation is a useful tool for monitoring minimal residual disease in acute myeloid leukemia. Eur J Haematol. 2009; 82(1):61-68.

16. Rezvani K, Yong A, Eniafe R, Jafarpour B, Abdul T, Mielke S, Savani BN, Kurlander R, Barrett AJ. Polyclonal memory CD8+ T-cell responses to PRAME-specific peptides occur in patients with acute leukemias and chronic myeloid leukemia. Blood. 2009;113:2245-55

17. Park E, Gang E, Hsieh Y, Schaefer P, Chae S, Klemm L, Huantes S, Loh M, Conway E, Kang, E, Koo H, Hofmann W, Heisterkamp N, Pelus L, Keerthivasan G, Crispino J, Kahn M, Muschen M, Kim, Y. Targeting survivin overcomes drug resistance in acute lymphoblastic leukemia. Blood. 2011 Aug 25; 118(8): 2191–2199.

18. Hont A, Cruz CR, Ulrey R, O'Brien B, Stanojevic M, Datar A, Albihani S, Saunders D, Hanajiri R, Panchapakesan K, Darko S, Banerjee P, Fernanda Fortiz M, Hoq F, Lang H, Wang Y, Hanley P, Dome J, Bollard CM, Meany HJ. Immunotherapy of relapsed and refractory solid tumors with ex vivo expanded multi-tumor associated antigen specific cytotoxic T lymphocytes: A phase I study. Journal of Clinical Oncology. 2019; 37 (26):2349-2357.

19. Stanojevic M, O'Brien S, Geiger A, Ulrey RK, Cruz CY, Hanley P, Keller M, Bollard C. Identification of novel HLA-Restricted PRAME peptides to facilitate “off-the-shelf” tumor-associated antigen-specific T-cells. Cytotherapy in press 2021

20. Weber G, Caruana I, Rouce RH, et al. Generation of tumor antigen-specific T cell lines from pediatric patients with acute lymphoblastic leukemia--implications for immunotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2013; 19(18): 5079-91.
21. Sili U, Leen AM, Vera JF, et al. Production of good manufacturing practice-grade cytotoxic T lymphocytes specific for Epstein-Barr virus, cytomegalovirus and adenovirus to prevent or treat viral infections post-allogeneic hematopoietic stem cell transplant. *Cytotherapy* 2012; **14**(1): 7-11.

22. Klebanoff C, Gattinoni L, Palmer D, Muranski P, Ji Y, Hinrichs C, Borman Z, Kerkar S, Scott C, Finkelstein S, Rosenberg S, Restifo N. Determinants of successful CD8+ T-cell adoptive immunotherapy for large established tumors in mice. *Clinical Cancer Research*. 2011. **17**(16); 5343-52.

23. Abraham AA, John TD, Keller MD, Cruz CRN, Salem B, Roesch L, Liu H, Hoq F, Grilley BJ, Gee AP, Dave H, Jacobsohn DA, Krance RA, Shpall EJ, Martinez CA, Hanley PJ, Bollard CM. Safety and feasibility of virus-specific T cells derived from umbilical cord blood in cord blood transplant recipients. *Blood Adv.* 2019 Jul 23;**3**(14):2057-2068. doi: 10.1182/bloodadvances.2019000201. Erratum in: *Blood Adv.* 2019 Aug 27;**3**(16):2453. PMID: 31292125; PMCID: PMC6650740.

24. Keller MD, Darko S, Lang H, Ransier A, Lazarski C, Wang Y, Hanley P, Davila BJ, Heimall JR, Ambinder RF, Barrett J, Rooney CM, Heslop HE, Douek DC, Bollard CM. T-cell receptor sequencing demonstrates persistence of virus-specific T cells after antiviral immunotherapy. *British Journal of Haematology*. 2019. **187**(2);206-218.

25. Hanley P, Melenhorst J, Nikiforow S, Scheinberg P, Blaney J, Demmler-Harrison G, Cruz CR, Lam S, Krance RA, Leung KS, Martinez CA, Lui H, Douek DC, Heslop HE, Rooney CM, Shpall EJ, Barrett AJ, Rodgers JR, Bollard CM. CMV-specific T cells generated from naive T cells recognize atypical epitopes and may be protective in vivo. *Science Translational Medicine*. 2015. **7**(285); 285-63.

26. Christopeit M, Kuss O, Finke J, Bacher U, Beelen DW, Bornhäuser M, Schwerdtfeger R, Bethge WA, Basara N, Gramatzki M, Tischer J, Kolb HJ, Uharek L, Meyer RG, Bunjes D, Scheid C, Martin H, Niederwieser D, Kröger N, Bertz H, Schrezenmeier H, Schmid C. Second allograft for hematologic relapse of acute leukemia after first allogeneic stem-cell transplantation
from related and unrelated donors: the role of donor change. J Clin Oncol. 2013 Sep 10;31(26):3259-71. doi: 10.1200/JCO.2012.44.7961. Epub 2013 Aug 5. PMID: 23918951.

27. Tomonari A, Iseki T, Ooi J, Nagayama H, Sato H, Takahashi T, Ito K, Nagamura F, Uchimaru K, Takahashi S, Shirafuji N, Tojo A, Tani K, Asano S. Second allogeneic hematopoietic stem cell transplantation for leukemia relapse after first allogeneic transplantation: outcome of 16 patients in a single institution. Int J Hematol. 2002 Apr;75(3):318-23. doi: 10.1007/BF02982050. PMID: 11999364.

28. Weisdorf D. The role of second transplants for leukemia. Best practice and research clinical hematology. 2016; 29: 359-364.

29. Christopher M, Petti A, Rettig M, Miller C, Chendamarai E, Duncavage E, Klco J, Helton N, O’Laughlin M, Fronick C, Fulton R, Wilson R, Wartman L, Welch J, Heath S, Baty J, Payton J, Graubert T, Link D, Walter M, Westervelt P, Ley T, DiPersio J. Immune escape of relapsed AML cells after allogeneic transplantation. N Engl J Med 2018; 379:2330-41.

30. Lulla P, Naik S, Vasileiou S, Tzannou I, Watanabe A, Kuvalekar M, Lulla S, Carrum G, Almeida Ramos C, Kamble R, Hill LC, Randhawa JK, Gottschalk S, Krance R, Tao W, Wu M, Robertson C, Gee AP, Mi-Yung Chung B, Grilley B, Brenner M, Heslop H, Vera JF, Leen AM; Clinical effects of administering leukemia-specific donor T cells to patients with AML/MDS post-allogeneic transplant. 2020. Blood. doi: https://doi.org/10.1182/blood.2020009471

31. Yao Y, Zhou J, Wang L, Gao X, Ning Q, Jiang M, Wang J, Wang L, Yu L. Increased PRAME-Specific CTL Killing of Acute Myeloid Leukemia Cells by Either a Novel Histone Deacetylase Inhibitor Chidamide Alone or Combined Treatment with Decitabine. 2013. PLOS ONE 8(8): e70522. https://doi.org/10.1371/journal.pone.0070522

32. Almstedt M, Blagitko-Dorfs N, Duque-Afonso J, Karbach J, Pfeifern D, Jäger E, Lübbert M. The DNA demethylating agent 5-aza-2’-deoxycytidine induces expression of NY-ESO-1 and other cancer/testis antigens in myeloid leukemia cells. 2013. Leukemia Research 34 (7); 899-905. https://doi.org/10.1016/j.leukres.2010.02.004.
33. Melenhorst JJ, Leen AM, Bollard CM, Quigley MF, Price DA, Rooney CM, Brenner MK, Barrett AJ, Heslop HE. Allogeneic virus-specific T cells with HLA alloreactivity do not produce GVHD in human subjects. Blood. 2010 Nov 25;116(22):4700-2. doi: 10.1182/blood-2010-06-289991. Epub 2010 Aug 13. PMID: 20709906; PMCID: PMC2996125.

34. Leen AM, Christin A, Myers GD, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. Blood 2009; 114(19): 4283-92.

35. Schade AE, Schieven GL, Townsend R, Jankowska A, Susulic V, Zhang R, Szpurka H, Maciejewski J. Dasatinib, a small-molecule protein tyrosine kinase inhibitor, inhibits T-cell activation and proliferation. Blood. 2008;111(3):1366-1377.

36. Weichsel R, Dix C, Wooldridge L, Clement M, Fenton-May A, Sewell A, Zezula J, Greiner E, Gostick E, Price D, Einsele H, Seggewiss R. Profound inhibition of antigen-specific T-cell effector functions by dasatinib. Clin Cancer Res. 2008; 14(8); 2484-2491.

37. Miller JS, Weisdorf DJ, Burns LJ, Slungaard A, Wagner JE, Verneris MR, Cooley S, Wangen R, Fautsch SK, Nicklow R, DeFor T, Blazar BR. Lymphodepletion followed by donor lymphocyte infusion (DLI) causes significantly more acute graft-versus-host disease than DLI alone. Blood. 2007; 110;7:2761-2763.

38. Yan CH, Wang JZ, Liu DH, Xu LP, Chen H, Liu KY, Huang XJ. Chemotherapy followed by modified donor lymphocyte infusion as a treatment for relapsed acute leukemia after haploidentical hematopoietic stem cell transplantation without in vitro T-cell depletion: superior outcomes compared with chemotherapy alone and an analysis of prognostic factors. Eur J Haematol. 2013 Oct;91(4):304-14.

39. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 2015;5:1282–95.
40. Gardner R, Wu D, Cherian S, Fang M, Hanafi LA, Finney O, et al. Acquisition of a CD19-negative myeloid phenotype allows immune escape of MLL-rearranged B-ALL from CD19 CAR-T-cell therapy. Blood. 2016;127:2406–10.
FIGURE LEGENDS

Figure 1. Characterization of the TAA-T product by phenotype and TCR clonotype diversity. (A) Variable composition of the TAA-T products by phenotype [CD8+, CD4+, NK (natural killer), TCRγδ (gamma delta T cells), NKT (natural killer T cells)] presented as percent of total cells in product as determined by 12-color flow cytometry (n=23). (B, C) Memory phenotype described as central memory (TCM - CD3+CD4/8+CD45RO+CCR7+CD62L+), effector memory (TEM - CD3+CD4/8+CD45RO+CCR7-CD62L-), effector T cells (TEFF - CD3+CD4/8+CD45RO-CCR7-CD62L) for evaluable samples (n=11). (D) Diversity of T cell receptor (TCR) sequences of TAA-T products shown for representative patients in the relapsed group (P9, P3) and patients treated pre-emptively with TAA-T products (P12, P13).

Figure 2. Antigen specificity as measured by anti-IFNγ ELISpot, TNFα and IFNγ intracellular cytokine staining and cytolytic function of the TAA-T products. (A) Target antigen specificity of the TAA-T product (n=25) as determined by IFNγ production, measured by ELISpot. Target antigens were WT1, PRAME, survivin and TAA (WT1, PRAME and survivin pepmixes combined). The bottom dotted line denotes the median for negative control (actin = 12 SFU), the top dotted line denotes the median for positive control (SEB = 732 SFU). Mean antigen responses were statistically significantly different from actin for WT1 (\(p=0.0469\)), PRAME (\(p=0.0001\)) and TAA (\(p<0.0001\)) but not for survivin (\(p=0.7028\)). (B) TNFα and IFNγ ICS demonstrates antigen specificity for WT1 and PRAME shown for products (P6, P9). Antigen specificity measured by TNFα and IFNγ ICS of CD8+ T cells (C) and CD4+ T cells (D) of the TAA-T-cell product in evaluable samples (n=7). SEB is used as the positive control, actin as negative control. (E) In vitro cytolytic activity of the HLA A*02+ TAA-T product against an HLA A*02+ AML cell line (THP-1) as compared to a donor lymphocyte infusion (donor PBMCs) product. (F) Superior cytolytic activity against THP-1 Violet+ CD33+ cells of the TAA-T product as compared to donor lymphocyte infusion (PBMC) is reproducible in the majority of A*02+ donor TAA-T products evaluated (as shown for P2, P6, P12).
Figure 3. Clinical outcomes for patients treated with TAA-T for relapsed disease after BMT (n=11). (A) Swimmer plot showing clinical outcomes following salvage therapy and TAA-T infusion in patients with relapsed/refractory disease after BMT, categorized by dose level (1-4). Hematologic remission was achieved in 9/11 patients prior to TAA-T infusion with post-infusion clinical outcomes defined as continued complete remission (CCR), partial response (PR), stable disease (SD), progressive disease (PD) and relapse. Patients in hematologic remission with MRD are noted as CCR*. Patients who did not achieve hematologic remission are noted (+; P3, P11). The dotted line denotes 1-year post-infusion. (B) Kaplan-Meier curve estimating leukemia-free survival (LFS) post-infusion of relapsed patients. 1-year LFS 27.3%, median LFS was 64 days. Patients characterized as responders (CCR within 3 months of first TAA-T infusion; n=4) had prolonged median LFS (839 days) compared to non-responders (PD/R within 3 months of first TAA-T infusion; n=7), median LFS 42 days (P=0.003). (C) Kaplan-Meier curve estimating overall survival (OS) post-infusion of relapsed patients. 1-year OS was 36.36% with median survival of 255 days post-TAA-T infusion. Responders had prolonged median OS (1150 days) compared to non-responders (150 days) (P=0.003). (D) 1-year post-relapse OS was 42% in early relapsers (patients with relapse within 6 months of transplant; n=7) who received TAA-T infusion. (E) Qualitative grading of immunoflorescence expression of TAA targets (WT1, PRAME and survivin) on blast population and clinical outcomes following TAA-T of evaluable patients with relapsed AML post-transplant. The paraffin embedded tissues were deparaffinized and incubated post-antigen retrieval with anti-survivin, anti-Wilms Tumor protein (abcam) and anti-PRAME (Sigma) followed by Alexa Fluor568 (Texas red channel) donkey anti-rabbit IgG secondary antibody for survivin and PRAME (abcam) and AlexaFluor488 (FITC) donkey anti-mouse IgG secondary antibody for WT1 (abcam). The sections were mounted with DAPI staining solution (abcam) and the images were captured at 20x magnification on an Olympus BX53-DP73 microscope using cellSens software. Clinical outcomes characterized as responder
and non-responder (as above). (F) Disease course and TCR unique clonotype frequencies over time for P5 with MDS/AML, relapsed 117 days post-transplant and subsequently achieved CR with salvage therapy (azacitidine) prior to TAA-T infusion. Hematologic relapse with peripheral blasts cleared with a second TAA-T infusion, azacitidine and lenalidomide, though remained MRD+. (G) Disease course and unique TCR clonotype frequency over time for P8, pediatric patient with Ph+ B cell ALL with persistent bcr/abl positivity post-transplant despite treatment with dasatinib. Briefly achieved bcr/abl negativity following 1st TAA-T infusion followed by rise in bcr/abl quantification ratio following the 2nd TAA-T infusion.

Figure 4. Clinical outcomes for patients with high-risk disease treated pre-emptively with TAA-T after BMT (n=12). (A) Swimmer plot showing clinical outcomes of patients treated pre-emptively with TAA-T infusion for high-risk disease after BMT, categorized by dose level (1-4). All patients were in continued complete remission (CCR) at the time of TAA-T infusion. The dotted line denotes 1-year post-infusion. (B) Kaplan-Meier curve estimating leukemia-free survival (LFS) post-infusion of pre-emptively treated patients. Median LFS has not been reached for all patients. Patients who relapsed in the first 6 months post-TAA-T infusion (n=2) had median LFS of 99 days, median LFS for patients in persistent remission (no relapse or PD within 6 months of TAA-T infusion (n=9) has not been reached. (C) Kaplan-Meier curve estimating overall survival (OS) post-infusion of pre-emptively treated patients. Median OS has not been reached for all patients.
| Patient ID | Age/ Sex  | Diagnosis | Indication for BMT | Donor and transplant type | Time to evidence of disease after transplant (days) | Status at relapse | Post-relapse treatment | Status at TAA-T cell infusion (evaluation timepoint pre-infusion) | Day post-BMT at time of TAA-T cell infusion (k/IU) | ALC at first TAA-T cell infusion (k/IU) | TAA-T dose level (Number of doses) | Best response post infusion | Time to Relapse post infusion (days) | Survival post infusion (days) |
|------------|-----------|-----------|--------------------|---------------------------|-------------------------------------------------|-----------------|----------------------|-------------------------------------------------|---------------------------------|-------------------------------|---------------------------------|-----------------------------|-----------------------------|-------------------------------|
| 1          | 24 Years/M| B-ALL Ph+ | TBI MA             | HLA=sibling               | 75                                             | CNS3 BM 15% blasts | Azacitidine, DLI, XRT, TKI, IT chemotherapy | BCR/ABL positive, CNS negative (34 days) | +96                             | 0.27                          | 1 (1)                          | SD                           | 21                            | 28                            |
| 2          | 64 Years/M| MDS/AML  | NMA                | HLA=sibling               | 155                                            | BM 27% blasts      | HIDAC x2                                          | CR (9 days)                       | +330                           | 0.77                          | 1 (1)                          | PD                           | 42                            | 169                           |
| 3          | 48 Years/F| B-ALL    | NMA                | HLA=sibling               | 56                                             | Extramedullary leukemia | VP16/MTX x1, blinatumomab, Cytarabine HIDAC x2 | PD (3 days)                       | +423                           | 2.21                          | 2 (1)                          | SD                           | 19                            | 255                           |
| 4          | 54 Years/M| MDS/AML  | tri8,13,20 MLL RUNX1T1 | HLA=sibling               | 107                                            | BM 80% blasts      | CR (17 days)                                     | +184                             | 0.37                           | 2 (1)                          | PD                           | 36                            | 137                           |
| 5          | 68 Years/F| MDS/AML  | tri8, 5q-, mo7     | HLA=sibling               | 117                                            | BM 5% blasts       | Azacitidine x3                                   | CR (9 days)                       | +222                           | 1.43                          | 3 (3)                          | CCR                          | 167                           | 422                           |
| 6          | 70 Years/M| MDS/AML  | tri8, 14           | HLA=sibling               | 179                                            | BM 80% blasts      | ACDVP16 x1                                       | CR (53 days)                      | +283                           | 0.86                          | 3 (1)                          | PD                           | 53                            | 95                            |
| 7          | 58 Years/M| AML      | 9q-;tri21,Mut CEBPa,Kit, IK2F | HLA=sibling               | 289                                            | BM 60% blasts      | ACDVP16 x1 HIDAC x2 | CR (123 days)                      | +455                           | 1.81                          | 3 (4)                          | CCR                          | 518                           | 1150                          |
| 8          | 9 Years/F | B-ALL Ph+ | MA                 | HLA=sibling               | N/A                                            | Persistent positive BCR/ABL | TKI | Persistent positive BCR/ABL, MRD negative (15 days) | +231                             | 2.82                           | 4 (2)                          | PR (MRD negative, BCR/ABL undetectable) | N/A (MRD positive day 105) | 1160+                         |
| 9          | 21 Years/M| AML     | FLT3 positive      | Haplo mother NMA          | N/A                                            | No relapse after 2 transplant | Marrow morphology "suspicious" for AML | Second BMT, azacitidine | CR (28 days)                      | +167                           | 1.09                          | 4 (1)                          | CCR                          | N/A                          | 812+                         |
| 10         | 4 Years/F | AML     | MLL rearrangement  | Haplo father RI           | 271 post 2 transplant | BM blasts 0.08% (0.48% 3 months later) | iFNa, DLI | MRD 0.01% (6 days) | +460                             | 2.12                           | 4 (1)                          | PR                           | 90                           | 323                           |
| 11         | 61 Years/M| MDS/AML  | EBB1, p53 mutation | Mini-Haplo son NMA        | 115                                            | BM blasts 20%      | Azacitidine (post TAA-T cells) | Persistent blasts (20 days) | +135                           | 0.71                          | 4 (1)                          | SD                           | 64 (PD)                       | 150                           |
ACDVP16 (cytarabine, daunorubicin, etoposide), AML (Acute myeloid leukemia), B-ALL (B cell acute lymphoblastic leukemia), BM (bone marrow), CCR (continued complete remission), CNS (central nervous system), CR (complete remission), DLI (donor lymphocyte infusion), Haplo (haploidentical), HIDAC (high dose cytarabine), “HLA=sibling” (HLA matched sibling), IT (intrathecal), MA (myeloablative), MDS (myelodysplastic syndrome), MRD (minimal residual disease), MTX (methotrexate), NMA (non myeloablative), PD (progressive disease), Ph+ (Philadelphia chromosome positive), PR (partial response), RI (reduced intensity), SD (stable disease), TBI (total body irradiation), TKI (tyrosine kinase inhibitor), XRT (radiation therapy).
Table 2. Patient characteristics and disease response in patients treated pre-emptively with TAA-T infusion.

| Patient ID | Age/ Sex | Diagnosis and High-Risk Features | Donor and transplant type | Post-transplant treatment | Day post-BMT at time of TAA-T infusion | TAA-T dose level (Number of doses) | ALC at first infusion (k/IU) | Best response post infusion | Time to relapse post infusion (days) | Survival post infusion (days) |
|------------|----------|-----------------------------------|---------------------------|--------------------------|----------------------------------------|-----------------------------------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|
| 12         | 9 Years/F | AML, MLL, NPM1                    | HLA=sibling NMA           | Azacitidine maintenance  | +189                                   | 4 (1)                            | 0.52                          | CCR                           | 134                             | 728                           |
| 13         | 31 Years/M| AML MLL 11:16 t:11 1q partial trisomy 11q | HLA=sibling MA            | None                     | +337                                   | 4 (1)                            | 1.82                          | CCR                           | N/A                             | 806+                          |
| 14         | 67 Years/F| AML/MDS                           | MiniHaplo NMA             | None                     | +167                                   | 1 (1)                            | 3.27                          | CCR                           | N/A                             | 561+                          |
| 15         | 31 Years/M| AML NPM1, PTPN1 mutation          | HLA=sibling NMA           | None                     | +261                                   | 2 (1)                            | 0.98                          | CCR                           | N/A                             | 368+                          |
| 16         | 25 Years/M| AML FLT3                          | HLA=sibling MA            | Gilteritinib             | +212                                   | 4 (1)                            | 1.30                          | CCR                           | N/A                             | 383+                          |
| 17         | 1 Years/F | AML MLL rearrangement             | Haplo MA                  | None                     | +131                                   | 4 (1)                            | 4.09                          | CCR                           | N/A                             | 771+                          |
| 18         | 59 Years/M| AML Monosomy 7                    | Haplo NMA                 | None                     | +92                                    | 4 (1)                            | 0.51                          | CCR                           | 700                             | 707+                          |
| 19         | 47 Years/F| AML TP53 complex karyotype        | Haplo NMA                 | None                     | +149                                   | 4 (1)                            | 0.64                          | PD (MRD+ day 35)               | 275                            | 644                           |
| 20         | 7 Years/F | AML inv(16)                       | HLA=sibling MA            | None                     | +55                                    | 4 (1)                            | 1.30                          | CCR                           | N/A                             | 380+                          |
| 21         | 28 Years/F| AML Leukemia cutis                | Haplo NMA                 | None                     | +148                                   | 4 (1)                            | 2.20                          | CCR                           | 64                              | 228                           |
| 22         | 18 Years/F| AML GATA2, germline mutation      | Haplo NMA                 | None                     | +83                                    | 3 (1)                            | 1.10                          | CCR                           | 126                             | 126+                          |
| 23         | 23 Years/M| AML Relapsed pre-transplant       | HLA=sibling RI            | None                     | +148                                   | 4 (1)                            | 0.57                          | CCR                           | N/A                             | 189+                          |

ALL (acute lymphoblastic leukemia), AML (acute myeloid leukemia), A (acute GVHD), C (chronic GVHD), CCR (continued complete remission), GVHD (graft versus host disease), Haplo (haploidentical donor), “HLA=sibling” (HLA matched sibling), MA (myeloablative BMT), MDS (myelodysplastic syndrome), NMA (non myeloablative BMT), PD (progressive disease), RI (reduced intensity BMT).
Figure 3

A

| Dose Patient Level | Patient Type | Maximum Disease Burden at Relapse after BMT |
|--------------------|--------------|------------------------------------------|
| 1                  | B-ALL/R/Post-HSCT | BM 60% blasts | 0 |
| 2                  | AML/R/R Post-HSCT | BM 80% blasts | 0 |
| 3                  | AML/R/R Post-HSCT | BM 5% blasts | 0 |
| 4                  | AML/R/R Post-HSCT | BM 0.4% | 0 |
| 5                  | AML/R/R Post-HSCT | 3% Blasts Post Second BMT | 0 |
| 6                  | AML/R/R Post-HSCT | BM 20% blasts | 0 |
| 7                  | AML/R/R Post-HSCT | BM 60% blasts | 0 |
| 8                  | B-ALL/R/Post-HSCT | BM 27% blasts | 0 |
| 9                  | AML/R/R Post-HSCT | BM 80% blasts | 0 |
| 10                 | AML/R/R Post-HSCT | BM 20% blasts | 0 |

KEY:
- Relapse
- PD
- CCR
- PR
- SD
- T cell infusion

1-year

B

RESPOIDER (n=4)
NON-RESPONDER (n=7)

Probability of Survival at 1 year from diagnosis of TAA-T1 translocation

p=0.003

C

RESPOIDER (n=4)
NON-RESPONDER (n=7)

Probability of Survival post-TAA-T1 translocation

p=0.003

D

Probability of Survival from time of Relapse post transplant

E

Positive Controls
Example of grading; PRAME

Immunofluorescence staining for available relapsed patient samples

| WT1 | SURVIVIN | PRAME | Response |
|-----|----------|-------|----------|
| P7  | +        | +++   | R        |
| P6  | -        | ++    | NR       |
| P5  | ++       | ++    | R        |
| P10 | +        | +++   | NR       |
| P2  | +        | -     | NR       |
| P4  | -        | ++    | NR       |

F

TCRB Clonotype Frequency (blood)

% Peripheral Blasts

Days post BMT

G

TCRB Clonotype Frequency (blood)

Bcr/Abl

Threshold of quantification for Bcr/Abl

Days post BMT
Figure 4.