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Chimeric antigen receptor T cells (CAR-T) for the treatment of T-cell malignancies

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ABSTRACT

At present, the only curative therapy for patients with T-cell malignancies is allogeneic stem cell transplant, which has associated risks and toxicities. Novel agents have been tried in relapsed T-cell acute lymphoblastic leukemia (T-ALL), but only one, with 20%–30% complete remission rates, has been approved by the US Food and Drug Administration. T-ALL is a heterogeneous disease, but it has universal overexpression of CD7 as well as several other T-cell markers, such as CD2 and CD5. T cells engineered to express a chimeric antigen receptor (CAR) are a promising cancer immunotherapy. Such targeted therapies have shown great potential for inducing both remissions and even long-term relapse-free survival in patients with B-cell leukemia and lymphoma. UCART7 for $CD7^+$ T-cell malignancies is in development for treatment of relapsed T-ALL in children and adults. It may also have potential in other $CD7^+$ hematologic malignancies that lack both effective therapies and targeted therapies. The challenges encountered and progress made in developing a novel fratricide-resistant "off-the-shelf" CAR-T (or UCART7) that targets $CD7^+$ T-cell malignancies are discussed here.

Introduction

T-cell malignancies represent a class of devastating hematologic cancers with high rates of relapse and mortality in both children and adults for which there are currently no effective or targeted therapies [1,2]. Despite intensive multi-agent chemotherapy regimens, fewer than 50% of adults [3,4] and 75% of children [5] with T-cell acute lymphoblastic leukemia (T-ALL) survive beyond 5 years. For those who relapse after initial therapy, salvage chemotherapy regimens induce remissions in 20%–40% of cases. Allogeneic stem cell transplant, with its associated risks and toxicities, is the only curative therapy [6].

T cells engineered to express a chimeric antigen receptor (CAR) are a promising cancer immunotherapy. Such targeted therapies have shown great potential for inducing both remissions and even long-term relapse-free survival in patients with B-cell leukemia and lymphoma [7–9]. T-ALL represents a genetically diverse group of diseases but all with universal overexpression of CD7 as well as several other T-cell markers such as CD2 and CD5 [10]. Outcomes in children are significantly worse than in children with B-ALL [11] and relapse is often associated with death in spite of many of these patients progressing to allogeneic stem cell transplant. Between 2007 and 2015 at St Louis Children's Hospital, there were 68 cases of T-ALL, of which 8 patients relapsed and 7 of these 8 went on to die of relapsed disease in spite of most receiving an allogeneic stem cell transplant. T-ALL has a slightly better prognosis in adults compared to adult B-ALL, but still, over 50% of adults will relapse after standard-of-care chemotherapy and only ~20% of those will achieve a second remission with the best salvage chemotherapy. These rates of second remissions after salvage chemotherapy for T-ALL (nelarabine, GlaxoSmithKline) in adults stand in sharp contrast to the rates of second remissions in patients with $CD19^+$ relapsed

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B-ALL receiving new targeted antibodies (inotuzumab, Pfizer; 60%–80%), bispecific T-cell engager therapy (blinatumomab, Amgen; ~40%–60%), and especially CD19 CAR-T cell therapy (~80%–95%) [9,12,13]. In fact, there are a number of patients with relapsed B-ALL who, after receiving CD19 CAR-T therapy, have remained in remission for > 3 years without the need of an allogeneic transplant.

Of note is that between 2007 and 2015 there were 57 cases of T-ALL diagnosed in the adult population at Barnes-Jewish Hospital, of which 39 relapsed and only 19 achieved a second remission and were able to proceed to transplant. Only 10 of the 19 remain alive and disease free. Six of the 10 still alive have ongoing issues with acute and chronic graft-vs-host disease (GVHD), further limiting their quality of life. A recent review by Dores et al. used the SEER-17 data to assess frequency and outcomes of all patients in the United States with T-ALL between 2001 and 2007 (covering ~28% of the US population primarily in 8 states) [14]. Based on this data, there are ~2500 cases of T-ALL and T-lymphoblastic lymphoma diagnosed in the US each year. Approximately 55% are children and 45% are adults. Survival ranges between 70% for children and young adults to 30%–40% for adults. Thus T-ALL represents a significant national, regional, and local unmet medical need especially for those patients who relapse where no effective or targeted therapies currently exist. Although novel agents have been tried in relapsed T-ALL, none with the exception of nelarabine (CR rates of only 20%–30% for relapsed T-ALL) has been approved by the US Food and Drug Administration. Recent efforts to develop Notch-1 inhibitors targeting the most common mutation in T-ALL (NOTCH) have been limited by high rates of GI toxicity and low response rates limiting their continued clinical development [15]. Recent observations that cyclin D3 is essential for the induction of T-ALL has led to efforts to target cyclin D-CDK4/6 inhibitors in T-ALL using drugs such as palbociclib (Pfizer), ribociclib (Novartis), and abemaciclib (Lilly), which have demonstrated only modest activity in preclinical models and have yet to be tested in the clinic [16]. These data further support the need for novel targeted therapies such as our UCART7 for $CD7^+$ T-cell malignancies. Although this paper is focused on the development UCART7 for treatment of relapsed T-ALL in children and adults, it is important to recognize that there are other $CD7^+$ hematologic malignancies that lack both effective therapies and targeted therapies, such as 20%-30% of AML which is $CD7^+$, NK and NKT lymphomas (~100% $CD7^+$), and all T-NHLs (~90% $CD7^+$) with the exception of cutaneous T-cell lymphomas that are $CD2^+$, $CD5^+$, and universally $CD7^-$. Thus, our approach may have broad applications to many other diseases in addition to T-ALL.

Significance

T-ALL and non-Hodgkin T-cell lymphomas (T-NHLs) are devastating cancers with high relapse and mortality rates in both children and adults for which there are no effective or targeted therapies. A targeted therapy against such T-cell malignancies represents a significant unmet medical need. CAR-T have demonstrated remarkable clinical efficacy against T-cell malignancies. However, several limitations have prevented the development of a clinically viable CAR-T targeting T-cell neoplasms. First, the shared expression of target antigens on both normal T cells and malignant T cells results in fratricide of CAR-T. Second, the inability to isolate normal T cells from malignant T cells precludes the use of autologous donor T cells for the generation of CAR-T. Harvesting adequate numbers of autologous T cells without contamination by malignant cells is, at best, technically challenging and prohibitively expensive. Third, the use of genetically modified CAR-T cells from allogeneic donors may result in life-threatening GVHD when infused into immune-compromised HLA-matched or mismatched recipients.

CD7 is highly expressed on T-ALL (98%) and other T-cell malignancies (including NK cell leukemia/lymphomas) and proves to be an attractive target for immunotherapy of T-cell cancers. We have generated a CD7-deleted and T-cell receptor alpha chain (TRAC)-deleted CAR-T targeting CD7 (UCART7). UCART7 efficiently kill human T-ALL cell lines and patient-derived primary T-ALL in vitro and in vivo without resulting in xenogeneic GVHD. UCART7 represents an “off-the-shelf” fratricide resistant CAR-T for the treatment of T-cell hematologic malignancies and is the first clinically viable targeted therapy for T-cell neoplasms. The studies proposed in this application will further the development of CAR-T targeting T-cell malignancies and provide the framework for performing the first clinical trial using CAR-T therapy against T-cell malignancies.

Preliminary data

Many T-cell malignancies overexpress CD7, providing an attractive target for immunotherapy of T-cell cancers [17–19]. However, normal T cells, including those used to engineer CAR-T, also express CD7 (> 86%) [20]. Thus, CD7-targeted CAR-T cells induce T-cell fratricide, limiting therapeutic potential. We hypothesized that deletion of CD7 and the T-cell receptor alpha chain (TRAC) using CRISPR/Cas9 while also transducing these same T cells with a CD7 targeting CAR would result in the efficient targeting and killing of malignant T cells without significant effector T-cell fratricide. TRAC deletion blocks TCR-mediated signaling, permitting the safe use of allogeneic T cells as the source of CAR-T without inducing life-threatening GVHD and without risk of contamination by CD7-deleted malignant cells, resistant to CART7 therapy. Using high-efficiency CRISPR/Cas9 gene-editing, we generated CD7 and TRAC-deleted CAR-T targeting CD7 (UCART7) (Fig. 1). These UCART7 cells efficiently kill human T-ALL cell lines and patient-derived primary T-ALL in vitro and in vivo without resulting in xenogeneic GVHD. Accordingly, for the first time, we have generated data for a clinically viable off-the-shelf strategy to effectively treat T-cell malignancies using CAR-T therapy.

CD7-targeted CAR-T

To generate the CD7-CAR-T (CART7), an anti-CD7 single chain variable fragment (scFv) was commercially synthesized and cloned into a 3rd generation CAR backbone with CD28 and 4-1BB internal signaling domains (Fig. 2). The extracellular domain of hCD34

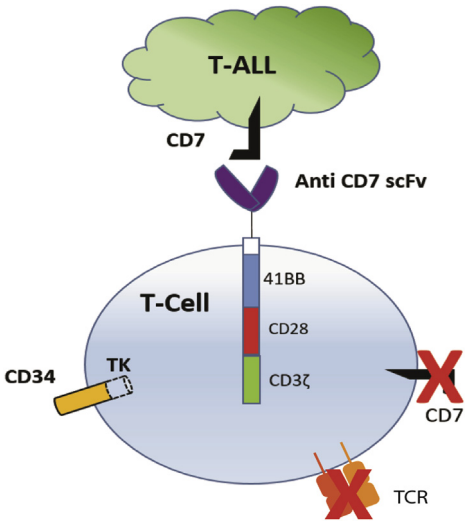

Fig. 1. CRISPR/Cas9 gene editing of CD7 and TRAC will prevent fratricide and enable use of allogeneic donor CAR-T without causing GvHD.

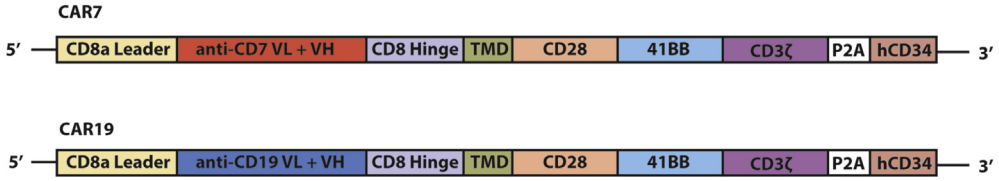

Fig. 2. Schematic of anti-CD7-CAR and anti-CD19-CAR (control) constructs.

was added after a P2A peptide to enable both detection of CAR following viral transduction and purification using anti-hCD34 magnetic beads. CAR-T targeting CD19 (CART19) were used as an irrelevant CAR-T control. Following transduction, there were significantly fewer CART7 than CART19 due to the fratricide of $CD7^+$ CAR-T cells by CART7.

$CD7^{\Delta}$CART7 prevents fratricide and effectively kills T-ALL in vitro and in vivo

We performed CRISPR/Cas9 gene-editing of CD7 followed by transduction of CD7-edited T cells ($CD7^{\Delta}$) with the CART7 construct to generate $CD7^{\Delta}$CART7. Activated T cells were electroporated with spCas9 mRNA and CD7g4 one day prior to viral transduction with either CAR7 or CAR19 control on Day 3. Cells were cultured for an additional 6 days. We anticipated that there would be a low level of fratricide resulting from residual CD7 surface expression following gene-editing, and this was confirmed by a moderate reduction in $CD7^{\Delta}$CART7 yield relative to $CD7^{\Delta}$CART19 (7.5-fold vs 12.6-fold expansion over 6 days). Autologous T cells transduced with GFP were effectively killed by $CD7^{\Delta}$CART7, but not $CD7^{\Delta}$CART19, confirming the requirement for CD7 deletion when CAR-T target CD7. Finally, in contrast to $CD7^{\Delta}$CART19, $CD7^{\Delta}$CART7 effectively killed $CD7^+$ T-ALL cell lines MOLT-4 (70% $CD7^+$), MOLT-3 (96% $CD7^+$), and HSB-2 (99% $CD7^+$) as determined by 4hr Cr release assays. To assess the activity of $CD7^{\Delta}$CART7 in a xenogeneic model of T-ALL, 1×10^5 click beetle red (CBR) luciferase-labeled CCRF-CEM T-ALL (99% $CD7^+$ by FACS) cells were injected intravenously into NSG recipients prior to infusion of 2×10^6 $CD7^{\Delta}$CART7 or non-targeting $CD7^{\Delta}$CART19 control cells on day + 1. In contrast to mice receiving $CD7^{\Delta}$CART19, or mice injected with tumor only, mice receiving $CD7^{\Delta}$CART7 had significantly prolonged survival and reduced tumor burden as determined by bioluminescent imaging (BLI) (Fig. 3) [21].

To assess the efficacy of $CD7^{\Delta}$CART7 against patient primary T-ALL cells, CAR-T were tested against patient-derived xenografts. However, T-ALL blasts were detectable only in mice receiving tumor only and were eliminated in mice receiving either $CD7^{\Delta}$CART7 or $CD7^{\Delta}$CART19 or unmanipulated T cells. This suggests that $CD7^{\Delta}$CART7 maintained similar levels of alloreactivity in vivo in NSG mice as both non-transduced human T cells and $CD7^{\Delta}$CART19, suggesting allogeneic $CD7^{\Delta}$CART7 in humans would maintain capacity to induce GVHD.

Double deletion of TRAC and CD7 in CART7 prevents fratricide, GVHD, and maintains robust CD7-directed T-ALL

To overcome alloreactive barriers that limit the use of non-self T cells, due to the risk of lethal GVHD, we generated CAR-T in which both CD7 and TRAC were genetically deleted. The gRNA sequence targeting TRAC was obtained from Osborn et al. [22]. T cells were activated using anti-CD3/CD28 beads for 2 days prior to bead removal and electroporation with 20 μg of CD7g4, 20 μg of TRACg, and 15 μg of Cas9 mRNA. Multiplex CRISPR/Cas9 gene-editing resulted in the simultaneous deletion of CD7 and TRAC in

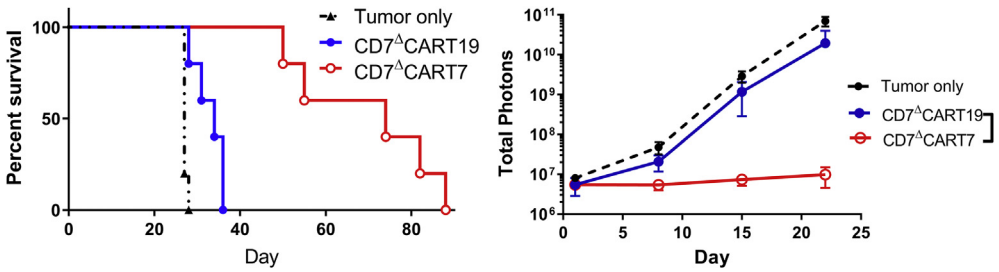

Fig. 3. NSG mice were injected with CCRF-CEM$^{CBR\text{-}GFP}$ cells on day 0, then infused with 2×10^6 CD7$^{\Delta}$CART7 or CD7$^{\Delta}$CART19 on day +1. Mice were imaged weekly using the IVIS50. CD7$^{\Delta}$CART7-treated mice had significantly prolonged survival relative to those injected with CD7$^{\Delta}$CART19 (median survival CD7$^{\Delta}$CART19 treated mice, 34 days vs. CD7$^{\Delta}$CART7 treated mice, 74 days; $p = 0.0027$) and significantly reduced tumor burden as determined by BLI imaging (Cooper et al. *Leukemia*, 2018 [21]).

72.8% ± 1.92 of cells, as determined by FACS analysis (Fig. 4) using anti-CD7 and anti-CD3 antibodies (CD3 is a surrogate marker for TRAC expression) [21].

In keeping with recent nomenclature in the field, our CD7$^{\Delta}$TRAC$^{\Delta}$CART7 was termed universal CART7 or UCART7. UCART7 was as effective as CD7$^{\Delta}$CART7 at killing T-ALL cell lines in vitro. UCART7 had no proliferation defect when compared to CD7$^{\Delta}$CART7; however, as observed with CD7$^{\Delta}$CART7, UCART7 resulted in moderately reduced CAR-T proliferation and yield relative to the CD19 control CAR-T. Since incomplete gene-editing of TRAC leaves residual potentially alloreactive CD3$^+$ CAR-T, these were depleted by negative selection using anti-CD3 magnetic beads on Day +8. Both UCART7 and CD7$^{\Delta}$CART7 killed CD7$^+$ T-ALL cell lines, MOLT3, CCRF-CEM, and HSB-2 in vitro with equally high efficiencies, demonstrating no loss of efficacy upon double deletion of CD7 and TRAC. We next tested the ability of UCART7 to kill primary T-ALL blasts in vitro. Both CD7$^{\Delta}$CART7 and UCART7 killed an average of 95% of T-ALL blasts across all three primary samples relative to the respective CD19 control CAR-T, thus demonstrating exceptional efficacy against human primary T-ALL in vitro. We tested the capacity of UCART7 to kill primary T-ALL in vivo without alloreactive graft-vs-leukemia effect (GVL) or xenogeneic GVHD. Primary T-ALL were obtained from David Weinstock (Dana-Farber Cancer

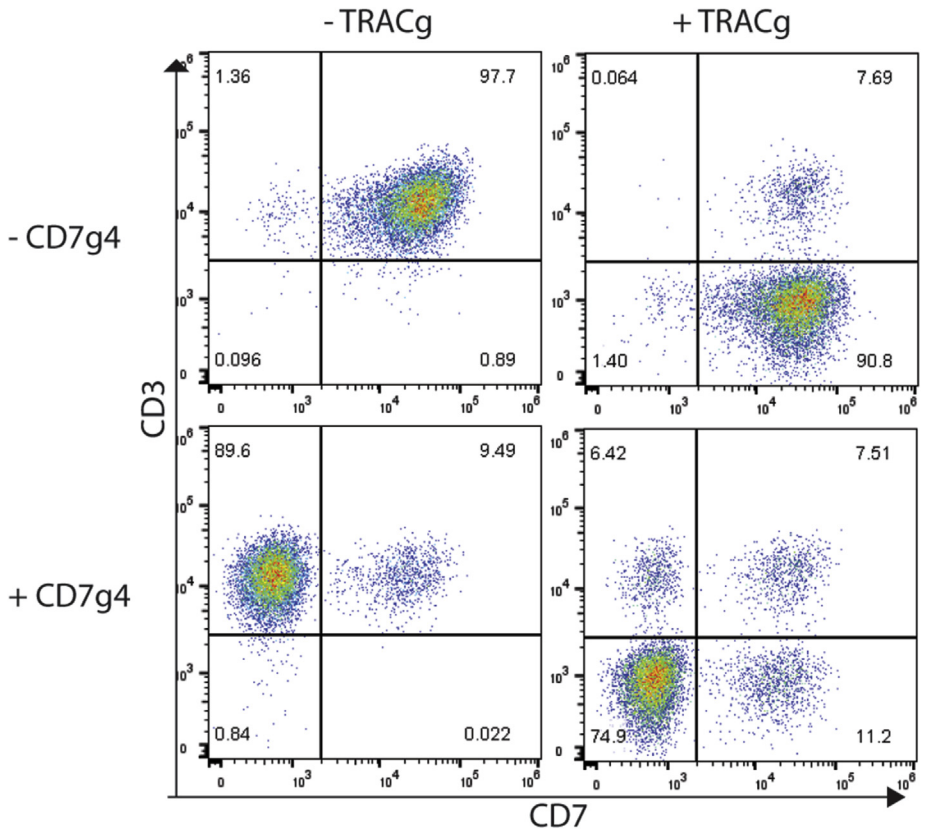

Fig. 4. Multiplex CRISPR/Cas9 gene editing results in high efficiency double deletion of TRAC and CD7 as determined by FACS (Cooper et al. *Leukemia* 2018 [21]).

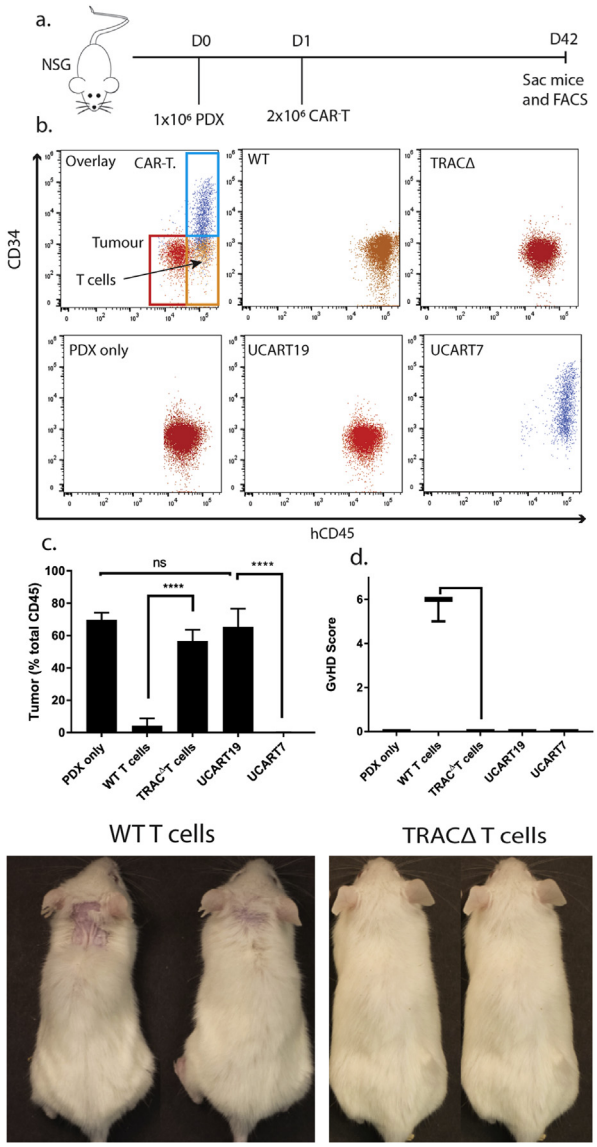

Fig. 5. UCART7 kills primary patient T-ALL blast in vivo without inducing xenogeneic GvHD. (A). NSG were engrafted with 1×10^6 T-ALL PDX cells on day 0 followed by infusion of 2×10^6 UCART7, UCART19, TRAC$^{\Delta}$ or WT T on day +1. Mice were bled 6 weeks post CAR-T injection. (b) Representative flow cytometry plots of blood analysis presented to show both tumor and T cells (C) Percentage of tumor cells out of total mouse and human CD45 cells in the blood UCART7 is effective at clearing PDX relative to UCART19 (blood, $P < 0.0001$) (d) Clinical GVHD scores. (e) Unlike TRAC$^{\Delta}$ T cells, WT T cells induce GVHD (Cooper et al. *Leukemia* 2018 [21]).

Institute), curator of the largest repository of hematologic malignancy patient-derived xenografts, and infused into NSG mice followed by treatment with either WT T cells, TRAC$^{\Delta}$ T cells, UCART7, or UCART19 (Fig. 5a) [21]. T-ALL blasts were absent in peripheral blood of mice receiving UCART7 in comparison to mice receiving UCART19, with T-ALL comprising > 56% of total human CD45^{+} cells in these mice ($P < 0.0001$) (Fig. 5b and c). Furthermore, unlike UCART19, UCART7 were detectable 6 weeks post infusion as detected by the hCD34 epitope, demonstrating the persistence of UCART7 in vivo (Fig. 5b). In contrast to recipients of TRAC$^{\Delta}$ T cells, UCART7 and UCART19, only those mice infused with WT T cells developed GVHD (mean clinical GVHD score = 5.66 (Fig. 5d and e). These data demonstrate that UCART7 are completely resistant to fratricide, exhibit no alloreactivity or GVHD potential, expand and persist and efficiently eliminate CD7^{+} T-ALL in vivo, providing a platform for the first clinically feasible adoptive T-cell gene therapy for T-cell malignancies.

Translation to the clinic

Although the exact design and eligibility criteria for the first trial of off-the-shelf UCART7 is outside the scope of this paper, we do

plan to include both children and adults, with relapsed refractory $CD7^+$ T-ALL, in either a single trial of two parallel studies in pediatric patients. Phase 1 clinical trials will include a lymphodepletion conditioning regimen prior to infusion of UCART7. This should insure in vivo expansion as well as a significant reduction in the likelihood of rejection (Host versus Graft effect). Unlike UCART19, UCART7 would have the additional advantage of directly killing recipient alloreactive T cells and NK cells (both $CD7^+$ and targets for UCART7), thus potentially inhibiting or negating the rejection of donor UCART7 by host T and NK cells.

Disclosures

Drs. Cooper and DiPersio are cofounders of WUGEN, Inc., a biotechnology company developing a novel CAR-T therapy platform for fratricide-resistant CAR-T cell therapy.

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