Alloimmune Defense Receptor Combined with Genetic Ablation of Adhesion Ligand CD58 is a Comprehensive Approach to Promote Functional Persistence of Allogeneic Cell Therapies without Conditioning Chemotherapy

Alan M. Williams[1], Rina M. Mbofung[1], Daniel Morales-Mantilla[1], Bi-Huei Yang[1], Shilpi Chandra[1], Brian Groff[1], Alison O'Connor[1], Jonatan Tuncel[1], Betsy Rezner[1], Ramesh Janani[1], Binzhong Li[1], Yijia Pan[1], Mark Jelcic[1], Tom T. Lee[1], Karl-Johan Malmberg[3,4], Maksim Mamonkin[2], John Goulding[1], Eigen Peralta[1], Jode P. Goodridge[1], Bahram Valamehr[1]

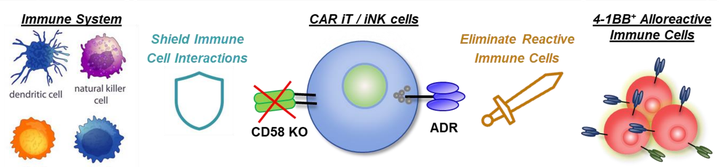
[1]Fate Therapeutics, San Diego, CA; [2]Baylor College of Medicine, Houston, TX; [3]Karolinska Institute, Stockholm, Sweden; [4]University of Oslo, Norway

Introduction

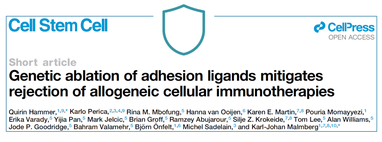
Clinical administration of chimeric antigen receptor (CAR) NK cell and CAR T-cell therapies require conditioning chemotherapy (CCT) to deplete the host immune system, maximize access to homeostatic cytokines, and promote cell expansion and functional persistence. However, CCT also elicits pan-immune cell cytopenia, increases susceptibility to infection and malignancy, and requires patient hospitalization and administration of therapy in costly tertiary healthcare settings. The administration of off-the-shelf CAR NK cell and CAR T-cell therapies to patients without requiring CCT has the potential to significantly reduce toxicities and cost of care, and, importantly, extend patient reach by enabling treatment in primary or outpatient community healthcare settings.

We have developed a unique approach to promote the functional persistence of cell therapy in the absence of CCT through the cooperative action of a novel Alloimmune Defense Receptor (ADR), which eliminates 4-1BB+ alloreactive immune cells, and the genetic disruption of CD58 (CD58KO), a unidirectional synapse-stabilizing ligand which is required for effector:target cell association.

Unique Strategy to Reduce or Eliminate the Need for Conditioning Chemotherapy



Strategy	Combination with Intense CCT	KO of HLA-I & -II	KO of HLA-I & -II + HLA-E + HLA-G	KO of HLA-I & -II + CD47	Fate's Approach ADR Expression CD58 Knockout
Avoidance of rejection by host CD8 T cells	+	+	+	+	+++
Avoidance of rejection by host CD4 T cells	+	+	+	+	+++
Avoidance of rejection by host NK cells	+	-	+/-	+/-	+++
Avoidance of suppression by host Tregs	+	-	-	-	+++
Induction of proliferation signal	+	-	-	-	+++
Creation of endogenous space	+	-	-	-	+++
Avoidance of toxicity associated with immunosuppression	x	✓	✓	✓	✓


Cell Stem Cell — CellPress OPEN ACCESS

Short article

Genetic ablation of adhesion ligands mitigates rejection of allogeneic cellular immunotherapies

Quinn Hammer,[1,6,*] Karlo Perica,[2,3,4,5] Rina M. Mbofung,[1] Hanna van Oojen,[3] Karen E. Martin,[1,4] Pouria Momayyezi,[1] Erika Varady,[1] Yija Pan,[1] Mark Jelcic,[1] Brian Groff,[1] Ramzey Abujarour,[1] Silje Z. Krokeide,[1,7] Tom Lee,[1] Alan Williams,[1] Jode P. Goodridge,[1] Bahram Valamehr,[1] Björn Önfelt,[3,4] Michel Sadelain,[1,*] and Karl-Johan Malmberg[1,3,4,8,9,*]


nature biotechnology — ARTICLES

Engineered off-the-shelf therapeutic T cells resist host immune rejection

Feiyan Mo[1,2], Norihiro Watanabe[1], Mary K. McKenna[1], M. John Hicks[3], Madhuwanti Srinivasan[1], Diogo Gomes-Silva[1], Erden Atilla[1], Tyler Smith[1], Pinar Ataca Atilla[1], Royce Ma[1,4], David Quach[1], Helen E. Heslop[1,2], Malcolm K. Brenner[1,2] and Maksim Mamonkin[1,2,3]

Summary & Conclusions

Cell-based immunotherapies that can be administered without the need for conditioning chemotherapy (CCT) can facilitate broader clinical application across multiple lines of therapy, including combination with standard-of-care agents that are used for treatment of patients with newly-diagnosed disease.

Collectively, through the examination of novel stealth strategies that operate effectively in the presence of an intact endogenous immune compartment, we highlight:

- ADR/CD58KO edited CAR iT cells are resistant to immune cell-mediated depletion through selective suppression and evasion of 4-1BB+ alloreactive immune cells.
- ADR/CD58KO edited CAR iT cells maintain extended persistence and durable tumor control *in vitro* and *in vivo,* even under supraphysiological challenge with primed alloreactive T cells.
- ADR/CD58KO edited CAR iT cells do not impede the anti-tumor activity of primary T cells (non-alloreactive to CAR iT cells) despite robust 4-1BB expression.
- Collectively, we demonstrate the potential of off-the-shelf cell therapy, through the novel combination of ADR and CD58KO, to elicit a robust cytotoxic response against diseased cells in the presence of an intact endogenous immune compartment.

Results

CAR iT cells armed with ADR and CD58^{KO} show functional persistence and continued antitumor activity in an allogeneic setting

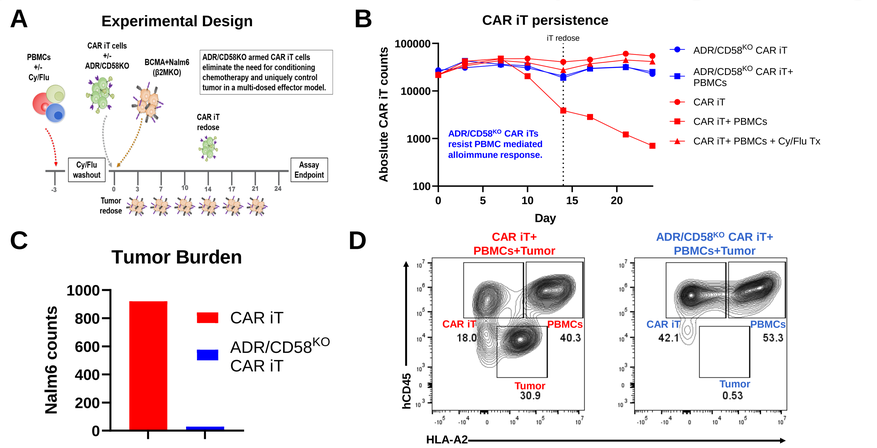


Figure 1. The Sword and Shield (ADR and CD58^{KO}) technology fully protects CAR iT from allo-rejection similar to cyclophosphamide and fludarabine (cy/flu) treatment. A) Experimental schematic demonstrating the design, setup, and timeline of the in vitro mixed lymphocyte reaction +/- cy/flu treated PBMCs and anti-BCMA CAR iT +/- ADR/CD58^{KO}. BCMA expressing Nalm6 tumor was periodically dosed as indicated. **B)** Graph summarizes the persistence of CAR iT +/- ADR/CD58^{KO} in co-cultures with or without cy/flu treated PBMCs. **C)** Graph summarizes the remaining tumor burden from co-cultures of CAR iT+/- ADR/CD58^{KO} at the end of the assay (Day 24). **D)** Flow plots indicating the gating strategies for detecting CAR iT cells +/- ADR/CD58^{KO}, BCMA+ Nalm6 tumor, and allo PBMCs at the end of the assay (Day 24).

CAR iT cells armed with ADR and CD58^{KO} combine immune evasion and selective targeting to maximize suppression of alloreactivity

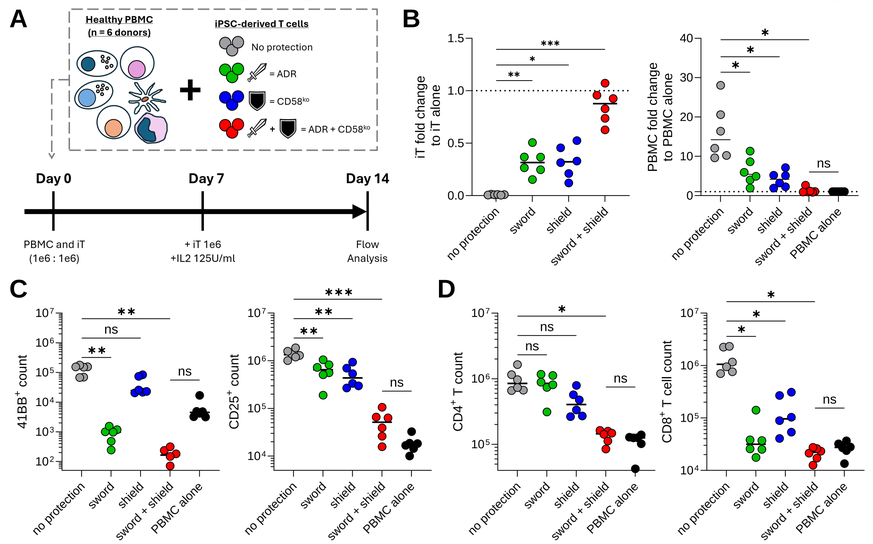


Figure 4. The Sword and Shield (ADR and CD58^{KO}) technology fully protects iT cells from allo-rejection and prevents allo PBMC expansion *in vitro*. **A)** Illustrations demonstrate the design, the setup and the timeline of the in vitro mixed lymphocyte reaction. **B)** Graphs summarize the iT (left) or PBMC (right) fold changes normalized to their cell alone controls. **C)** Graphs summarize the absolute 41BB+ (left) or CD25+ (right) cell counts, respectively. **D)** Graphs summarize the absolute CD4+ (left) or CD8+ (right) T cell counts, respectively. RM one-way ANOVA, multiple comparison was used for statistical analysis of all graphs, showing indicated comparisons with *p<0.05, **p<0.001, ***p<0.0001, and ns = not significant.

CAR iT cells armed with ADR and CD58^{KO} avoid allo T cell response similar to MHC-I/II null controls without NK cell activation

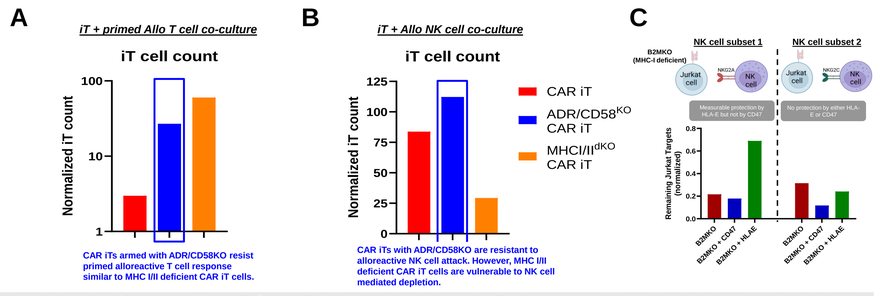


Figure 2. Sword and Shield (ADR and CD58^{KO}) CAR iT cells resist primed alloreactive T cell responses while avoiding induction of NK cell missing-self response. CAR iT cells (control, or ADR/CD58^{KO}, or MHC I/II^{dKO}) were co-cultured for 48 hours with either **A)** primed alloreactive T cells, or **B)** expanded allo pbNK cells and analyzed by flow cytometry for iT cell persistence (Fig.2 A and B). **C)** Graphs summarize Jurkat cell persistence after the indicated 96 hour co-culture conditions. Jurkat cells were genetically engineered for MHC-I deficiency and further transduced with lentivirus expressing CD47 or HLA-E as indicated. Engineered Jurkat cells were co-cultured with NK cell subsets from healthy donors found to be enriched for either NKG2A or NKG2C expression as indicated at a Jurkat:NK cell ratio of 4:1.

ADR exhibits selective targeting of alloreactive T cells and uniquely facilitates activity of anti-tumor T cells

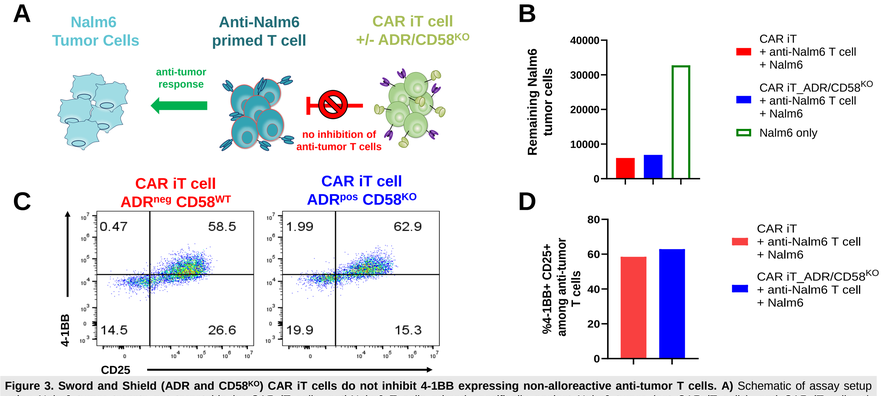


Figure 3. Sword and Shield (ADR and CD58^{KO}) CAR iT cells do not inhibit 4-1BB expressing non-alloreactive anti-tumor T cells. A) Schematic of assay setup using Nalm6 tumor targets not targetable by CAR iT cells, anti-Nalm6 T cells primed specifically against Nalm6 tumor (not CAR iT cells), and CAR iT cells +/- ADR/CD58^{KO}. Anti-Nalm6 T cells, Nalm6 tumor, and CAR iT cells were cultured at a 1:1:1 ratio for 48 hours. **B)** Remaining Nalm6 tumor cells in indicated culture conditions at 48 hours post seeding. **C)** Representative FACS plots of T cells cultured at a 1:1:1 anti-Nalm6 T cell: Nalm6 tumor: CAR iT cell +/- ADR/CD58^{KO} condition for 48 hours. **D)** Percent 4-1BB+CD25+ T cells among total anti-Nalm6 T cells in indicated culture conditions at 48 hours.

CAR iT cells armed with ADR and CD58^{KO} demonstrate improved functional persistence and durable anti-tumor efficacy *in vivo* in an allogeneic xenograft model of disseminated aggressive leukemia

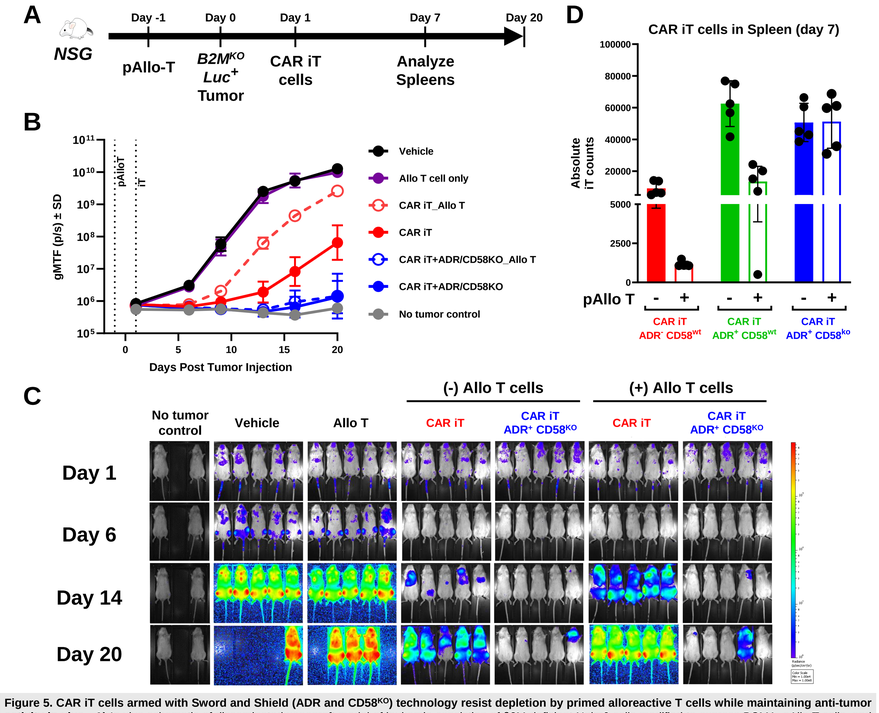


Figure 5. CAR iT cells armed with Sword and Shield (ADR and CD58^{KO}) technology resist depletion by primed alloreactive T cells while maintaining anti-tumor activity in vivo. A) In vivo schematic of disseminated xenograft model of leukemia consisting of β2M-deficient Nalm6 cells modified to express BCMA, pAllo T cells, and anti-BCMA CAR iT cells +/- ADR/CD58^{KO} with 5 replicates per group. **B)** Bioluminescence-based tumor quantification by total flux was measured to monitor tumor growth in mice. **C)** Representative images depicting tumor burden in all mice through to study termination (Day 20). **D)** Absolute iT cell counts among total splenocytes on Day 7 in the indicated conditions.