

levels in an array of malignancies including mesothelioma, ovaria, non-small cell lung cancer, and pancreatic cancers and is an attractive target antigen for immune-based therapies. Early clinical evaluation of autologous MSLN-targeted chimeric antigen receptor (CAR)-T cell therapies for malignant pleural mesothelioma has shown promising acceptable safety¹ and have recently evolved with incorporation of next-generation CAR co-stimulatory domains and armoring with intrinsic checkpoint inhibition via expression of a PD-1 dominant negative receptor (PD1DNR).² Despite the promise that MSLN CAR-T therapies hold, manufacturing and commercial challenges using an autologous approach may prove difficult for widespread application. EBV T cells represent a unique, non-gene edited approach toward an off-the-shelf, allogeneic T cell platform. EBV-specific T cells are currently being evaluated in phase 3 trials [NCT03394365] and, to-date, have demonstrated a favorable safety profile including limited risks for GvHD and cytokine release syndrome.³⁻⁴ Clinical proof-of-principle studies for CAR transduced allogeneic EBV T cell therapies have also been associated with acceptable safety and durable response in association with CD19 targeting.⁵ Here we describe the first preclinical evaluation of ATA3271, a next-generation allogeneic CAR EBV T cell therapy targeting MSLN and incorporating PD1DNR, designed for the treatment of solid tumor indications.

Methods We generated allogeneic MSLN CAR+ EBV T cells (ATA3271) using retroviral transduction of EBV T cells. ATA3271 includes a novel 1XX CAR signaling domain, previously associated with improved signaling and decreased CAR-mediated exhaustion. It is also armored with PD1DNR to provide intrinsic checkpoint blockade and is designed to retain functional persistence.

Results In this study, we characterized ATA3271 both in vitro and in vivo. ATA3271 show stable and proportional CAR and PD1DNR expression. Functional studies show potent antitumor activity of ATA3271 against MSLN-expressing cell lines, including PD-L1-high expressors. In an orthotopic mouse model of pleural mesothelioma, ATA3271 demonstrates potent antitumor activity and significant survival benefit (100% survival exceeding 50 days vs. 25 day median for control), without evident toxicities. ATA3271 maintains persistence and retains central memory phenotype in vivo through end-of-study. Additionally, ATA3271 retains endogenous EBV TCR function and reduced allototoxicity in the context of HLA mismatched targets.

Conclusions Overall, ATA3271 shows potent anti-tumor activity without evidence of allototoxicity, both in vitro and in vivo, suggesting that allogeneic MSLN-CAR-engineered EBV T cells are a promising approach for the treatment of MSLN-positive cancers and warrant further clinical investigation.

REFERENCES

1. Adusumilli PS, Zauderer MG, Rusch VW, *et al.* Abstract CT036: A phase I clinical trial of malignant pleural disease treated with regionally delivered autologous mesothelin-targeted CAR T cells: Safety and efficacy. *Cancer Research* 2019;**79**:CT036-CT036.
2. Kiesgen S, Linot C, Quach HT, *et al.* Abstract LB-378: Regional delivery of clinical-grade mesothelin-targeted CAR T cells with cell-intrinsic PD-1 checkpoint blockade: Translation to a phase I trial. *Cancer Research* 2020;**80**:LB-378-LB-378.
3. Prockop S, Doubrovina E, Suser S, *et al.* Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplantation. *J Clin Invest* 2020;**130**:733–747.
4. Prockop S, Hiremath M, Ye W, *et al.* A Multicenter, Open Label, Phase 3 Study of Tabelecleucel for Solid Organ Transplant Subjects with Epstein-Barr Virus-Driven

Post-Transplant Lymphoproliferative Disease (EBV+PTLD) after Failure of Rituximab or Rituximab and Chemotherapy. *Blood* 2019; **134**: 5326–5326.

5. Curran KJ, Sauter CS, Kernan NA, *et al.* Durable remission following 'Off-the-Shelf' chimeric antigen receptor (CAR) T-Cells in patients with relapse/refractory (R/R) B-Cell malignancies. *Biology of Blood and Marrow Transplantation* 2020;**26**:S89.

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STRUCTURAL OPTIMIZATION OF ANTI-CEA-GITR-CAR TO REDUCE TONIC SIGNALING AND IMPROVE ANTIGEN-SPECIFIC REACTIVITY

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Background Adoptive immunotherapy using chimeric antigen receptor (CAR) is recently reported as one of the effective cancer therapy. Especially CAR-T cell therapy targeting CD19 antigen in B-cell tumors have shown impressive clinical results and CAR-T cell products targeting CD19 have already approved. However as the high relapse rate is still the problem and the clinical efficacy of CAR-T cell therapy for solid tumors is currently inadequate, further improvement of CAR design is required. It is known that the design of CAR construct affects the function of CAR-T cells. For example co-stimulatory domain such as CD28 and 4-1BB is used in the second generation CARs, CD28z-CAR-T cells show higher anti-tumor activity, whereas 4-1BBz-CAR-T cells demonstrate superior in vivo persistence. To enhance survival of T cells, several attempts had been made to optimize the signaling domains. Recently, we have developed the novel CARs incorporated GITR (glucocorticoid-induced tumor necrosis factor receptor family-related protein) intracellular domain for T cell survival prolongation and inhibition of regulatory T cells' suppressive activity. It is also reported that the antigen-nonspecific activation of CAR-T cells (tonic signaling) is influenced by the CAR design, and excessive T cell activation leads exhaustion of CAR-T cells. Previously, we have found that the design of CAR, not only single chain variable fragments (scFvs), affect the strength of tonic signaling. Thus, the optimization of CAR construct is essential to induce antigen-specific response with minimal non-specific activation, which results in maximal efficacy.

Methods We have optimized the structure of anti-CEA-GITR-CAR targeting CEA antigen expressing solid tumor such as gastric and pancreatic cancer. We have constructed several CARs with the different composition such as hinge region, transmembrane domain, and the order of VL/VH in scFV region, and compared the tonic signaling and antigen-specific activity in CAR-T cells.

Results The property of CAR-T cells was largely affected by the CAR constructs, especially the hinge region. The CAR-T cells with CD8 α hinge showed strong tonic signaling, the CAR-T cells with short hinge-CAR lost antigen specificity, and elimination of hinge region lowered the CAR expression level and antigen reactivity. Furthermore, GITR-CAR-T cells showed higher proportion of CCR7+CD45RA+ cells and lower expression of exhaustion markers (PD1, Tim3, and LAG3) compared to CD28z-CAR-T cells.

Conclusions Our CEA-GITR-CAR with the optimized scFV design and CD28-hinge demonstrated improved antigen-

specific response with reduced tonic signaling, potentially indicating that our novel CAR-T cells may show improved clinical efficacy on solid tumor.

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DRUG-REGULATABLE ENGINEERED T CELLS ELIMINATE CD33+ AND CD33ΔE2+ AML

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Background Bioengineered T cell treatments for acute myeloid leukemia (AML) are challenged by near universal expression of leukemia antigens on normal hematopoietic stem/progenitor cells.¹⁻² ‘on target/off tumor’ activity may cause myelosuppression while sustained antigen exposure can lead to T cell exhaustion.³ In addition, splicing variants may allow antigen escape. We hypothesize that by using a novel CD33-C2-specific single domain VHH antibody as the antigen targeting domain in dimerizing agent-regulated immunoreceptor complex T cells (DARIC T cells), we will enable pharmacologically-controllable targeting of CD33, allowing eradication of leukemia expressing either of the major splice variants of CD33: i.e., full-length CD33 or CD33ΔE2.

Methods We engineered DARIC-expressing lentiviral vectors containing encoding separated CD33-C2-specific antigen binding and 41BB-CD3zeta signaling chains that heterodimerize following addition of rapamycin via embedded FKBP12 and FRB* domains.⁴ Peripheral blood mononuclear cells were stimulated with IL-2, anti-CD3, and anti-CD28 antibodies 24h prior to transduction with DARIC33 lentiviral vector. Surface expression of antigen binding or signaling chains was assessed using biotinylated CD33, or antibodies to VHH-domains or FRB* respectively. Rapamycin-dependent in vitro activity was measured by IFNγ release. To evaluate in vivo activity, NSG mice injected with 1×10^5 MOLM-14/luc cells were treated 5-7 days later with 1×10^7 DARIC33 T cells in the presence or absence of rapamycin and tumor progression followed by luciferase activity.

Results DARIC33+ T cells bound biotinylated-CD33, anti-VHH and anti-FRB* antibodies. Rapamycin addition increased expression of both signaling and antigen-recognition chains, suggesting augmented receptor stability in the presence of dimerizing drug. In the presence of rapamycin, coculture of DARIC33 T cells with cell lines expressing either full length or CD33ΔE2⁵ showed equivalent rapamycin-dependent activation, demonstrating DARIC33 responds to both splice variants. Titration experiments showed rapamycin-dependent activation with $EC_{50} = 25\text{pM}$. Negligible IFNγ release was observed in the absence of drug. DARIC33 T cells significantly extended survival of AML-bearing mice, but only when treated with rapamycin. The DARIC33 T cells were activated in vivo by sub-immunosuppressive rapamycin dosing, as weekly or 0.1 mg/kg QOD dosing led to similar levels of tumor suppression.

Conclusions DARIC33 T cells appear to be potent antileukemic agents: they are activated by AML cell lines in vitro as demonstrated by cytokine release and cytotoxicity, and significantly extend survival in an aggressive xenograft model.

Temporal control provided by the DARIC architecture promises to enhance safety and potentially efficacy of CAR T therapy for AML, for example by enabling hematopoietic recovery or providing T cell rest.

REFERENCES

1. Perna F, Berman SH, Soni RK, Mansilla-Soto J, Eyquem J, Hamieh M, *et al*. Integrating proteomics and transcriptomics for systematic combinatorial chimeric antigen receptor therapy of AML. *Cancer Cell* 2017 Oct 9;**32**(4):506–519.e5.
2. Haubner S, Perna F, Köhnke T, Schmidt C, Berman S, Augsberger C, *et al*. Co-expression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. *Leukemia*. 2019 Jan;**33**(1):64.
3. Lamarche C, Novakovsky GE, Qi CN, Weber EW, Mackall CL, Levings MK. Repeated stimulation or tonic-signaling chimeric antigen receptors drive regulatory T cell exhaustion. *bioRxiv*. 2020 Jun 28;2020.06.27.175158.
4. Leung W-H, Gay J, Martin U, Garrett TE, Horton HM, Certo MT, *et al*. Sensitive and adaptable pharmacological control of CAR T cells through extracellular receptor dimerization. *JCI Insight* [Internet]. 2019 Jun 6 [cited 2019 Jun 11];4(11). Available from: <https://insight.jci.org/articles/view/124430>
5. Pérez-Oliva AB, Martínez-Esparza M, Vicente-Fernández JJ, Corral-San Miguel R, García-Peñarrubia P, Hernández-Caselles T. Epitope mapping, expression and post-translational modifications of two isoforms of CD33 (CD33M and CD33m) on lymphoid and myeloid human cells. *Glycobiology* 2011;**21**(6):757–770.

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ENGINEERING GAMMA/Delta T CELLS WITH THE T-CELL ANTIGEN COUPLER RECEPTOR EFFECTIVELY INDUCES ANTIGEN-SPECIFIC TUMOR CYTOTOXICITY IN VITRO AND IN VIVO

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Background Engineered T cell therapies have revolutionized treatment of relapsed refractory haematological malignancies, however the cost of treatment for autologous products remains a significant challenge to their widespread use. The high cost is driven largely by the need for personalized manufacturing of autologous cell products. A non-conventional class of T cells, the gamma/delta T cell, can be safely transplanted into an unrelated recipient without inducing graft-versus host disease,¹ making them an ideal candidate for mass-manufactured off-the-shelf T cell therapies. We have previously described a novel method of directing conventional alpha/beta T cells towards tumour targets by co-opting the T cell receptor using the T cell Antigen Coupler (TAC) receptor.² Here, we describe the use of TAC receptors to engineer antigen-specific reactivity into gamma/delta T cells, resulting in highly potent anti-tumor cytotoxicity.

Methods Engineered gamma/delta T cells were manufactured by activating PBMCs with Zoledronate and IL-2. The TAC transgene was introduced into T cells using either VSV-G pseudotype lentivirus or GALV-psuedotyped gamma-retrovirus vectors. Through optimization studies, we determined transduction was highest 24 hours post-activation for lentivirus and 72 hours post-activation for gamma-retrovirus. Cultures were fed with IL-2 supplemented media every 2 – 3 days and enriched on Day 14 to >99% gamma/delta T cell purity using CD4/CD8 magnetic-activated cell sorting depletion (Miltenyi Biotec).

Results Both methods of gene transfer tested for our pilot study yielded excellent gene transduction (40% - 70%). Using lentivirus-engineered gamma/delta T cells, we demonstrated that the TAC receptor re-directs gamma/delta T cells to attack tumors in an antigen-specific manner. The presence of the TAC receptor did not interfere with lysis of tumor cells via the natural tumor-reactive gamma/delta T cell receptors.