ONCOIMMUNOLOGY
2018, VOL. 7, NO. 1, e1368604 (6 pages)
https://doi.org/10.1080/2162402X.2017.1368604

BRIEF REPORT

OPEN ACCESS

mSA2 affinity-enhanced biotin-binding CAR T cells for universal tumor targeting

Jason J. Lohmueller[a,†], James D. Ham[a,b,†], Michael Kvorjak[a], and Olivera J. Finn[a]

[a]University of Pittsburgh School of Medicine, Department of Immunology, Pittsburgh, PA USA; [b]Carnegie Mellon University, Department of Biomedical Engineering, Pittsburgh, PA USA

ABSTRACT

Chimeric antigen receptor T cells (CAR-Ts) are promising cancer therapeutics. However, since cancer cells can lose the CAR-targeted antigen and avoid destruction, targeting multiple antigens with multiple CARs has been proposed. We illustrate here a less cumbersome alternative, anti-tag CARs (AT-CARs) that bind to tags on tumor-targeting antibodies. We have created novel AT-CARs, using the affinity-enhanced monomeric streptavidin 2 (mSA2) biotin-binding domain that when expressed on T cells can target cancer cells coated with biotinylated antibodies. Human T cells expressing mSA2 CARs with CD28-CD3ζ and 4–1BB-CD3ζ signaling domains were activated by plate-immobilized biotin and by tumor cells coated with biotinylated antibodies against the tumor-associated antigens CD19 and CD20. Furthermore, mSA2 CAR T cells were capable of mediating cancer cell lysis and IFNγ production in an antibody dose-dependent manner. The mSA2 CAR is a universal AT-CAR that can be combined with biotinylated tumor-specific antibodies to potentially target many different tumor types.

ARTICLE HISTORY
Received 14 June 2017
Revised 10 August 2017
Accepted 12 August 2017

KEYWORDS
Chimeric antigen receptor; CAR; adoptive cell therapy; tag-CAR; cancer immunotherapy; synthetic biology

Chimeric antigen receptors (CARs) are synthetic T cell receptors used to re-direct T cell effector functions toward tumor associated antigens (TAAs).[1,2] Commonly consisting of a TAA-specific antibody single chain variable fragment (scFv) fused via a spacer and transmembrane domain to intracellular T cell signaling domains, when a CAR binds to its target antigen, T cell signaling is initiated leading to target cell lysis, cytokine production and cell proliferation.[3] Clinical trials with adoptively transferred CAR T cells targeting the B cell antigen CD19 have been highly successful in treating refractory acute lymphoblastic leukemia, and there is much interest in expanding the antigens targeted by CARs and the number of cancers that can be treated.[1,4-7]

Despite this success and interest, many challenges remain to expanding the application of CAR therapy. The single antigen specificity of CARs can render them ineffective against tumors that lack expression of the target antigen due to antigen loss or tumor heterogeneity.[8] Additionally, the unregulated persistence of CAR activity can cause cytokine release syndrome and other toxicities.[9] For targeting new cancers, the creation of new CARs is needed, which is technically challenging and requires extensive engineering and safety testing to rule out off-tumor toxicities.[10]

One alternative approach that addresses some of these challenges is the creation of CARs that bind to common tag molecules – such as fluorescein isothiocyanate (FITC), peptide neo-epitopes (PNE), Fcγ, and biotin – that are conjugated to TAA-specific antibodies.[11-14] So-called anti-tag CAR (AT-CAR) therapy would be designed so that patients are infused with a tagged, TAA-specific antibody that binds to tumor cells, followed by T cells expressing AT-CARs that react with the tagged antibodies on tumor cells (Fig. 1A). This approach has the potential to allow for sequential or simultaneous targeting of multiple tumor antigens with different antibodies.[15] Additionally, the activity of AT-CARs can be regulated by altering the concentration of tagged antibodies or halting antibody administration for better control over potential toxicities.[16]

Here we report the construction and characterization of a new AT-CAR with potent activity composed of the affinity-enhanced monomeric streptavidin (mSA2) protein, engineered to have high affinity for biotin compared to other monomeric and dimeric avidins ($K_d = 5.5 \times 10^{-9}$ at 37°C).[17] Previous studies have shown that higher affinity can lead to greater T cell activation and antitumor response in the AT-CAR format.[16] We find that mSA2 CAR T cells are efficiently stimulated by plate-immobilized biotin and that they are capable of potent target cell lysis and cytokine production when combined with biotinylated TAA-specific antibodies.

We first constructed lentiviral vectors encoding two mSA2 CAR signaling domain variants, mSA2-41BBζ and mSA2-CD28ζ (Fig. 1B). Driven by the EF1α promoter, CAR-coding regions consisted of the murine Igκ leader sequence, the codon-optimized mSA2 protein domain, the CD8α-hinge spacer domain, the CD28 transmembrane domain, either the CD28 or the 4-1BB cytoplasmic domain and the CD3ζ cytoplasmic

CONTACT Olivera J. Finn ojfinn@pitt.edu University of Pittsburgh School of Medicine, E1040 BST, Pittsburgh, PA 15232, USA.
Supplemental data for this article can be accessed on the publisher's website.
† These authors contributed equally to this work.
© 2018 Jason J. Lohmueller, James D. Ham, Michael Kvorjak and Olivera J. Finn. Published with license by Taylor & Francis Group, LLC
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

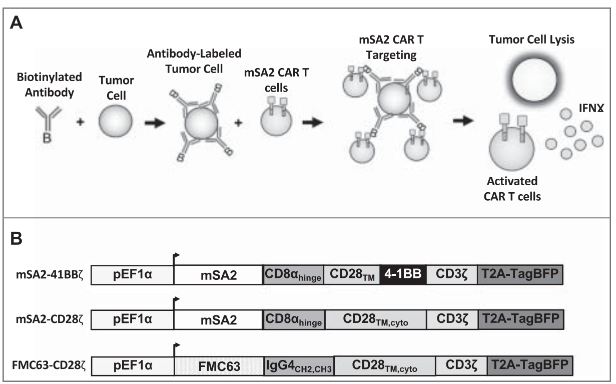

Figure 1. Schematic of the function and vector design of mSA2 anti-biotin CARs. (A) A tumor-specific antibody that is biotinylated encounters and binds to its target antigen on a tumor cell while simultaneously providing biotin as a new target. Avidin-bearing mSA2 CAR T cells recognize the biotin as their specific target, which leads to their activation, cytokine production and tumor cell lysis. (B) Designs of the mSA2-41BBζ and mSA2-CD28ζ CAR lentiviral expression constructs and the positive control FMC63-CD28ζ anti-CD19 CAR construct.

domain. Additionally, to mark transduced cells, we added the TagBFP marker gene via a T2A co-translation peptide.[18] An anti-CD19 CAR, FMC63-CD28ζ, which consisted of the FMC63 scFv, the IgG4 extracellular spacer, CD28 transmembrane and co-signaling domains and CD3ζ, was also constructed as a positive control CAR.[19] All the vectors were packaged into lentiviruses and transduced into primary human T cells.

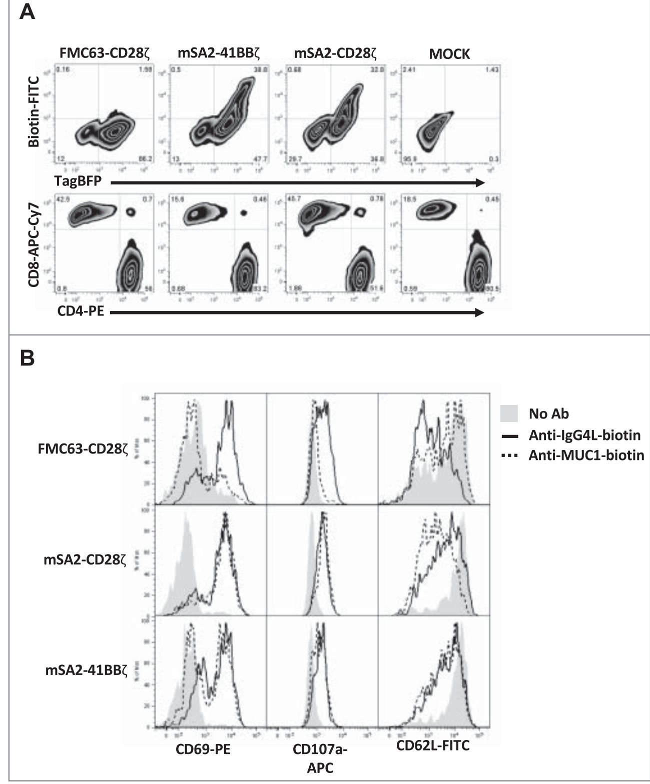

Figure 2. Cell surface expression of mSA2 CARs and activation of primary human T cells. (A) Primary human T cells were transduced with lentiviruses encoding the mSA2–41BBζ CAR, the mSA2-CD28ζ CAR or the FMC63-CD28ζ CAR. After one stimulation cycle, cells were sorted by flow cytometry for TagBFP expression and stained with biotin-FITC or for CD4 and CD8 expression. (B) CAR T cells were plated in the presence of the indicated plate-immobilized biotinylated antibody for 18 hours and assayed by flow cytometry for T cell activation markers CD69, CD107a, and CD62L (down-regulated upon activation).

We found that the mSA2 CARs were efficiently expressed on the cell surface (Fig 2A) and that the mSA2 CAR T cells could be activated by plate-bound biotin (Fig 2B). Following one stimulation cycle, transduced T cells were sorted for TagBFP expression and then stained with biotin-FITC. The staining with biotin-FITC was specific to mSA2 CAR T cells and correlated with TagBFP expression (Fig. 2A). T cells were also evaluated for helper and cytotoxic populations based on CD4 and CD8 expression. Next, we found that the mSA2 CAR T cells could be efficiently activated by plate-immobilized biotin (Fig. 2B). Specifically, we incubated CAR T cells or control cells on plates coated with biotinylated antibodies for 18 hours and then assayed cells for T cell activation markers by flow cytometry. Incubation with plate immobilized biotin led to the upregulation of T cell activation markers CD69 and CD107 a as well as the downregulation of CD62 L. We found that FMC63-CD28ζ CAR T cells were activated by the plate immobilized anti-hIgG4 antibody that can bind to the IgG4 extracellular spacer but not by a biotinylated antibody against an irrelevant target (anti-MUC1 antibody H15K6).

Next, we tested if mSA2 CAR T cells could be combined with anti-tumor antibodies to mediate specific T cell effector functions. We co-incubated mSA2–41BBζ or mSA2-CD28ζ T cells with CD20+ Raji target cells (Fig. S1) in the presence of varying amounts of biotinylated Rituximab (anti-CD20). The mSA2 CAR T cells responded by up-regulating T cell activation markers in a dose-responsive manner to the biotinylated Rituximab (Fig. 3A). They also produced IFNγ (Fig. 3B) and performed target cell lysis (Fig. 3C) in a dose-responsive manner. We found that mSA2-CD28ζ T cells produced vastly more IFNγ compared to the mSA2–41BBζ T cells, however, both showed comparable levels of specific target cell lysis. This result is consistent with previous findings for traditional CARs with CD28 versus 4–1BB co-signaling domains from several research groups.[20-23] It is likely the result of established differences in CD28 and 4–1BB signaling pathways which signal via Akt and TRAFs, respectively.[21,24] Notably, incubating the mSA2 CAR T cells with biotinylated antibody and off-target cells lead neither to T cell activation nor any significant induction of T cell effector functions, indicating that immobilizing the antibodies on the surface of the target cells was necessary to initiate CAR T cell receptor signaling and that soluble antibody alone could not induce activation. As an additional control, we found that MOCK transduced T cells, when combined with biotinylated antibodies, were not activated nor induced to produce cytokines or lyse tumor cells (Fig. S2).

We then tested the activity of mSA2–41BBζ and mSA2-CD28ζ CAR T cells when combined with various antigen-positive and antigen-negative target cell lines, in the presence of biotinylated Rituximab, FMC63 (anti-CD19), or biotinylated Cetuximab (anti-EGFR) antibodies. Biotinylated Cetuximab served as a negative control as its target antigen, EGFR, is not expressed on any of the targeted cell lines. Target cell lines included Jurkat cells which are negative for both CD19 and CD20, K562 cells which are negative for both CD19 and CD20, K562 cells which are engineered to express CD19 (K562+CD19), and Raji cells which are naturally positive for both CD19 and CD20 (Fig. S1). Following co-incubation of T cells and target cells with the different biotinylated antibodies, we found that the mSA2-CAR T cells were induced to express

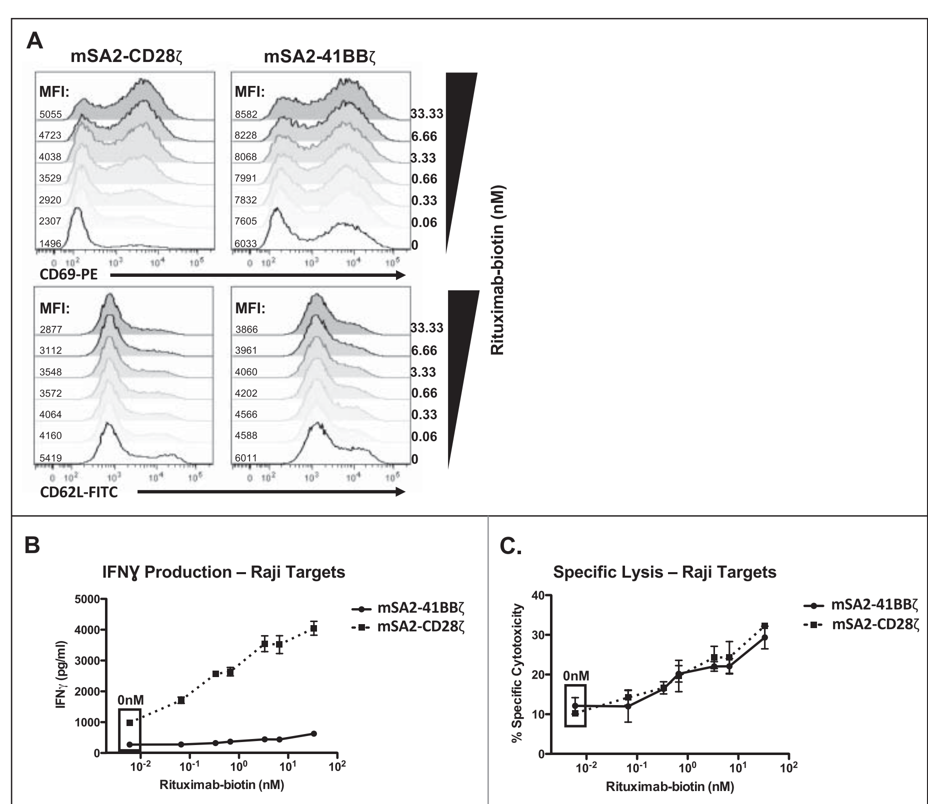

Figure 3. mSA2 CAR T cells display biotinylated antibody dose-responsive effector functions. mSA2-CD28ζ or mSA2–41BBζ cells were incubated at an E:T ratio of 10:1 with CD20+ Raji tumor targets in the presence of increasing concentrations of biotinylated anti-CD20 antibody Rituximab for 18 hours. (A) Cells were stained for activation markers CD69 (upper panels) and CD62 L (lower panels) and evaluated by flow cytometry. (B) Supernatants from co-incubations were evaluated by ELISA for IFNɣ production. (C) Co-incubations were performed similar to (A) and (B) however target cells were labeled with Cell Trace Yellow were further evaluated by flow cytometry for viability by staining with Ghost Dye Red. For (B) and (C), multiple ANOVA comparisons were performed. As the data did not have homogeneity of variance (Levene's test), Tukey's HSD was used for post hoc analysis between antibody conditions. "*" denotes a significance of $p < .01$ and "NS" stands for not significant. Error bars represent standard deviations for $n = 3$ replicates.

activation markers (Fig. 4A), produce IFNγ (Fig. 4B) and lyse target cells (Fig. 4C). Importantly, the presence of both the biotinylated antibody and the targeted antigen on the cancer cells was required for mSA2 CAR cells to be activated and functional. We found that the lytic abilities of mSA2-CD28ζ CAR cells were comparable to the FMC63-CD28ζ positive control CAR.

Future studies will include *in vivo* testing of mSA2–41BBζ and mSA2-CD28ζ CAR T cells in mice bearing human tumor xenografts with different biotinylated antibody doses and schedules. The potency of the biotinylated antibodies and mSA2 CAR T cells could potentially be further improved by performing site-specific biotinylation on the antibodies at various amino acid positions and selecting for sites that give the most potent lysis, likely optimizing the formation of the T cell synapse.[16]

For future therapeutic applications one concern is the potential immunogenicity of the mSA2 protein. While it is known that tetrameric avidin can elicit antibody and cellular responses, additional studies will be necessary to determine immunogenicity of the monomeric mSA2.[25] The monomeric form with fewer repeating structures could be expected to elicit a more diminished antibody response. Nevertheless, mutations have been reported that can make tetrameric avidin less immunogenic, which could be applied to the mSA2 protein domain.[26] As many CARs in the clinic have immunogenic mouse antibody domains including the most common CAR FMC63, there could be a similar therapeutic window in which the mSA2-CAR T cells could function before being rejected. In comparison to other tag-CARs, the biotin tag may be better tolerated than PNE or FITC, which are entirely foreign molecules.

The mSA2-CAR is a new AT-CAR with potential for targeting several different antigens by T cells engineered to express a single CAR. Complementary to previously developed tag-CARs, the mSA2 avidin binding domain has a unique compact structure (122 amino acids in length) that may make it suitable to targeting particular new antigens. This CAR has great promise for use in combination with the ever-increasing list of FDA-approved tumor-targeting antibodies and antibodies in clinical development.[7] It is also ripe for combining with antibodies that display tumor-specificity but lack anti-tumor therapeutic activities on their own. In addition to future promise as an off-the-shelf therapeutic, which will be tested in preclinical animal models, the mSA2-CAR can already be used as an off-the-shelf reagent for preselecting *in vitro* the best candidate antibodies for antigen binding domains of traditional CARs before proceeding with their construction.

Materials and methods

Lentiviral vector construction and virus production

The CAR coding regions listed in Supplementary Table S1. were synthesized (Integrated DNA Technologies) and cloned into the pSICO-EF1 vector using Gibson Assembly.[18] Virus

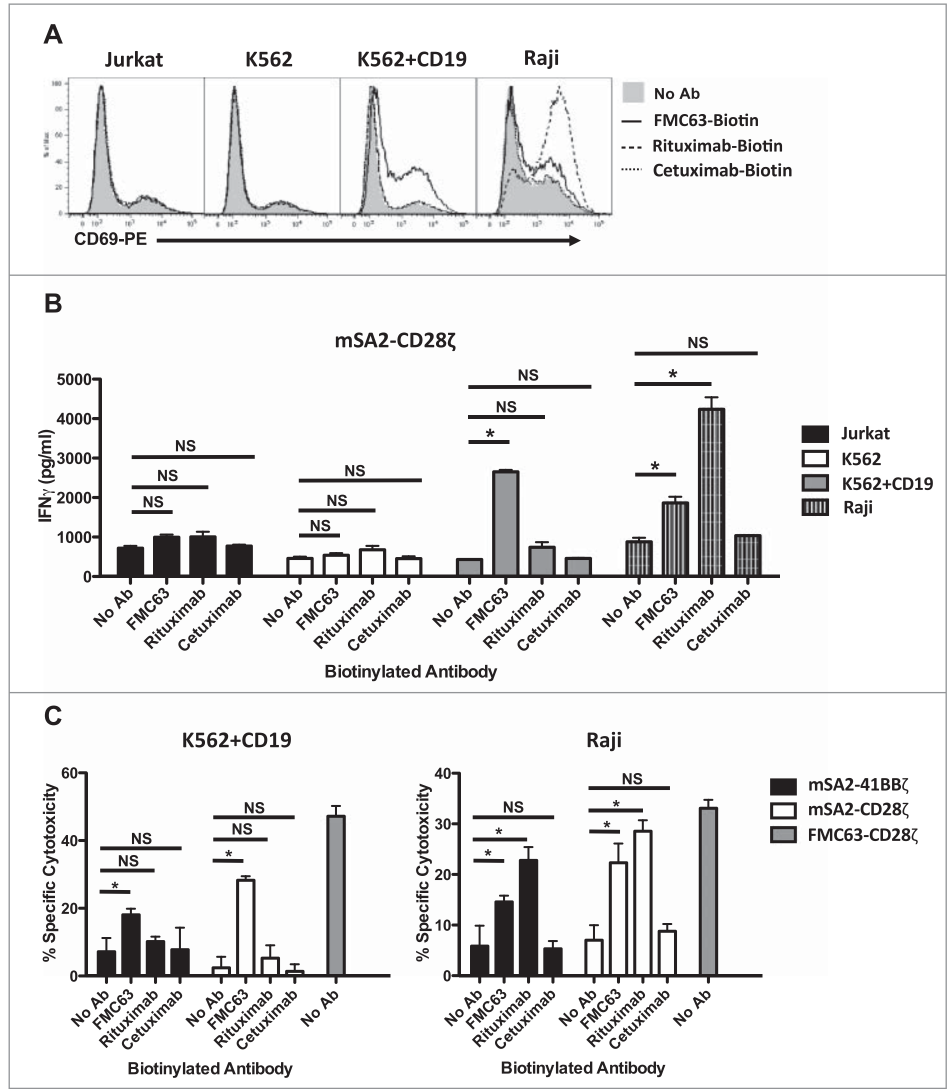

Figure 4. mSA2 CAR T cells display biotinylated-antibody mediated effector functions against various target cell lines. Tumor target cells, CAR T cells, and 5 μg/ml (33.33 nM) of the indicated antibodies were incubated for 18 hours and (A) cells were stained for the activation marker CD69 and evaluated by flow cytometry. (B) Supernatants from co-incubations were evaluated by ELISA for IFNγ production. (C) Co-incubations were performed similar to (A) and (B) however target cells were labeled with Cell Trace Yellow were further evaluated by flow cytometry for viability by staining with Ghost Dye Red. For (B) and (C), multiple ANOVA comparisons were performed. As the data did not have homogeneity of variance (Levene's test), Tukey's HSD was used for post hoc analysis between antibody conditions. "*" denotes a significance of $p<.01$ and "NS" stands for not significant. Error bars represent standard deviations for $n = 3$ replicates.

was generated using the above described transfer vectors following methods described previously in detail.[18]

Antibody biotinylation

Antibodies FMC63, Rituximab and Cetuximab were purchased (Absolute Antibody) and biotinylated using the EZ-Link NHS biotin kit (ThermoFisher Scientific) and were determined by HABA assay to contain an average of 3–4 molecules of biotin per antibody.

Cell line culture

Human tumor cell lines Jurkat Clone E6–1 (TIB-152), K562 (CCL-243), and Raji (CCL-86) were obtained from American Type Culture Collection (ATCC) and cultured at 37°C in RPMI medium supplemented with 1X MEM amino acids solution, 10 mM Sodium Pyruvate, 10% fetal bovine serum (FBS) and Penicillin-Streptomycin (Life Technologies). K562+CD19 cells that stably express full-length CD19 were generated by transducing K562 cells with CD19-expressing lentivirus and sorting for cells positive for CD19 expression. HEK293 T (human embryonic kidney) cells (ATCC) were cultured at 37°C in DMEM supplemented with 10% FBS, and Penicillin-Streptomycin.

Primary human T cell culture and lentiviral transduction

All experiments were performed on PBMC isolated from de-identified human Buffy Coat samples purchased from the Pittsburgh Central Blood Bank fulfilling the basic exempt criteria 45 CFR 46.101(b)(4) in accordance with the University of Pittsburgh IRB guidelines. Human T cells were cultured in supplemented RPMI media as described for cell lines above, however, 10% Human AB serum (Gemini Bio Products) was used instead of FBS, and the media was further supplemented with

100 U/ml human IL-2 IS and 1 ng/ml IL-15 (Miltenyi Biotec). PBMC were isolated from a Buffy Coat from healthy volunteer donors using Ficoll centrifugation and total human T cells were isolated using the Human Pan T cell isolation kit (Miltenyi Biotec). T cells were stimulated and expanded using TransAct Human T cell Activation Reagent (Miltenyi Biotec). For transduction, two days after addition of TransAct, lentivirus was added to cells at an MOI of 10–50 in the presence of 6 μg/ml of DEAE-dextran (Sigma Aldrich). After 18 hours, cells were washed and resuspended in fresh T cell media containing 100 U/ml IL-2 and 1 ng/ml IL-15. After an additional 12 days of stimulation and expansion, CAR+cells were flow-sorted by TagBFP expression. To obtain sufficient numbers of cells for experiments, sorted CAR+ cells then underwent an additional TransAct stimulation cycle prior to being assayed.

Flow cytometry staining

Cells were stained using the indicated antibodies and diluted in flow cytometry buffer (PBS + 2% FBS), for 30 minutes at 4 °C followed by two washes with flow cytometry buffer. Live cells were gated based on forward and side scatter and CAR+ cells were gated on TagBFP expression. 50,000 total events were recorded per sample.

Plate-immobilized biotin stimulation assay

High protein-binding 96 well flat-bottom plates (Corning) were coated with 10 μg/ml of biotinylated antibody in PBS or with PBS alone for 2 hours at 37°C and washed 2 times with PBS. 100,000 CAR T cells were incubated on the plate for 18 hours. After incubation, cells were stained with antibodies against T cell activation markers CD69-PE (BD Biosciences), CD62L-FITC (BD Biosciences) and CD107a-APC (BD Biosciences) and evaluated for marker expression by flow cytometry.

CAR T cell and target cell antibody-mediated activation co-incubation assay

100,000 primary T cells were co-cultured with 10,000 target cells and the indicated amounts of biotinylated antibodies for 18 hours. After incubation, cells were stained with antibodies against T cell activation markers CD69-PE (BD Biosciences) and CD62L-FITC (BD Biosciences) and evaluated for activation marker expression by flow cytometry. Supernatants from these co-cultures were also collected and analyzed for the presence of IFNɣ by ELISA (BioLegend). Assays were performed in triplicate and average IFNɣ production was plotted with standard deviation.

Target cell lysis assay

Target cells were stained with Cell Trace Yellow following manufacturer's recommendation (ThermoFisher), re-suspended in DMEM-media and plated at 10,000 cells per well in 50 μl in a 96 well V-bottom plate. 50 μL of CAR T cells were added at E:T ratio of 10:1 (100,000 effector cells). Plates underwent a quick spin to collect cells at the bottom of the wells and were incubated at 37°C for 18 hours. To identify lysed cells, samples were stained with Ghost Dye Red Viability Dye (Tonbo Biosciences) and analyzed by flow cytometry. Target cells were identified by Cell Trace Yellow and lysed target cells were identified by positive Ghost Dye staining. Specific cytotoxicity was calculated by the equation: 100 * (% experimental lysis – % target-only lysis) / (100 – % target-only lysis).

Disclosure statement

The authors declare no conflicts of interest.

Acknowledgments

The authors would like to acknowledge the members of the Finn lab and DB Thompson for helpful discussions. The authors would also like to acknowledge LC Lohmueller for performing the statistical analysis of the data.

Funding details

This work was supported by the National Institutes of Health grant R35 CA210039 (O.J.F.); the Undergraduate Research Office of Carnegie Mellon University (JDH); and by the American Cancer Society grant PF LIB 125429 (J.L.). This work benefitted from SPECIAL BD LSRFORTESSA funded by NIH 1S10OD011925-01.

References

1. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371:1507-17. https://doi.org/10.1056/NEJMoa1407222. PMID:25317870
2. Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci U S A. 1989;86:10024-8. https://doi.org/10.1073/pnas.86.24.10024. PMID:2513569
3. Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013;3:388-98. https://doi.org/10.1158/2159-8290.CD-12-0548. PMID:23550147
4. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, Teachey DT, Chew A, Hauck B, Wright JF, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. N Engl J Med. 2013;368:1509-18. https://doi.org/10.1056/NEJMoa1215134. PMID:23527958
5. Park JH, Riviere I, Wang X, Bernal YJ, Yoo S, Purdon T, et al. CD19-Targeted 19–28z CAR Modified Autologous T Cells Induce High Rates of Complete Remission and Durable Responses in Adult Patients with Relapsed, Refractory B-Cell ALL. Blood 2014;124:382.
6. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365:725-33. https://doi.org/10.1056/NEJMoa1103849. PMID:21830940
7. Lohmueller J, Finn OJ. Current modalities in cancer immunotherapy: Immunomodulatory antibodies, CARs and vaccines. Pharmacol Ther 2017;pii:S0163-7258(17)30088-8. https://doi.org/10.1016/j.pharmthera.2017.03.008. PMID:28322974
8. Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, Klichinsky M, Aikawa V, Nazimuddin F, Kozlowski M, et al. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest. 2016;126:3814-26. https://doi.org/10.1172/JCI87366. PMID:27571406
9. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124:188-95. https://doi.org/10.1182/blood-2014-05-552729. PMID:24876563
10. Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The future of cancer treatment: immunomodulation, CARs and combination

immunotherapy. Nat Rev Clin Oncol. 2016;13:394. https://doi.org/10.1038/nrclinonc.2016.65. PMID:27118494

11. Rodgers DT, Mazagova M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR, Schulman A, Du J, Wang F, Singer O, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proc Natl Acad Sci U S A. 2016;113:E459-68. https://doi.org/10.1073/pnas.1524155113. PMID:26759369
12. Tamada K, Geng D, Sakoda Y, Bansal N, Srivastava R, Li Z, Davila E. Redirecting gene-modified T cells toward various cancer types using tagged antibodies. Clin Cancer Res. 2012;18:6436-45.https://doi.org/10.1158/1078-0432.CCR-12-1449. PMID:23032741
13. Urbanska K, Lanitis E, Poussin M, Lynn RC, Gavin BP, Kelderman S, Yu J, Scholler N, Powell DJ Jr. A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. Cancer Res. 2012;72:1844-52. https://doi.org/10.1158/0008-5472.CAN-11-3890. PMID:22315351
14. Kudo K, Imai C, Lorenzini P, Kamiya T, Kono K, Davidoff A, Chng WJ, Campana D. T lymphocytes expressing a CD16 signaling receptor exert antibody-dependent cancer cell killing. Cancer Res. 2014;74:93-103. https://doi.org/10.1158/0008-5472.CAN-13-1365. PMID:24197131
15. Urbanska K, Powell DJ. Development of a novel universal immune receptor for antigen targeting: To Infinity and beyond. Oncoimmunology 2012;1:777-9. https://doi.org/10.4161/onci.19730. PMID:22934280
16. Ma JS, Kim JY, Kazane SA, Choi SH, Yun HY, Kim MS, Rodgers DT, Pugh HM, Singer O, Sun SB, et al. Versatile strategy for controlling the specificity and activity of engineered T cells. Proc Natl Acad Sci U S A. 2016;113:E450-8. https://doi.org/10.1073/pnas.1524193113. PMID:26759368
17. Lim KH, Huang H, Pralle A, Park S. Stable, high-affinity streptavidin monomer for protein labeling and monovalent biotin detection. Biotechnol Bioeng. 2013;110:57-67. https://doi.org/10.1002/bit.24605. PMID:22806584
18. Lohmueller JJ, Sato S, Popova L, Chu IM, Tucker MA, Barberena R, Innocenti GM, Cudic M, Ham JD, Cheung WC, et al. Antibodies elicited by the first non-viral prophylactic cancer vaccine show tumor-specificity and immunotherapeutic potential. Sci Rep. 2016;6:31740. https://doi.org/10.1038/srep31740. PMID:28442756
19. Kochenderfer JN, Feldman SA, Zhao Y, Xu H, Black MA, Morgan RA, Wilson WH, Rosenberg SA. Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. J Immunother. 2009;32:689-702. https://doi.org/10.1097/CJI.0b013e3181ac6138. PMID:19561539
20. Brentjens RJ, Santos E, Nikhamin Y, Yeh R, Matsushita M, La Perle K, Quintás-Cardama A, Larson SM, Sadelain M. Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. Clin Cancer Res. 2007;13:5426-35. https://doi.org/10.1158/1078-0432.CCR-07-0674. PMID:17855649
21. Campana D, Schwarz H, Imai C. 4-1BB chimeric antigen receptors. Cancer J. 2014;20:134-40. https://doi.org/10.1097/PPO.0000000000000028. PMID:24667959
22. Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhal M, Suhoski MM, Varela-Rohena A, Haines KM, Heitjan DF, Albelda SM, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc Natl Acad Sci U S A. 2009;106:3360-5. https://doi.org/10.1073/pnas.0813101106. PMID:19211796
23. Finney HM, Akbar AN, Lawson AD. Activation of resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. J Immunol. 2004;172:104-13. https://doi.org/10.4049/jimmunol.172.1.104. PMID:14688315
24. Kane LP, Andres PG, Howland KC, Abbas AK, Weiss A. Akt provides the CD28 costimulatory signal for up-regulation of IL-2 and IFN-gamma but not TH2 cytokines. Nat Immunol. 2001;2:37-44. https://doi.org/10.1038/83144. PMID:11135576
25. Weir C, Hudson AL, Moon E, Ross A, Alexander M, Peters L, Langova V, Clarke SJ, Pavlakis N, Davey R, et al. Streptavidin: a novel immunostimulant for the selection and delivery of autologous and syngeneic tumor vaccines. Cancer Immunol Res. 2014;2:469-79. https://doi.org/10.1158/2326-6066.CIR-13-0157. PMID:24795359
26. Yumura K, Ui M, Doi H, Hamakubo T, Kodama T, Tsumoto K, Sugiyama A. Mutations for decreasing the immunogenicity and maintaining the function of core streptavidin. Protein Sci. 2013;22:213-21. https://doi.org/10.1002/pro.2203. PMID:23225702