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TITLE: Engineering Next-Generation CAR T Cells to Treat Pediatric AML: Enhancing Safety Through Dynamic Control and Specificity

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13. SUPPLEMENTARY NOTES

14. Over the course of the last year we have developed synthetic cellular circuitry to: 1) titrate cytotoxic activity of a CAR T cell using a gamma secretase inhibitor drugs. Specifically, we have successfully engineered CAR T cells with a reversible OFF switch by using a synthetic Notch (synNotch) receptor that drives expression of a CAR. The synNotch receptor is a new type of antigen triggered receptor that can induce a transcriptional response. SynNotch induction of CAR expression can be tightly regulated by inhibition of the intracellular gamma secretase cleavage site by gamma secretase inhibitors. We have shown that CAR expression and cytotoxic activity can be titrated by this class of small molecules. We have demonstrated the cytotoxic activity, specificity and dynamics of this titratable CAR T cell in vitro and have developed a protocol to test the OFF-switch in immunocompromised mice. From last year's data, we have not been able to test Nirogacestat *in vivo* as the price has been prohibitive ($1,000/mouse/experiment). We therefore turned towards another GSI, crenigacestat, which was evaluated safe in a phase 1 clinical trial and is currently being tested in association with BCMA CAR T cell therapy in a clinical trial. Our preliminary *in vitro* data suggest that this new drug could be a great alternative to Nirogacestat. The cost of the drug for the in vivo application is affordable ~ $50/mouse/experiment. 2) demonstrate *in vivo* that we can target abnormal combinations of cell surface antigens using combinatorial recognition approach using mouse model of glioblastoma. Using a conceptually similar circuit based of a synNotch driving a CAR for glioblastoma cancer treatment, we were able to show that synNotch-CAR T cells showed less differentiation, less exhaustion *in vitro* and additionally showed that this circuit efficiently and durably cleared the glioblastoma cancer *in vivo*. This work is conceptually similar to the issues facing AML for recognition as it requires combinatorial reignition of antigen which on their own are imperfect and could lead to off-target toxicity but when used in combination overcome these issues. Therefore, we feel confident that this approach could be very fruitful for AML using our previously identified combinatorial AML antigen pairs that are specific for AML cells and will translate into a greater efficacy *in vivo*, overcoming the major challenges faced by current CAR T cell treatments for blood cancer.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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1. INTRODUCTION:

Pediatric myeloid hematopoietic malignancies represent a spectrum of diseases that are particularly challenging to treat. The majority of pediatric myeloid leukemias are acute myeloid leukemia (AML). Although progress has been made in the treatment of pediatric AML, the 5-year survival rate is still approximately 50%.[2] There is an urgent need to develop more effective and less toxic treatments for this deadly disease. Chimeric Antigen Receptor (CAR) T cell therapies have emerged as a powerful class of anti-cancer therapeutics, particularly for B-cell acute lymphoblastic leukemia (B-ALL), where unprecedented rates of remission have been achieved in the multiply relapsed and refractory population. CARs are a synthetically engineered receptor that are expressed in an autologous T cell and allow for re-direction of cytotoxic T cell activity towards an antigen of choice. presents a particularly attractive candidate for T cell therapy for a number of reasons: extensive knowledge of AML cell surface expression exists, relative ease of sampling tumor from peripheral blood draws or bone marrow aspirates, and a natural preference for T cells to home to hematologic organs such as the blood, bone marrow and lymph nodes. However, there are no leukemia-specific surface antigens in AML. Although these CAR T cells are capable of effectively eradicating AML in vitro, they would likely lead to profound and potentially fatal myelosuppression via on-target/off-tumor myeloid progenitor cell depletion.

2. KEYWORDS:

Acute myeloid leukemia, immunotherapy, pediatric oncology, chimeric antigen receptor T cells, synthetic notch receptor, hematologic malignancies, immune-oncology, synthetic biology

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1(specified in proposal)	Proposed Timeline	Percent Completed
Major Task 1: Engineer anti-AML CAR T cells with a controllable ON/OFF-switch	Months	50% **("60%", see explanation using a new concept)**

Subtask 1: ON/OFF CAR scFv design and cloning	1-2	50%
Subtask 2: Confirm CAR T cell recognition and cytotoxic activity in vitro in AML cell lines and patient samples	3-6	100%
Subtask 3: Assay toxicity in vitro in human cord blood and bone marrow cells	6-9	50%
Subtask 4: In vivo assay to assess AML cell clearance and healthy myeloid toxicity	9-18	10% **("40%", see explanation using a new concept)**
Major Task 2: Generate dual-receptor, AND-gate T cells with enhanced myeloid leukemic specificity		40% **("50%", see explanation using a new concept)**
Subtask 1: design and clone AND-gate T cell circuitry with CD33 synNotch and aberrant antigen B (CD123, CD15, CD64, CD19) CAR	1-2	60%
Subtask 2: design and clone AND-gate T cell circuitry with CD33 or CD123 synNotch and CD33 or CD123 CAR to target antigens that are over-expressing AML cells	3-4	40%
Subtask 3: design and clone AND-gate T cell circuitry with CD33 synNotch and stress antigen B (CD47 or NKG2D ligand) CAR	5-6	30%
Specific Aim 2	**Proposed Timeline**	**Percent Completed**
Major Task 3: test dual-receptor, AND-gate T cells for AML specificity and cytotoxicity as well as healthy myeloid toxicity		25% **("50%", see explanation using a new concept)**
Subtask 1: Confirm dual-receptor, AND-gate T cell recognition and cytotoxic activity in vitro in AML cell lines and patient samples	6-12	25%
Subtask 2: Assay toxicity in vitro in human cord blood and bone marrow cells	12-15	0%
Subtask 4: In vivo assay to assess AML cell clearance	15-24	0%

What was accomplished under these goals?

Major Conclusions:

- Major Task 1: We have generated a novel ON/OFF switch which is controlled by a new small molecule, Crenigacestat which is more affordable than Nirogacestat. Here we use a synNotch receptor to induce expression of a CAR. Crenigacestat is also a gamma-secretase inhibitor, that blocks cleavage and output of the synNotch receptor. Preliminary results show that this can be used to achieve titratable and reversible control of CAR T cell activity. *In vitro* this circuit achieves tumor clearance similar to the constitutive CAR expression and shows less T cell differentiation as well as less exhaustion. These characteristics have been positively correlated with better in vivo performance in mice and humans. We further provide *in vivo* data on glioblastoma model that demonstrates longer T cell persistence and enhanced clearance (see tasks below).
- Major Task 2: While no major change since last year are reporter, the use of a conceptually similar circuit on glioblastoma models demonstrates that this approach can be successful for combinatorial recognition and improved tumor clearance as detailed below.
- Major Task 3: No major change since last year but see Major Task 2 above as this also applies to this Major task.

Major activities, specific objectives, significant results or key outcomes:

On Specific Aim 1, we are working rapidly towards completion of the ON/OFF switch using a new GSI: We have also developed an additional, more effective switch with great promise for the treatment of AML. Below, we further extend our characterization of this novel circuit. Additionally, This switch is controlled by the small molecule Crenigacestat which is more affordable than the previously used one Nirogacestat and will allow us to proceed with planned *in vivo* experiments.

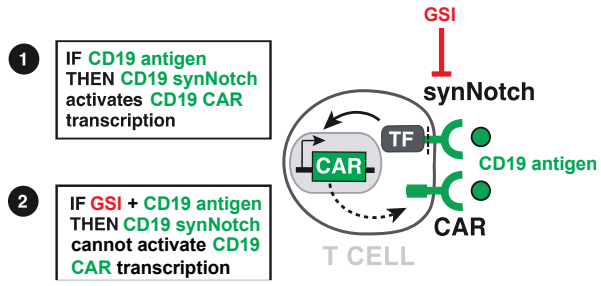

Figure 1: Titratable approach for cytotoxic therapy administration.

Our approach uses an autoregulatory synthetic circuit that is capable of dynamically controlling chimeric antigen receptor (CAR) cytotoxic T cell activity. This T cell circuit is dependent on using two receptors to recognize the target disease antigen – first a synthetic notch (synNotch) receptor recognizes the B-cell specific antigen, CD19, on the surface of B-cell leukemia and lymphoma cells, and in response, induces the gene expression of a CAR that also recognizes the target antigen, CD19 (**Figure 1.1**). This circuit is capable of autoregulation – the cytotoxic activity enabled by the CAR is only induced when the synNotch receptor first detects cells with CD19. Conversely, once malignant cells expressing CD19 cells are killed, CAR expression and cytotoxic activity will autonomously decrease in response to decreasing antigen burden. This two-receptor circuit has two main advantages over the conventional, constitutively expressed CAR T cell: 1) in the absence of antigen, CAR is not expressed, minimizing tonic signaling during CAR T cell manufacturing and expansion (tonic signaling is known to lead to T cell exhaustion), and 2) in the presence of antigen, CAR expression is dependent on antigen detection by synNotch and therefore CAR expression is dynamic, proportional to antigen load, and

expressed only at the level required to clear antigen, features that are likely to lead to reduced T cell exhaustion in vivo (and longer overall persistence of T cells) and reduced toxic adverse side effects, such as strong cytokine release syndrome.

Building upon our previous data we further characterized the T cell phenotypes using a multiple challenge assay and by looking at their differentiation and their exhaustion (**Figure 2A** for a detail of the assay). Tonic signaling has been shown to increase exhaustion and induce effector T-cell differentiation resulting in poor *in vivo* performance. Central memory T cells and stem cell-like memory T cells are known to promote sustained proliferation and persistence after T-cell therapy. Additionally, lack of CAR-T cell *in vivo* persistence was due to a lower percentage of naïve T cells vs. effector memory T cells prior to *in vivo* delivery as *ex vivo* cultivation and expansion of the T cell skews the proportions of these subpopulations. To evaluate the extent of differentiation in our engineered T cells, we used CD62L and CD45RA cell surface markers to distinguish between the different states of differentiation: naïve ($CD45RA^{+}CD62L^{+}$), central memory ($CD45RA^{-}$ $CD62L^{+}$), effector memory ($CD45RA^{-}$ $CD62L^{-}$), and terminally differentiated effector memory ($CD45RA^{+}$ $CD62L^{-}$). Strikingly, autoregulatory CAR T cells showed less differentiation and a more naïve/central memory phenotype compared to the constitutive CD19 CAR even after multiple challenges with CD19+ cells (Nalm6) (**Figure 2B**).

Importantly, this suggests that autoregulatory CAR T cells may exhibit decreased exhaustion, enhanced durability and ultimately, improved efficacy. Thus, we extended our analysis to the exhaustion state of the T cells after exposures to the CD19+ Nalm6 cells by evaluating a panel of exhaustion makers (programmed cell death protein 1: PD1, lymphocyte-activation gene 3: LAG3, T-cell immunoglobulin and mucin-domain containing-3: TIM3) expressed on T cells. T cells constitutively expressing CAR had higher levels of exhaustion compared with the non-transduced T cells or synNotch-CAR T cells, the later were not that different from the non-transduced T cells (**Figure 2C**).

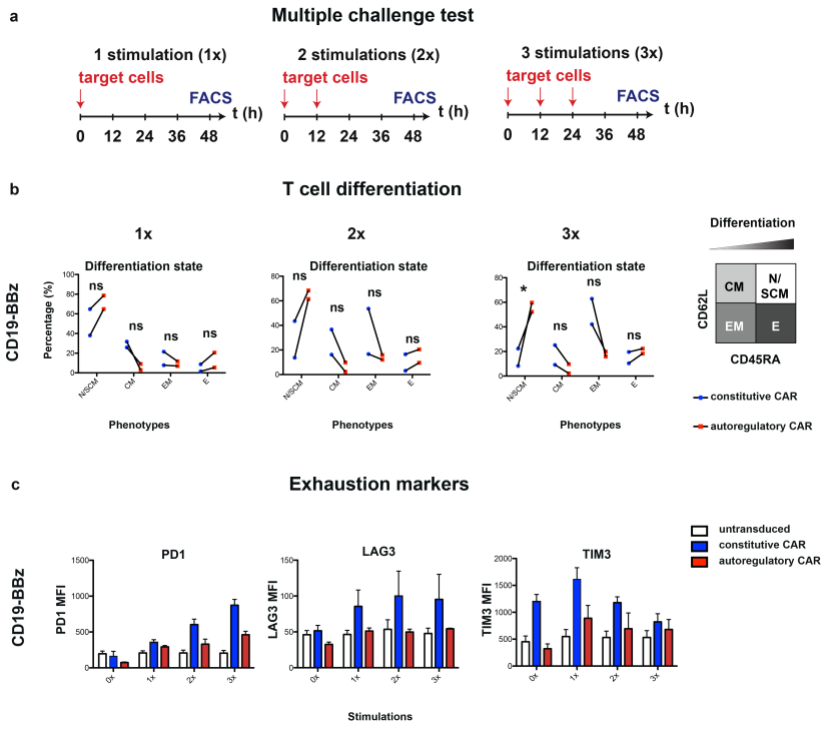

Figure 2: The autoregulatory CARs reduce antigen-induced differentiation and exhaustion. A. schematic of the multiple challenge assay. Quantification of CAR T cells differentiation states in **B**, and exhaustion marker levels in **C**, after 1, 2 or 3 stimulations with target cells over a 48h period for constitutive and autoregulatory CD19-BBz (n=2) CARs. N/SCM: Naïve/Sem Cell Memory, CM: Central Memory: EM, Effector Memory, E: Effector. Represented are means +/-SEM of biological replicates. * denotes statistical difference p-value < 0.05, ns: non-significant. An ANOVA followed by a Bonferroni port-hoc teste was used for **B**. No statistical tests were done for **C**.

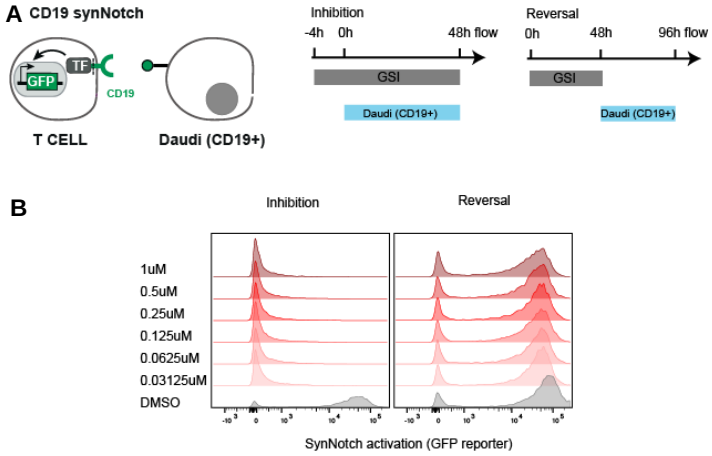

Figure 3: GSI Allows for Titratable Induction. A. CD19 synNotch T cells driving expression of inducible GFP were used to quantify synNotch induction at varying concentrations of Nirogacestat using a inhibition and a reversal protocol **B**. GFP reporter induction histograms for both inhibition and reversal.

In addition, this dual receptor circuit can be controlled by an exogenous drug – the synNotch receptor can be tightly regulated by the class of small molecule inhibitors, gamma secretase inhibitors (GSI) (**Figure 1.2**). This regulation is titratable and user-controlled, so that the user can fine-tune the amount of cytotoxicity that the CAR T cell imposes. Importantly, the OFF-switch is reversible. Upon removal of the GSI, cytotoxic activity of the T cell resumes. This has the potential to be used to enhance the safety of CAR T cells. We therefore tested the more affordable GSI, Crenigacestat and showed potent inhibition of the synNotch activity induction and its reversibility using a reporter assay (**Figure 3**).

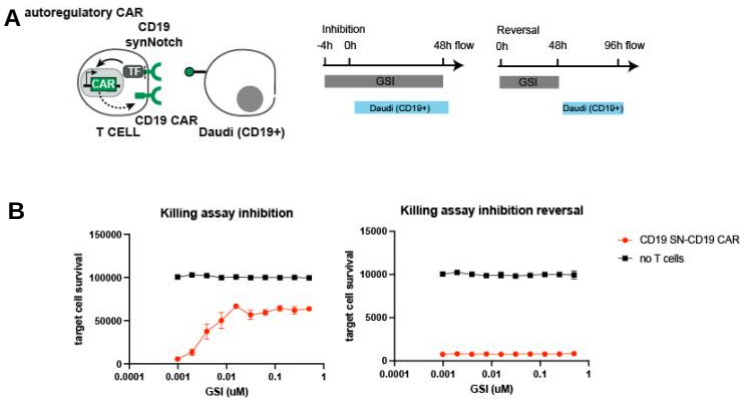

Figure 4: Cytotoxic CAR T Cell Activity is Titratable with GSI Treatment. A: CD19 synNotch T cells driving expression of a CD19 CAR were treated with varying concentrations of GSI and co-incubated with CD19 expressing cancer cell lines to assess inhibition and reversal. **B:** Cancer survival was quantified by cell line fluorescence at varying concentrations as described in **A**.

Consistent with these observations we also demonstrated that the cytotoxic activity was also inhibited by Crenigacestat and that upon washout of the drug the cytotoxic activity was restored (**Figure 4**). Importantly, Crenigacestat did not change the levels of CD19 surface expression on the target cells (data no shown).

All together, these data support that we can fine tune the activity of the CAR activity by using a small molecule.

<u>On Specific Aims 2 and 3, we have validated *in vivo* the combinatorial approach using disease relevant antigen on a glioblastoma mouse model.</u>

We deployed a similar approach based on synNotch-CAR circuit in T cells, as described for this proposal, to successfully treat patient-derived xenograft glioblastoma in mice. While this is not directly part of the AML work, this important finding supports the approach we proposed in the grant as it overcomes off-target toxicity by using unperfect target antigens. These T cells show less tonic signaling, less differentiation and less exhaustion. Our data suggest that this approach can be generalized to AML as described in this proposal using the previously identified target antigens.

SynNotch-CAR circuit is a general platform for wide-range of cancer treatments. Using the same core-basis as described in the grant proposal, here we describe another synNotch-CAR circuit for glioblastoma treatment where engineered T cells are first primed by a synNotch receptor that recognizes a cancer-specific but heterogeneous antigen, EGFRvIII, to then induce the expression of a CAR that kills by recognizing a homogenous though imperfectly tumor-specific antigens, such as EphA2 or IL13Rα2 (**Fig. 5**). The strategy in this circuit design is to take advantage of the specificity of the priming antigen combined with the homogeneity of the killing antigens, in principle yielding specific and complete tumor killing.

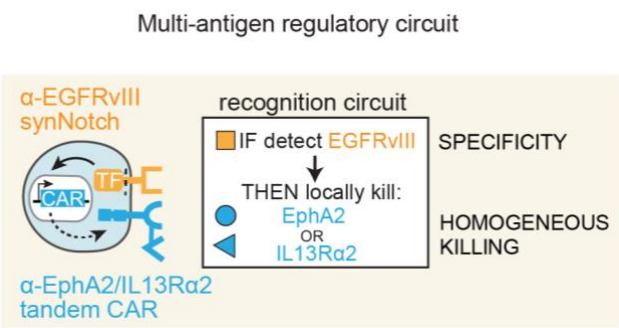

Figure 5: synNotch-CAR circuit: a same core for wide-range of cancer treatment. The design of synNotch-CAR circuit priming on EGFRvIII neoantigen for glioblastoma. α-EGFRvIII synNotch receptor induces expression of a tandem α-EphA2/IL13Rα2 CAR. All these antigens are imperfect to be used as CARs on their own. Indeed, EGFRvIII is not homogenously expressed on the GBM cells, while EphA2 or IL13Ra2 are also expressed on healthy tissues. We are facing the similar limitations for AML choice of antigens to target.

EGFRvIII-triggered synNotch-CAR T cells efficiently and durably clear heterogeneous GBM6 PDX tumors better than constitutive CAR T cells. We evaluated the efficacy of synNotch-CAR T cells in a tumor model which exhibits naturally occurring heterogeneity of EGFRvIII expression (GBM6 patient-derived xenograft: PDX) but homogenous expression of EphA2 and IL13Rα2. We implanted GBM6 tumors in the brains of immunodeficient NCG mice and treated them with T cells bearing α-EGFRvIII synNotch-α-EphA2/IL13Rα2 CARs (**Fig. 6A**). As controls, we treated mice with non-transduced T cells or T cells constitutively expressing α-EphA2/IL13Rα2 tandem CAR. Strikingly, all of the mice treated with the synNotch-CAR T cells showed complete and long-term remission of the GBM6 tumors in contrast to the largely ineffective constitutive CAR (**Fig. 6B**), despite their equivalent cytolytic activity in vitro against the same target cells (data not shown).

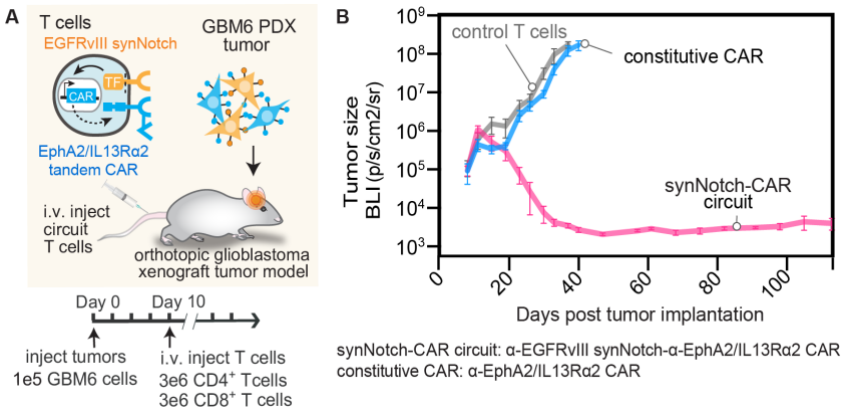

Figure 6: SynNotch-CAR circuit outperforms conventional CARs. **(A)** The timeline for in vivo tumor experiments with GBM6 tumors is shown. GBM6 PDX tumors expressing luciferase were orthotopically implanted in brains of NCG mice. Ten days after tumor implantation, mice were infused intravenously (i.v.) with 3 million each of $CD4^+$ and $CD8^+$ T cells expressing no construct (control) (n=5), α-EGFRvIII synNotch-α-EphA2/IL13Rα2 CAR circuit (n=6) or constitutively expressed α-EphA2/IL13Rα2 tandem CAR (n=5). **(B)**Time course of tumor size as measured by bioluminescence. p=0.0263, two-way ANOVA followed by a Dunnett's test non-transduced vs synNotch-CAR T cells at day 37). Error bars represent mean ± SEM of 5-6 individual mice.

A careful evaluation of post-mortem brain slices by immunofluorescence of mice over 100 days post treatment with the synNotch-CAR T cells showed complete elimination of GBM6 tumor cells and revealed persistent CAR T cells in the brain parenchyma and meninges (data not shown).

In summary, these PDX mouse experiments showed that the synNotch-CAR circuit outperforms conventional CAR with improved killing and persistence.

SynNotch-induced CAR T cells show a more stem-like phenotype, reduced exhaustion, and improved in vivo persistence compared to the constitutive CAR. One of the most surprising findings of these in vivo studies was the improved tumor clearance capacity of the synNotch-CAR T cells compared to the constitutive CAR T cells, since both sets of T cells used the same CAR molecule despite being able to equally kill in vitro (**data not shown**). This led us to hypothesize that there were additional features of synNotch-induced CAR circuits that yielded improved anti-tumor activity in vivo.

Similar to the challenges in liquid tumors treatment, a general challenge in treating solid cancers with CAR T cells is exhaustion of the T cells preventing persistent anti-tumor activity. Tonic signaling by constitutively expressed CARs can play a major role in increasing their susceptibility to exhaustion, leading to poor in vivo T cell persistence. We therefore examined the differentiation state of the different types of engineered T cells by flow analysis, and found that the synNotch-CAR circuit T cells contained a higher fraction of cells in a naïve-like state ($CD62L^+$ $CD45RA^+$, naïve or stem central memory) a phenotype associated with greater in vivo anti-tumor activity, compared to the conventional CAR T cells (**Fig. 7A**).

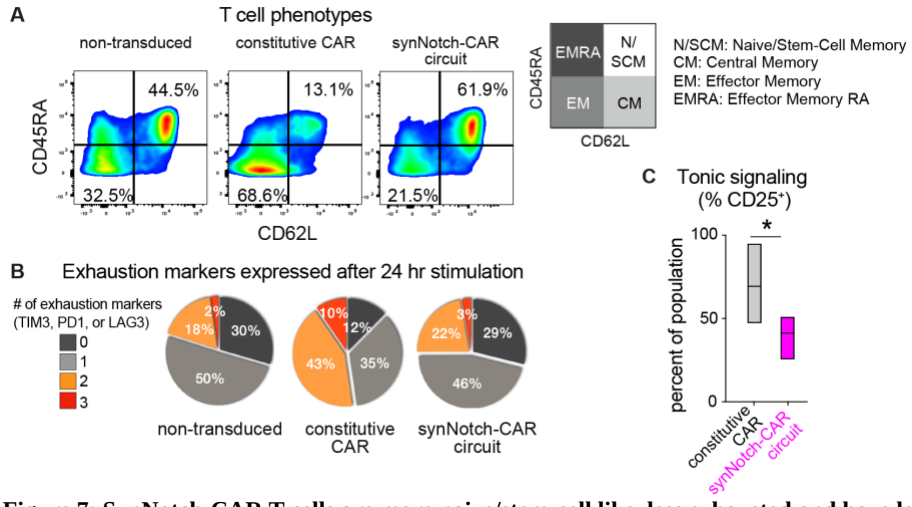

Figure 7: SynNotch-CAR T cells are more naive/stem-cell like, less exhausted and have less tonic signaling. (**A**) Representative flow cytometry plots show surface expression of CD45RA and CD62L to distinguish naïve-like cells ($CD45RA^+CD62L^+$), central memory cells ($CD45RA^-CD62L^+$), effector memory cells ($CD45RA^-CD62L^-$), and effector memory RA cells ($CD45RA^+CD62L^-$) (representative of 3 experiments from different donors). T cells were rested for 10 days in vitro after transfection before phenotypic analysis. (**B**) Expression of exhaustion markers by indicated CAR and synNotch-CAR T cells after 24h stimulation by target cells. Pie chart shows percentage of cells that express 0, 1, 2, or 3 exhaustion markers (PD1, LAG3, or TIM3). Data shown are an average of 3 different donors. (**C**) Tonic signaling in constitutive vs synNotch-CAR T cells was measured based on percent of $CD25^+$ cells (one-way ANOVA followed by a Dunnett's test, $p < 0.05$, $n = 5$ different donors). Boxes represent min to max with median center line.

We extended our analysis to the exhaustion state of the T cells after exposure to the GBM6 cells by evaluating a panel of exhaustion makers (programmed cell death protein 1: PD1, lymphocyte-activation gene 3: LAG3, T-cell immunoglobulin and mucin-domain containing-3: TIM3) expressed on T cells. T cells constitutively expressing CAR had

significantly more cells expressing two or more exhaustion markers compared with the non-transduced T cells ($p < 0.05$) while synNotch-CAR T cells were not significantly different from the non-transduced T cells (**Fig. 7B**).

Tonic signaling by constitutively expressed CARs has been shown to lead to increased T cell exhaustion. Thus, we hypothesized that the synNotch-CAR circuits might show reduced exhaustion and increased stem-like phenotypes because of reduced tonic signaling. Indeed, the synNotch-CAR T cells showed lower tonic signaling than constitutive CAR T cells as measured by CD25 expression in the absence of any stimulation ($p=0.037$, **Fig. 7C**). Hence, synNotch regulation of the CAR appears to prevent tonic signaling and subsequent T cell differentiation and exhaustion.

A more naïve-like and less exhausted phenotype is linked to stronger T cell proliferation and persistence in vivo. We directly investigated T cell persistence in vivo at 6 days after infusion into the tumor-bearing mice, and found abundant synNotch-CAR T cells in the brain. In contrast, we found no surviving constitutive CAR T cells in parallel experiments (data not shown). Together, these findings are consistent with a model in which synNotch-CAR circuits prevent tonic signaling normally observed in constitutively expressed CARs, thereby allowing the T cells to maintain a more naïve, stem-like state less prone to differentiation and exhaustion. Thus, restricting CAR expression locally to the tumor (where priming signals are present) not only increases T cell targeting specificity, but also yields a much more potent and persistent T cell state.

These data strongly support that we can use disease relevant antigens which on their own would be imperfect targets for CAR treatment but when used in combination through the synNotch-CAR circuit can overcome off-target toxicity and improve clearance.

Stated Goals Not Met

We have not been able to test Nirogacestat in vivo as the price has been prohibitive so far using our regular drug suppliers ($1,000/mouse/experiment). Unfortunately, our efforts to obtain it for free or discounted through Spring Works therapeutics, the company synthetizing FDA approved Nirogacestat failed has the legal department could not give us their approval.

What opportunities for training and professional development has the project provided?

Multiple training opportunities are provided to those students and postdocs involved in this project. In addition to close one-on-one mentoring, the trainees participate and present in several regular meetings – the Lim Lab group meeting, the UCSF Systems and Synthetic Biology Center monthly meeting, and the UCSF Cell Therapy club (once a month). This gives them a good exposure in quantitative biology, cell engineering, and translational medicine. The Center for Systems and Synthetic Biology (of which I am Director) also offers training courses for professional development, in terms of helping trainees with grant writing, preparing for lab management, etc. We also support workshops for increasing diversity among trainees. There are also ample opportunities for trainees to mentor high school, undergraduate or rotation students. We are developing IDPs for all of our trainees in which we will regularly review goals for the year, as well as plan for their evolving long- term career objectives.

How were the results disseminated to communities of interest?

Disseminating the results of our research to the community is integral to keep the field moving forward. Our efforts include:

Education. Dr. Lim has developed programs on cell engineering and therapy with local K-12 schools. The iGEM summer research program is a long-term partnership with Biotechnology classes in the San Francisco Unified School District, which has been featured on TV, the SF Chronicle and the NYTimes. Top students come to the lab and work on cell engineering projects that they help to develop. We have also developed demonstrations on cell engineering for middle school biology classes and high school science teachers.

Exhibits/Demos. We have presented demonstrations on cell engineering and therapy at the Exploratorium of San Francisco.

Fundraising. We are central participants in the UCSF Capital Campaign, one of the largest fundraising efforts ever set by a U.S. university ($5 billion). The Lim Lab has led top donors through an exercise engineering “self-driving” cells from a person’s own immune system to kill cancer.

What do you plan to do during the next reporting period to accomplish the goals?

- Test different GSIs for the ON/OFF switch circuits
- Initiate *in vivo* studies for ON/OFF switch circuits.
- Test and optimize AND-gate circuits to enhance dual antigen targeting specificity

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

The Lim lab has developed several innovative strategies to tackle this obstacle, including designing an ON/OFF switch CAR and a dual-receptor, AND-gate CAR T cell that enhances on-target specificity. We have made significant progress towards implementing these techniques to tackle AML using cellular therapies. The completion of this project could result in new "living drugs" that will harness never before used technologies to advance the field of immunotherapy for AML.

What was the impact on other disciplines?

This invention improves the safety of engineered cellular therapies. There are already several engineered cellular therapies that have been approved by the FDA (axicabtagene ciloleucel, tisagenlecleucel) and a staggering number in clinical trials. This invention could be used to enhance the safety and efficacy of all existing and future cellular therapies by providing a safe and effective means to control the cytotoxic activity of cellular therapies.

What was the impact on technology transfer?

This invention improves the safety of engineered cellular therapies. There are already several engineered cellular therapies that have been approved by the FDA (axicabtagene ciloleucel, tisagenlecleucel) and a staggering number in clinical trials. This invention could be used to enhance the safety and efficacy of all existing and future cellular therapies by providing a safe and effective means to control the cytotoxic activity of cellular therapies.

Cell Design Labs was founded based on the synNotch receptor and split CAR ON-switch technology. Cell Design Labs was acquired by Kite Pharma who was acquired by Gilead Sciences, Inc. The technology has also been licensed out to other inventors by Gilead Sciences, Inc.

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Wendell Lim*
Project Role:	*PD/PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4052-8056*
Nearest person month worked:	*0.4*
Contribution to Project:	*Dr. Lim is responsible for overseeing all aspects of the project.*
Funding Support:	*Howard Hughes Medical Institute*
Name:	*Mignon Loh*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4099-4700*
Nearest person month worked:	*0.3*
Contribution to Project:	*Dr. Loh is responsible for co-overseeing all aspects of this project, particularly those aspects associated with AML cell lines and AML xenograft models.*
Funding Support:	*Leukemia and Lymphoma Society, Developmental and Hyperactive Ras Tumor SPORE, Cookies of Kids' Cancer, Dana-Farber Cancer Institute, Incyte Corporation, NIH/NCI, Alex's Lemonade Stand Foundation, NIH/NIGMS, St. Bladrick's Consortium*
Name:	*Michael Broeker*
Project Role:	*Staff research associate III*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*9*
Contribution to Project:	*Assistant Specialist support in cell culture and cell analysis.*
Funding Support:	*Defence Advanced Research Projects Agency; Cell Design Institute*

Name:	*Milos Simic*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Simic has performer work in the area of synthetic and molecular biology as well as testing of primary human T cell circuits*
Funding	*N/A*
Name:	*Jason Duecker*
Project Role:	*Junior specialist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Mr. Duecker has helped characterize the human T cell circuits*
Funding	*N/A*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

New Awards:

Title: **Recognizing the tumor ecosystem: Integrating stromal and cancer antigen signals to achieve precision recognition of solid tumors by CAR T cells**
Major goals: The goal of this project is to engineer therapeutic immune cells that are substantially safer and more effective
in treating solid tumors.

Status of Support: Active
Project Number: R01 CA249018
Name of PD/PI: Lim, Wendell A.
Source of Support: NIH/NCI
Project/Proposal Start and End Date: 12/01/2020-11/30/2025
Total Award Amount (including Indirect Costs):
Effort 1.80 cal mos

Title: **Synthetic epigenetic circuits: tunable cell fate switches controlled by dynamic and combinatorial inputs**.
Major goals: Cellular differentiation is controlled by epigenetic regulatory systems that integrate multiple

sensory inputs over the proposed research aims will establish a versatile circuit design platform that leverages epigenetic regulation to elicit memory and temporally regulated expression programming in mammalian cells, such as cellular timers to orchestrate gene expression sequences, recorders for monitoring and appropriately responding to disease in the host and artificial cell differentiation to drive cells to adopt distinct and complementary functional roles. These capabilities will be critical for engineering novel cell therapies to effectively tackle complex diseases and address future therapeutic challenges.

Status of Support: Active
Project Number: R01 CA253017
Name of PD/PI: Lim, Wendell A.
Source of Support: NIH/NCI
Project/Proposal Start and End Date: 04/01/2021-03/31/2026
Total Award Amount (including Indirect Costs):
Effort 1.80 cal mos

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: