AWARD NUMBER: W81XWH-18-1-0202

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

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CONTRACTING ORGANIZATION: University of California, San Francisco

REPORT DATE: April 2022

TYPE OF REPORT: FINAL REPORT

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
APRIL 2022	FINAL REPORT	01Jul2018 - 31DEC2021

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER
W81XWH-18-1-0202

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
0011171383

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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SAN FRANCISCO CA 94103-4241

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: This proposal will address at least two focus areas specified in the application guidelines: Immunotherapy and Cancer in Children, Adolescents, and Young Adults. Acute myeloid leukemia (AML) in children, adolescents and young adults is only curable in 50% of patients. Immunotherapeutic approaches are changing the landscape of treatments for patients with B-lineage malignancies but have not yet been effective in myeloid malignancies due to concerns over on-target/off-tumor effects on healthy myeloid cells leading to lifethreatening myelosuppression. No AML-specific cell surface antigens have been identified. However, there are abnormal cell surface immunophenotypes that are specific to AML cells. There has not been a successful immunotherapy approach targeting the abnormal cell surface phenotypes that differentiate AML from normal myeloid cells. In the Lim lab, we have developed two novel receptors that we believe have the potential to overcome the obstacles of on-target/off-tumor toxicity.

Objective/Hypothesis: Our goal is to improve the treatment of pediatric acute myeloid leukemia (AML) by developing next generation immunotherapy with enhanced AML specificity and decreased toxicity. This is a collaborative venture between a leader in T cell therapy engineering (Lim) and a leader in childhood cancers (Loh). Specifically, we will be taking two approaches using recently developed, novel receptors to generate a chimeric antigen receptor (CAR) T cell with titratable cytotoxic activity and an AND-gate, combinatorial antigen receptor CAR T cell. Using these approaches, we believe that we will be able to 1) titrate myelosuppression and 2) develop T cell circuitry to target abnormal combinations of cell surface antigens that are specific to leukemic cells and will spare toxicity to healthy myeloid cells.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRDC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	20	

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Acute myeloid leukemia (AML) is curable in less than 30% of patients. Immunotherapeutic approaches have changed the landscape of treatments for patients with B-lineage malignancies but have not yet been effective in myeloid malignancies due to concerns over on-target/off-tumor effects on healthy myeloid cells. No AML-specific cell surface antigens have been identified and using conventional chimeric antigen receptor (CAR) T cell therapy to target myeloid antigens would likely lead to life-threatening myelosuppression. In the Lim lab, we have developed two novel receptors that we believe have the potential to overcome the obstacles of on-target/off-tumor toxicity.

Our goal is to improve the treatment of AML by developing next generation immunotherapy with enhanced AML specificity and decreased toxicity. This is a collaborative venture between a leader in T cell therapy engineering (Lim) and a leader in childhood leukemia (Loh). We have made significant progress towards accomplishing the goals of this project. We have taken two approaches using recently developed, novel receptors to generate a CAR T cell with titratable cytotoxic activity and a dual receptor, AND-gate CAR T cell. Over the course of the last few months, we have developed synthetic cellular circuitry to:

1) titrate cytotoxic activity of a CAR T cell using a gamma secretase inhibitor drug.

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 (gamma secretase inhibitors: GSIs). 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. We previously tested two GSIs: nirogacestat and crenigacestat. Both gave promising *in vitro* results. We further tested new GSIs such as YO-01027, BMS-708163 and LY-411575. YO-01027 gave the best results *in vitro*.

2) demonstrate *in vivo* that we can target CD19+ cells with our autoregulatory circuit that contains a CD19 synNotch and a CD19 CAR. We showed previously that this autoregulatory circuit provided *in vitro* advantages such as a more naïve/stem cell like state and less exhaustion upon multiple

challenges compared to the constitutively expressed CD19 CAR alone. *In vivo* testing of this circuit showed similar performance as the constitutive CAR.

3) demonstrate *in vivo* control of the autoregulatory CAR with GSIs. While *in vivo* testing of this circuit showed similar performance as the constitutive CAR, we discovered that cycling administration of GSI resulted in increased killing efficiency. While we expected that this cycling regiment would shut down the circuit completely and stop the killing, it turned out that this succession of alternating ON/OFF states probably resulted in invigorating the T cells consistent with previous work from the Mackall lab in Stanford. We believe that nevertheless it is possible to turn off the circuit by administrating the GSI more often and at higher doses than the regiment we had. Indeed, due to the cost of crenigacestat we could not afford increasing the dosing and the frequency of administration of the drug.

Innovation: 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.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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:** *Provide a brief list of keywords (limit to 20 words).*

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

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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	100% using another cell model
Subtask 1: ON/OFF CAR scFv design and	1-2	100% using another

cloning		cell model
Subtask 2: Confirm CAR T cell recognition and cytotoxic activity in vitro	3-6	100%
Subtask 3: Assay toxicity in vitro with GSI titration	6-9	100%
Subtask 4: In vivo assay to assess cancer cell clearance	9-18	100%
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?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Conclusions:

- Major Task 1: We have generated a novel ON/OFF switch which is controlled by a class of new small molecules, gamma secretase inhibitors (GSIs). In addition to the previously tested Crenigacestat which is more affordable than Nirogacestat, we further tested YO-01027, BMS-708163 and LY-411575, other relatively cheaper alternatives. Here we use a synNotch receptor to induce expression of a CAR. These drugs are gamma-secretase inhibitors, that blocks cleavage and output of the synNotch receptor. Our results show that this can be used to achieve titratable control of CAR T cell activity. *In vitro* this circuit achieves tumor clearance similar to the constitutive CAR expression.
  We further provide *in vivo* data on a human B cell leukemia model in mice (see tasks below). This autoregulatory circuits clear the cancer cells similarly to the constitutive CAR. By administrating the GSI crenigacestat every other day (ON/OFF cycle for 14 days), we were able to improve the clearance of the tumor cells above the constitutively expressed CAR levels. This improvement is probably the result of a mechanism that allows the T cells to rest and get reinvigorated. This mechanism was described by the Mackall lab in Stanford.
- Major Task 2: No major change since last year.
- Major Task 3: No major change since last year.

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

On Specific Aim 1, we have completed our study 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 *in vivo*. Additionally, this switch is controlled by the small molecule Crenigacestat and others which are more affordable than the previously used one Nirogacestat and will allow us to proceed with planned *in vivo* experiments.

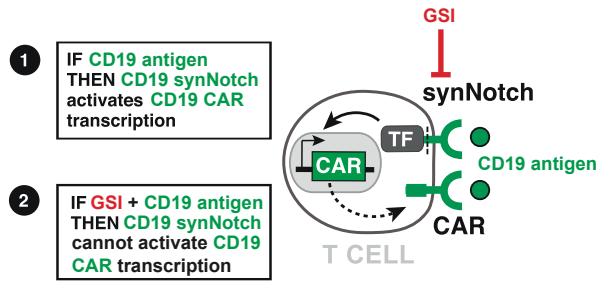

Figure 1: Titratable approach for cytotoxic therapy administration.

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.

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 have previously shown that both Nirogacestat and Crenigacestat were both capable of potent, titratable and reversible inhibition of synNotch. We additionally explored more affordable GSIs such as YO-01027, BMS-708163 and LY-411575. Crenigacestat (aka LY-303047) and YO-01027 showed the best inhibition of the synNotch activity induction and therefore the cytotoxic activity of the T cells associated to the CAR (**Figure 2**).

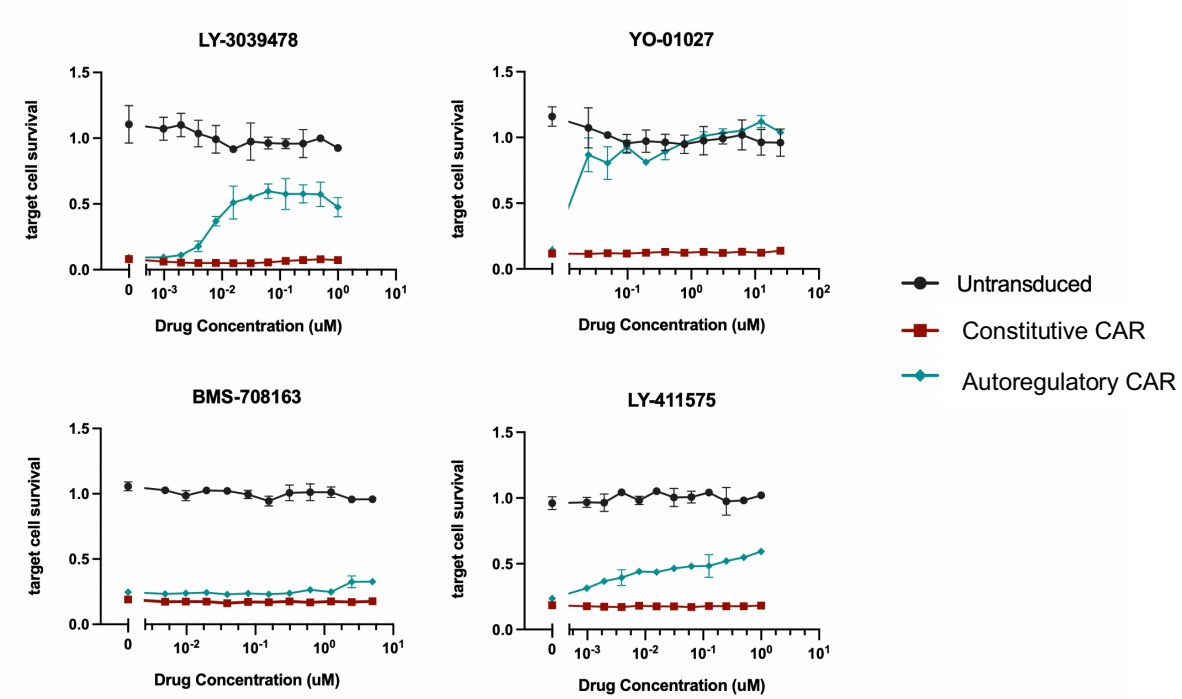

Figure 2: Cytotoxic CAR T Cell Activity is Titratable with GSI Treatment. Untransduced, constitutive CAR and autoregulatory CAR (CD19 synNotch T cells driving expression of a CD19 CAR) T cells were treated with varying concentrations of GSI and co-incubated with Daudi (CD19 expressing cancer cells) to assess inhibition of their cytotoxic activity. Target survival was normalized to 1 (=100% survival of cancer cells).

Our previous *in vitro* data showed that the autoregulatory CAR T cells had less differentiation and a more naïve/central memory phenotype compared to the constitutive CD19 CAR even after multiple challenges with CD19+ cells (Nalm6), as well as less exhaustion markers. We therefore moved *in vivo* to test if the autoregulatory CAR T cells would outperform the constitutive CD19 CAR T cells. In order to save on resources, we combined this experiment with the testing of the GSI (below).

To explore the ON/OFF switch using GSIs, we used the data from the bioavailability of the GSI Crenigacestat, to set up a gavage protocol with feeding every other day for 2 weeks (**Figure 3**). This should completely inhibit the synNotch and result in preventing the cytotoxic activity of the CAR therefore we expect to see prevention of tumor clearance.

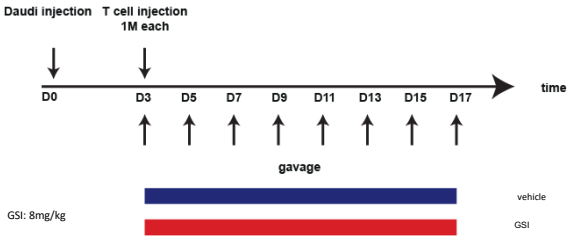

Figure 3: In vivo testing of autoregulatory CAR control with GSI. Daudi cells are CD19+ cells Burkitt's lymphoma cell line.

Looking at the vehicle control first, we show that the autoregulatory CAR T cells had very similar performance as the constitutively express ones (**Figure 4, top panels**). This is in contrast to other synNotch-CAR systems we previously published on another cancer model (glioblastoma) that uses a combinatorial recognition approach. This could be explained by the fact that the sensitivity of the synNotch detection of CD19 is lower than the CAR which also detects the same antigen. Therefore, we hypothesize that the tumor recurrence might be due to low CD19 expression Daudi cells that survive making it harder for the synNotch to be activated as we expect the CAR to kill easily the high CD19 expressing Daudi cells first. This would not happen in the case of a combinatorial antigen recognition where the synNotch and the CAR recognize different antigens, which is the case for AML.

Now looking at the role of the GSI, to our surprise, we saw the opposite of what we expected. Instead of completely shutting down the synNotch and therefore the CAR activity, this feeding regiment resulted in improved clearance of the cancer cells, better than the constitutive CAR (**Figure 4, bottom panels**). This could be interpreted in the eyes of the recent work from the Mackall lab in Stanford, that used a different approach to turn OFF their CAR system. They showed that ON/OFF cycles of the CAR resulted in invigorating the CAR T cells by a mechanism they called "transient rest". This mechanism allows the T cells to improve their phenotype by lowering exhaustion markers for example. We believe this is what we are observing too. Nevertheless, we believe that by increasing the gavage dosing and frequency would result in completely shutting down the synNotch and therefore the CAR activity, similar to what the Mackall lab has showed. We could not test these different optimizations due to cost of the crenigacestat drug despite being already much cheaper than nirogacestat.

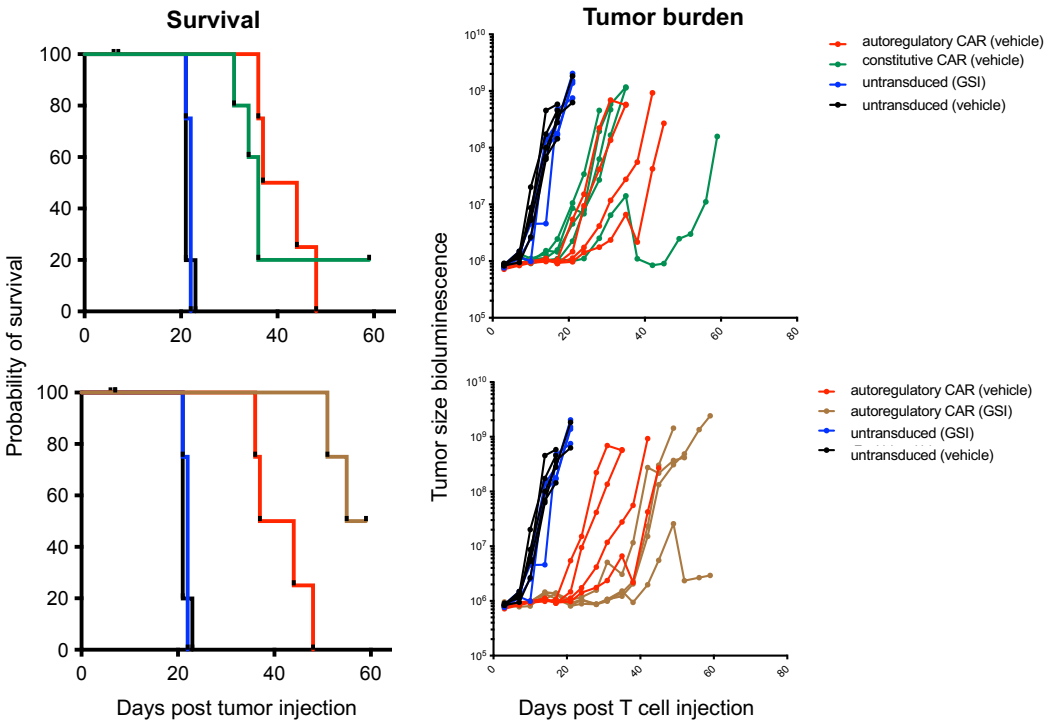

Figure 4: The GSI crenigacestat extends survival of autoregulatory CAR treated mice. Top panels show the comparison between autoregulatory and constitutively expressed CAR, treated with vehicle. Bottom panels show the improved survival and tumor burden when treated with ON/OFF cycles of crenigacestat. N=5 mice in each group.

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>

As a reminder from last report, 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 and improved *in vivo* persistence compared to the constitutive CAR while preventing off-target toxicity. Our data suggest that this approach can be generalized to AML as described in this proposal using the previously identified target antigens.

<u>Stated Goals Not Met</u>

Aims 2 and 3 were not completed to 100%.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

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?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- This is the final report, nothing to report

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

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. 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

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

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of

compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	*Wendell Lim*
Project Role:	*PD/PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4052-8056*
Nearest person month worked:	*0.12*
Contribution to Project:	*Dr. Lim was responsible for overseeing all aspects of the project.*
Funding Support:	*N/A*
Name:	*Jason Duecker*
Project Role:	*Junior specialist*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*7.03*
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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: ***N/A***

9. APPENDICES: *N/A*