

Award Number: W81XWH-19-1-0243

TITLE: CD133-Targeted Natural Killer Cell Therapy for Aggressive Prostate Cancer

PRINCIPAL INVESTIGATOR: Aaron M. LeBeau, PhD

CONTRACTING ORGANIZATION: University of Minnesota, Twin Cities, Minneapolis, MN

REPORT DATE: August 2021

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE			<i>Form Approved</i> <i>OMB No. 0704-0188</i>		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE August 2021		2. REPORT TYPE Annual		3. DATES COVERED 01Aug2020-31Jul2021	
4. TITLE AND SUBTITLE CD133-Targeted Natural Killer Cell Therapy for Aggressive Prostate Cancer			5a. CONTRACT NUMBER PC180269		
			5b. GRANT NUMBER W81XWH-19-1-0243		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Aaron M. LeBeau, PhD E-Mail: alebeau@umn.edu			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Minnesota Department of Pharmacology 312 Church SE Minneapolis, MN 55455			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 The goal of this project is to develop a novel chimeric antigen receptor natural killer cell (CAR NK cell) therapy for the treatment of highly aggressive androgen receptor negative prostate cancer by targeting the cell surface antigen CD133. The CAR NK cells were going to be made from primary NK cells isolated from the peripheral blood of healthy donors using adeno associated virus (AAV) and CRISPR/cas9. The objective was to create these CAR NK cells and then subsequently test them using in vitro and in vivo models of highly aggressive prostate cancer. Thus far we have encountered much difficulty manipulating the primary NK cells to successfully express CARs. Coupled with the COVID-19 pandemic, little progress has been made on this project thus far.					
15. SUBJECT TERMS CD133, castration resistant prostate cancer, natural killer cells					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRDC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			11

Table of Contents

	<u>Page</u>
Introduction.....	2
Body.....	3
Key Research Accomplishments.....	5
Reportable Outcomes.....	6
Conclusion.....	7
References.....	8
Appendices.....	9

Introduction

This project represents the convergence of two important areas for the development of chimeric antigen receptor natural killer cell (CAR NK cell) therapies for prostate cancer (PCa): 1) target identification and 2) NK cell engineering. In our preliminary studies, we developed a novel antibody for the cancer stem cell surface antigen CD133 and found that it was overexpressed in aggressive variant prostate cancer (AVPC) and in the PCa of African-American (AA) men. These are two patient populations typified by poor overall survival who would benefit from the development of new therapies. Additionally, we identified CAR constructs that performed better than a standard 3rd generation CAR T-cell construct in NK cells and developed methods to efficiently and site-specifically introduce CAR constructs into the genomes of readily abundant peripheral blood primary NK cells – something that was previously unattainable. Natural killer cell therapies have been woefully understudied in solid tumors and we are not aware of any research programs developing CAR NK cells for the treatment of PCa. This study has the potential to be the most comprehensive preclinical evaluation to date of CAR NK cell therapeutics in PCa. The impact of this project in the short term will be proof-of-concept documenting that CAR NK cells are effective in solid tumors.

Body

While we have recently won some significant battles in the fight against prostate cancer (PCa), the war to improve survival in men with aggressive forms of PCa continues. With the recent success of cell-based immunotherapy in hematological diseases, there is great interest in applying similar strategies to the treatment of PCa. Much of the immunotherapy research conducted currently in PCa is focused on either vaccine development or chimeric antigen receptor (CAR) T-cell therapy. Few laboratories, if any at all, are actively developing CAR natural killer (NK) cell therapies for PCa. CAR NK cells represent an unexplored resource that could have a dramatic impact on prolonging the life expectancy of, or even curing, men with aggressive PCa. NK cells are cytotoxic lymphocytes of the innate immune system that do not require antigen priming and kill in the absence of MHC presentation. They are also effector cells in antibody-dependent cell-mediated cytotoxicity - the main mechanism of action for monoclonal antibody drugs. They are arguably a better cell type for immunotherapy since they do not require donor matching like T cells and they do not contribute to graft-versus-host disease. Since allogeneic NK cells can be used for adoptive transfer, CAR NK cell therapies represent affordable off-the-shelf products that can be administered to vast patient populations. Attempts to engineer primary NK cells to express CARs have largely been unsuccessful. The studies outlined in this proposal build on major discoveries we have made in the development CAR NK cell therapies for PCa. Recently, we optimized engineering methods using a modified CRISPR/Cas9 system to manipulate the genome of primary NK cells to site-specifically introduce transgenes. This has allowed us to develop a facile methodology for creating CAR NK cells from primary NK cells, an accomplishment that was previously unattainable. Additionally, we identified a high-affinity antibody for the cancer stem cell antigen CD133 by human antibody phage display. Using this antibody for immunohistochemistry, we discovered that CD133 was overexpressed in men who failed second-generation anti-androgen therapy and developed non-androgen receptor driven disease and in African-American (AA) men – two patient populations who exhibit aggressive disease with poor overall survival.

Objective: The potential of CAR NK cell therapies to treat aggressive PCa have not been realized. We hypothesize that CAR NK cells represent an untapped arsenal that can turn the tide of the war against PCa in our favor. Our objective is to establish a new cell-based immunotherapy paradigm for aggressive PCa by developing a CD133-CAR NK cell therapy that can be translated swiftly into the clinic.

The aims of the proposal are as follows:

1. Identify a lead CD133-CAR NK construct for in vivo testing in mouse models of human PCa
2. Evaluate the therapeutic efficacy of the lead CD133-CAR construct in vivo

Study Design: We recently identified CAR constructs that performed better than a standard 3rd generation CAR T cell construct (T-CAR) in NK cells. Using a modified CRISPR/Cas9 system, the NK-specific CARs targeting CD133 will be site-specifically inserted into the genome of primary NK cells at a safe harbor locus. The CD133-CAR NK cells will be tested for therapeutic efficacy in vitro against cell lines that express different levels of CD133. Positron emission tomography imaging will be performed using a reporter gene encoded by the CAR construct to evaluate the biodistribution, tumor infiltration, and expansion of the CAR NK cells in tumor bearing mouse models. A lead CD133-CAR NK construct demonstrating high in vitro cytotoxicity, favorable biodistribution, high tumor infiltration, and expansion in vivo upon cytokine stimulation will be selected for subsequent in vivo testing. The lead CD133-CAR construct, expressed in primary NK cells, will first be tested in subcutaneous xenografts models derived from PCa cell lines in addition to patient-derived xenograft models. The ability of the CD133-CAR NK cells to eliminate small-dispersed lesions in complex microenvironments will be tested using cardiac dissemination mouse models. In all of the in vivo studies, the CD133-CAR NK cells will be tested against T-CAR NK cells, non-targeted CAR NK cells and unmodified control NK cells.

For this reporting period, our goal was to complete Specific Aim 1 and generate CAR-NK cells for in vivo testing (months 1-16).

Key Research Accomplishments

The objective of this reporting period was to create, characterize and optimize CAR NK cells that target CD133 in NEPC. We experienced multiple difficulties in creating the CAR NK cells. The primary obstacle encountered was poor quality virus for transducing the primary NK cells to create the CAR NK cells. We outsourced virus to production to what ended up being several companies due to a lack of consistency in viral titer. None of the virus used was able to efficiently transduce to the NK cells. Virus production takes an average of two months each time and we spent a great deal of time waiting for virus production. We were making headway with manipulating NK cells and then COVID-19 hit. A university wide shutdown was ordered on March 16, 2020 as a result of the COVID-19 pandemic. As required by University decree, all non-COVID-19 related research was forced to stop. We had to end operations until further notice. During the summer of 2020 the University of Minnesota instituted a multi-step reopening plan to resume research. The first step allowed for no more than 50% research productivity. Employees were also allowed to opt out of returning to work if they felt unsafe. This was exacerbated by the fact that the University of Minnesota did not – and still does not – offer COVID-19 testing to employees. The second wave of COVID-19 that occurred that fall and winter resulted in a number of employees of the laboratory opting out and returning to home. Several laboratory members, including the PI, acquired COVID-19 with some missing months of work. Research was difficult during this time and progress was slow. To further encumber research efforts on this project, the LeBeau laboratory moved from the University of Minnesota to the University of Wisconsin in June 2021. It took several months for research and animal protocol to be completed. The funds for this grant have still not been fully transferred to the University of Wisconsin.

In spite of this, we did acquire highly quality recombinant adeno-associated virus for transducing NK cells with CARs. We decided to mainly focus on using our second generation CAR for making CAR NK cells given the difficulty we experienced with modifying NK cells with the other CARs. We were able to efficiently modify NK cells to express CARs. These CAR NK cells could be then be expanded to homogeneity in quantities suitable for in vitro and in vivo studies which will be conducting once the laboratory has relocated to the University of Wisconsin. We anticipate performing in vivo studies in the spring of 2022 once all of the funds have been transferred. Additionally, the University of Minnesota recently decommissioned and sold their microPET/CT due to low use making the CAR NK cell imaging study impossible to perform in Minnesota before we relocated. The University of Wisconsin has a state-of-the-art imaging facility that can easily accommodate our needs for this study.

Reportable Outcomes

- An efficient protocol for making CAR NK cells with recombinant adeno-associated virus has been optimized.

Conclusions

As with many facets of life, COVID-19 has had a dramatic and deleterious impact on this project. However, we have gained much needed traction in manufacturing the CAR NK cells. This will allow for the rapid completion of *in vivo* studies at the University of Wisconsin.

References

None

Appendices
None