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TITLE: STING-Activating CAR-NK Cell Therapy for the Systemic Treatment of Rare Gynecological Cancers

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CONTRACTING ORGANIZATION: University of Southern California

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14. ABSTRACT

Uterine sarcomas are rare and lethal cancers with inadequate screening and treatment options and dismal clinical outcomes. These cancers also disproportionately impact service members, relative to the general population, adversely impacting mission readiness and highlighting the urgent need for curative therapies.

Uterine sarcomas typically escape immune surveillance and are largely unresponsive to current immunotherapies that have revolutionized the standard of care for other tumor types. A central cause for the lack of efficacy of current immunotherapies is the immunosuppressive tumor microenvironment in these tumors, where there is limited infiltration and activation of effector cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells within the tumor core. Our *long-term goal* is to develop engineered NK cells designed to generate STING-activating cyclic dinucleotide alarm signals specifically in response to tumor-released surface antigens, thereby converting the immunologically “cold” microenvironment into an immunoreactive one that drives immune tumor rejection.

15. SUBJECT TERMS

NONE LISTED

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TABLE OF CONTENTS

Page

1. Introduction

Uterine sarcomas remain a major cause of cancer-related mortality and a significant public health problem, with few treatment options and dismal long term survival rates. Immunotherapies that have revolutionized the standard of care for other cancer types are often ineffective against ovarian cancer, underscoring the urgent need for transformative therapies that can deliver lasting disease remission.
A central cause for the lack of efficacy of current immunotherapies is the immunosuppressive tumor microenvironment of ovarian tumors, where there is limited infiltration and activation of effector cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells within the tumor core.

The major goal of this proposal is to develop engineered, chimeric antigen receptor (CAR)-expressing NK cells (CAR-NK) designed to sense and respond to ligands expressed by malignant uterine sarcomas through the acute generation of STING-activating cyclic dinucleotide immune alarm molecules. Thus, the investigational CAR-NK cells developed for this proposal are designed to convert the immunologically 'cold' tumor micro-environment into an immunoreactive one that supports immune tumor rejection, resulting in long-term or even permanent disease remission.

2. Keywords

immunotherapy, STING, cyclic dinucleotide, CAR-NK

3. Accomplishments

Specific Aim 1. To investigate the therapeutic efficacy of STING-activating CAR-NK cells for the treatment of uterine sarcomas.

Major Task 1: Demonstrate induction of in vitro anti-tumor immune responses by STING-activating CAR-NK cells.

% accomplished: 100

Description and summary of the efforts toward this task: We utilized lentivirus transduction to generate NK cell lines stably expressing engineered CAR constructs, and confirmed that these constructs generate CDN alarm signals upon stimulation with recombinant MICA, a tumor-shed ligand of NKG2D. Representative results are shown in F**igure 1.**

Major Task 2: Determine the in vivo therapeutic efficacy of STING-activating CAR-NK cells.

% accomplished: 100

Description and summary of the efforts toward this task: Using an in vivo mouse tumor xenograft model, we have successfully demonstrated of in vivo anti-tumor therapeutic efficacy of STING-activating CAR-NK cells against uterine tumor xenografts in mouse hosts. Representative results are shown in F**igure 2.**

Major Task 3: Compare the in vitro tumoricidal effects of STING-activating CAR-NK and STING agonist drugs.

% accomplished: 100

Description and summary of the efforts toward this task: We compared the in vivo antitumor efficacy of CAR-NK against investigational STING-activating small molecule drugs

Our results show that a single bolus administration of CAR-NK cells resulted in superior stimulation of lymphocyte infiltration and activation, as judged by a combination of immunohistochemistry and tissue histopathology. This pro-immune effect corresponded to higher response rates relative to STING-agonist drugs. and diminished tumor growth.

Specific Aim 2: To determine the synergistic therapeutic effect of combining STING-activating CAR-NK cell therapy and NK checkpoint blockade.

Major Task 1: In preliminary studies, we have demonstrated synergistic anti-tumor effects by combining NK checkpoint blocking antibodies with CAR-NK cell therapy.

% accomplished: 90

Description and summary of the efforts toward this task: In preliminary studies, we have demonstrated synergistic anti-tumor cytotoxic effects in vitro by combining NK checkpoint blocking antibodies with CAR-NK cell therapy.

Major Task 2: Demonstrate the synergistic therapeutic effect of combining CAR-NK cell therapy and NK checkpoint blockade.

% accomplished: 50

Description and summary of the efforts toward this task: Experiments examining the potential for synergistic anti-tumor effects by combining NK checkpoint blocking antibodies with CAR-NK cell therapy are now underway and are nearing completion. Final results will be presented in the next progress report.

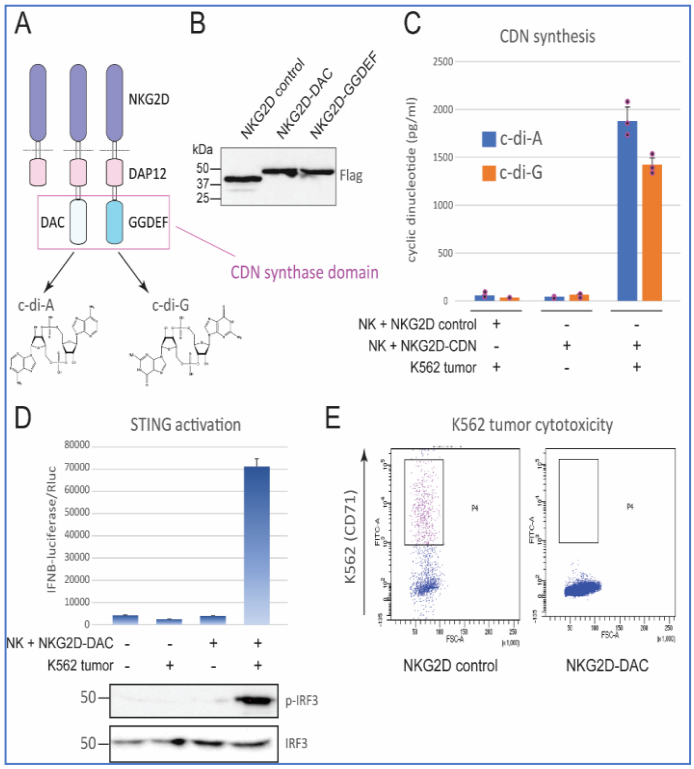

Figure 1. NKG2D-CDN synthase fusion CAR potentiates CDN generation and STING activation. **A.** Schematic of NKG2D control (left) or NKG2D-CDN synthase CARs. All constructs contain a C-terminal Flag epitope tag. **B.** Expression of CAR constructs in NK cells detected by Flag immunoblot. **C.** CDN generation measured by ELISA assay following 8 hr co-culture with K562 target cells. **D.** STING activation transcriptional reporter (top) and immunoblot (lower panels) showing p-IRF3 levels relative to total IRF3. **E.** FACS analysis showing depletion of CD71^{+} K562 target cells following co-culture with CAR-NK cells.

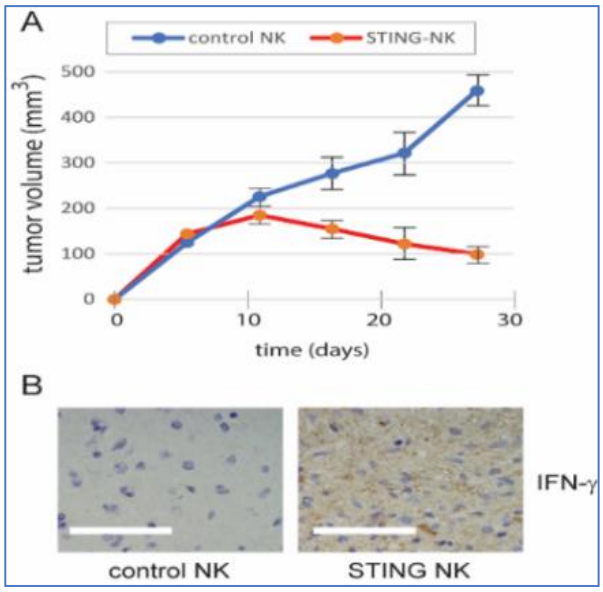

Figure 2. In vivo anti-tumor efficacy of STING-activating CAR-NK cells. A. Tumor growth of MES-SA cells (1x10^6) following murine host implantation, values show mean ± s.d. Control or STING-activating CAR-NK cell bolus was administered on day 5. *P* <0.005 for all comparisons from day 16, *t*-test. **B.** Representative IHC microscopy of inflammatory cytokine IFN-gamma in resected tumors at day 20 following treatment with the indicated NK control or STING-activating CAR-NK cell lines. Scale bars, 100 mm.

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

  Nothing to Report. The project was not intended to provide training and professional development.

- **How were the results disseminated to communities of interest?**

  Nothing to Report for Year 1. An abstract and poster for presentation at a national cancer immunotherapy conference is now in preparation.

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

  Complete in vivo experiments as set forth in SA 2, Major Task 2, of the SOW. Prepare and submit manuscript detailing results and conclusions.

4. Impact

Our long-term vision is to bring rare gynecological cancers into the list of diseases that can be treated and cured using the power of immunotherapy. We seek to achieve this objective through the development of powerful, NK-based cellular immunotherapies that merge the tumor-infiltrating and anti-tumor cytotoxic capabilities of NK cells with first-in-class synthetic CARs designed to trigger potent anti-tumor immune responses through the rapid and massive production of immunoreactive CDN alarm molecules in response to tumor-shed ligands, specifically within tumors and metastatic lesions**.** Therefore, the proposed work is significant on several levels:

A. Our research has provided direct evidence that immune evasion in rare tumors can be overcome using STING-activating CAR-NK cells. By combining the tumor ligand-sensing extracellular domain of human NKG2D with an intracellular CDN synthase, designed to generate intratumoral cyclic dinucleotide (CDN) immune alarm signals, our results advance our understanding of approaches to overcome tumor immunosuppression, and will accelerate translational studies.

B. Our results have validated STING-activating CAR-NK cell therapy as a unique, efficacious and scalable platform to trigger innate immune rejection of ovarian tumors, resulting in corresponding reductions in morbidity and mortality. The cell lines developed for this study will serve as a powerful tools for follow-on lead optimization and IND-enabling advanced preclinical studies.

5. Changes/Problems

Nothing to Report

6. Products

Nothing to Report

7. Participants

Name:	*Douglas E. Feldman, Ph.D.*
Project Role:	PD/PI
Researcher Identifier (e.g. ORCID ID):	*0000-0003-0659-0788*
Nearest person month worked:	*10*
Contribution to Project:	*Dr. Feldman performed experiments, analyzed the data, and prepared data for publication.*

Funding Support:	*Department of Pathology gift funds*

8. Special Reporting Requirements
Nothing to report

9. Appendices
None