AWARD NUMBER: W81XWH-21-1-0297

TITLE: Nanotechnology to Genetically Reprogram Tumor Cells for Treatment of Metastatic Ovarian Cancer

PRINCIPAL INVESTIGATOR: Jordan Green

CONTRACTING ORGANIZATION: Johns Hopkins University

REPORT DATE: May 2022

TYPE OF REPORT: Annual Report

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

Form Approved
OMB No. 0704-0188

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	**2. REPORT TYPE**	**3. DATES COVERED**
MAY 2022	Annual	1MAY2021 - 30APR2022

4. TITLE AND SUBTITLE

Nanotechnology to Genetically Reprogram Tumor Cells for Treatment of Metastatic Ovarian Cancer

5a. CONTRACT NUMBER OC200222

5b. GRANT NUMBER W81XWH-21-1-0297

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jordan J. Green, Ph.D.

E-Mail: green@jhu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Johns Hopkins University
3910 Keswick Road Baltimore,
Maryland 21211

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

Ovarian cancer is the second most common gynecological cancer and has the lowest survival rate. Immunotherapy holds promise for the treatment of ovarian cancer, in which high numbers of cytotoxic T cells correlate with better prognosis. Antigen-presenting cells (APCs) normally activate T cells by coordinated presentation of SIGNAL 1 (tumor antigen in the context of major histocompatibility complex class I, MHC I), SIGNAL 2 (surface-bound co-stimulatory molecule; here, 4-1BBL), and SIGNAL 3 (secreted cytokines; here, IL-12). We are working on using nanoparticles (NPs) based on biodegradable poly(beta-amino ester)s (PBAEs) to deliver key genetic factors to ovarian cancer cells *in vivo* to reprogram them into immunostimulatory cells, mimicking the expression patterns of APCs to promote productive antigen presentation to T cells. This is accomplished by transfecting signal 1-expressing cells with signals 2 and 3, leading to T-cell activation in an antigen-restricted manner. During the first reporting period, we focused on optimizing NP-mediated transfection of tumor cells *in vitro* and *in vivo* which will allow us to genetically engineer them with immunostimulatory genes in the next reporting period.

15. SUBJECT TERMS

Ovarian Cancer, Metastatic Cancer, Nanoparticles, Immunotherapy, Non-Viral Gene Therapy, Tumor-Associated Antigen-Presenting Cells

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	14	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

Page

1. **Introduction………………………………………………………………………..4**
2. **Keywords ………………………………………………………………………….4**
3. **Accomplishments…………………………………………………………………4-9**
4. **Impact……………………………………………………………………………… 10**
5. **Changes/Problems……………………………………………………………...11-12**
6. **Products……………………………………………………………………………12**
7. **Participants & Other Collaborating Organizations…………………………….13**
8. **Special Reporting Requirements…………………………………………………14**
9. **Appendices………………………………………………………………….…...14**

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

Ovarian cancer is the second most common gynecological cancer and has the lowest survival rate, due in part to the fact that patients with ovarian cancer are often diagnosed at late stages when tumors have metastasized. Immunotherapy holds promise for the treatment of ovarian cancer, in which high numbers of cytotoxic T cells correlate with better prognosis. Antigen-presenting cells (APCs) normally activate T cells by coordinated presentation of SIGNAL 1 (tumor antigen in the context of major histocompatibility complex class I, MHC I), SIGNAL 2 (surface-bound co-stimulatory molecule; here, 4-1BBL), and SIGNAL 3 (secreted cytokines; here, IL-12). However, engineering APCs to express these signals is costly, time-consuming, and technically challenging. In this project, we are developing nanoparticles (NPs) based on biodegradable poly(beta-amino ester)s (PBAEs) to deliver key genetic factors to ovarian cancer cells *in vivo* to reprogram them into immunostimulatory cells, mimicking the expression patterns of APCs to promote productive antigen presentation to T cells. This is accomplished by transfecting signal 1-expressing cells with signals 2 and 3, leading to T-cell activation in an antigen-restricted manner. Not only is this approach designed to cause a local T-cell response, but to also lead to cell-mediated, antigen-specific killing of metastatic cells. Moreover, even if ovarian cancer cells downregulate signal 1, induced expression of signals 2 and 3 will activate natural killer (NK) cells, further increasing the therapy's efficacy. During the first reporting period, we focused on optimizing NP-mediated transfection of tumor cells *in vitro* and *in vivo* which will allow us to genetically engineer them with immunostimulatory genes in the next reporting period.

2. **KEYWORDS:** (limit to 20 words).

Ovarian Cancer
Metastatic Cancer
Nanoparticles
Immunotherapy
Non-Viral Gene Therapy
Tumor-Associated Antigen-Presenting Cells

3. **ACCOMPLISHMENTS:**

What were the major goals of the project?

Specific Aim 1: Engineer non-viral PBAE/DNA NPs to transfect ID8 ovarian cancer cells *in vivo* in subcutaneous (s.c.) and intraperitoneal (i.p.) tumor models [Months 0-6]: 100% complete

Specific Aim 2: Demonstrate *in vivo* anti-tumor efficacy after direct administration of reprogramming NPs to s.c. flank tumors or i.p. tumors. [Months 6-14]: 30% complete

Specific Aim 3: Evaluate the effect of local injection of PBAE/DNA NPs on distant metastases *in vivo*. [Months 0-36]: 0% complete

What was accomplished under these goals?

Synthesize and screen poly(beta-amino ester)s (PBAEs) for transfection of ID8 mouse ovarian cancer cells in vitro

Traditional linear PBAEs and next-generation lipophilic PBAEs were synthesized and used to transfect ID8 cells *in vitro*. Polymers were complexed with enhanced green fluorescent protein (GFP) plasmid DNA to form nanoparticles (NPs) at different mass ratios of PBAE to DNA (w/w) and used to treat ID8 cells in culture at a range of doses, using high-throughput screening procedures to test a wide variety of different polymers. Transfection efficacy was measured as the percent of total live cells expressing GFP (% GFP^+) as well as the geometric mean fluorescence intensity of the GFP signal among live cells, normalized to untreated GFP^- cells (Normalized MFI). Cell viability was measured as the number of live cells per well normalized to the average

number of live cells per well in the untreated control group. Among linear PBAEs with <30% non-specific toxicity *in vitro*, lead polymers included 4-4-49, 4-5-6, 4-5-7, 5-3-6, and 5-3-49 (**Fig. 1**).

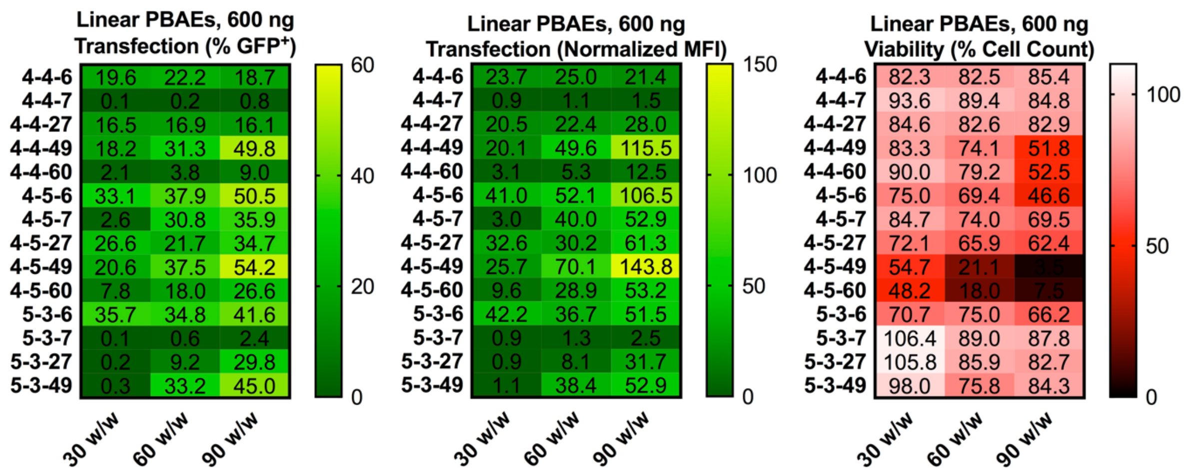

Figure 1. Linear PBAEs could transfect >40% of ID8 cells *in vitro* without excessive toxicity due to the NPs. Lead polymers that caused <30% toxicity at the dose tested were used for further *in vivo* study.

We have recently found that lipophilic PBAEs can bind nucleic acid more tightly to encapsulate it and may be more efficient in transfecting cells with DNA or mRNA. Because the lipid tail incorporated into the PBAE structure makes the NPs more effective but also may contribute to higher toxicity, lower doses were evaluated (**Fig. 2**). By titrating the dosage, we identified a structure, 7-90,c12-63 (80% lipophilic side chain) that could transfect a large percentage of cells, >50% of ovarian cancer cells with low *in vitro* toxicity.

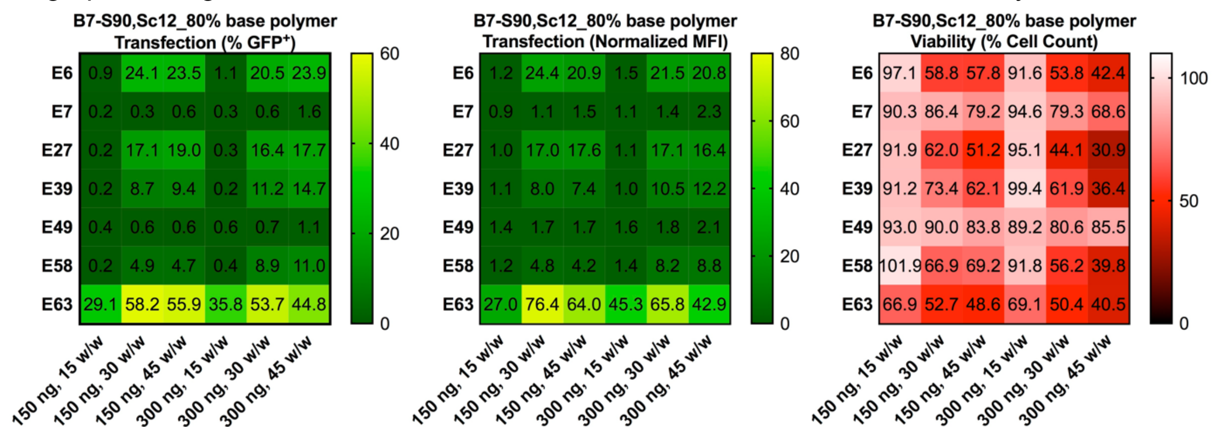

Figure 2. Lipophilic PBAEs could transfect >50% of ID8 cells *in vitro* without excessive toxicity due to the NPs. The lead polymer was used for further *in vivo* study.

The lead linear and lipophilic PBAE structures (**Fig. 3**) were used for further studies *in vivo*.

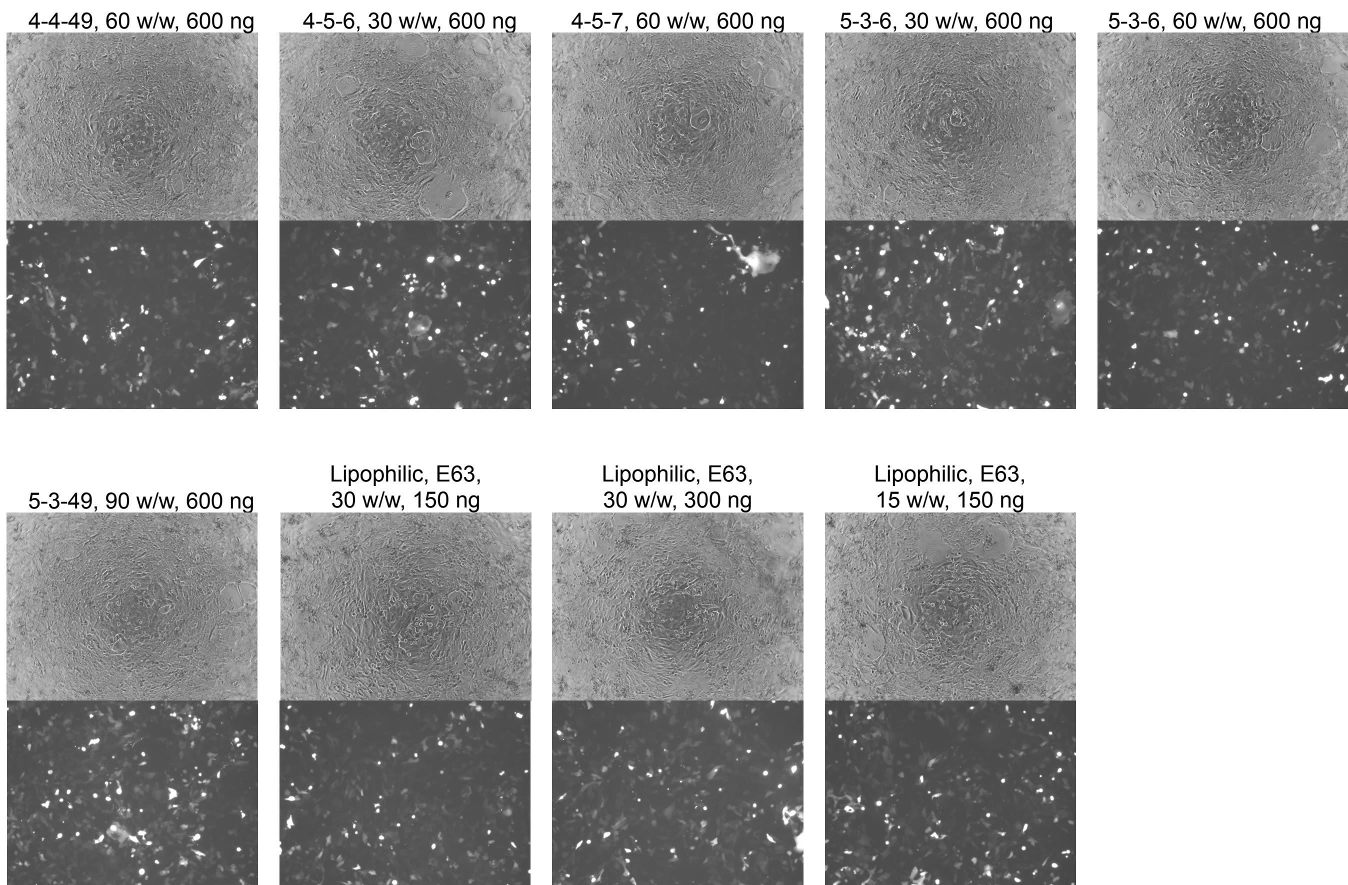

Figure 3. ID8 cells transfected with GFP DNA using linear or lipophilic PBAEs. Top: brightfield. Bottom: GFP.

Engineer ID8 cells to express fluorescent (tdTomato) and luminescent (firefly luciferase, fLuc) genes for in vivo tracking of metastatic cells

Using PBAE NPs, ID8 ovarian cancer cells were co-transfected *in vitro* with plasmids encoding a Piggybac transposase and a tdTomato=fLuc transposon. In order to achieve high gene expression, PBAE 4-5-27 was used at 90 w/w; although 4-5-27 was found to have some toxicity (**Fig. 1**), because the NPs were being administered *in vitro* only and would not affect cells in a living organism, slight toxicity was considered acceptable in order to maximize gene expression, which was required for insertion of the gene into the ID8 genome. Fourteen days after transfection, 0.33% of ID8 cells were found to contain the transgene and were collected by flow activated cell sorting (FACS) using a Sony SH800 (**Fig. 4**). Because the plasmids delivered via the NPs are normally episomal and non-integrating, we continued to culture the sorted cells and to re-sort them over time to ensure that the only remaining tdTomato$^+$ cells were those that exhibited genetic integration due to the transposase. After 2 months of culture, the ID8 cells were almost entirely tdTomato$^+$, and populations were collected with high and moderate fluorescence to ensure that one of these populations would have a good level of reporter gene expression to be able to be used *in vivo*. These cells were then called "ID8-tdT=fLuc-HI" and "ID8-tdT=fLuc-MED," respectively.

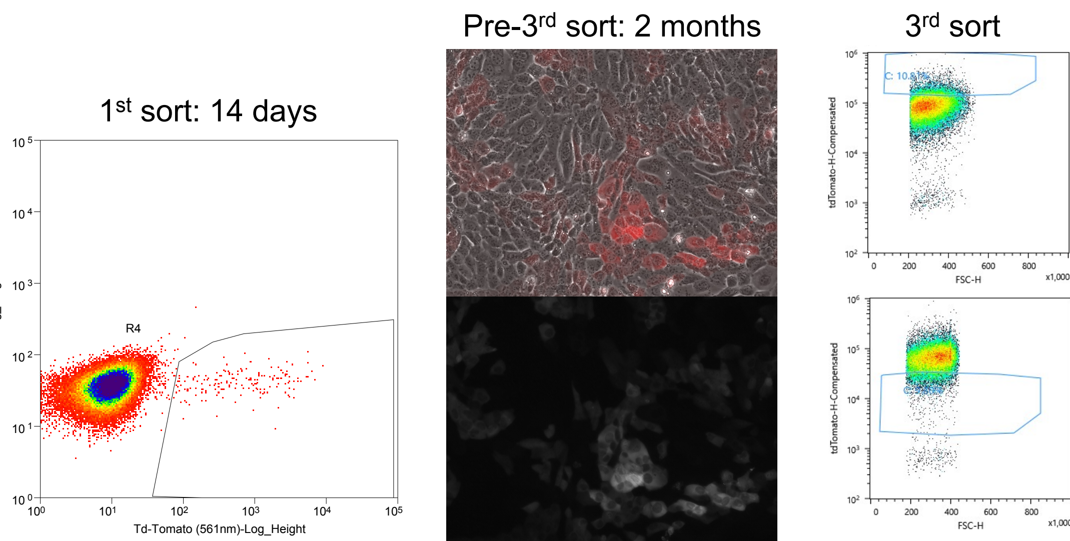

Figure 4. Two weeks after the initial transfection, a small population of tdTomato⁺ cells (and therefore fLuc⁺ cells) was sorted by flow cytometry. After repeated FACS and culture for 2 months, stable populations with high or moderate levels of reporter gene expression were sorted by FACS.

ID8-tdT=fLuc cells were injected intraperitoneally (IP) into mice to test whether they could establish detectable lesions in the IP space. Because cells expressing xenogenic reporter proteins like fLuc or tdTomato may induce an undesirable immune response after injection that can lead to clearing of the tumor, we conducted this test using the ID8-tdT=fLuc-HI cells. **Fig. 5** shows that cells injected IP can be detected by In Vivo Imaging System (IVIS) within days and that these lesions are not cleared by the animals' immune systems. In order to see greater contrast between large and small tumor lesions, we plan to use ID8-tdT=fLuc-MED cells for anti-tumor efficacy studies.

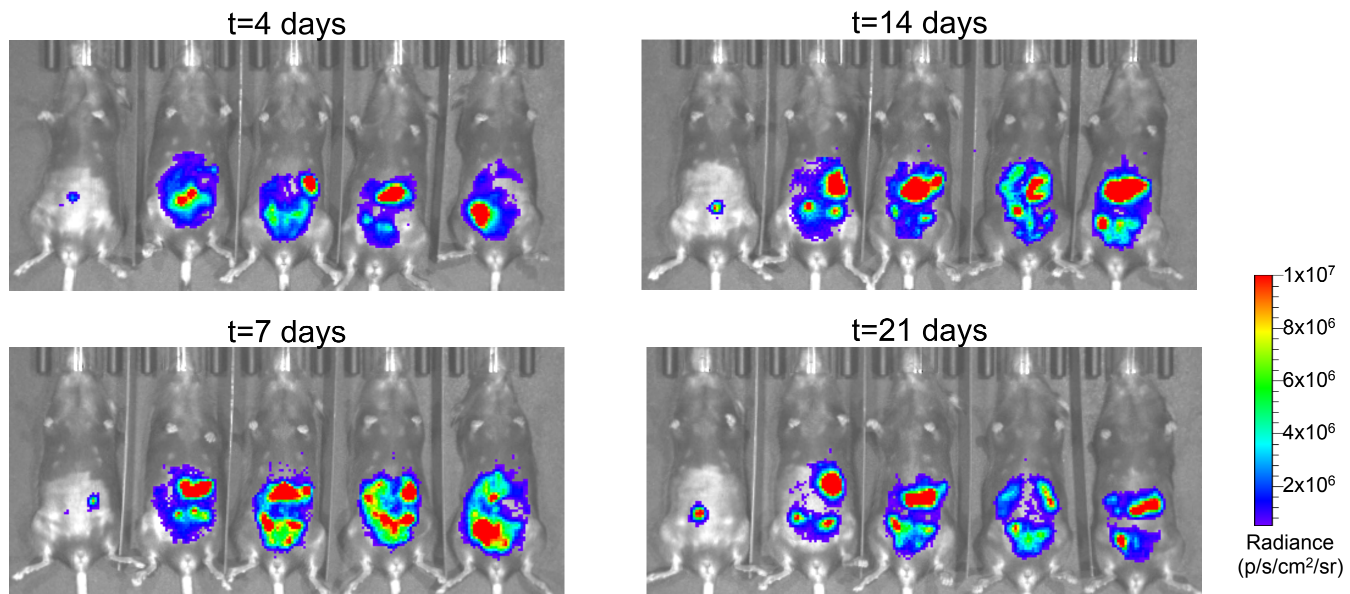

Figure 5. Female C57BL/6 mice were injected IP with 5×10^6 cells per mouse and imaged by IVIS over time to track the establishment and growth of ovarian cancer in the IP space.

Optimize PBAE NPs for in vivo *transfection of subcutaneous (SC) or IP ovarian tumors*

The lead PBAE structures from *in vitro* screens were formulated with fLuc DNA into NPs for either intratumoral (IT) injection into SC flank ID8 tumors or IP injection. For flank tumors, 5×10^6 ID8 cells were injected SC per mouse. It was noted that approximately 50% of the mice either did not form palpable tumors or only formed small tumors that regressed without external intervention; however, tumors were successfully established in enough of the mice to carry out NP optimization studies. One group (7-90,c12-63 with 1% PEG) only included 2 mice for this reason. Although this lower number of mice would not have been sufficient for

strong statistical analysis, it was concluded that the low transfection seen in this group was enough to eliminate it from future studies. This inconsistency in tumor formation will be addressed further below.

To ensure that NPs transfected tumor tissue but not surrounding healthy tissue, which could lead to off-target immunological side effects, the same NPs were also injected SC on the opposite flank of the mice (**Fig. 6**). PBAEs 4-5-6 and 5-3-6 were identified as the leading structures that consistently transfected tumor tissue after IT injection with low or no transfection of healthy tissue after SC injection; 5-3-49 was also identified as a candidate based on a low level of tumor transfection as well as its successful use in other tumor models in our lab.

No Treatment SC
No Treatment IT
7-90,c12-63_80, no PEG, SC
7-90,c12-63_80, no PEG, IT
7-90,c12-63_80, 5% PEG, SC
7-90,c12-63_80, 5% PEG, IT
5-3-6, SC
5-3-6, IT
5-3-49, SC
5-3-49, IT
4-4-49, SC
4-4-49, IT
4-5-6, SC
4-5-6, IT
7-90,c12-63_80, 1% PEG, SC
7-90,c12-63_80, 1% PEG, IT
5x10⁴
4x10⁴
3x10⁴
2x10⁴
1x10⁴
Radiance (p/s/cm²/sr)

Figure 6. Female C57BL/6 mice were inoculated SC on the right flank with 5×10^6 cells per mouse. NPs containing 5 μg DNA per injection were injected either IT or SC on the left flank. After 24 hr, gene expression was assessed by IVIS.

To test NP efficacy after IP injection, mice were first injected IP with unlabeled ID8 cells to ensure that transfection could occur in the context of the tumor. Mice were then injected IP with 5-3-6, 4-5-6, or 5-3-49 NPs containing 10 μg DNA per injection. As stated in the proposal, transfection of non-tumor cells (such as macrophages or other immune cells) is not a criterion for exclusion for this study, so transfection of tumor-bearing mice compared to non-tumor-bearing mice was not critical. As shown in **Fig. 7**, only polymer 4-5-6 showed strong transfection of cells in the IP space in most of the mice after injection. Thus, 4-5-6 was selected as the polymer to be used for future efficacy studies.

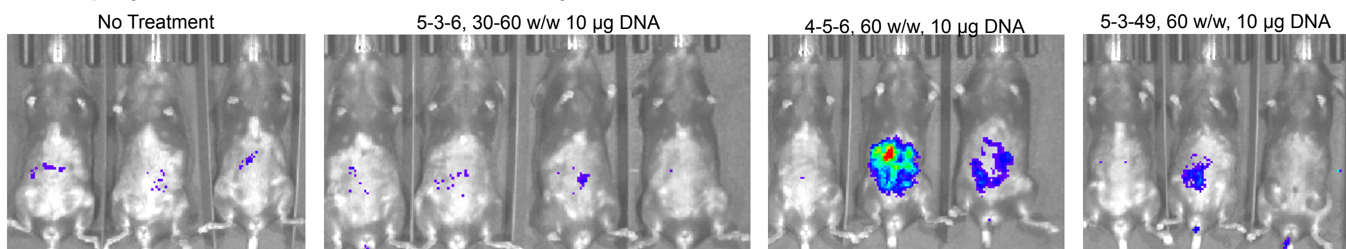

Figure 7. Female C57BL/6 mice were injected IP with 5×10^6 unlabeled ID8 cells per mouse. NPs containing 10 μg DNA per injection were injected IP. After 24 hr, gene expression was assessed by IVIS.

Demonstrate anti-tumor efficacy after direct administration of NPs to SC tumors

According to literature, ID8 cells can form SC flank tumors after injection of a high number of cells per mouse. In the absence of matrix materials like Matrigel, we have been able to form SC tumors in a percentage of the mice injected with 5×10^6 ID8 cells/mouse, allowing us to carry out preliminary transfection studies (e.g., **Fig. 6**). However, when we began our anti-tumor efficacy study, the tumor take rate was too low for the number of mice needed for a properly powered experiment. **Fig. 8** shows the tumor growth rate that was recorded from some of the mice that did form tumors, demonstrating that (1) tumor initiation is slow and (2) tumors that are still palpable after approximately 1 month do eventually grow to the full size needed for survival studies. This study is planned to be repeated in the next months with the adjustments described below.

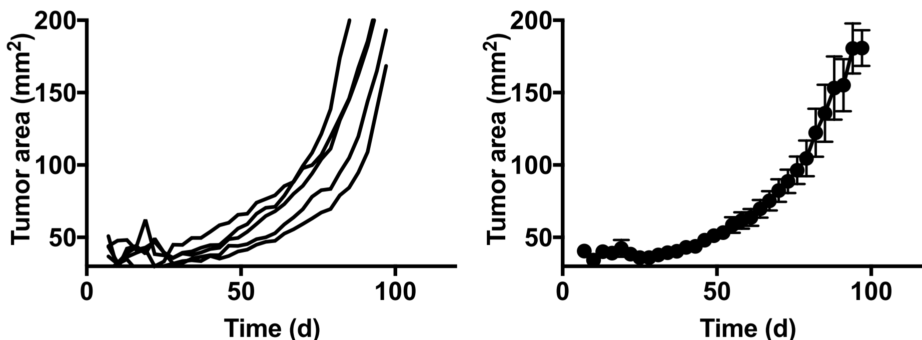

Figure 8. Female C57BL/6 mice were inoculated SC on the right flank with 5×10^6 cells per mouse. Tumor growth was slow but continued to completion in 27% of mice.

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."

Nothing to Report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

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.

As the NP formulation has been optimized here, the next reporting period will focus on functional anti-tumor studies.

1. Assess the ability of ID8 cells to cause immune activation *in vitro* after transfection with 4-1BBL and IL-12 DNA
2. Demonstrate anti-tumor efficacy after direct IT injection of NPs into SC tumors
3. Demonstrate anti-tumor efficacy after direct IP injection of NPs into mice with IP tumors
4. Demonstrate reduced IP tumor growth after only direct IT injection of NPs in SC tumors

4. **IMPACT:** Describe 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 results from this reporting period have established the following:

1. Polymeric NPs can successfully transfect ovarian cancer cells both *in vitro* and *in vivo*
2. Polymeric NPs can be used to stably engineer murine ovarian cancer cells to express fluorescent and luminescent reporter genes. This allows the cells to be detected over time in live animals as well as to be detected with high sensitivity and precision *ex vivo*. These engineered cells are also able to form stable tumor lesions in mice and do not elicit enough of an immune response against the xenogeneic proteins to cause regression.
3. ID8-bearing mice can be established with tumors at multiple different sites, though further optimization of the mouse model is needed to improve consistency.

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.

Nothing to report

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.*

Nothing to report

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 Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer 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.

During early stages of the project, it was found that higher doses of NPs are needed for significant transfection after IP injection. Although this is an approach that we have used in other animal models, it was not included in the protocol that we originally submitted to ACURO for approval. Thus, we submitted an amendment and received ACURO approval for increased NP dosing to the IP space, resulting in stronger transfection in this model.

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.

Although most published papers use Matrigel to encourage ID8 SC tumor formation, due to supply chain delays as part of the COVID-19 pandemic, we and the labs around us have not been able to acquire enough Matrigel for tumor studies from any source since mid 2021. The current estimated availability date for our open orders of Matrigel is August of 2022, though this has changed since late 2021, when availability was estimated for January and then May of 2022. As we have had difficulty establishing enough consistent SC tumors in mice for survival studies, we would like to use an aid like Matrigel; however, since it is unclear when this exact option will be possible, we will explore two other strategies to improve tumor take:

1. Increase the number of cells injected per mouse from 5×10^6 to 1×10^7. This number of cells per mouse is approved in our institutional ACUC protocol as well as our ACURO protocol.
2. Use a synthetic alternative to mimic the physical advantages of Matrigel [described in Grosskopf, A.K., Correa, S., Baillet, J. et al. Consistent tumorigenesis with self-assembled hydrogels enables high-powered murine cancer studies. Commun Biol 4, 985 (2021)].

As option #2 is still being tested in our hands and may require an additional ACURO approval, we plan to try option #1 first (increased cell number per mouse). In addition, we plan to inoculate a higher number of mice in order to account for some percentage of mice that may not form robust tumors. Because ID8 tumors are relatively slow-growing compared to many other common murine tumors, these issues based on supply chain limitations may cause a small delay of up to 6 months in the completion of the project; however, we are confident that the anti-tumor studies will be able to be completed.

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. Only a minor change in NP dosing was needed, and this was quickly approved by our institutional ACUC and subsequently by ACURO.

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.
  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?

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."

Name: *Dr. Jordan Green*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID): *0000-0003-4176-3808*
Nearest person month worked: *1*
Contribution to Project: *Dr. Green led and oversaw the activity of the team, including the initial planning of mouse experiments.*

Name: *Dr. Stephany Tzeng*
Project Role: *Co-I*
Researcher Identifier (e.g. ORCID ID): *NA*
Nearest person month worked: *1*
Contribution to Project: *Dr. Tzeng oversaw the activity of the team, including conducting initial transfection data and the performance of animal experiments.*

Name: *Karlsson , Johan*
Project Role: *Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID): *N/A*
Nearest person month worked: *8*
Contribution to Project: *Dr. Karlsson conducted experiments including polymer and nanoparticle synthesis and evaluation.*

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?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Nothing to Report

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys.

Nothing to Report