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

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CONTRACTING ORGANIZATION: Johns Hopkins University

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

Johns Hopkins University
3910 Keswick Road Baltimore,
Maryland 21211

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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
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
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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. We propose to use 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 will this approach cause a local T-cell response, but it will 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 second reporting period, we focused on improving ovarian tumor cell engraftment *in vivo* in order to test the therapeutic efficacy of the optimized NPs.

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 6-36]: 33% complete

Specific Aim 3: Evaluate the effect of local injection of PBAE/DNA NPs on distant metastases *in vivo*. [Months 0-36]: 33% 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**).

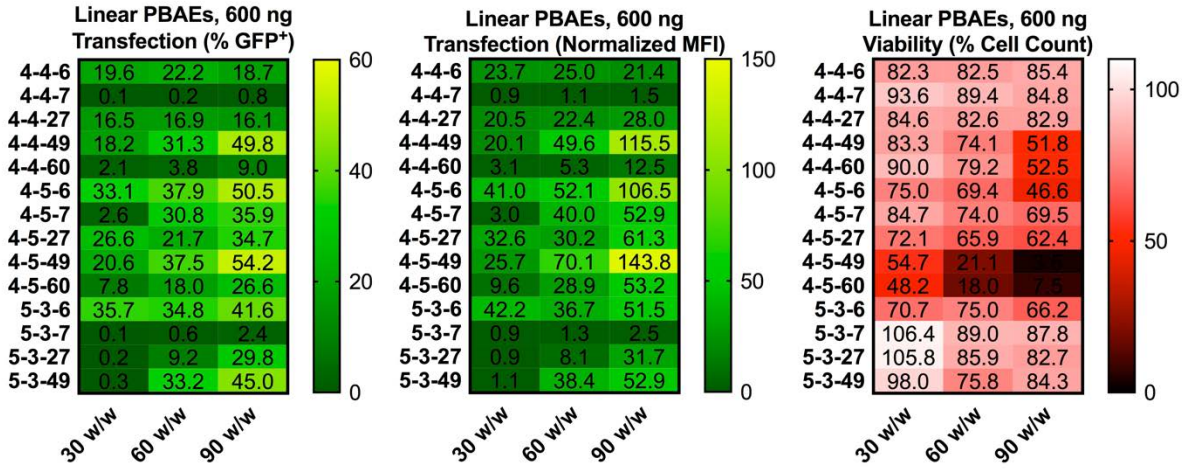


Figure 1. Linear PBAEs could transfect >40% of ID8 cells *in vitro* without excessive toxicity due to the NPs. Lead polymers that caused <30% toxicity at the dose tested were used for further *in vivo* study.

We have recently found that lipophilic PBAEs can bind nucleic acid more tightly 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 tested (**Fig. 2**). However, by titrating the dosage, we identified a structure, 7-90,c12-63 (80% lipophilic side chain) that could transfect >50% of cells with low *in vitro* toxicity.

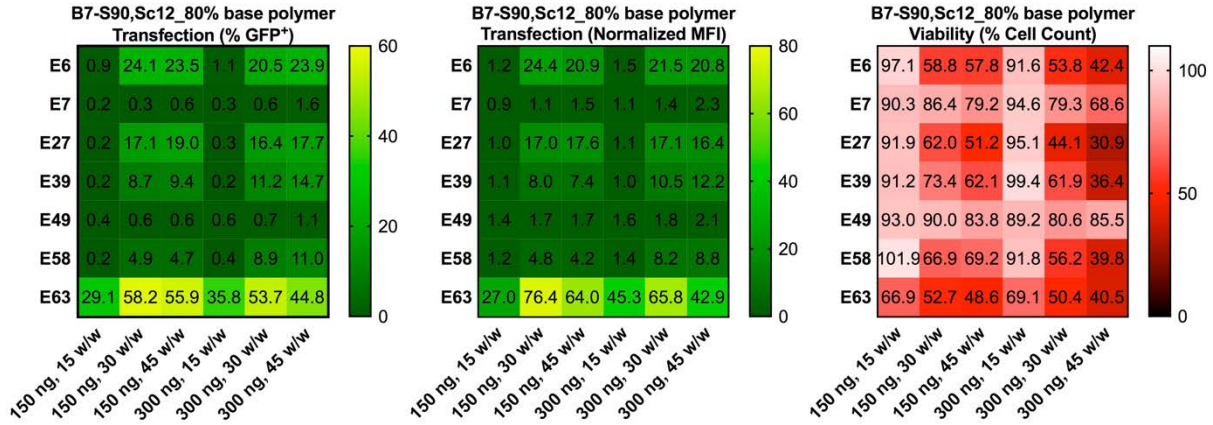


Figure 2. Lipophilic PBAEs could transfect >50% of ID8 cells *in vitro* without excessive toxicity due to the NPs. The lead polymer was used for further *in vivo* study.

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

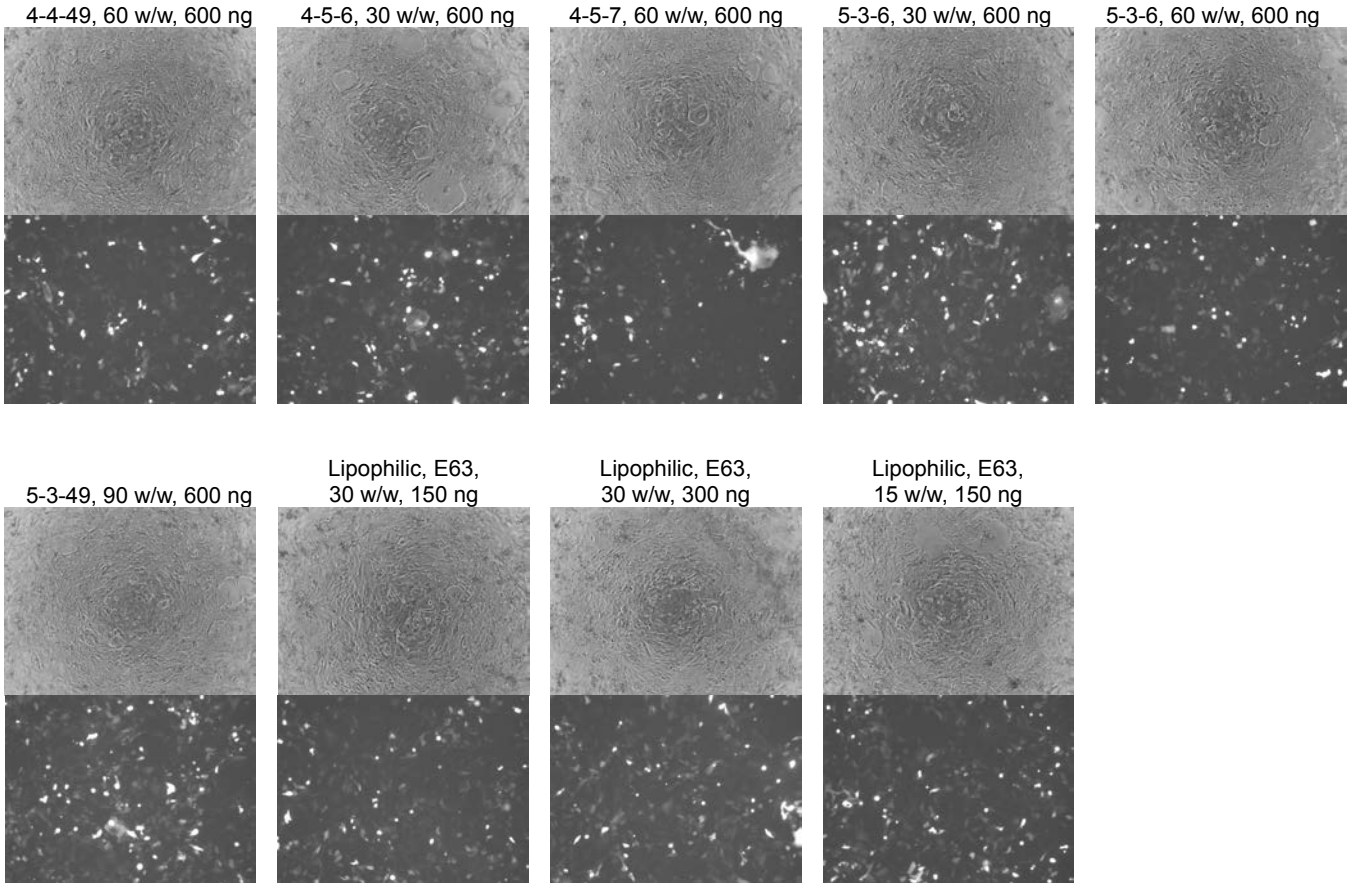


Figure 3. ID8 cells transfected with GFP DNA using linear or lipophilic PBAEs. Top: brightfield. Bottom: GFP.

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

Using PBAE NPs, ID8 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.

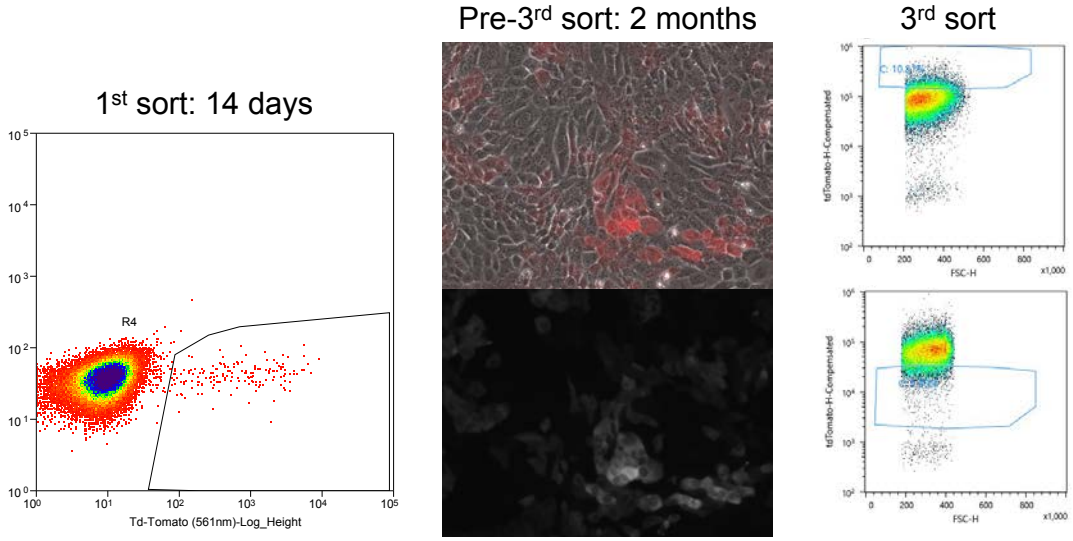


Figure 4. Two weeks after the initial transfection, a small population of tdTomato$^+$ cells (and therefore fLuc$^+$ cells) was sorted. After repeated FACS and culture for 2 months, stable populations with high or moderate levels of reporter gene expression were sorted by FACS.

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.

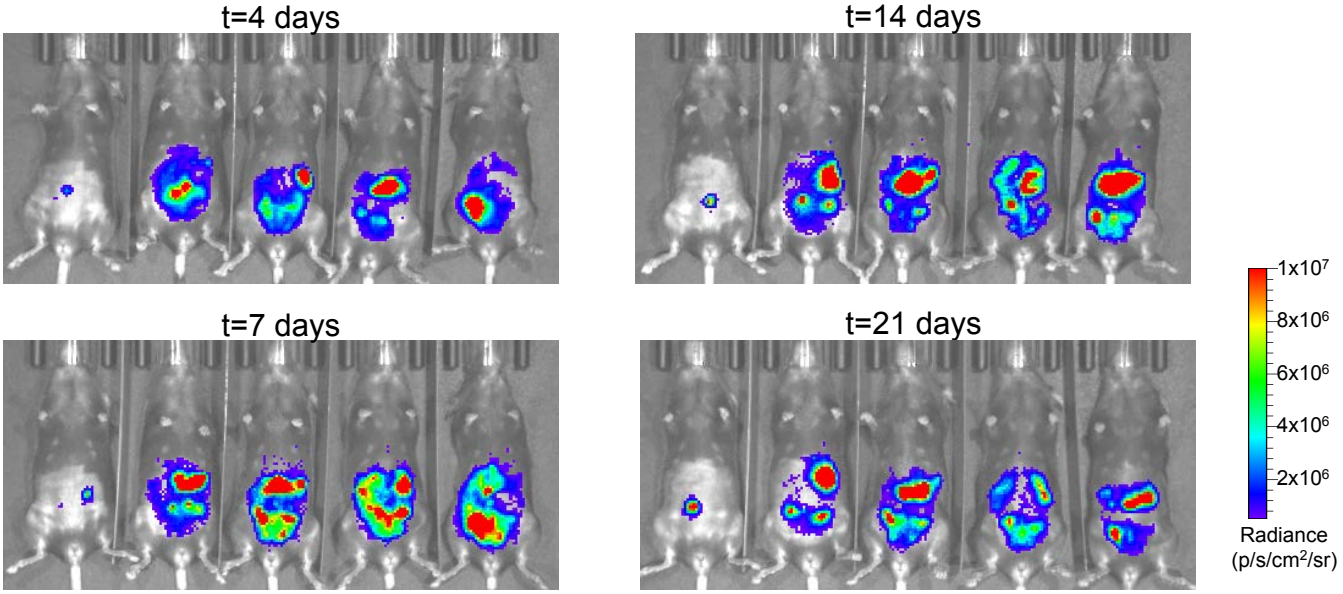


Figure 5. Female C57BL/6 mice were injected IP with 5×10^6 cells per mouse and imaged by IVIS over time to track the establishment and growth of ovarian cancer in the IP space.

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.

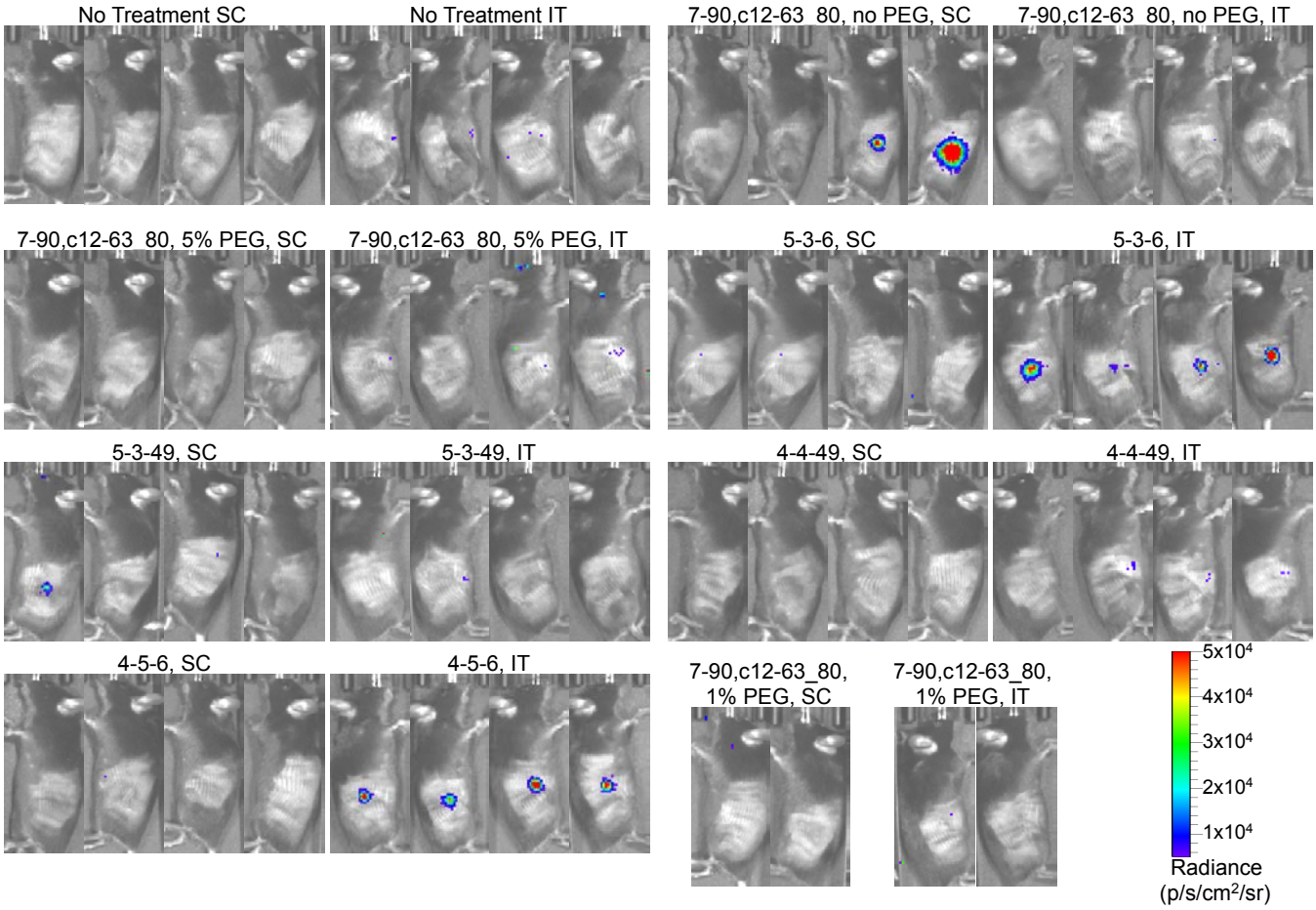


Figure 6. Female C57BL/6 mice were inoculated SC on the right flank with 5×10^6 cells per mouse. NPs containing 5 µg DNA per injection were injected either IT or SC on the left flank. After 24 hr, gene expression was assessed by IVIS.

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.

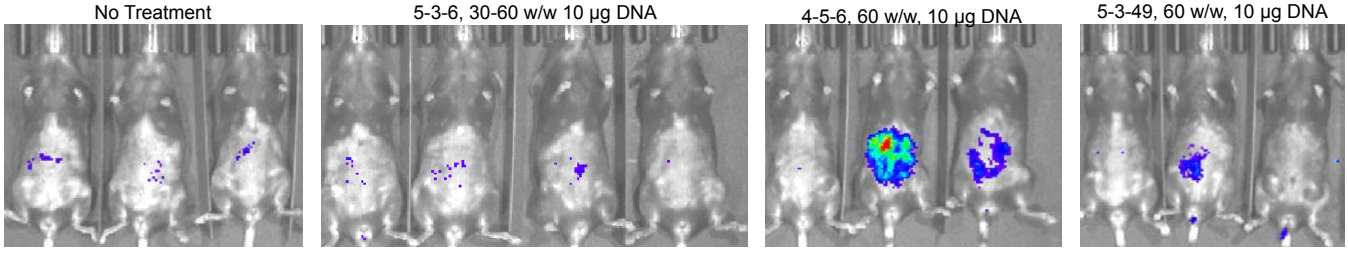


Figure 7. Female C57BL/6 mice were injected IP with 5×10^6 unlabeled ID8 cells per mouse. NPs containing 10 µg DNA per injection were injected IP. After 24 hr, gene expression was assessed by IVIS.

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**). Nevertheless, we were pleased to see find that the locally administered NPs did not cause measurable off-target toxicity, measured by an AST (aspartate transferase) assay performed on the blood from injected mice (**Fig. 9**).

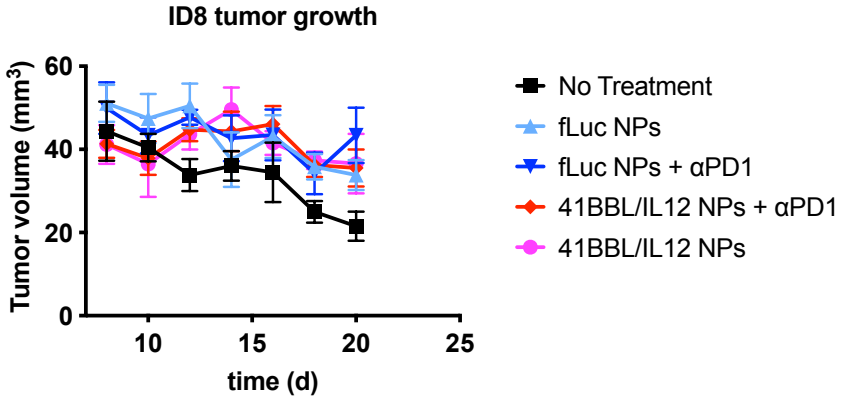
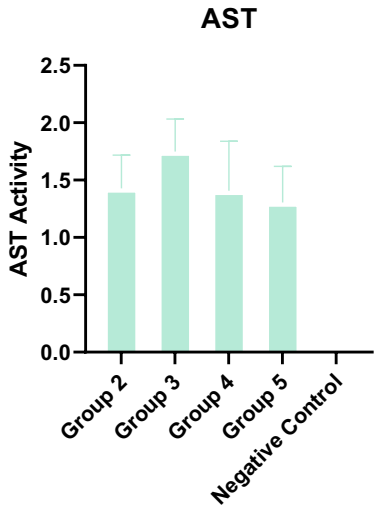


Figure 8. Female C57BL/6 mice were inoculated SC on the right flank with 5×10^6 cells per mouse. NPs were injected intratumorally, and anti-PD-1 antibody was administered systemically (IP) in some groups. Unfortunately, tumors in all groups, including the untreated group, decreased in average size over the course of the study, obscuring any differences that may have been caused by the NPs treatments themselves.



Treatment Groups:

	GFP	41BBL	IL12	αPD1
Group 2	+	-	-	-
Group 3	+	-	-	+
Group 4	-	+	+	+
Group 5	-	+	+	-

Figure 9. Blood was drawn from mice that had been treated intratumorally with NPs, with or without anti-PD-1. No liver toxicity was measured by AST assay.

Fig. 10 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.

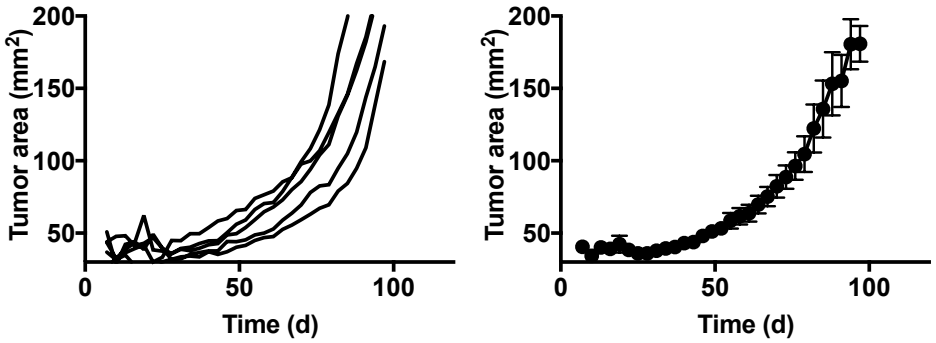


Figure 10. Female C57BL/6 mice were inoculated SC on the right flank with 5×10^6 cells per mouse. Tumor growth was slow but continued to completion in 27% of mice.

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

Similarly, mice were inoculated with (fLuc+) ID8 tumors IP, and NPs and/or anti-PD-1 were injected IP for treatment of tumors in that space. Mice were imaged over time by IVIS to measure tumor growth. Unfortunately, these tumors also regressed even in the control group(s), and differences between the groups could not be measured (**Fig. 11**).

Treatment Groups:

	41BBL	IL12	αPD1
Group 2	-	-	-
Group 3	-	-	+
Group 4	+	+	+
Group 5	+	+	-

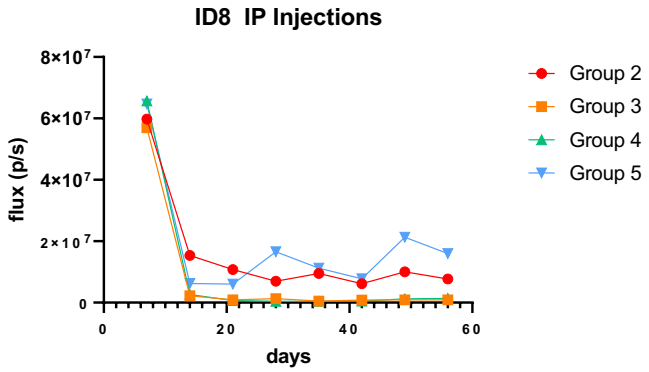


Figure 11. Female C57BL/6 mice were inoculated IP with 5×10^6 cells per mouse, then treated with NPs and/or anti-PD-1 antibody IP. IP tumors regressed too much for differences to be measured among groups

Improve ID8 tumor cell engraftment in mice by co-injecting cells with a synthetic Matrigel-like substrate

Using the synthetic substrate described in the literature [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)], we again injected fLuc$^+$ ID8 cells subcutaneously into the flanks of mice. This *in situ*-forming gel is meant to allow better tumor formation. However, while the gel did improve the viability of the tumor cells, the tumor eventually began to regress and eventually disappeared. Early tumor measurements did indicate increased tumor survival at the injection site, but the tumor failed to luminesce after multiple weeks, demonstrating that, while a palpable lump remained measurable, the tumor cells themselves had largely died within that time (**Fig. 12**). Thus, the alginate-based gel can form a palpable tumor on its own and seems to improve tumor cell survival at early time points, but it does not facilitate long-term or permanent tumor engraftment.

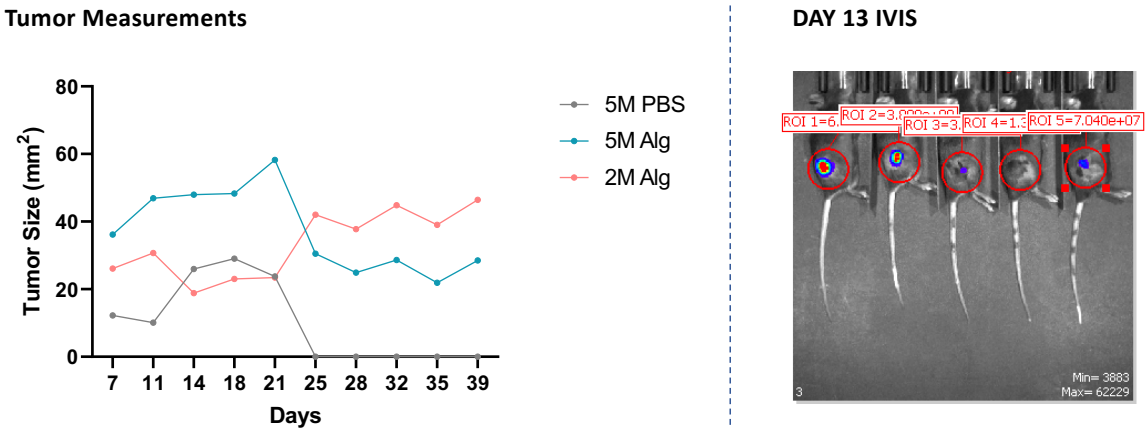


Figure 12. Female C57BL/6 mice were inoculated SC on the right flank with 2×10^6 or 5×10^6 cells per mouse, with or without the alginate gel (Alg or PBS, respectively). Tumor growth was improved by the gel, but luminescence decreased to zero in all of the groups tested.

Engineer an alternative mouse ovarian cancer cell line for in vivo *cellular engraftment*

We have since sourced an alternative ovarian cancer cell line, OV2944-HM-1, which has been reported in literature to be less immunogenic and therefore to form more consistent tumors in mice. We have completed the necessary paperwork to obtain this cell line and aim to use it in place of ID8. Although our *in vitro* screening experiments in ID8 cells have allowed us to obtain the necessary data to select an optimal formulation for transfection of ovarian cancer cells, and our truncated *in vivo* experiments in ID8 demonstrate nanoparticle transfection efficacy *in vivo*, we aim to complete the functional *in vivo* work using the new OV2944-HM-1 cell line.

First, PBAE NPs were tested to find formulations that could successfully transfect OV2944-HM-1 cells. It was found that polymer 4-5-39, formulated under similar conditions to those used for ID8 cells, could transfect some of these cells *in vitro* with GFP (**Fig. 13**).

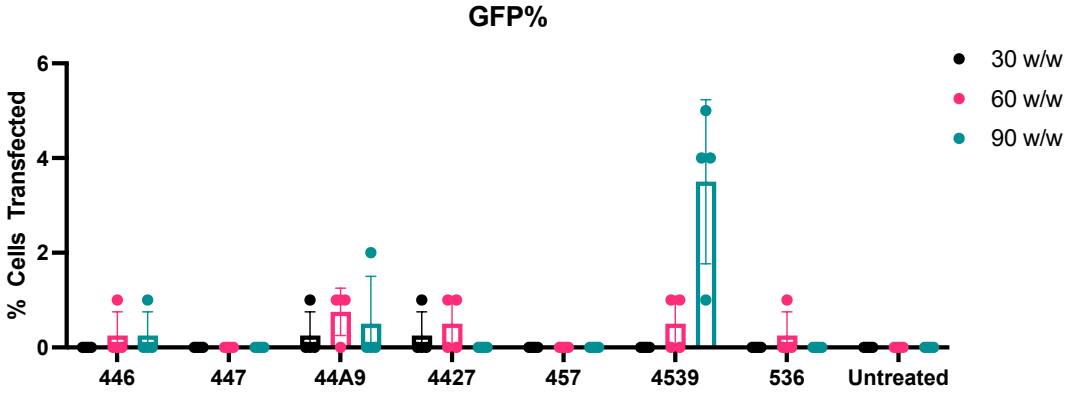


Figure 13. OV2944-HM-1 cells were transfected *in vitro* with GFP DNA using the PBAE structures shown on the graph above. 4-5-39 was the leading polymer of the ones tested.

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.

The next reporting period will focus on *in vivo* functional studies.

1. Submission of a revised experimental plan on mice with OV2944-HM-1 to ACURO for approval
2. Treatment of subcutaneous flank tumors of OV2944-HM-1 using nanoparticles with or without checkpoint inhibition, using the same experimental groups proposed for ID8 studies
3. Treatment of subcutaneous flank tumors of OV2944-HM-1 using nanoparticles with or without checkpoint inhibition in order to prevent the growth of OV2944-HM-1 cells in the intraperitoneal (IP) space, using the same experimental groups proposed for ID8 studies
4. Immunological analysis of flank and IP tumors after treatment of flank tumors with nanoparticles

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 multiple ovarian cancer cell lines both *in vitro* and *in vivo*
2. ID8-bearing mice can be established with tumors at multiple different sites, though further optimization of the mouse model is needed to improve consistency.
3. Polymeric NPs can be injected into tumors in order to engineer cells locally while causing no detectable systemic toxicity

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.

Although most published papers use Matrigel to encourage ID8 SC tumor formation, due to supply chain delays and the global pandemic, we and the labs around us have not been able to acquire enough Matrigel for a tumor study from any source since mid 2021. As an alternative, we have used a synthetic gel matrix for tumor engraftment, which improved tumor take but did not prevent eventual regression of the tumors. We have since identified and obtained another ovarian cancer cell line, OV2944-HM-1, which has been reported to engraft well in mice. We aim to use this cell line as a replacement for ID8 cells for *in vivo* functional studies.

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.

The animal facilities at Johns Hopkins have experienced a series of pathogen outbreaks over the last year, including tropical blood mites, pinworms, rotavirus, and mouse parvovirus. As these pathogens and/or the medications that the facility uses to treat them could affect the immune response, and some of the medications are even being tested as anti-cancer agents, we were severely limited in our ability to conduct experiments in the animal facilities. Although there is unfortunately not much that can be done on our part to ameliorate this problem, we have since gained access to a room that is no longer quarantined due to these pathogens, and we anticipate being able to carry out the planned experiments in that room over the next year.

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 all animal experiments as well as supervising trainees

Name: Tiarra Warren
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Ms. Warren synthesized materials to be used in ovarian cancer cell transfection screens.

Name: Joanna Yang
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 4
Contribution to Project: Ms. Yang conducted in vitro transfection experiments on ID8 cells as well as working on studies on optimize tumor inoculation in mice.

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