AWARD NUMBER: W81XWH-20-1-0544

TITLE: "Designer Nanoparticles" for Immunotherapeutic Treatment of Bladder Cancer

PRINCIPAL INVESTIGATOR: Jared Brown

CONTRACTING ORGANIZATION: University of Colorado, Aurora, CO

REPORT DATE: October 2023

TYPE OF REPORT: Annual

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**
October 2023	Annual	30Sep2022-29Sep2023

4. TITLE AND SUBTITLE

"Designer Nanoparticles" for Immunotherapeutic Treatment of Bladder Cancer

5a. CONTRACT NUMBER
W81 WH-20-1-0544

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jared Brown, PhD

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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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

Bladder cancer is a common cancer with more than 70,000 new cases and 15,000 deaths annually in the United States. Bladder cancer is the 4th most common cancer in veterans at 3.6%, behind only prostate, lung and colon cancer. For metastatic, advanced stage bladder cancer, 5 immune checkpoint inhibitors have been approved in the last several years. These drugs have revolutionized the treatment of metastatic bladder cancer, providing durable, and well tolerated responses in ~20% of patients. However, the treatment of localized disease remains unchanged and immune checkpoint inhibitors have not been integrated into the care of these patients. Objective/hypothesis: In this application, we propose to develop a "designer nanoparticle" based on bacteriophage lambda where we have coopted the non-infectious capsid which can be decorated with targeting antibodies, bioactive proteins, DNA, fluorophores, etc., in defined ratios. Specifically, we propose to develop theranostic 'phage-like particles' or PLPs which are decorated with fluorescein, anti-epidermal growth factor receptor (EGFR) and/or CD47 targeting capabilities, and interferon stimulatory DNAs (ISDs) that activate the stimulator of interferon genes (STING) pathway to promote an anti-tumor immune response. We hypothesize that PLPs can be optimized to activate the STING pathway resulting in production of Type I interferons (IFN alpha & beta) leading to a robust and durable anti-tumor immune response. We have generated preliminary data demonstrating engineering of the PLPs and specific uptake into bladder cancer cells. Impact: There is a substantial clinical gap in the treatment of early-stage bladder.

15. SUBJECT TERMS
None listed.

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
Unclassified	Unclassified	Unclassified	Unclassified	18	**19b. TELEPHONE NUMBER** *(include area code)*

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

TABLE OF CONTENTS

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

   Bladder cancer is a common cancer with more than 70,000 new cases and 15,000 deaths each year in the United States. It is common in both in active and retired military personnel, estimated to be the 4th most common cancer in the Veterans Affairs (VA) medical system. To address this, we will develop a novel immunotherapeutic approach using a "designer nanoparticle" platform that is based upon bacteriophage lambda, a virus that infects bacteria but that is non-infectious in humans. We have coopted the λ capsid which is an icosahedral shaped particle in the nanometer size range that we can then decorate with targeting antibodies, small molecules (e.g. drugs), DNA, bioactive proteins, etc., in a user-defined manner. We propose to develop this designer nanoparticle, a.k.a. 'phage-like particle' or PLP, for targeting bladder cancer and activating an anti-tumor immune pathway for treatment of this disease. We are specifically targeting a pathway which was recently recognized to contribute to immune responses against cancer cells. This pathway, the stimulator of interferon genes (STING), when activated, produces anti-tumor proteins termed Type I interferons that promote a robust and durable anti-tumor response by recruitment of other immune cells to attack the tumor.

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

   Bladder cancer, bacteriophage, interferon, STING, immunotherapy

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.

   The major goals of the project are:

Year 1	Months	Completion date	Percent completed
1. Production of phage-like particles	1-3	8/30/2021	100%
2. Decoration of phage-like particles (antibodies, ISDs)	1-3	8/30/2021	100%
3. Physicochemical characterization of phage-like particles (SEC, AUC, DLS, EM)	2-4	N/A	100%
4. Assessment of phage-like particle uptake in MB49 and HTB9 cells	4-6	10/15/2021	100%
5. Determine the optimal conditions for activation of the STING pathway in bladder cancer cells (MB49 and HTB9) - Measure interferon responses - Measure phosphorylated IRF3 and TBK1	6-18	10/1/2023	100%

- Measure NF-κB levels and nuclear translocation			
6. Modifications to PLP synthesis based on uptake and activation studies (e.g. decorations, density of antibodies and/or ISDs, etc)	6-18	10/1/2023	100%

Timeline for Aim 2

1. Regulatory review and approval by the USAMRMC Animal Care and Use Review Office (ACURO)	1-6	8/30/2021	100%
2. Treat mice with an orthotopic model of bladder cancer with phage-like particles to determine binding	12-24	N/A	0%
3. Assess tumor implantation and growth with bioluminescence and pathologically - Successful tumor implantation will be confirmed by measuring bioluminescence with an *in vivo* imaging system (IVIS) via luciferase expression from the transfected MB49 cells at 7 days post implantation. - Following treatment with PLPs, bioluminescence will be measured for tumor growth and infrared fluorescence for PLP delivery (which are labelled with IRDye 750). The same IVIS imaging system will be used. - Each group of mice will be euthanized at each described time point in the experimental design and bladder, kidney, lung, liver, heart, spleen and regional lymph nodes will be collected for routine histology (H&E staining and markers of inflammation including T cells and NK cells).	12-24	N/A	0%
4. Assess STING pathway activation *in vivo* after treatment with phage-like particles - Measure phosphorylated IRF3 and NF-κB expression and nuclear translocation - Assess IFNα and β expression in bladder tissue by quantitative PCR and ELISA	12-24	N/A	0%
5. Modifications to PLP synthesis based on in vivo targeting and STING activation (e.g. decorations, density of antibodies and/or ISDs, etc)	18-24	N/A	0%

6. Use bladder tissue to exam infiltration of key immune cells by flow cytometry (T cells, NK cells, macrophages, dendritic cells, etc).	14-26	N/A	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 Activities:

During this reporting period, we completed production and decoration of the phage like particles (PLPs). We have completed the characterization of the PLPs and assessed cellular uptake in MB49 and HTB9 bladder cancer cells and determined IFNβ production. We also have gone back to using the interferon stimulatory DNA (ISD) instead of the D-cGAMP as previously reported. The D-cGAMP agonist of the STING pathway was not working due to steric hindrance of the molecule and we were unable to get production of IFNβ despite successful decoration. However, the ISD appears to be working much better and we are moving forward with it for the upcoming animal studies that will completed during this no-cost extension period.

Specific Objectives:

The objectives for this reporting period were to: 1) synthesize, decorate and characterize the PLPs; 2) optimize cellular uptake through modifications of decoration proteins; 3) assess STING pathway activation in mouse and human bladder cancer cells.

Significant Results:

As shown in Figures 1-4, we have completed decoration and characterization of the phage like particles (PLPs). Figure 1 demonstrates completion of decoration of PLPs with cetuximab and fluorescein (i.e. the targeting antibody and detection fluorophore). In addition to fluorescein, we have also decorated the PLPs with IVIsense 680 fluorophore. This fluorophore will be used for the animal studies that will be completed in this no-cost extension period. We will be using an IVIS imager to detect the PLPs in mouse bladders and the original fluorescein dye we porposed is not detected in the IVIS imager, hence we will be using the new IVIsense 680 fluorophore to detect PLPs in mouse bladder. Figure 3 demonstrates successful decoration of the PLPs with interferon stimulatory DNA (ISD). Lastly, figure 4 is an electron micrograph of the PLPs illustrating their structure and confirmation.

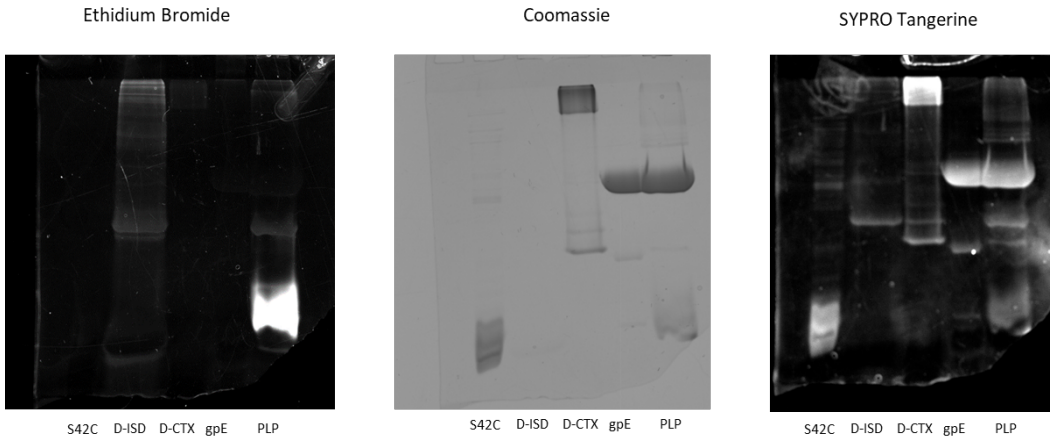


Fig 1. Decoration of 20% ISD phage like particle: 15% SDS PAGE gel visualizing the components of decorated phage like particles with 20% ISD 5% Cetuximab and 25% fluorescein. Gel was stained with Ethidium Bromide to visualize nucleic acid and stained with Coomassie and SYPRO Tangerine to visualized protein components.

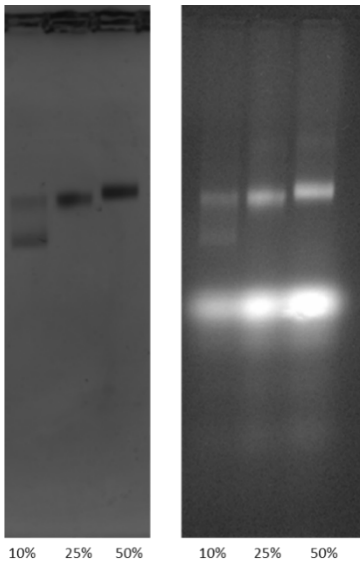
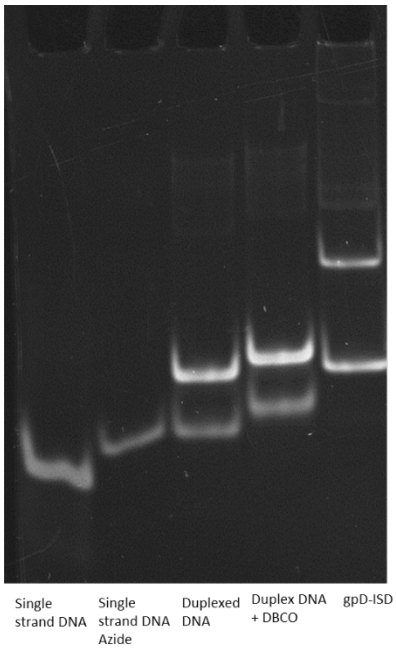


Fig 2. Decoration with IVISense fluorophore: 1% agarose gel depicting PLP's decorated with 10%,25% and 50% IVISense 680 maleimide fluorophore. (Left) is coomassie stained image and (Right) is the gel visualized with red epiluminesence

Fig 3. Conjugation of Interferon stimulating DNA with gpD: 10% native TBE PAGE gel stained with ethidium bromide and visualized with UV light.



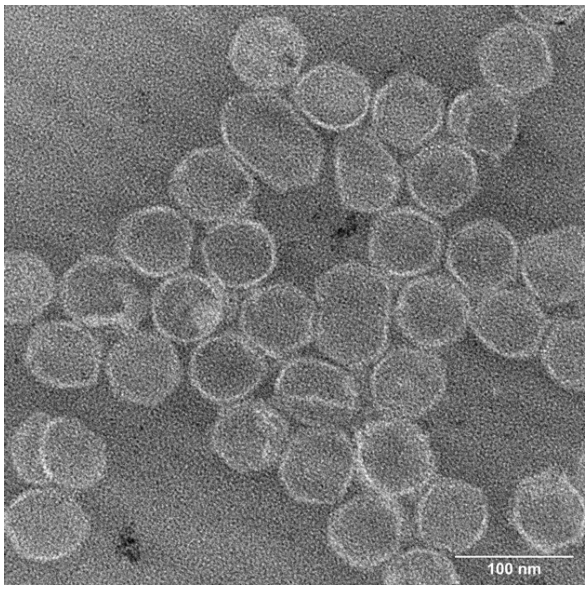


Fig 4. Transmission electron microscopy images of 5% cetuximab phage like particles: Images taken with 120,000X magnification and a 1.5um defocus.

Figures 5 and 6 demonstrate successful uptake of the PLPs in both human (HTB9) and mouse (MB49) bladder cancer cells respectively. The PLPs were decorated with 20% ISD and 5% Cetuximab for these studies and labeled with the fluorescein dye which shows up in the FITC channel (last column on each image). Overall, we have successful and reliable uptake of PLPs in bladder cancer cells further confirming our plans to move forward to animal studies during the no-cost extension period.

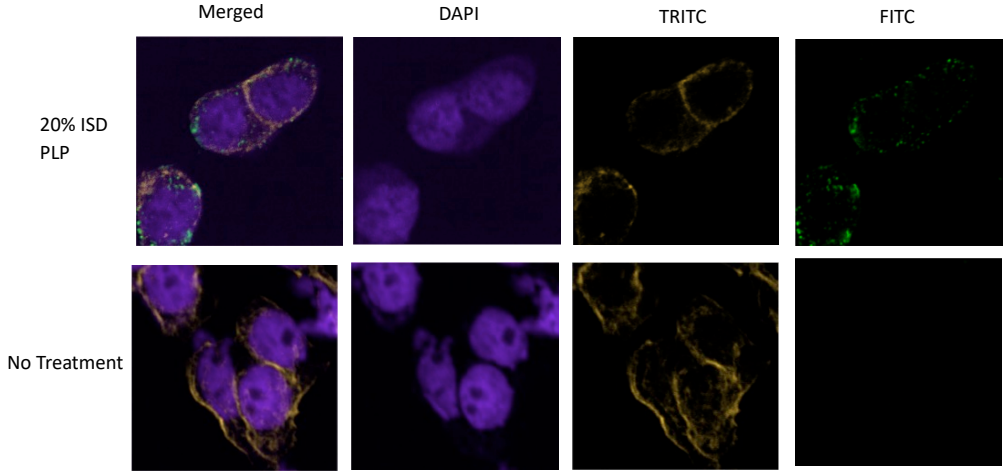


Fig 5. Representative Confocal microscopy images of HTB9 cells: Images were taken with a 20x air objective and 9x zoom. Cells were fixed and prepared for imaging 3 hours after treatment with 20% ISD 5% CTX phage like particles. Images are shown representing the merged image the DAPI, TRITC, and FITC channels.

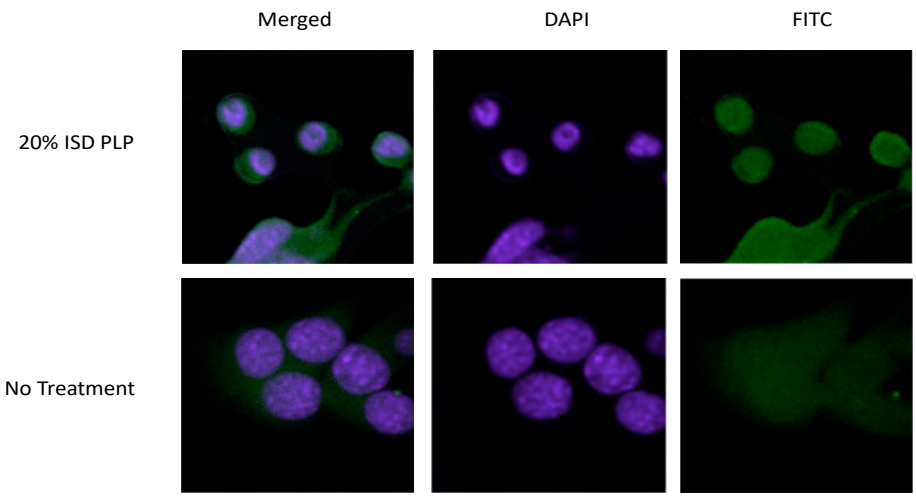
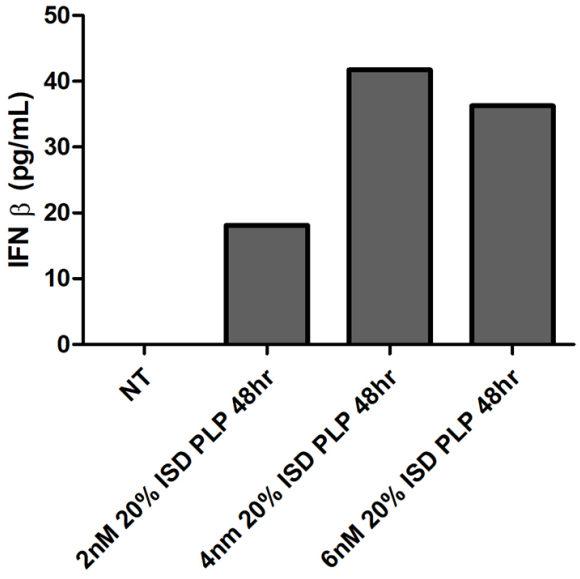


Fig 6. Representative Confocal microscopy images of MB-49 cells: Images taken on a 20x air objective with a 9x zoom. Cells were fixed and prepared for imaging 2 hours after treatment. Images are shown representing the merged image and the DAPI and FITC channels.

Lastly, as shown in Figure 7, we have successful production of IFNβ in mouse bladder cancer cells. In these studies, we have performed a dose response study to determine the optimal concentration of PLPs to elicit an IFNβ response. In this case, we found that 4 nM of the PLPs resulted in a consistent IFNβ response in MB49 cells. This concentration will be utilized in our mouse studies.

Fig 7. IFN-β activation of MB-49 cells: MB-49 cells were treated with phage like particles with a surface density of 5% cetuximab and 20% Interferon Stimulating DNA. Samples were evaluated by ELISA and samples were collected 48 hours after treatment.



1. Methods

1. *Cell Culture:* We cultured MB-49 and HTB9 cells in media with complete DMEM (high glucose with sodium pyruvate), 10% FBS, 10mL of 10X Pen-Strep. We started cultures with 1 million cells from liquid nitrogen. These are quickly thawed and then carefully added to a 15mL tube with 9mL of media. Cells are then spun at 300g for 3 minutes then resuspended and plated in a T-75 flask with 15mL of fresh media. To subculture these cells we collected and spun down the media in the flask at 300g for 5 minutes while trypsinizing the cells in the flask with 5mL of .25X trypsin EDTA for 5 min at 37C. After trypsinization 5mL of fresh media is added to the flask and then all the media is collected in a 15mL tube and spun down at 300g for 5 min. Media from the spins is aspirated from the tube without disturbing the pellet. The cells are then resuspended in fresh media and combined before counting. Cells are then counted by acridine orange staining and 150,000 or 300,000 cells are passed into 15 or 25mL of media depending on if using a T-75 or T-175 respectively. We cultured RAW Cells in media with complete DMEM (high glucose with sodium pyruvate), 10% FBS, 10mL of 10X Pen-Strep. We started cultures with 1 million cells from liquid nitrogen. These are quickly thawed and then carefully added to a 15mL tube with 9mL of media. Cells are then spun at 300g for 3 minutes then resuspended and plated in a T-75 flask with 15mL of fresh media. To subculture these cells we collected and spun down the media in the flask at 300g for 5 minutes while trypsinizing the cells in the flask with 5mL of 0.25X trypsin EDTA for 5 min at 37C. After trypsinization 5mL of fresh media is added to the flask and then all the media is collected in a 15mL tube and spun down at 300g for 5 min. Media from the spins is aspirated from the tube without disturbing the pellet. The cells are then resuspended in fresh media and combined before counting. Cells are then counted by acridine orange staining, and 100,000 cells are than added to 15mL of media in a T-75 flask.

2. *Construction of the PLP:* The desired plasmid (pNu3_E) is transformed into BL21(DE3) component cells, and following with DNAse I (0.04mg/mL) and lysozyme (0.4mg/mL) to increase the protein extraction efficacy from cell lysates. By applying 10%-40% sucrose gradient untracentrification, the PLPs are collected at the third band under bright light, and buffer-exchanged to PLP wash buffer (20mM Tris (pH 8.0 @ 4°C), 15mM $MgCl_2$, 1mM EDTA, 7mM βME) by 100kDa Amicon centrifugal filter units (100kDa), and dialyzed with PLP was buffer overnight. Proteins will be fractionated by cation exchange chromatography with 55mL diethylaminoethyl column and eluted by PLP elution buffer (PLP wash buffer + 1M NaCl) with 10-column volume linear gradient. The confirmation of the fractions is analyzed by SDS-PAGE and Agarose gel electrophoresis. PLP-containing fractions are pooled and dialyzed with PLP storage buffer (50mM HEPES (pH7.4 at room temperature), 100mM NaCl, 10mM $MgCl_2$), and the PLP particles are stored in 4°C until future use.

3. *Conjugation and purification of gpD::Cetuximab:* Reducing gpD (S42C) by incubating with 5 fold TCEP for 1hr at room temperature in maleimide conjugation buffer (50mM HEPES, 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6). We then buffer exchanged the antibody into succinimide conjugation buffer (10mM Na_2HPO4, 1mM KH_2PO_4, 137mM NaCl, 2.7mM KCl, 1mM EDTA, pH 7.2) with 30KDa spin filters. The antibody is then incubated with 5 fold excess of the PEG crosslinker reagent for 30 minutes at room temperature. We then pass the antibody reaction through two zeba spin desalting columns to remove any excess crosslinker reagent before

adding to the reduced gpD for 1 hour at room temperature. We then used a Superose 6 10/300 size exclusion column for purification.

4. *Conjugation and purification of gpD::F5M (fluorescent-5-Maleimide):* Reducing gpD (S42C) by incubating with 5 fold TCEP for 1hour at room temperature in maleimide conjugation buffer (50mM HEPES (pH 6.6), 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6) and fill up the final volume to 1mL with 50mM HEPES (pH 6.6). Fluorescence-5-maleimide is conjugated to the reduced gpD with a concentration of 350μM and following with 5mM of EDTA. The conjugation is stored in 4°C overnight, and purified by Zeba™ Spin Desalting Columns twice. The purified conjugation can be stored in 4°C until future use.

5. *Conjugation and purification of gpD::ISD (interferon stimulatory DNA):* The double stranded DNA is constructed by adding one to one ratio of the leading strand and lagging strand together, and the mixture of the leading strand and the lagging strand is incubated at 95°C for 2 minutes and cooled down to room temperature. Reducing gpD (S42C) by incubating with 5 fold TCEP for 1hr at room temperature in maleimide conjugation buffer (50mM HEPES, 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6). $DBCO_{(PEG)24}$-4-Maleimide linker is conjugated in 5 fold volume to the reduced gpD, with 1mM of EDTA, and incubated at 37°C for 2 hours. The conjugation is purified by Zeba™ Spin Desalting Columns twice, and buffer-exchanged to annealing buffer (10mM Tris, 50mM NaCl, 1mM EDTA). The double stranded DNA is incubated with 5 fold volume of the linker conjugation at room temperature for 1 hour, and fractionated by anion exchange chromatography with three 5mL HiTrap Q HP columns connected in tandem. The ISD-conjugation fractions are collected and stored in 4°C until future use.

6. *PLP Expansion and decoration:* To expand phage like particles we first buffer exchanged them into (2.5M urea, 10mM HEPES) and incubate for 1hr on ice. We then added a 504 fold excess of gpD (wild type and conjugations) in the desired ratio depending on desired final percentages of different components. The gpD conjugations are added individually with the largest being added first and incubated with the particles for 20 minutes. At the end the particles are incubated for 1hr and then stored at 4°C.

7. *Confocal Microscopy:* For confocal microscopy cells were plated on coverslips in 6 well plates one day before fixing with a goal confluency of 50-70%. Cells were then treated with PLP and fixed at time points 0, 30min and 3hr. Following treatment media is removed from the wells and slides are washed with cold PBS. After washing PBS is aspirated and cells are fixed with 1mL of 3.7-4.0% paraformaldehyde in PBS and rocked for 12min. After fixing, cells are washed with PBS before they are permeabilized by the addition of 1mL of 0.1% v/v triton X-100 in cold PBS rocking for 12 minutes. Cells are then washed and 1mL of 1ug/uL DAPI and phalloidin in cold PBS are added to the cells and rocked in the dark for 1 hour. After washing the coverslips are ready to be added to the slide with a 1:4 mixture of diamond anti-fade and supermount mixture. Cells are then imaged by confocal microscopy with the 10X air objective and images are analyzed for integrated density in the FITC channel to determine relative uptake of the particles.

8. *IFN-β ELISA*: For ELISA experiments we treated cells with different concentrations of the secondary messenger cGAMP and particles decorated with Cetuximab and cGAMP. 500,000 cells were plated the day before treatment and were treated in serum free media for 3 hours. After the

3-hour treatment time serum supplemented media was added into each well for the remaining 24 hour period. After 24 hours media from each well was collected and centrifuged at 600g for 5 minutes. After centrifugation supernatant was collected from each sample and then tested using standard ELISA protocol.

4. Stated Goals Not Met: We did not meet our goal of performing the proposed animal studies in the last reporting period. As noted above, while we observed robust cellular uptake of the PLPs decorated with D-cGAMP in bladder cancer cells, we have not observed significant STING activation as expected. We believe this issue was due to steric hindrance of the D-cGAMP molecule and we have instead gone back to decorating the PLP's with ISD molecules. These have been successful, but we just recently completed these studies, therefore we have requested a no-cost extension on this project to complete the animal studies in 2024.

Other Achievements:

Nothing to report.

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.

A Masters student, Robert Canfield, who has been working on this project just recently joined our PhD program in Pharmaceutical Sciences and is continuing to work on this project for his dissertation. He completed the decoration studies and bladder cancer cell studies in the past year. For the upcoming perios, he is going to receive training from Dr. Tom Flaig's lab (co-I) on animal studies and will assist with those in 2024.

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 No-Cost Extension period plans include the following:

1) Complete animal studies in an orthotopic model of bladder cancer
    a. Animal studies will be based on optimized PLPs as determined from the in vitro activation studies

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).

Nothing to report.

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 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.

We ran into some issues with conjugating the interferon stimulatory DNA on the PLPs and made the switch to using cGAMP as a STING agonist which we have had more success in conjugation. We have also run into some issues with activation of the bladder cancer cells by the PLPs, so we have also been examining macrophages which can produce significant amounts of type 1 interferons. We are also examining different bladder cancer cell lines. Our initial studies were with murine cell lines and we have begun experiments with human bladder cancer cells to determine if they have improved IFN-β production. Lastly, we are adjusting the amount of targeting antibody on the PLPs to improve uptake in the bladder cancer cells which should improve immune activation.

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 (no human subjects).

Significant changes in use or care of vertebrate animals

Nothing to report (animal studies have not yet been started).

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".

Name:	*Jared Brown*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1.2*
Contribution to Project:	*Oversaw entire project.*
Funding Support:	*This grant CA190078*
Name:	*Carlos Catalano*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1.2*
Contribution to Project:	*Oversaw PLP synthesis.*
Funding Support:	*This grant CA190078*
Name:	*Robert Canfield*
Project Role:	*Ph.D. student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Synthesized PLPs and performed cellular uptake studies.*
Funding Support:	*Immunology/Microbiology Master's program at CU Anschutz from Oct 2021 – July 2022. From July 2022 to present Robert has been supported by this grant CA190078.*

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: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (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.*

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, etc.*