AWARD NUMBER: W81XWH-15-1-0382

TITLE: Selective activation of a Perforin-Granzyme B fusion protein toxin by PSA as therapy for metastatic prostate cancer

PRINCIPAL INVESTIGATOR: Samuel Denmeade

RECIPIENT: Johns Hopkins University
Baltimore, MD 21287

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Fort Detrick, Maryland 21702-5012

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Selective Activation of a Perforin-Granzyme B Fusion Protein Toxin by PSA as Therapy for Metastatic Prostate Cancer

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5b. GRANT NUMBER
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

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The Johns Hopkins University
600 North Wolfe Street
Baltimore, MD 21287

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Fort Detrick MD 21702-5012

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11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; Distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Protein toxins represent a class of agents that can kill cells in a proliferation independent manner. Many such proteins, derived primarily from bacterial sources, have been identified that are highly potent cytotoxins. While this approach has great potential, the major limitation is the fact that the protein toxin proves to be highly immunogenic and not amenable to repeated dosing to achieve maximal antitumor effect. This obstacle can be overcome through the use of human protein toxins. The goal of this proposal, therefore, is to develop a targeted cytotoxic agent that can selectively kill both proliferating and non-proliferating prostate cancer cells within a metastatic site without significant host toxicity. To achieve this goal, we propose to modify Granzyme B, the major cell-killing components present in cytotoxic T lymphocyte (CTL) granules to a form that is selectively targeted to prostate cancer cells. Granzyme B is a zymogen that must be proteolytically activated and then must penetrate cell membranes to proteolytically activate intracellular pro-apoptotic factors. We describe a targeting approach that recapitulates this dual activation but redirects it to prostate cancer cells. We will replace the native two amino acid propeptide of granzyme B with a peptide recognized as a substrate by PSA. To facilitate granzyme B internalization, we will couple a potent small molecule inhibitor of PSMA to the C-terminus of granzyme B. This granzyme B toxin will only be activated in the prostate cancer microenvironment while remaining inactive against normal tissues lacking both PSA and PSMA

15. SUBJECT TERMS
Granzyme B, PSA, PSMA, protoxin, protease, zymogen

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	35	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

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

While prostate cancer can grow slowly, it is not curable with current therapies once it has metastasized outside of the prostate gland. Since prostate cancer cells have a remarkably low proliferation rate, novel therapies designed to selectively target and kill non-proliferating prostate cancer could be highly effective in this disease. Protein toxins represent a class of agents that can kill cells in a proliferation independent manner. Many such proteins, derived primarily from bacterial sources, have been identified that are highly potent cytotoxins. Attempts have been made to redirect the potent, but non-specific cytotoxicity of these toxins to produce tumor or tissue specific cell killing. This is typically accomplished through the creation of fusion proteins in which a cell-type specific ligand (e.g. cytokines) or antibody is fused to the toxic portion of the bacterial protein. While this approach has great potential, the major limitation of this approach is the fact that, in all cases described, the protein toxin proves to be highly immunogenic and not amenable to repeated dosing to achieve maximal antitumor effect. This obstacle can be overcome through the use of human protein toxins. The goal of this proposal, therefore, is to develop a targeted cytotoxic agent that can selectively kill both proliferating and non-proliferating prostate cancer cells within a metastatic site without significant host toxicity. To achieve this goal, we propose to modify Granzyme B, the major cell-killing components present in cytotoxic T lymphocyte (CTL) granules to a form that is selectively targeted to prostate cancer cells. Granzyme B is a zymogen that must be proteolytically activated and then must penetrate cell membranes to proteolytically activate intracellular pro-apoptotic factors. We describe a targeting approach that recapitulates this dual activation but redirects it to prostate cancer cells. We will replace the native two amino acid propeptide of granzyme B with a peptide recognized as a substrate by PSA. To facilitate granzyme B internalization, we will couple a potent small molecule inhibitor of PSMA to the C-terminus of granzyme B. This granzyme B toxin will only be activated in the prostate cancer microenvironment while remaining inactive against normal tissues lacking both PSA and PSMA.

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

Granzyme B, PSA, PSMA, protoxin

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: **Aim 1:** Synthesize, purify, and characterize modified GZMB proteins and analyze for PSA activation and PSMA binding and stability in mouse and human plasma. **Aim 2:** Demonstrate the selectivity of modified GZMB proteins against PSA/PSMA producing prostate cancer cells but not PSA/PSMA negative non-prostate cancer cells in vitro. **Aim 3:** Evaluate toxicity and efficacy of the lead modified GZMB proteins in vivo against PSA-producing prostate cancer xenografts.

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/specific objectives

Aim1 Activities

1) Generated a mutant form of Granzyme B containing a C-Terminal cysteine
2) Coupled a maleimide-containing PSMA inhibitor to the C-terminal cysteine of Granzyme B to generate a PSMA-targeted Granzyme B protein
3) Demonstrated PSMA-Granzyme B selectively binds to PSMA and becomes internalized into recycling endosome.
4) Generated a mutant form of Pseudomonas exotoxin PE35
5) Coupled a maleimide-containing PSMA inhibitor to the C-terminal cysteine of PE35 to generate a PSMA-targeted PE35 protein (PE35-MU2)
6) Demonstrated PE35-MU2 selectively binds to PSMA and becomes internalized into recycling endosome.
7) Attempted to generate human BID and BAD pro-apoptotic proteins conjugated to maleimide-containing PSMA inhibitor

Aim 2 Activities

1) Demonstrated selective uptake of PSMA-Granzyme B in PC-3 cells transfected to produce PSMA but no uptake in PC-3 transfected with vehicle control.
2) Generate fluorescent PSMA-Granzyme B to show specific uptake and concentration of protein in the recycling endosome.
3) Performed cytotoxicity assays with PSMA-Granzyme B against PSMA producing LNCaP and PSMA transfected PC3 vs. PSMA negative PC3 cells.
4) Demonstrated selective uptake of PE35-MU2 in PC-3 cells transfected to produce PSMA but no uptake in PC-3 transfected with vehicle control.
5) Generate fluorescent PE35-MU2 to show specific uptake and concentration of protein in the recycling endosome.
6) Performed cytotoxicity assays with PE35-MU2 against PSMA producing LNCaP and PSMA transfected PC3 vs. PSMA negative PC3 cells.
7) Performed cytoxtoxic assasys with PSMA-BID protein against PSMA producing LNCaP and PSMA transfected PC3 vs. PSMA negative PC3 cells.

Aim 3 Activities

1) Demonstrated that intratumoral injection of PSA-activated Granzyme B had limited antitumor activity against PSA-producing xenografts
2) Demonstrated that intratumoral injection of PE35-MU2 produces significant antitumor effect against PSMA producing xenografts but minimal effect against PSMA-negative xenografts
3) Demonstrated that intravenous administration of PE35 produced marked antitumor effect against PSMA-producing human prostate cancer xenografts without significant host toxicity

4) Significant Results

Over the course of the grant we generated a series of PSA and PSMA targeted toxins. Data from these studies is presented below. Protein toxins that were generated and studies during this proposal were:

1) **PSMA-Granzyme B**
2) **PSA-Granzyme B**
3) **PSMA-Pseudomonas exotoxin**
4) **PSMA-BID**
5) **PSMA-BAD**
6) **PSMA-Cytolethal distending toxin (Cdt) (induces DNA double strand breaks)**

Figure 1: Synthesis and production of protein-urea drug conjugates. (A). Chemical synthesis scheme taken to make a thiol-reactive maleimide-linked urea separated by two PEG units (MU2) This PSMA-targeted maleimide-linked urea was used to generat PSMA targeted protein toxins. (B). Mass spectrometry plot of MU2 product. (C). Diagram depicting the protein-MU2 conjugation reaction (D). Ellman's reagent assay of BSA +/- MU2 under non-reducing conditions (E).Non-reducing SDS PAGE gel of C-terminal reactive GZMB +/- MU2 following dialysis (F). ABD-F fluorescent assay of PE35 +/- MU2

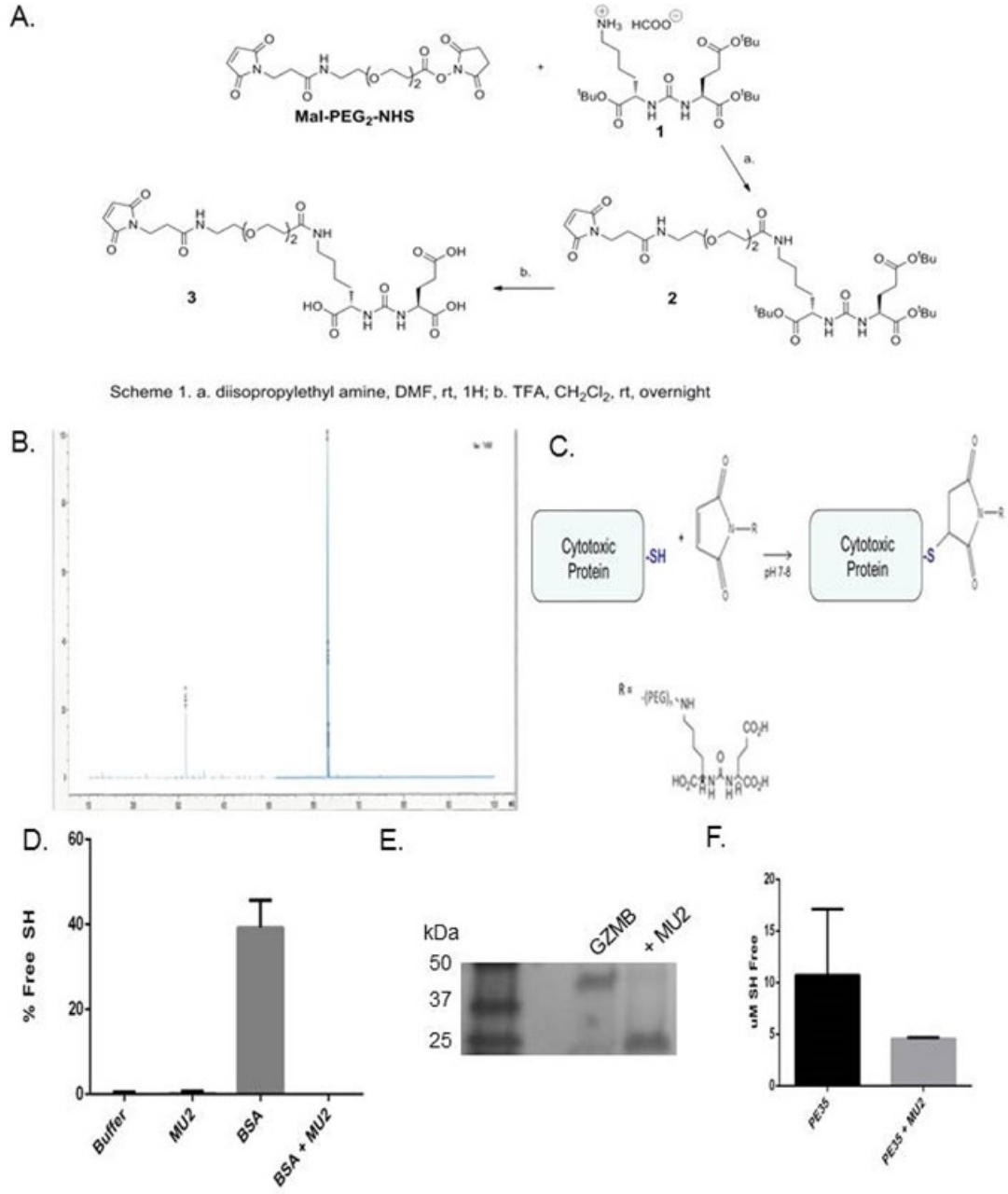
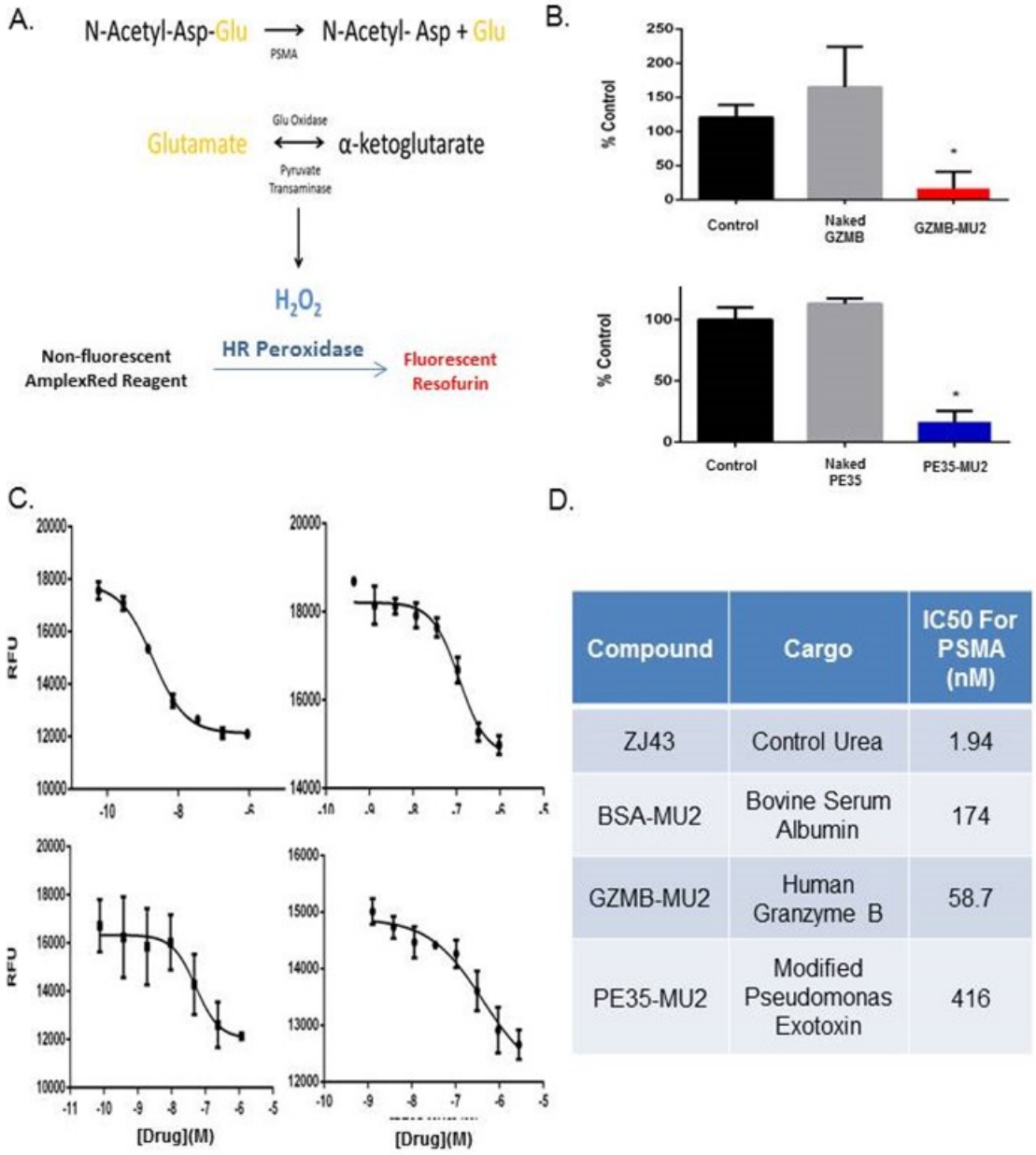


Figure 2: Protein-urea conjugates bind and inhibit PSMA. Scheme of the enzyme-coupled PSMA enzymatic assay utilized to detect urea-conjugate binding (A). Inhibition of PSMA by coupled or naked cytotoxic proteins represented as a percentage of the control reaction (B). Dose response curves of ZJ43 (top left), BSA-MU2 (top right), GZMB-MU2 (bottom left), and PE35-MU2 (bottom right) (C). Table describing the IC50 values for PSMA obtained for each compound (D).



Compound	Cargo	IC50 For PSMA (nM)
ZJ43	Control Urea	1.94
BSA-MU2	Bovine Serum Albumin	174
GZMB-MU2	Human Granzyme B	58.7
PE35-MU2	Modified Pseudomonas Exotoxin	416

Figure 3: GZMB-MU2 internalizes into PIP cells but does not induce cell death. Enzymatic activity of GZMB or GZMB-MU2 using a GZMB-specific fluorescent substrate (A). Cytotoxicity of purified GZMB-MU2 on PIP-PC3 (top left), Flu-PC3 (top right), LAPC4 (bottom left), or CWR22 Rv1 (bottom right) (B). Confocal microscopy of PIP or Flu-PC3 cells treated with Flor-GZMB-MU2 for 1 hour at 20X magnification (left) or 60X (right) (C).

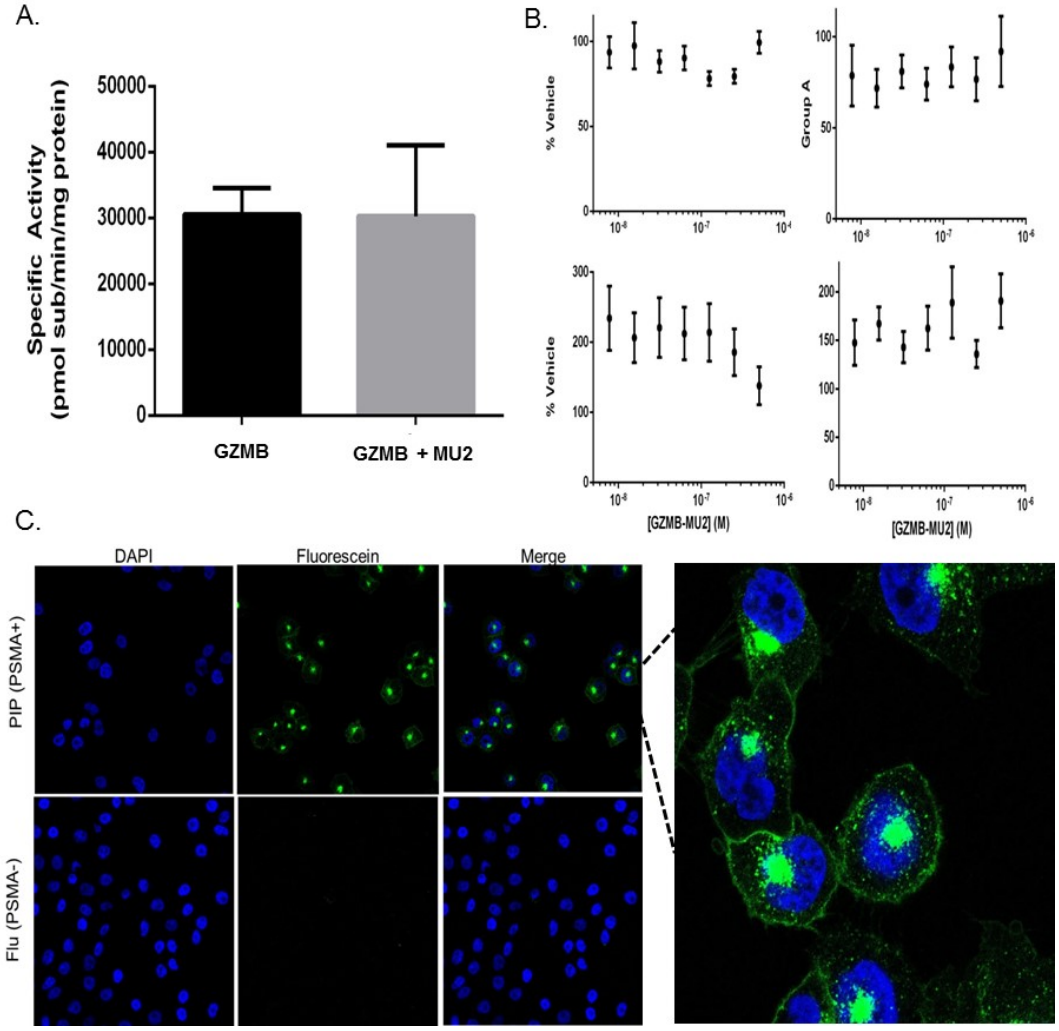
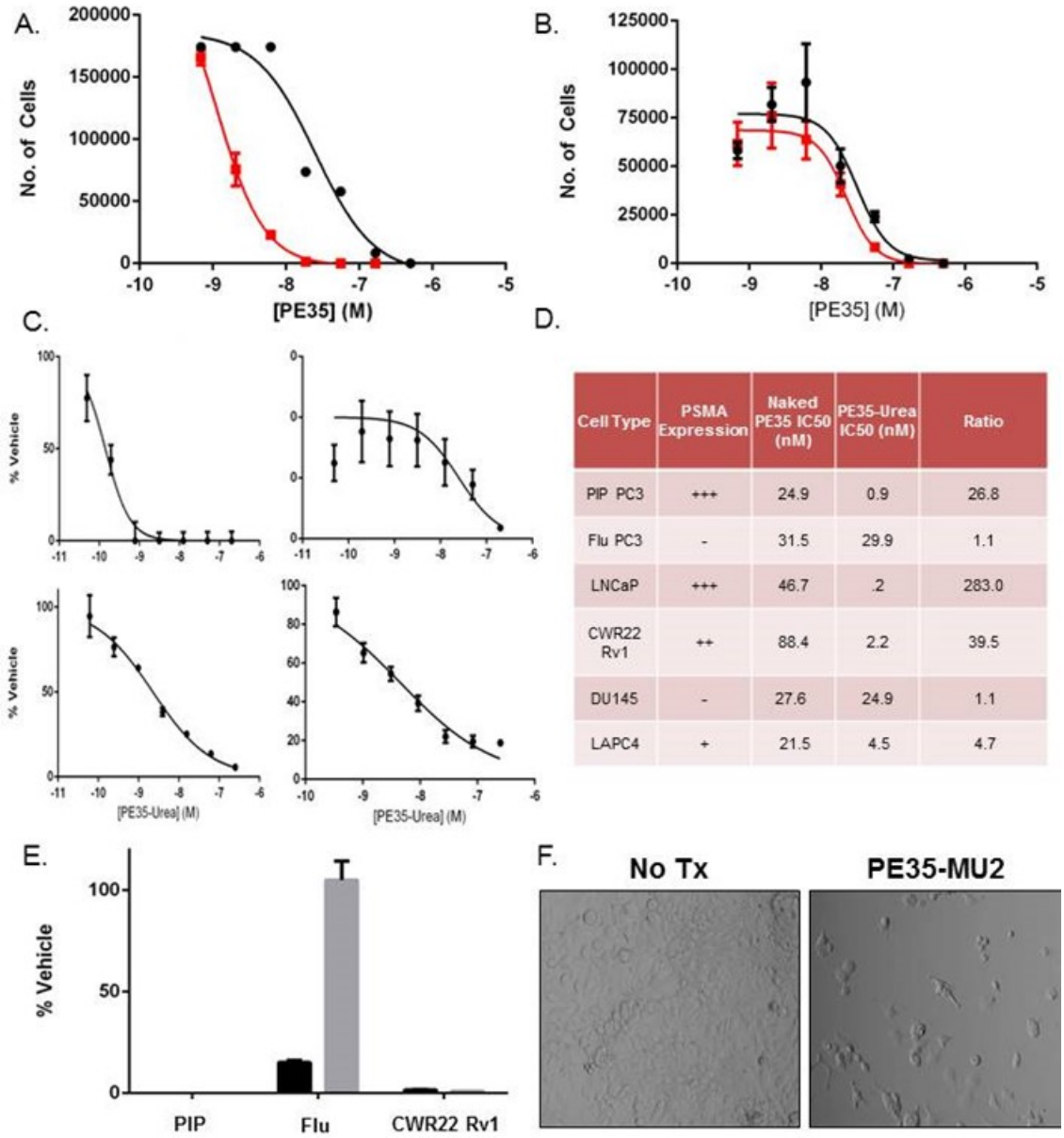


Figure 4: PE35-MU2 is selectively toxic to PSMA producing cells. Viability of PIP-PC3 (A) or Flu-PC3 (B) cells treated with naked PE35 (black) or PE35-MU2 (red). Dose response curve LNCaP (top left), DU145 (top right), CWR22 Rv1 (bottom left) or LAPC4 (bottom right) (C). Table depicting the IC50 values obtained on various cell lines with naked or conjugated PE35 (D). Pulse-soak experiment in which PIP-PC3, Flu-PC3, or CWR22 Rv1 were dosed with 250 nM PE35-MU2 for 5 days (black bars) or pulsed for 24 hours (grey bars) (E). Light microscopy images of CWR22 Rv1 cells treated with 3 nM PE35-MU2 for 5 days at 10x magnification (F).



Cell Type	PSMA Expression	Naked PE35 IC50 (nM)	PE35-Urea IC50 (nM)	Ratio
PIP PC3	+++	24.9	0.9	26.8
Flu PC3	-	31.5	29.9	1.1
LNCaP	+++	46.7	.2	283.0
CWR22 Rv1	++	88.4	2.2	39.5
DU145	-	27.6	24.9	1.1
LAPC4	+	21.5	4.5	4.7

Figure 5: PE35-MU2 is selectively internalized by PSMA expressing cells. Confocal microscopy images of PIP-PC3 treated with Flor-PE35-MU2 for two hours plus or minus PSMA inhibitor 10 uM 2PMPA

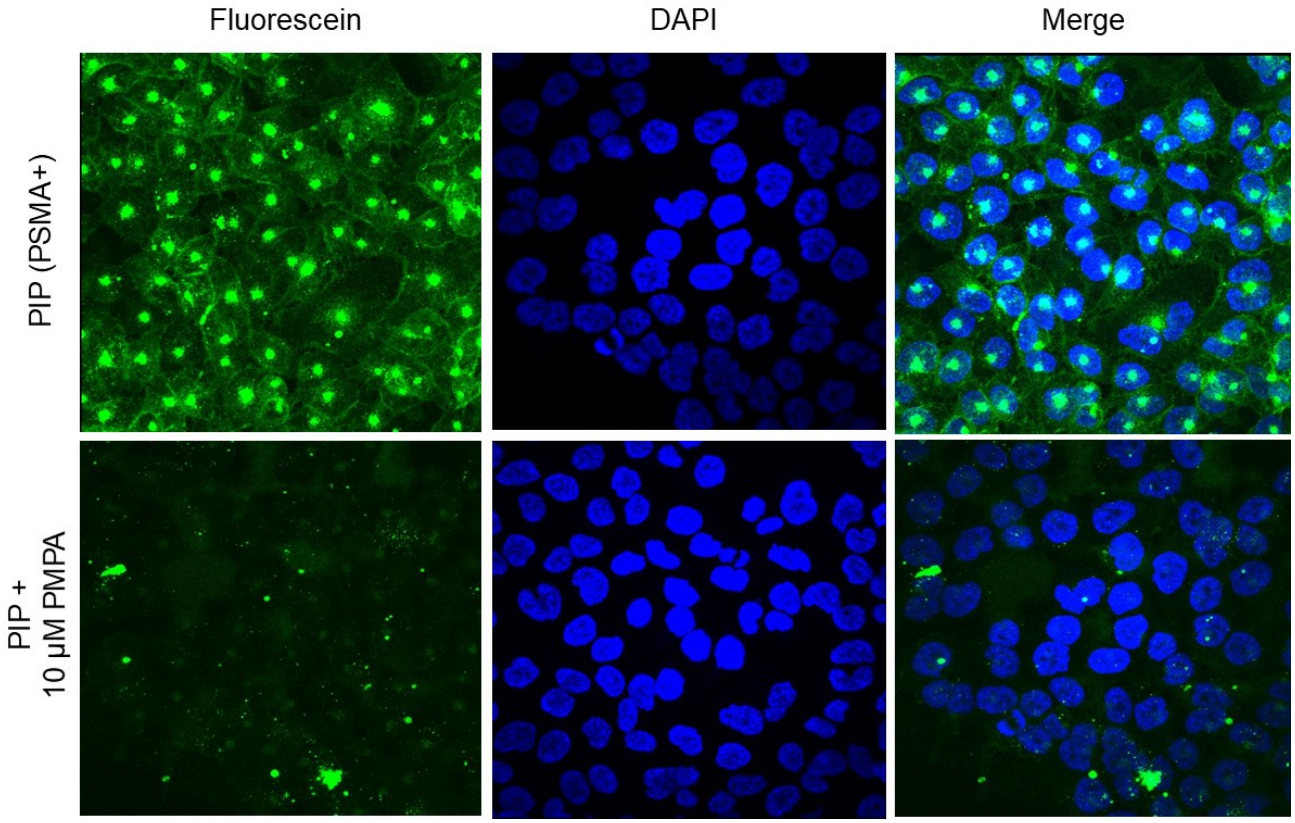


Figure 6: PE35-MU2 regresses PSMA expressing xenografts when injected intratumorly. Growth of PIP-PC3 (A) and Flu-PC3 (B) following two injections of vehicle (black) or 20 ug PE35-MU2 (red). LNCaP xenograft growth following two injections of either vehicle (black), 20 ug uncoupled PE35 (blue) or 20 ug PE35-MU2 (red) (C). PSA levels determined via ELISA of LNCaP-bearing nude mice after 3 weeks of treatment (D). H & E staining of a PE35-MU2- injected LNCaP tumor after 3 weeks treatment at 4x (left or 10x (right) (E).

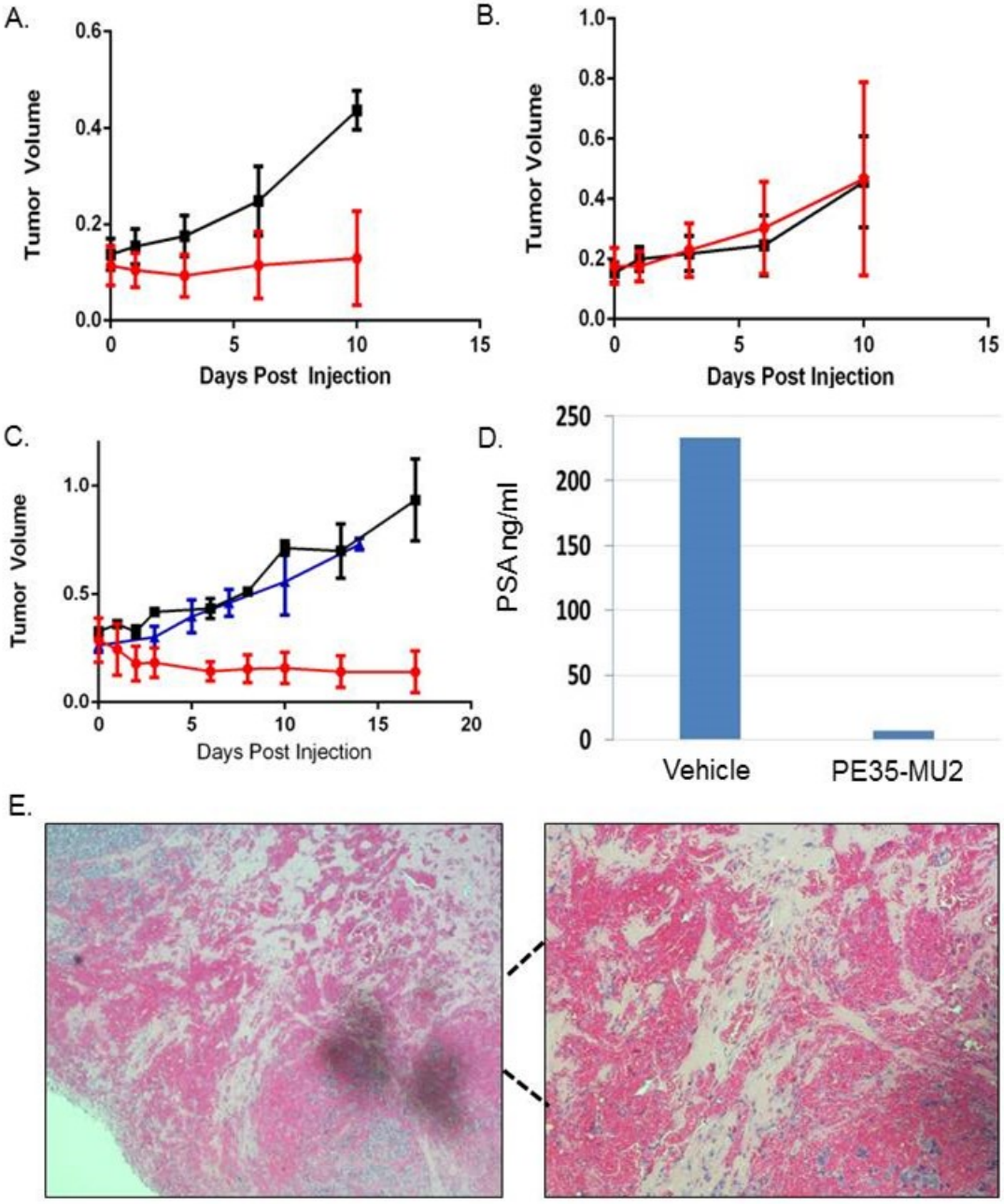


Figure 7. Intravenous injection of PE35-MU2 daily x 5 produces significant antitumor effect against PSMA-producing human LNCaP prostate cancer xenografts.

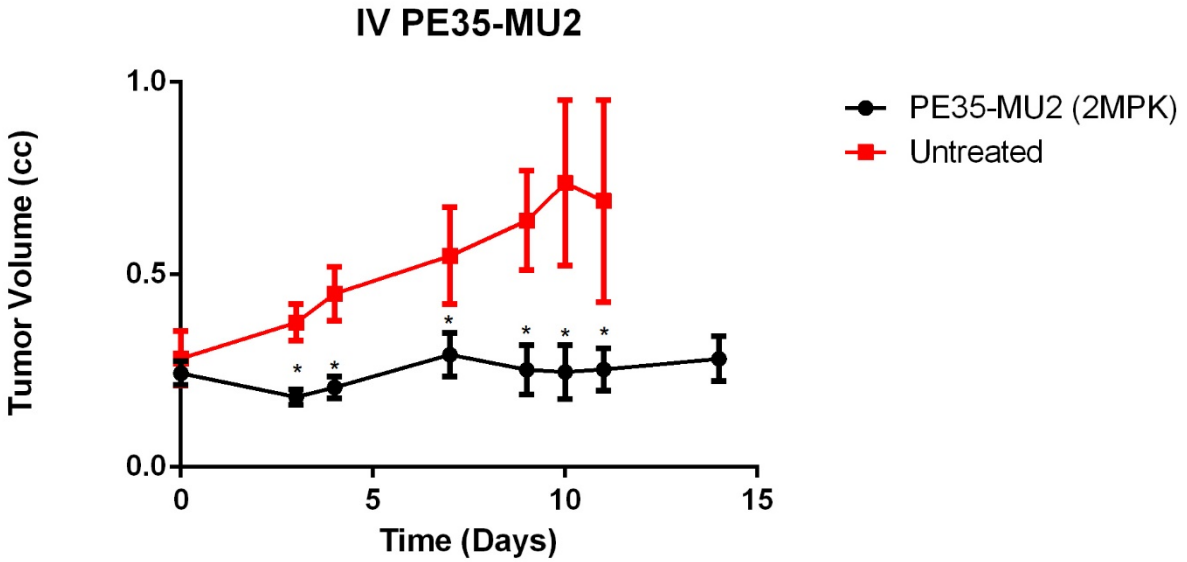
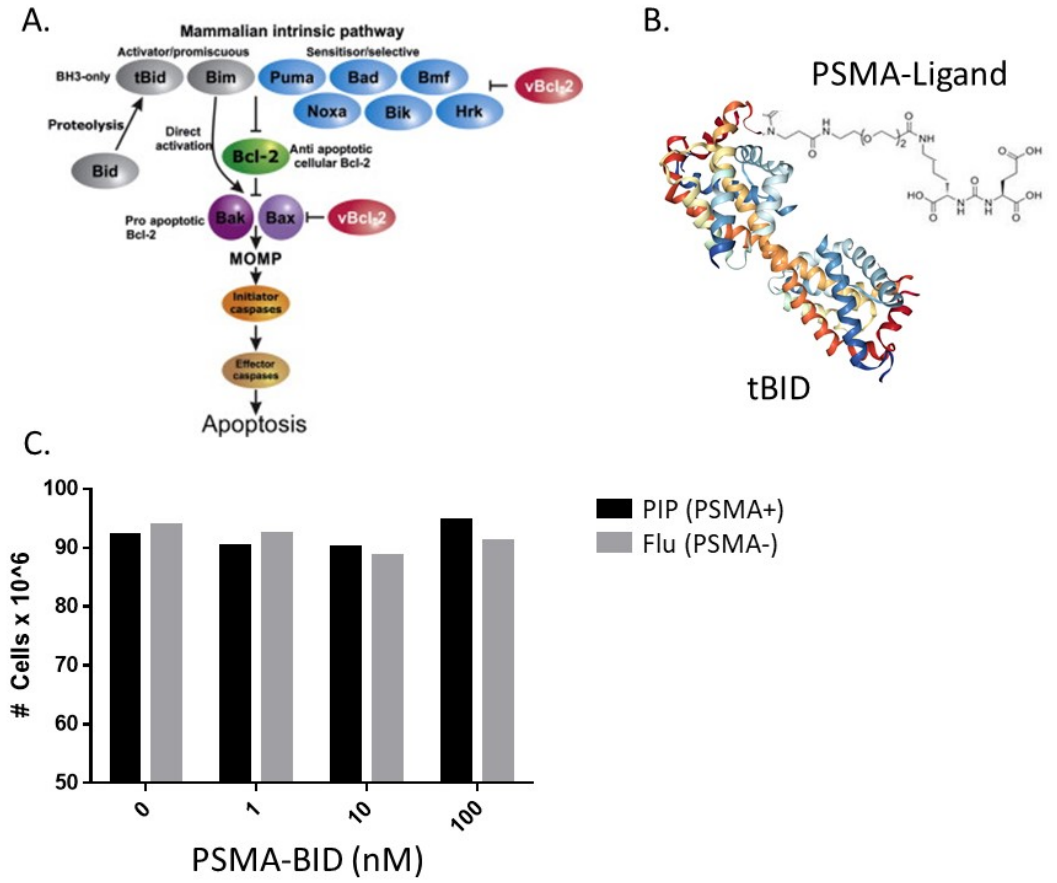


Figure 8. Targeting Human BID to PSMA positive prostate cancer cells. (A) BID and BAD as BH-3 family member proteins that induce apoptosis; (B) Schematic of PSMA-targeted tBID; (C) PSMA-tBID peptide activity against PSMA+ and PSMA- prostate cancer cells.



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.

Training activities: The grant supported the graduate training of Oliver Rogers who is a graduate student in the Pharmacology and Molecular Science Department within the Johns Hopkins University School of Medicine. The project described in this grant represents this student's thesis project. The grant also supported graduate student Jamey Therres, who had to leave in mid-year due to health reasons.

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

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Poster and podium presentations at the meetings describe above.

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.

Nothing to Report

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).

We demonstrated that small-molecule PSMA ligand can be readily coupled to large proteins without disruptiong protein function. The PSMA-ligand allows for specific and efficient intracellular deliver of these toxic protein cargoes to prostate cancer cells producing the PSMA-target.

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.

We describe a novel method to delivery protein cargoes intracellularly using a small molecular weight compound that does not alter protein structure and does not require cleavage or removal from the protein for activation. This approach has significant advantages compared to antibody based targeting approaches which requires specific engineered labile linkers that can disrupt antibody function and non-specific activation by proteases outside of the target tissue.

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.*

We are working with the Technology Transfer office at Johns Hopkins to submit a Patent application on these findings.

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.

None

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.

None

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

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).*

  Rogers OC, Anthony L, Rosen DM, Brennen WN, Denmeade SR. PSA-selective activation of cytotoxic human serine proteases within the tumor microenvironment as a therapeutic strategy to target prostate cancer. Oncotarget. 2018 Apr 27;9(32):22436-22450.

  Rogers O, Yen H, Solomon A, Drake C, Denmeade S. An IL-2 proaerolysin fusion toxin that selectively eliminates regulatory t cells to enhance antitumor immune response. Prostate. 2019 Jul;79(10):1071-1078.

  Rogers O, Rosen M, Denmeade S. A PSMA-inhibitor pseudomonas exotoxin protein selectively targets PSMA-expressing human prostate cancer cells. Manuscript submitted May 2020.

  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.*

See above for description of presentations

- **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:	*Samuel Denmeade*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Denmeade is the PI who has designed and oversighted all of the work described in the proposal*
Name:	*Oliver Rogers*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Rogers has performed all of the laboratory studies described in this proposal under the supervision of Dr. Denmeade.*

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.