Award Number: W81XWH-15-1-0090

TITLE: Testing ER-Beta Agonist Synergy with B7-H1 and mTOR Inhibitors as Novel and Effective Treatments for Ovarian Cancer

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CONTRACTING ORGANIZATION: University of Texas Health Science Center
San Antonio, TX 78229

REPORT DATE: Oct 2018

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Oct 2018	Final Technical Report	07/20/2015 - 07/19/2018

Testing ER-beta Agonist Synergy with B7-H1 and mTOR Inhibitors as Novel and Effective Treatments for Ovarian Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0090

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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San Antonio, TX 78229

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

Factors driving growth of epithelial ovarian cancer cells are poorly understood. Estrogen receptor-β (ERβ) is a tumor suppressor gene that reduces growth of ovarian cancer cells and to correlate with survival in ovarian cancer patients. We found that the immune co-signaling molecule B7-H1 regulates ERβ signals in cells. Using available agents to manipulate B7-H1, which is highly expressed in most ovarian cancers, we found that ERβ signals can be altered through mTOR signals to reduce tumor growth. We will test if manipulating ERβ signals through B7-H1 can treat ovarian cancer effectively in the well-established ID8 ovarian cancer model, test improvements through simultaneous mTOR inhibition, and assess effects in human ovarian cancer cells. **Innovation:** B7-H1 is an immune modulator, but we found that it modulates estrogen signals through ERβ, which is an unknown function for B7-H1 or any immune co-signaling molecule. We found that ERβ-mediated tumor growth inhibition can potentially be improved using anti-B7-H1 antibodies for direct effects on tumor cells and that mTOR inhibitors can augment this effect. This mTOR inhibitor effect is also novel as mTOR inhibitors are primarily used as direct modulators of tumor mTOR. **Impact:** Novel uses for drugs in clinical trials to treat ovarian cancer effectively would be a great benefit to patients.

15. SUBJECT TERMS
Ovarian cancer, B7-H1, PD-L1, ERβ, mTOR, treatment

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
Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

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

Factors driving growth of epithelial ovarian cancer cells are poorly understood. Estrogen receptor-β (ERβ) is a tumor suppressor gene that reduces growth of ovarian cancer cells and to correlate with survival in ovarian cancer patients. We found that the immune co-signaling molecule B7-H1 regulates ERβ signals in cells. Using available agents to manipulate B7-H1, which is highly expressed in most ovarian cancers, we found that ERβ signals can be altered through mTOR signals to reduce tumor growth. We will test if manipulating ERβ signals through B7-H1 can treat ovarian cancer effectively in the well-established ID8 ovarian cancer model, test improvements through simultaneous mTOR inhibition, and assess effects in human ovarian cancer cells. **Innovation:** B7-H1 is an immune modulator, but we found that it modulates estrogen signals through ERβ, which is an unknown function for B7-H1 or any immune co-signaling molecule. We found that ERβ-mediated tumor growth inhibition can potentially be improved using anti-B7-H1 antibodies for direct effects on tumor cells and that mTOR inhibitors can augment this effect. This mTOR inhibitor effect is also novel as mTOR inhibitors are primarily used as direct modulators of tumor mTOR. **Impact:** Novel uses for drugs in clinical trials to treat ovarian cancer effectively would be a great benefit to patients.

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

Ovarian cancer, experimental therapy, ERβ, B7-H1, PD-L1, pre-clinical

2. **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.

Aim 1 Test the hypothesis that ERβ agonists treat OC better when combined with an mTOR inhibitor.
1.1 Treat WT mice bearing ID8 tumors with ERβ agonist (LY) alone vs control. 100% completed by April 2017
1.2 *Treat WT mice bearing* ID8 tumors with mTORC1 inhibitor (rapamycin) ± LY + controls. 70% completed by August 2017
1.3 Treat WT mice bearing ID8 tumors with mTORC1/2 inhibitor ZD ± LY + controls. 50% completed by August 2017

Aim 2 Test the hypothesis that blocking tumor B7-H1 improves ERβ agonist treatment of OC
2.1. Test αB7-H1 + ERβ agonists in WT mice bearing ID8 tumors. 100% completed by April 2017
2.2. Test αB7-H1 + ERβ agonists + mTOR inhibitors in WT mice bearing ID8 tumors. 70% completed by August 2017

Aim 3 Identify ERβ/B7-H1 effects in human OC cells in WT mice bearing ID8 tumors. 100% completed by August 2017

What was accomplished under these goals?

1) major activities
A. We used transplantable, syngeneic mouse models of ovarian cancer (OC) to assess if ERβ and/or mTOR were useful therapeutic targets for OC treatment.

B. We used genetic engineering, immunoblots, flow cytometry, pharmacologic approaches, *in vitro* and *in vivo* studies to understand ERβ and mTOR signals in OC signals in relationship to B7-H1 signals.

2) specific objectives;
1 Test the hypothesis that ERβ agonists treat OC better when combined with an mTOR inhibitor.

2 Test the hypothesis that blocking tumor B7-H1 improves ERβ agonist treatment of OC

3 Identify ERβ/B7-H1 effects in human OC cells in WT mice bearing ID8 tumors

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

Please note that during the course of these studies, the name B7-H1 was changed to PD-L1 to conform with the nomenclature most used by the field. PD-L1 is equivalent to B7-H1 and is just an alternative name for the same molecule.

1 Test the hypothesis that ERβ agonists treat OC better when combined with an mTOR inhibitor.
We found that the ERβ agonist LY alone did not treat OC *in vivo* (**Fig. 1**).

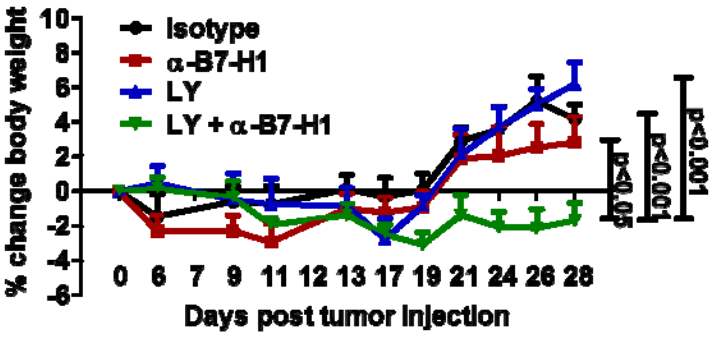


Fig. 1 The ERβ agonist LY improves tumor control in ID8agg tumor challenge. Groups of N=7 mice challenged with 4 x 10^6 ID8agg cells by intraperitoneal injection and treated with LY, αB7-H1, both or controls, as per the proposal protocol. Body weight is ascites, an accepted measure of tumor growth in this model.

Because LY was toxic in these *in vivo* studies, we also turned to *in vitro* approaches to complement *in vivo* work.

PD-L1^{lo} ID8agg cells grow slowly *in vivo*. We used shRNA to knock down B7-H1 expression in ID8agg cells to generate B7-H1^{lo} ID8agg (**Fig. 2A**). These cells grew slower than control ID8agg *in vitro* (**Fig. 2B**). We injected 4 x 10^6 cells into immune deficient NSG mice and demonstrated significantly slower growth versus control ID8agg cells (**Fig. 2B**). We used Western blots to validate the B7-H1knock down (**Fig. 2C**).

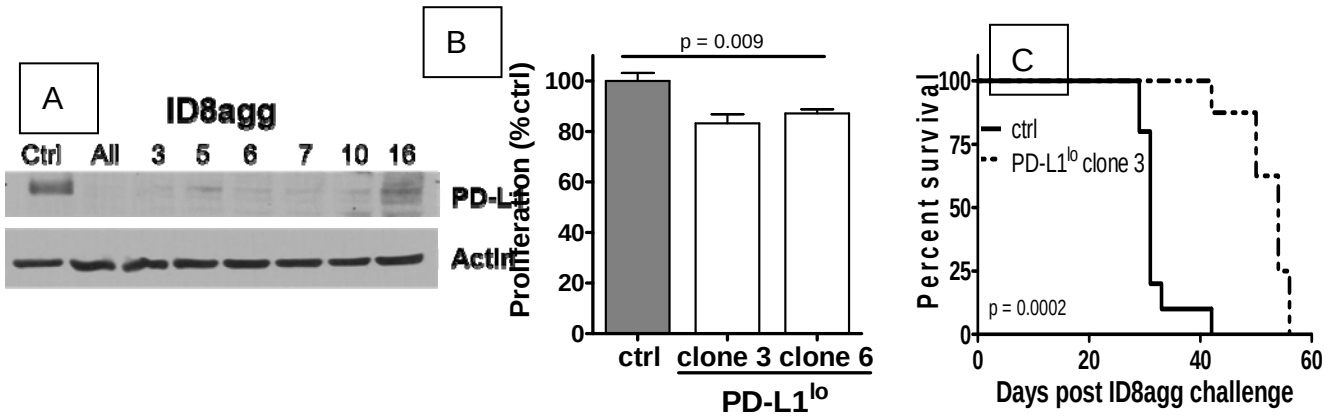
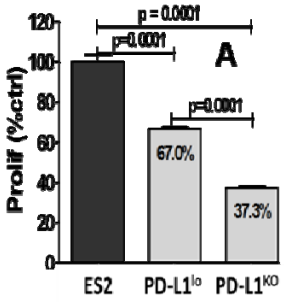


Fig. 2 B7-H1^{lo} ID8agg cells grow slowly *in vitro* and *in vivo*. A. Western blot validation of the B7-H1 knockdown. Lane numbers refer to distinct clones. **B** Cells cultured 48 hours *in vitro* and proliferation measured by MTT assay. **C.** 4 x 10^6 of the indicated cells injected IP into NSG mice (N=6/group) and survival assessed.



B7-H1 signals in ES2 human ovarian cancer cells drive proliferation. We also showed that human ES2 ovarian cancer cells are B7-H1^{+} and B7-H1^{lo} ES2 cells proliferate more slowly than control ES2 cells. We generated and validated B7-H1^{KO} ES2 (CRISPR/Cas9). Proliferation of B7-H1^{KO} cells *in vitro* was slower than B7-H1^{lo} ES2 cells, which were slower than control (**Fig. 3**), demonstrating cell-intrinsic B7-H1 control of human ovarian cancer cell proliferation and a B7-H1 dose response effect, consistent with our ID8agg data.

Fig. 3 PD-L1 increases proliferation in human ES2 cells. Human ES2 ovarian cancer cells incubated for 72 hours *in vitro*. Data normalized to control ES2 set at 100% proliferation.

PD-L1 in ovarian cancer cells regulate mTOR signals. To test mammalian target of rapamycin complex 1 (mTORC1) signaling effects of B7-H1 in human ovarian cancer cells, we performed Western blots of parentas versus PD-L1^{lo} cells (ID8 agg data shown in **Fig. 4**). We found that PD-L1 promoted mTORC1 based on relative abundance of pP70S6K^{473}.

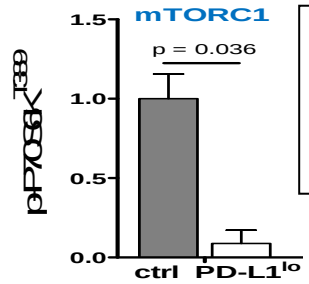


Fig. 4 ID8agg-intrinsic PD-L1 promotes mTORC1 signals. Representative immunoblot of the mTORC1 target p-P70S6K to total P70S6K (ctrl normalized to 1.0) with full details of all mTORC1 targets in our manuscript(2).

Mammalian target of rapamycin complex 1 (mTORC1) in OC pathology. Activation of mTORC1 promotes tumor growth and virulence(4), but mTORC1 inhibitors have had only limited clinical utility (5). We found that PD-L1 in ID8agg cells made them resistant to the mTORC1 inhibitor, rapamycin (**Fig. 5**).

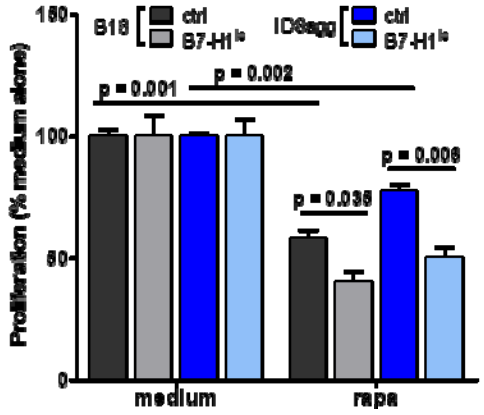


Fig. 5 PD-L1 promotes resistance to the mTORC1 inhibitor rapamycin (rapa). Cells cultured with rapamycin 5 nM, or control for 72H and proliferation determined bt MTT assay. B16 melanoma cells shown for comparison.

2 Test the hypothesis that blocking tumor B7-H1 improves ERβ agonist treatment of OC

ERβ agonist improves αPD-L1 immunotherapy for OC. The transplantable mouse ID8agg OC model is completely refractory to αPD-L1 immunotherapy, but adding the ERβ agonist LY produced significant clinical utility (**Fig. 1,** above).

Because we found that ERβ agonists were too toxic for further *in vivo* use in these studies, we focused on B7-H1 effects, in which we made very significant discoveries including showing that OC PD-L1 promotes tumor mTORC1 signals, the target of rapamycin (**Fig. 5**). We showed that tumor PD-L1 made OC cells resistant to rapamycin (**Fig. 4,** above).

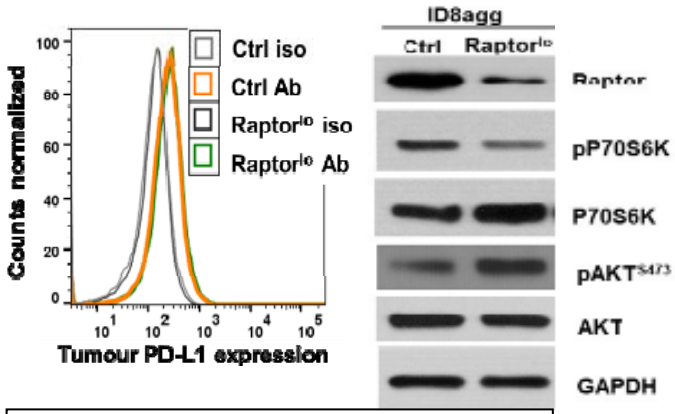


Fig. 6 Tumor mTORC1 does not affect PD-L1. Typical Raptor^{lo} ID8agg clone. Flow (left) and immunoblot (right).

Tumor-intrinsic mTORC1 is critical for tumor *in vitro* growth but not PD-L1 expression. Raptor is required for mTORC1 signals(4). We used shRNA to generate ID8agg populations with stable *Raptor* knock down (Raptor^{lo}) to reduce mTORC1-specific signals, and validated Raptor and mTORC1 reduction in Raptor^{lo} cells by Western blots, with no effect on PD-L1 expression (**Fig. 6**).

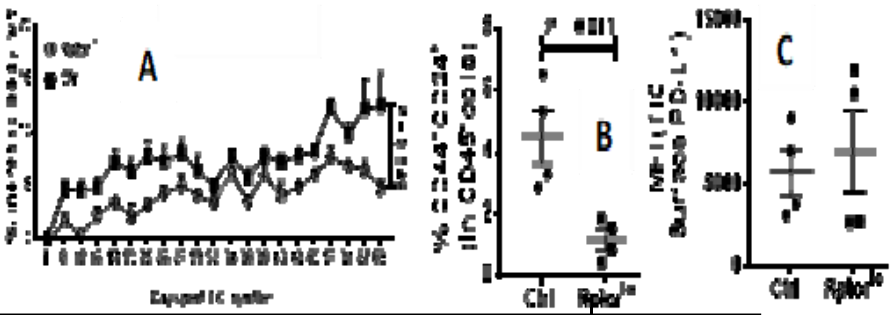
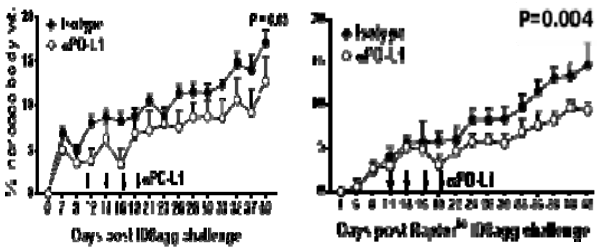


Fig. 7 Tumor mTORC1 regulates virulence. WT females challenged IP with 4 x 10^6 indicated ID8agg cells. **A.** weight gain (ascites) reflects tumor growth(3). P value, ANOVA. **B.** $CD44^+CD24^+$ tumor initiating cells in tumor (FACS; p value, unpaired *t*-test) and **C**. tumor mean fluorescence intensity (MFI, flow cytometry), day 69.

Tumor-intrinsic mTORC1 affects *in vivo* OC virulence but not tumor PD-L1. Raptor^{lo} versus control ID8agg cells were significantly less virulent

in orthotopically-challenged wild type mice, growing slower, producing smaller tumors and less ascites and fewer tumor initiating cells *in vivo* although Raptor^{lo} and control tumor and tumor PD-L1 expression were unaffected (**Fig. 7**).

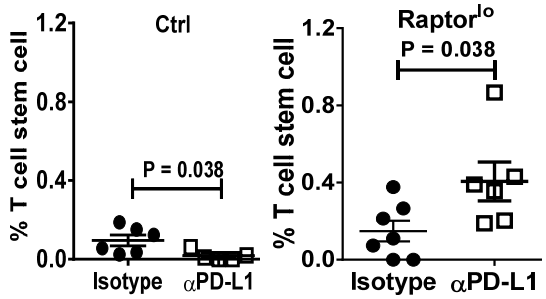


Tumor-intrinsic mTORC1 blunts αPD-L1 efficacy. αPD-L1 was ineffective against orthotopically-challenged control ID8agg in WT whereas Raptor^{lo} ID8agg cells responded significantly (**Fig. 8**), despite similar tumor PD-L1 expression in Raptor^{lo} and control cells after treatment, demonstrating αPD-L1 efficacy dependent on tumor mTORC1 signals.

Fig. 8 Tumor-intrinsic mTORC1 affects αPD-L1 efficacy. WT mice challenged with indicated ID8agg and treated with αPD-L1. N=5-7/group. Ascites and tumor weights were significantly lower in Raptor^{lo} (not

3 Identify ERβ/B7-H1 effects in human OC cells and in WT mice bearing ID8 tumors
We showed that ERβ agonist LY improved αPD-L1 efficacy in PD-L1-expressing tumors (**Fig. 1**).

4) other achievements.



Tumor-intrinsic mTORC1 alters αPD-L1-induced tumor-infiltrating lymphocytes (TIL). αPD-L1 increased tumor-infiltrating T cell stem cells (TSCS) significantly in Raptor^{lo} tumors versus significant reduction in control tumors (**Fig. 9**). Increased TCSC after αPD-1 or αPD-L1 predict treatment efficacy in other tumors (6,7). Our data establish tumor-intrinsic mTORC1 effects on α-PD-L1-induced TIL that could help explain treatment outcomes, and establish TCSC as mechanistic candidates for future investigations.

Fig. 9 Tumor-intrinsic mTORC1 affects αPD-L1 TIL generation. WT mice challenged with indicated ID8agg and treated with αPD-L1. N=5-7/group. Flow gated on CD45$^+$CD8$^+$TCF-1$^+$CXCR5$^+$PD-1$^+$Tim3$^-$ T cell stem cells.

Stated goals not met. Our efforts to assess treatment effects of pharmacologic ERβ agonists with mTOR inhibitors was not done due to toxicity of the ERβ agonist. Nonetheless, much useful treatment information and biology was uncovered as described above.

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.

The post-doctoral trainee Harshita Gupta, PhD learned how to use LY ERβ agonist, αPD-L1 *in vivo* in mice, and generated the engineered cell constructs for this work. She published some of these data in a first author manuscript in 2016 (1), as a co-author on a second paper in 2016 (2), presented these data at the American Association of immunologists Annual meeting in 2016 and is currently writing another manuscript on these data for peer-reviewed publication as first author.

How were the results disseminated to communities of interest?

We published some of these data in two manuscripts (1,2), presented some of these data at the American Association of immunologists Annual meeting in 2016 and are currently writing another manuscript for publication.

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:** 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).

We found that current doses and schedule of LY are toxic in this OC model, and that other drugs, or other doses and schedule of LY should be considered for further use.

αPD-L1 synergizes with an ERβ agonist to treat a refractory OC model, suggesting that this approach could be tested clinically. As αPD-L1 is FDA-approved and ERβ agonists are in clinical trials, these data are immediately translatable.

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 showed that tumor PD-L1 also affects treatment resistance in melanoma, breast cancer, bladder cancer.

We found that tumor PD-L1 also promotes mTORC1 melanoma, breast cancer, bladder cancer.

We found that ERβ agonists also treat melanoma.

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.*

Commercial ERβ agonists, mTOR inhibitors or αPD-L1 could be used in these novel applications described above.

The ERβ agonist data were seen by Ausio Biotech who is now considering using them to treat breast cancer and other cancers.

- *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.*

5.

We have developed novel treatment strategies to treat OC that we validated pre-clinically, which could be used to improve treatment for OC.

We defined novel treatment resistance mechanisms in OC that could be used to predict treatment responders and/or develop novel means to overcome such resistance, which would improve treatments for OC.

We showed that some of these findings could be applicable to other cancers for improving treatment response prediction or treatments, including in breast and bladder cancer and in melanoma.

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.

Nothing to report.

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.

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).*

1. Gupta, H. B., Clark, C. A., Yuan, B., Sareddy, G., Pandeswara, S., Padron, A. S., Hurez, V., Conejo-Garcia, J., Vadlamudi, R., Li, R., and Curiel, T. J. (2016) Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation and functions in melanoma and ovarian cancer. *Signal Transduct Target Ther* **1**
2. Clark, C. A., Gupta, H. B., Sareddy, G., Pandeswara, S., Lao, S., Yuan, B., Drerup, J. M., Padron, A., Conejo-Garcia, J., Murthy, K., Liu, Y., Turk, M. J., Thedieck, K., Hurez, V., Li, R., Vadlamudi, R., and Curiel, T. J. (2016) Tumor-Intrinsic PD-L1 Signals Regulate Cell Growth, Pathogenesis, and Autophagy in Ovarian Cancer and Melanoma. *Cancer Res* **76**, 6964-6974

Federal support acknowledged for both manuscripts.

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.*

2016 American Association of Immunologists

1. ***Tumor-intrinsic B7-H1 in melanoma and ovarian cancer regulates mTOR, autophagy and tumor growth.** Curtis A. Clark, Harshita Gupta, Srilakshmi Pandeswara, Gangadhara R. Sareddy, Bin Yuan, Vincent Hurez, Rong Li, Ratna Vadlamudi, Tyler J. Curiel

2. **Estrogen receptor β stimulation improves immune signal blockade therapeutic efficacy in melanoma and ovarian cancer**
   Harshita B. Gupta, Gangadhara Sareddy, Curtis A. Clark, Vincent Hurez, Ratna Vadlamudi, Rong Li, Tyler J. Curiel

- **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:	*Tyler Curiel, MD, MPH*
Project Role:	*PI*
Nearest person month worked:	*1*
Contribution to Project:	*Gave overall project guidance, developed and analyzed data. Wrote progress report*
Funding Support:	*NIH, CDMRP*
Name:	*Harshita Gupta, PhD*
Project Role:	*post-doc*
Nearest person month worked:	*1*
Contribution to Project:	*Performed all hands-on work. Developed and analyzed data.*
Funding Support:	*NIH, CDMRP*

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.*

No other organizations.

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.*

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