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TITLE: **Targeting the stem-like cells of chemoresistant high grade serous ovarian cancer: BMI1 in the spotlight**

PRINCIPAL INVESTIGATOR: **Dr. Resham Bhattacharya**

CONTRACTING ORGANIZATION:
Board of Regents of the University of Oklahoma Health Sciences Center

Oklahoma City, OK 73104-3609

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Board of Regents of the University of Oklahoma Health Sciences Center
865 Research Parkway, URP 865-450
Oklahoma City, OK 73104-3609

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Despite frequent initial responses to platinum/taxane therapy, most patients with high-grade serous ovarian cancer eventually develop resistance that leads to low responsiveness to any drug and shortened survival. We and others, have demonstrated that the polycomb protein BMI1 mediates a molecular stem-like phenotype and reprograms cellular metabolism leading to chemoresistance. Our goal is to elucidate the link between mitochondrial and nuclear functions of BMI1 that lead to therapy resistance and also evaluate targeting of this axis using the clinically relevant inhibitors. We have identified post-translational modifications of BMI1 that may affect its stability and function as a transcriptional regulator. These modifications may be instrumental in mediating oncogenic activity of BMI1, which are being investigated.

15. SUBJECT TERMS

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

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

Despite frequent initial responses to platinum/taxane therapy, most patients with high-grade serous ovarian cancer (HGSOC) eventually develop resistance that leads to low responsiveness to any drug and shortened survival. To design effective strategies that improve prognosis, there is a critical need to identify and target the mechanisms that lead to chemoresistance. In this context, investigating a role of the polycomb protein BMI1 that mediates a molecular stem-like phenotype and reprograms cellular metabolism leading to chemoresistance in HGSOC is highly significant. The principal purpose of this study is to evaluate how transcriptional and metabolic reprogramming by BMI1 is instrumental in mediating a molecular stem-like phenotype that causes chemoresistance. The mechanisms that govern metabolic or phenotypic conversion of cancer cells to stem-like cells post cytotoxic therapy is poorly defined precluding targeting of the stem-like phenotype. The main scope of this study is to elucidate the coordinate link between mitochondrial and nuclear functions of BMI1 leading to therapy resistance and also to evaluate targeting of this axis using the clinically relevant BMI1 inhibitors, which should facilitate clinical translation potentially impacting patient survival in the near-term.

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

BMI1, ovarian cancer, chemoresistance, stem-like phenotype

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.

Sp. Aim1: Determine how BMI1 mediates a molecular stem-like phenotype leading to chemoresistance:
Major Task 1: Characterize the role of BMI1 in ovarian cancer
Subtask 1: Evaluation of mitochondrial BMI1 in HGSOC cells. ~50% completed.
Subtask 2: Determining the role of nuclear BMI1 in HGSOC. ~50% completed.

Sp. Aim2: Evaluation of anti-BMI1 therapy in PDX model.
Major Task 1: Evaluation of BMI1 inhibitors in the chemoresistant PDX model: ~50%completed.
Major Task 2: Evaluation of BMI1 inhibitors in the relapse PDX model: To be initiated in the third year of the award.

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.

Accomplishments under Specific Aim1:

Major activities: Characterize the role of BMI1 in ovarian cancer

Specific objectives: Evaluation if BMI1 is imported in mitochondria of HGSOC cells.

Significant results and conclusions:

We have shown earlier that mitochondria isolated from BMI1 silenced cells have significantly reduced Complex I, II, IV activity, reduced ATP levels, enhanced mtROS and reduced mitochondria (mt) encoded mature transcript levels. Now, to determine the possible import of BMI1 into the isolated mitochondria, we performed a mt-import assay. Biotinylated lysine labelled BMI1 (preprotein) was generated from pcDNA3.1 FLAG-WT-BMI1 using the TnT®T7 Transcend™ Non-Radioactive Translation Detection Systems (Promega). Mitochondria isolated from CP20 cells were incubated with labeled BMI1 in presence or absence of trypsin and subjected to SDS-PAGE. After SDS-PAGE and immunoblotting, the biotinylated BMI1 was visualized by binding with Streptavidin-Horseradish Peroxidase (Streptavidin-HRP), followed by chemiluminescent detection. 10% of the labeled preprotein was used per import reaction (Fig. 1A). We expected to observe import of BMI1 into the mitochondria and emergence of lower molecular weight mature BMI1 protein (below 37KDa). Although our initial results (Fig. 1A) suggested the presence of lower mol. wt. mature BMI1 protein bands, however, similar sized protein bands in the mitochondrial fractions (without any labeled preprotein, Fig 1B, Mito only) lead us to conclude that in this assay we cannot reliably detect mitochondrial import of BMI1 or may require other cellular factors not present in this system.

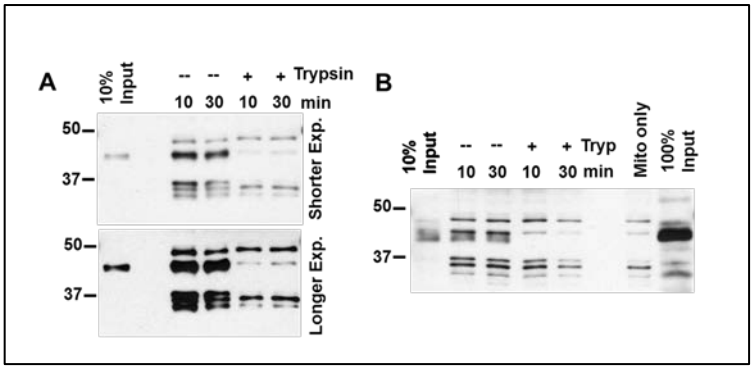

Figure 1. The biotinylated lysine labelled BMI1 preprotein was incubated with isolated CP20 mitochondria for 10 minutes for import. Next, samples were treated with 1.25 µg/ml trypsin for 10 or 30 min on ice. The trypsin digestion was terminated by adding soybean trypsin inhibitor to a final concentration of 250 µg/ml. Mitochondria were re-isolated and alkaline extraction was performed to obtain imported precursor protein. Mitochondrial proteins were separated by SDS-PAGE and detected by chemiluminescence. Indicated amount of the labeled preprotein used per import reaction.

Specific objectives: Evaluation of regulation of transcription by BMI1 in high-grade serous ovarian cancer

Significant results and conclusions:

Conventionally, BMI1 is known as a transcriptional repressor. However, we previously showed that BMI1 positively regulates the expression of MDR1 upon cisplatin treatment. We showed that cisplatin treatment upregulated BMI1 and MDR1 while concurrently downregulating HOXA9 at the mRNA level. We also showed that cisplatin treatment increased occupancy of BMI1 at the MDR1 promoter. Now to evaluate the possible occupancy of BMI1 at the HOXA9 promoter, we performed ChIP assays using PCR primers encompassing the ten different clusters of E boxes on the HOXA9 promoter (Figure 2).

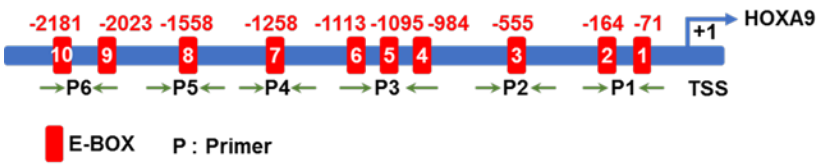

Figure 2: A representative schema of the E-box clusters (E1–E10) within the HOXA9 promoter. The transcription start site (TSS) is indicated by +1 and representative ChIP primer binding position is shown (P1-P10).

In non-treated cells, BMI1 bound to all of these E-box clusters within HOXA9 promoter but it was higher in E7-E10 clusters (as shown in Table1), encompassing primers P4-P6 and highest in E8. On the other hand, cisplatin treatment decreased this association significantly in each of the E-box clusters (P5) (Table:1). Antibody directed against IgG was used as a negative control. These results suggest a negative regulation of the HOXA9 by BMI1 in absence of cisplatin. However, in presence of cisplatin reduced occupancy by BMI1 suggests modulation likely by other polycomb members which is currently being pursued. We are currently performing ChIP for expanding evaluation of the active (trimethyl histone3 lysine4) and repressive marks (trimethyl histone3 lysine27) at both MDR1 and HOXA9 promoters.

ChIP primer No.	Sequence (Forward) (5' - 3')	Sequence (Reverse) (5' - 3')	Fold Enrichment (IgG=1)	
			NT	Cisplatin
P1	GGCACGATCCCTTTACATAAAAACA	ATTTCATGTAACAACTTGGTGGCA	2.91	0.71
P2	GCGGGTAAACTCGCCTCTC	AGTCGGAAACGACCAACAGA	2.28	0.70
P3	ACCGAACTTGCCTTCCATTCAT	GGTTGGTTGTGCGGCG	4.0	1.42
P4	CCCCGTAGGTAACCAAGGC	TGAATGGAAGGCAAGTTCGGT	5.21	1.31
P5	TTCTCCGCTTTCTAGGCACCA	TTAAGTGTCTGCAAATGGGCTG	5.94	2.03
P6	CTCGCCGCCAGGGAAG	GCCGCCACTCCGTTAATTG	5.03	1.74

Table 1: ChIP of the HOXA9 promoter. CP20 cells were treated with or without cisplatin (10uM) for 48h. After treatment, cells were subjected to ChIP analysis using EZ-Magna ChIP kit (Millipore), according to the manufacturer instructions. Cell lysates were subjected to ChIP analysis using anti-BMI or isotype IgG antibodies. Precipitated DNA was subjected to quantitative PCR amplification with various HOXA9 promoter primers. The relative amount of promoter DNA was normalized using fold values of the input samples (i.e. 1% of starting chromatin).

Major activities: Determining the role of nuclear and extra nuclear BMI1 in HGSOC.

Specific objectives: Evaluation of the role of extra-nuclear BMI1 to maintain a stem-like phenotype f HGSOC cells.

Significant results and conclusions:

To determine the role of nuclear and extra nuclear BMI1 in HGSOC, we constructed dual expression lentiviral plasmids that express both BMI1 shRNA and various mutant forms of BMI1. The shRNA sequences (Fig. 3A) were cloned in pZIP-TRE3GS plasmid, after successful transduction and selection with puromycin, cells were induced with Dox and GFP levels were evaluated using IF (not shown). Efficacy of shRNA for BMI1 targeting was evaluated using RTqPCR (Fig. 3B) and immunoblotting (Fig. 3C).

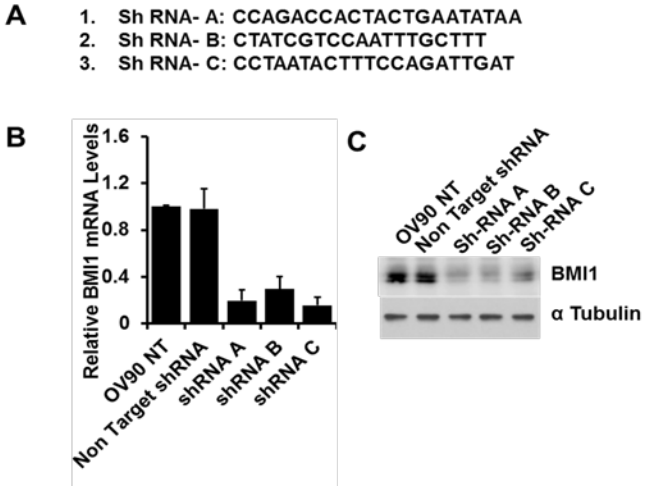

Figure 3. **(A)** Revised BMI1 shRNA sequences were cloned in pZIP-TRE3GS plasmid. **(B)** Lentivirus expressing these shRNA were made using HEK393 cells and used to transduce OV90 cells. After successful selection with puromycin, the efficacy of these shRNA was evaluated using RT-qPCR **(B)** and immunoblotting **(C)**. mRNA levels were normalized with B2M.

Two shRNA showing efficient BMI1 inhibition were selected, first shRNA (shRNA A) targets 3' UTR region of the BMI1 and the second shRNA (shRNA C) targets coding region of BMI1. To simultaneously knockdown endogenous BMI1 and re-express BMI1 in OV90 cells, the BMI1 coding sequence corresponding to shRNA C was changed using silent mutation and 3 copy flag sequence was added at the C-terminal of BMI1 to distinguish ectopic BMI1 from the endogenous BMI1. Clones were sequence verified, and to evaluate the flag expression, transiently overexpressed in HEK 293T cells. As shown in Fig. 4A, Dox treatment induced Flag overexpression. pZIP TRE3G plasmid along with shRNA A and different flag tagged BMI1 constructs were used to make stable OV90 cells. Post puromycin selection OV90 cells expressing shRNA A and either flag tagged wild type BMI1, NLS mutated BMI1 or HTH domain deleted BMI1 were Dox induced and lysates evaluated for the efficacy of shRNA and BMI1 re-expression using immunoblotting. While shRNA reduced endogenous BMI1 expression, re-expression, especially with respect to Flag could not be detected in the stable cells (Fig 4B) while it was evident in transient transfection. Therefore, we conclude that stable transduction using shRNA A and C can efficiently target endogenous BMI1 but dual stable expression poses some challenges. Therefore, for efficient re-expression of BMI1 constructs, we will utilize a separate pInducer20 system (Addgene #44012). Currently we are cloning WT-BMI1-Flag, Nuclear Localization Signal mutated BMI1 (NLS BMI1) flag, or helix-turn-helix (HTH) domain deleted BMI1flag in pInducer20. OV90 cells will be stably transduced with respective lentivirus containing either WT-BMI1, NLS BMI1, or dHTH BMI1. The efficacy of shRNA to target endogenous BMI1

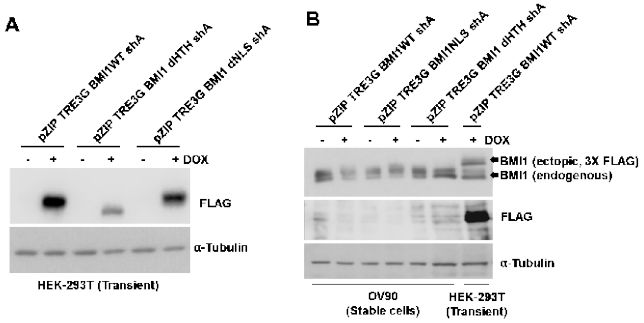

Figure 4. To achieve knockdown of endogenous BMI1 and overexpress BMI1 simultaneously, the GFP sequence of the pZIP-TRE3GS plasmid expressing shRNA-A was replaced with either wild type BMI1 sequence (WT), nuclear localization signal mutated (NLS) BMI1 or helix turn helix (HTH) domain deleted BMI1. shRNA targeting sequence from these constructs were modified and the ectopic BMI1 is fused with 3 copy of flag sequence. **(A)** HEK393T cells were transfected with the respective plasmids, post 24h transfection cells were treated with Dox and Flag expression was determined. **(B)** stable OV90 cells were generated using lentiviral transduction, post puromycin selection cells were induced with Dox and the efficacy of shRNA A to target endogenous BMI1 and the efficiency of ectopic BMI1 expression was evaluated. HEK293T cells transiently transfected with pZIP TRE3G BMI1WT shA was used as positive control.

and the efficiency of re-expression of respective BMI1 constructs will be evaluated using immunoblotting, once established these cells will be used to decipher the pathophysiological significance of nuclear and extranuclear BMI1.

Accomplishments under Specific Aim2:

Major activities: Evaluation of anti-BMI1 therapy in PDX model.

Specific objectives: To determine the therapeutic potential of BMI1 inhibitors in chemoresistant PDX model.

Significant results and conclusions:

We developed PDX-0113 as a chemo resistant HGSOC in-vivo model. PDX-0113 was successfully grown in NSG mice and showed resistance to the combination treatment of Carboplatin and Paclitaxel which is used as a standard first-line chemotherapy in patients with advanced ovarian cancer (Fig. 5). Due to the severe toxicities of the solvent (cremophor) used to solubilize Paclitaxel, FDA has recently approved a solvent-free formulation of paclitaxel (Abraxane®; nab-paclitaxel) which not only circumvents the requirement for solvents but also has an increased therapeutic index. Therefore, we used nab-paclitaxel (10mg/kg) along with carboplatin (50mg/kg) as standard chemotherapy regimen, for 2 cycles in PDX-0113. To determine the efficacy of BMI1 inhibitors (PTC-028 and its clinical analog PTC-596) along with standard chemotherapeutics, we performed a pilot experiment to determine feasibility of administration. Briefly, viably frozen P2-PDX-0113 was expanded sub-cutaneously to NSG mice and further randomized into 4 groups (5-9 mice per group) when tumors reach ~200 mm3. The groups were treated as: (a) Vehicle; (b) Carboplatin + nab-paclitaxel [C+T]; (c) PTC-028 (15mg/kg) + (C+T) [C+T+028]; (d) PTC-596 (10mg/kg) + (C+T) [C+T+596].

Both PTC-028 and PTC-596 were administered by oral gavage (2X/wk on D1 and D3) followed by carboplatin by I.P. (weekly on D5) and nab-paclitaxel by I.V. (weekly on D7) for 2 weeks. These mice were monitored for tumor volume [V=(LXW2)/2], tumor regression until 6 weeks after 2 weeks of therapy and their body weight was measured. Compared to standard chemotherapy (C+T) alone, combining with either of the BMI1 inhibitors (C+T+028) or (C+T+596) showed significant and similar tumor regression (Fig. 5A). However, body weight measurement showed a significant drop in body weight of mice in the (C+T+028) combination group (Fig. 5B). Based on results from this pilot study, we have selected PTC-596 as the BMI inhibitor to be used in our future PDX studies.

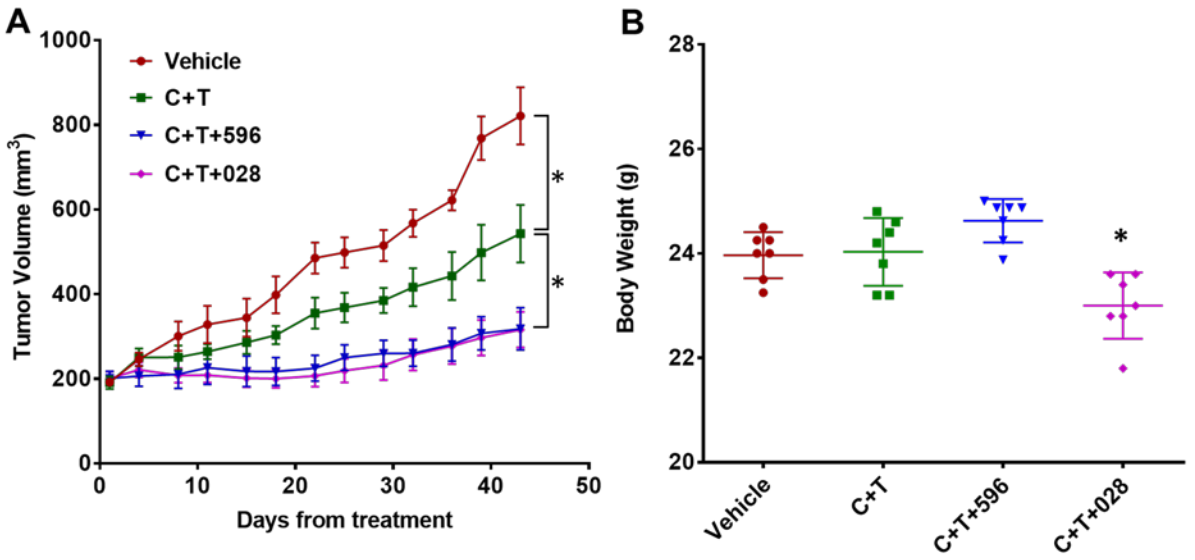

Figure 5. PDX-0113 was expanded sub-cutaneously to NSG mice and further randomized into 4 different groups (5-9 mice per group) when tumors reach ~200 mm^3. The groups were treated as: (a) Vehicle; (b) Carboplatin (50mg/kg) + nab-paclitaxel (10mg/kg) [C+T]; (c) PTC-028 (15mg/kg) + carboplatin (50mg/kg) + nab-paclitaxel(10mg/kg) [C+T+028]; (d) PTC-596 (10mg/kg) + carboplatin (50mg/kg) + nab-paclitaxel(10mg/kg) [C+T+596]. PTC-028 and PTC-596 was administered by oral gavage (2X/wk on D1 and D3) followed by carboplatin by I.P. (50mg/kg, weekly on D5) and nab-paclitaxel by I.V. (weekly on D7) for 2 weeks and tumor volume **(A)** and body weight **(B)** was monitored. Data represent mean ± SEM. *P<0.05.

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.

Nothing to report

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

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.

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.

We are currently pursuing ChIP assay to study the mechanisms of negative regulation of the HOXA9 by BMI1 and any potential involvement of other polycomb members e.g. EZH2, SUZ12, CBX2 etc. We are also performing ChIP for expanding evaluation of the active (trimethyl histone3 lysine4) and repressive marks (trimethyl histone3 lysine27) at both HOXA9 and MDR1 promoters. Currently we are cloning WT-BMI1-Flag, Nuclear Localization Signal mutated BMI1 (NLS BMI1) flag, or helix-turn-helix (HTH) domain deleted BMI1flag in HGSOC cells and will be stably transduced with respective lentivirus containing either WT-BMI1, NLS BMI1, or dHTH BMI1. The efficacy of shRNA to target endogenous BMI1 and the efficiency of over-expression of respective BMI1 constructs will be evaluated. These experiments will address the pathophysiological significance of nuclear and extranuclear BMI1. We have already standardized and evaluated PDX-0113 as the chemoresistant PDX model and also determined PTC-596 as our choice of BMI1 inhibitor. We have started our final experiments to determine the efficacy of PTC-596 alone and also in combination with standard chemotherapy regimen in this chemoresistant PDX model. Finally, experiments pertaining to anti-BMI1 therapy in relapse PDX model will be initiated.

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

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.

Due to the severe toxicities of the solvent (cremophor) used to solubilize Paclitaxel, FDA has recently approved a solvent-free formulation of paclitaxel (Abraxane®; nab-paclitaxel) which not only circumvents the requirement for solvents but also has an increased therapeutic index. As such, we used nab-paclitaxel (10mg/kg) along with carboplatin (50mg/kg) as standard chemotherapy regimen, for 2 cycles in PDX-0113. These changes have been approved by ACURO.

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.

Some Covid related delays occurred due to University guidelines regarding non-essential research related work that was suspended for ~4-6 weeks.

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).*

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

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".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Resham Bhattacharya
No Change

Name: Shailendra Dwivedi
Project Role: Research personnel
Nearest person month worked: 12
Contribution to the Project: Wet bench research

Name: Udayan Bhattacharya
Project Role: Research personnel
Nearest person month worked: 5
Contribution to the Project: Wet bench research

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*

Oklahoma Medical Research Foundation (OMRF), Dr. Magdalena Bieniasz. Reported results from the animal experiments for Specific Aim2 were performed at OMRF.

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.*