**AWARD NUMBER: W81XWH-18-1-0054**
**OC170378**

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

**PRINCIPAL INVESTIGATOR: Dr. Resham Bhattacharya**

**CONTRACTING ORGANIZATION:**
**UNIVERSITY OF OKLAHOMA,**
**BOARD OF REGENTS OF THE UNIVERSITY OF OK**
**865 RESEARCH PKWY STE 530**
**OKLAHOMA CITY OK 73104-3609**

**REPORT DATE: SEPTEMBER 2019**

**TYPE OF REPORT: Annual**

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

# REPORT DOCUMENTATION PAGE

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|---|---|---|
| SEPTEMBER 2019 | Annual | 15AUG2018 - 14AUG2019 |

**4. TITLE AND SUBTITLE**

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

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**5b. GRANT NUMBER**

OC170378

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

Dr. Resham Bhattacharya

**5d. PROJECT NUMBER**

0011144233-0001

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

UNIVERSITY OF OKLAHOMA,
BOARD OF REGENTS OF THE UNIVERSITY OF OK
865 RESEARCH PKWY STE 530
OKLAHOMA CITY OK 73104-3609

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**

USA Med Research ACQ Activity
820 Chandler St
Fort Detrick MD 217502-5014

**10. SPONSOR/MONITOR'S ACRONYM(S)**

USAMRAA

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

**12. DISTRIBUTION/AVAILABILITY STATEMENT:** **Approved for Public Release; Distribution Unlimited**

**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**

NONE LISTED

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
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## TABLE OF CONTENTS

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: Differential regulation of transcription by BMI1 in high-grade serous ovarian cancer. ~50% completed.
Subtask 2: Identification of lysine residues acetylated by TIP60 on BMI1 in various HGSOC. ~50% completed.
Subtask 3: Determining the role of nuclear BMI1 in HGSOC. ~30% completed.
Subtask 4 & 5 to be initiated in the second and third year of the award respectively.

Major Task 2: Evaluation of PTC-028 in the PDX model: To be initiated in the second 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:** Determining how BMI1 mediates a stem-like phenotype using HGSOC cells.

**Specific objectives:** Evaluation of differential regulation of transcription by BMI1 in high-grade serous ovarian cancer and identification of post-translational modifications of BMI1 in HGSOC cells.

**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. To evaluate this differential regulation we utilized RT-qPCR and promoter luciferase assays. We report that cisplatin treatment upregulated BMI1 and MDR1 while concurrently downregulating HOXA9, CDKN2A and PTEN at the mRNA level (Fig. 1).

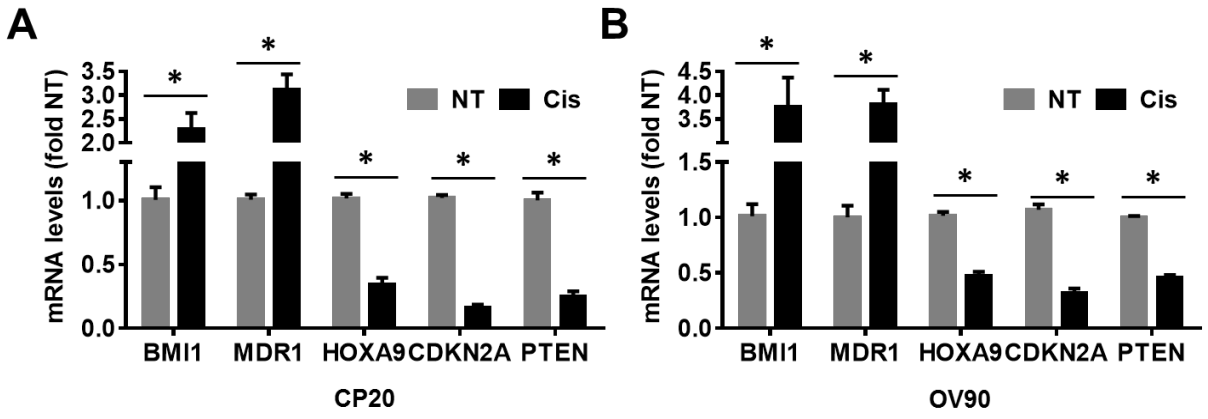
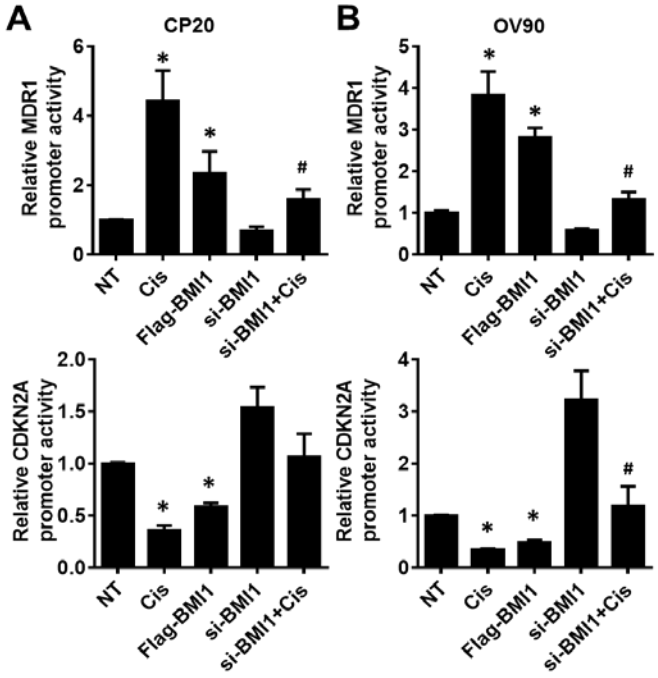



**Figure 1.** CP20 and OV90 cells were treated with or without Cisplatin (Cis: 10uM for CP20 and 6uM for OV90) for 48h. mRNA expression levels of BMI1, MDR1, HOXA9, CDKN2A and PTEN were determined by qPCR analysis. 18S rRNA was used as an internal control. Vehicle treated (NT) cells mRNA levels were set to 1 and data represent mean ± SD of three independent experiments performed in triplicate and *P<0.05 when compared with respective NT control.

We then determined that overexpression of BMI1 in untreated cells or cisplatin treatment which upregulates BMI1 increased MDR1 promoter luciferase activity. Simultaneously knockdown of BMI1 in cisplatin treated cells decreased MDR1 promoter luciferase activity confirming that MDR1 promoter activity is regulated by BMI1 in a positive manner. Conversely, the CDKN2A promoter was repressed by BMI1 in untreated or in cisplatin treated cells (Fig.2 and Fig.3).



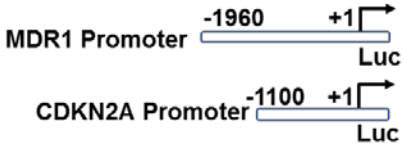




**Figure 2.** Schematic representation of MDR1 and CDKN2A promoter sequences. The MDR1 promoter sequence was cloned in hte PGL2basic vector. The CDKN2A promoter was a kind gift from Dr. Sabyasachi Sanyal (CSIR-CDRI, Lucknow, India)

**Figure 3. (A)** CP20 and **(B)** OV90 Cells were transfected with either scrambled si-RNA or empty vector plasmid (NT) or pCDNA3 Flag-BMI1 or si-BMI1 or treated with cisplatin (10uM for CP20 and 6uM for OV90). After 48h treatment surviving cells were counted and seeded in 96 well plate and further transfected with either PGL3-MDR1 promoter + pRLTK renilla or PGL3-CDKN2A promoter + pRLTK renilla construct. Firefly luciferase activity was measured 24h after transfection, normalized with renilla and compared with vehicle treated cells (NT). Data represent mean ± SD of three independent experiments performed in triplicate. P<0.05, * when compared with NT and # when compared with si-BMI1.

We previously reported that BMI1 and TIP60 co-precipitated in cisplatin treated cells. To determine if BMI1 regulated promoters were co-occupied by TIP60 we performed chromatin immunoprecipitation (ChIP) followed by qPCR. Cisplatin treatment increased occupancy of both BMI1 and TIP60 at the MDR1 promoter. Antibody directed against IgG was used as a negative control (Fig. 4). We are currently performing ChIP for the CDKN2A promoter and also expanding evaluation of the active and repressive marks at these promoters.

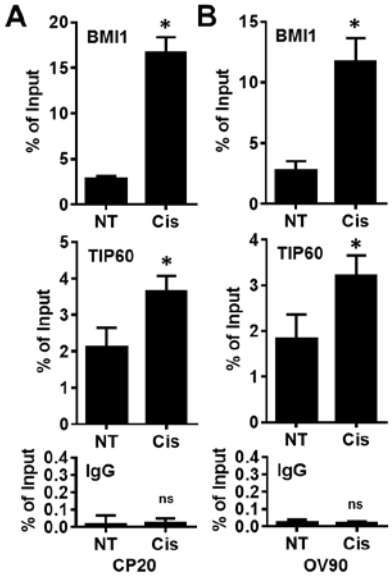



**Figure 4.** ChIP of the MDR1 promoter. **(A)** CP20 and **(B)** OV90 cells were treated with or without cisplatin (10uM for CP20 and 6uM for OV90) for 48h. After treatment, cells were subjected to ChIP analysis using Magna ChIP HiSens kit (Millipore), according to the manufacturer instructions. Cell lysates were subjected to ChIP analysis using anti-BMI (upper panel), or anti-TIP60 (middle panel) or isotype IgG (lower panel) antibodies. Precipitated DNA was subjected to quantitative PCR amplification with MDR1 promoter primers. The relative amount of promoter DNA was normalized using percentages of the input samples. With this method, signals obtained from the ChIP are divided by signals obtained from an input sample (i.e. 1% of starting chromatin). Data represent mean ± SD of three independent experiments performed in triplicate. P<0.05, # when compared with respective vehicle treated cells (NT).

We hypothesized that post-translational modification BMI1 could potentially impact its differential regulation of transcription. Using immunoprecipitation, we determined if endogenous BMI1 could be acetylated by TIP60. Acetylation of BMI1 in TIP60 overexpressing OV90 cells was moderate and could not be consistently observed in CP20 cells. Because TIP60 activity is increased in cisplatin treated cells, we are currently determining the acetylation status of endogenous BMI1 after cisplatin treatment.

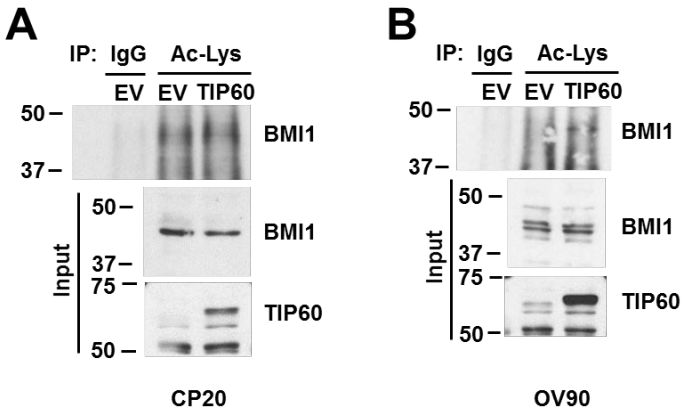



**Figure 5.** Detection of post-translational modification of endogenous BMI1 by TIP60 in HGSOC cells. Both CP20 **(A)** and OV90 **(B)** cells were transfected with either empty vector (EV) or FLAG-TIP60 (TIP60) plasmids and immunoprecipitated (IP) with anti-acetylated-Lysine (Ac-Lys) or isotype IgG antibody. Expression of BMI1 and TIP60 was determined by immunoblotting. All results represent three independent experiments.

Interestingly, we consistently observed O-GlcNAc, post-translational modification of BMI1 after cisplatin treatment. Such modifications may alter interaction of BMI1 with the chromatin and with partner proteins ultimately affecting transcriptional regulation of MDR1 and CDKN2A which are also being pursued.

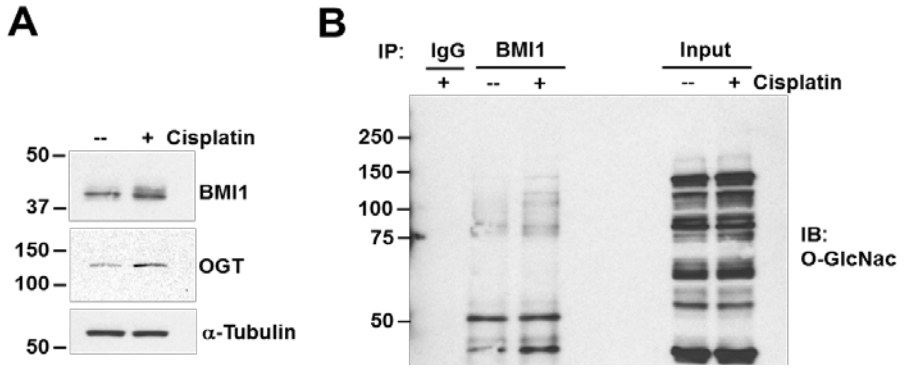



**Figure 6. (A)** OV90 cells were treated with 6uM cisplatin for 48h and expression of BMI1, OGT and α-Tubulin (used as loading control) was determined by immunoblotting. **(B)** OV90 cells were treated with 6uM cisplatin for 48h and immunoprecipitated with anti-BMI1 antibody or isotype IgG antibody. Expression of O-GlcNAc was determined by immunoblotting. All results represent three independent experiments.

To determine a role of nuclear and extra nuclear BMI1 in HGSOC we are constructing dual expression lentiviral plasmids that express both BMI1 shRNA and express various mutant forms of BMI1. Initial screen of three different BMI1 shRNA resulted in moderate 30% knockdown (not shown) and currently we are screening another three new shRNAs targeted against BMI1 according to the schema below (Fig. 7).

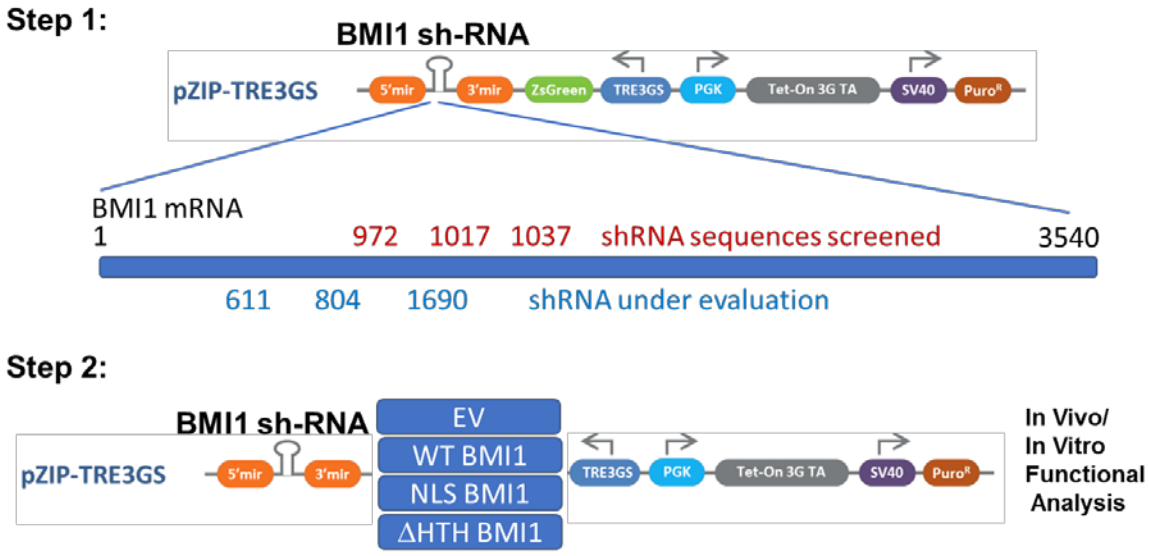



Figure 7: Functional evaluation of extra-nuclear BMI1: We are using pZIP-TRE3GS plasmid which is a 3rd generation Tetracycline (Tet) inducible plasmid. At first the shRNA targeting BMI1 will be evaluated once the effective BMI1 inhibition is achieved with shRNA, the GFP sequence will be replaced with either shRNA non-responsive BMI1 full length ORF or NLS or HTH domain deleted BMI1 and stable cell lines will be prepared expressing these constructs. Next, after induction with Tet these cells will express shRNA targeting endogenous BMI1 and at the same time shRNA- nonresponsive BMI1 and different BMI1 mutants will be expresses allowing us to evaluate the function of extra-nuclear BMI1.

In conclusion, our results indicate respective positive and negative regulation of the MDR1 and the CDKN2A promoter in a BMI1 dependent manner. ChIP studies support increased co-occupation of BMI1 and TIP60 with the MDR1 promoter which may impact either BMI1 acetylation or histone acetylation purportedly leading to transcriptional regulation. Interestingly in cisplatin treated cells we have consistently observed O-GlcNAc modification of BMI1 that may participate both in stabilization of BMI1 and in mediating transcriptional regulation which is also being pursued.

**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 performing experiments that address post-translational modifications of BMI1 in cisplatin treated conditions. These will be correlated with ChIP assays for evaluation of active or repressive marks at respective promoters. Construction of dual expression lentiviral plasmids is ongoing. Finally, experiments pertaining to mitochondrial role of BMI1 and anti-BMI1 therapy in 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.*

Regarding post-translational modifications of BMI1 that may impact its regulation of transcription, in addition to acetylation changes we are also pursuing O-GlcNAc modifications because cisplatin treated cells consistently demonstrate O-GlcNAc modification of BMI1.

**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).*

  | |
  |---|
  | Nothing to report |

  **Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  | |
  |---|
  | Nothing to report |

  **Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  | |
  |---|
  | Nothing to report |

- **Website(s) or other Internet site(s)**

  *List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

  | Nothing to report |
  |---|

- **Technologies or techniques**
  *Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

  | Nothing to report |
  |---|

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

  | Nothing to report |
  |---|

- **Other Products**
  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**
*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".*

*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
Project Role: Principal Investigator
Nearest person month worked: 1
Contribution to Project: Oversee

Name: Anindya Dey
Project Role: Research personnel
Nearest person month worked: 9
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

Name: Pooja Popli
Project Role: Research personnel
Nearest person month worked: 1
Contribution to the Project: Wet bench research

Name: Kai Ding
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to the Project: Biostatistician

Name: Sanam Husain
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to the Project: Pathologist

**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.*

* See attached updated Other Support

**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, Dr. Magdalena Bieniasz. Nothing to report for first year of award.

## 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.*