AWARD NUMBER: W81XWH-19-1-0509

TITLE: Targeting PLK-1 for Treating MYC-Driven Lymphomas

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Targeting PLK-1 for Treating MYC-Driven Lymphomas

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**6. AUTHOR(S)**
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**5e. TASK NUMBER**

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**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Roswell Park Comprehensive Cancer Center

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Medical Center

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Fort Detrick, Maryland 21702-5012

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**12. DISTRIBUTION / AVAILABILITY STATEMENT**

Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

The overall aim of the project is to determine the potential therapeutic value of targeting PLK-1 in the treatment of MYC-driven lymphomas. Polo-like kinase (PLK)-1 selective inhibitor, Volasertib (BI 6727), has been known as a potent anti-tumor effect in various cancer cells, including B cell lymphomas. However, Volasertib was withdrawn in Phase III clinical trials due to its adverse effects. In this study, we developed an Antibody-drug conjugates (ADC), which is anti-CD19 antibody (Inebilizumab)-Volasertib conjugate (V-ADC) to increase the specificity for B-cell lymphomas and minimize the toxic effects of Volasertib. We will test the specificity of V-ADC to B-cell lymphoma cells and the efficacy of therapeutic effects in B-cell lymphomas in vitro and in vivo. We found that V-ADC exhibits very little cytotoxic effects in various B-cell lymphoma cell lines. We hypothesized that the expression level and gene mutation of CD19 may affect the binding affinity which required for V-ADC mediated cytotoxicity. We have shown that most cell lines tested have low to modest level of CD19 expression. Overexpression of wild type CD19 in Z138 cells exhibited expected cytotoxic effect of Volarsertib in a dose-dependent manner. Furthermore, we have found most cell lines harbor CD19 L174V gene mutation which may significantly affect the CD19-binding. We are in the process of studying whether CD19 gene mutation affect V-ADC effect in lymphoma cell lines. Furthermore, we will further investigate the effects of CD19 expression and gene mutation in PDX models of aggressive B-cell lymphoma in vivo. All of these studies will be completed within this period of no-cost extension.

**15. SUBJECT TERMS**

NONE LISTED

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
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**Prescribed by ANSI Std. Z39.18**

## TABLE OF CONTENTS

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

Polo-like kinase (PLK)-1 selective inhibitor, Volasertib (BI 6727), has been known as a potent anti-tumor effect in various cancer cells, including B cell lymphomas. However, Volasertib was withdrawn in Phase III clinical trials because of its adverse effects, such as hypotension and myelosuppression. In this study, we developed an Antibody-drug conjugates (ADC), which is anti-CD19 antibody (Inebilizumab)-Volasertib conjugate (V-ADC) to increase the specificity for B-cell lymphomas and minimize the toxic effects. We will test the specificity of V-ADC to B-cell lymphoma cells and the efficacy of therapeutic effects in B-cell lymphomas in vitro and in vivo.

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

Antibody-drug conjugates (ADC), CD19 antibody-Volasertib conjugate (V-ADC), Apoptosis, B cell lymphoma

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.*

Original Statement of Works:

Major task #1: Determine PLK-1 mediated activation of oncogenic pathways in lymphoma cells.
- Completed

Major task #2: Determine the significance of PLK-1 in MYC-induced lymphomagenesis.
- Completed.

Major task #3: Determine if PLK-1 inhibition confers synthetic lethality to MYC-driven lymphoma.
- Completed.

Major task #4: To develop a practical method for targeting PLK-1
- Ongoing during this period of No-cost extension.

Major task #5: Determine the therapeutic effect of PLK-1 inhibition *in vivo*.
- Ongoing during this period of No-cost extension.

For major task #4: the subtask #1 is to establishment of antibody-drug-conjugate, we have successfully established the anti-CD19 antibody (Inebilizumab)-Volasertib conjugate (V-ADC), we are in the process of studying the specificity and efficacy of this ADC in lymphoma cell lines (Major task #4, subtask #2) and PDX animal models (Major task #5, subtask #2).

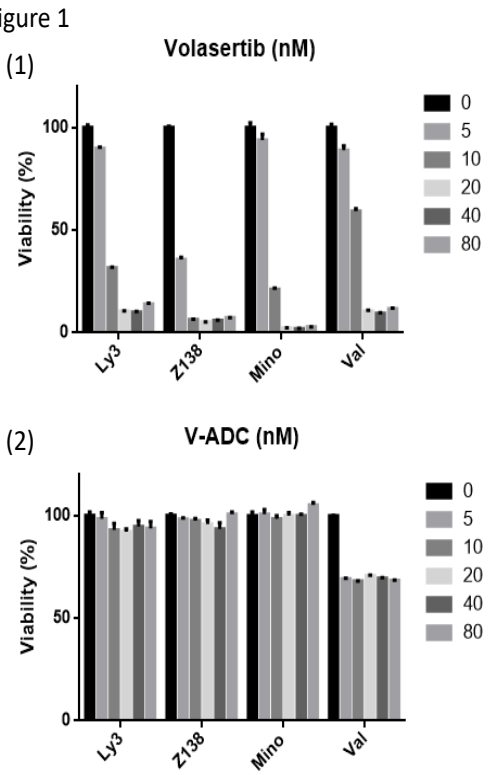
**What was accomplished under these goals?**

*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.*

We investigated the cytotoxic effect of Volasertib in B cell lymphoma cell lines using PrestoBlue HS Cell Viability Reagent (ThermoFisher, P50201). As shown in Figure 1(1), the viability of lymphoma cells was significantly reduced in a dose dependent manner 48 hours after volasertib treatment in B-cell lymphoma cell lines tested. We further shown that Volasertib induced apoptotic cell death in B cell lymphoma cells via G2/M arrest (data not shown). However, CD19 antibody-Volasertib conjugate (V-ADC) didn't show expected cytotoxic effect in these lymphoma cells (Figure 1 (2)). We hypothesized that the discrepancy seen between free drug versus V-ADC may due to 1) the expression level of CD19 on the surface of these B cell lymphoma cells may be too low to exert any therapeutic effect of ADC; 2) genetic mutation in CD19 molecules may prevent sufficient binding by Inebilizumab, an anti-CD19 antibody used in ADC. We first tested the CD19 expression on four B cell lymphoma cells by Flow cytometry using FITC Mouse Anti-Human CD19 antibody (BD, 555412). We found that the CD19 expression was extremely low in Ly3 and Z138 cells, and was modest in Mino and Val cells (Figure 1 (3)). Secondly, we examined whether these B cell lymphoma cell lines harbor CD19 gene mutation (L174V (C.520 C<G)) by Sanger sequencing (UNMC Core facility). We found that whileas Ly3 is a wild type for CD19 L174V, Z138 harbored a heterozygous CD19 L174V mutation; and both Mino and Val exhibited homozygous gene mutation of CD19 L174V. More recently, we have also established a stable Z-138 lymphoma cell line with overexpression of wild type CD19 (WT-CD19-OE) to further study the cytotoxic effect of V-ADC in lymphoma cells. We found that V-ADC significantly reduced the viability of WT CD19 OE Z138 cells in a dose dependent manner, suggesting that level of CD19 expression plays an important role in CD19-mediated V-ADC effects (Figure 2). We are in the process of studying the effect of V-ADC in cell lines with overexpression of mutated CD19 to investigate whether CD19 gene mutation may affect CD19-binding. Furthermore, we plan to further investigate these effects in a PDX model of aggressive B-cell lymphoma.

Figure 1



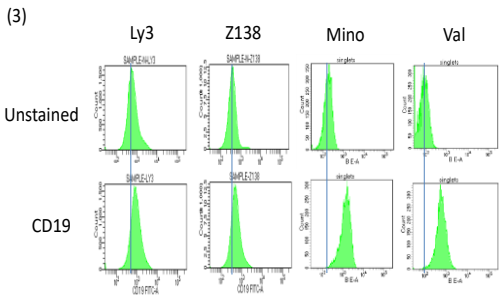
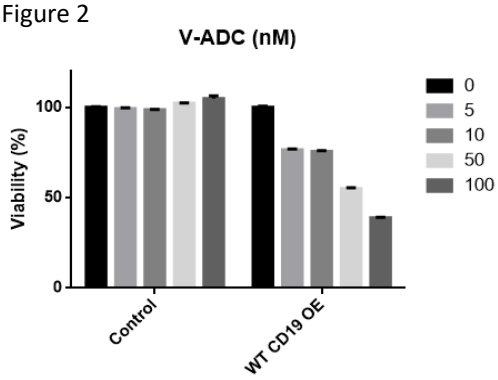




Figure 2



**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 project provided the opportunities for training activities, such as one-on-one work with a mentor to perform molecular biology, like cloning and virus infections.

**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.*

Our next plan is 1) investigate the effect of CD19 gene mutation in CD19-mediated V-ADC cytotoxic effect; and 2) determine the therapeutic efficacy of V-ADC in PDX model of B-cell lymphomas (Major task #5 subtask #2).

***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).*

The findings may have significant impact in future development of clinical trials in which both CD19 expression level and gene mutation may affect the therapeutic effect of anti-CD19 therapies.

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to Report

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Nothing to report.

**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.*

**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.*

V-ADC therapy didn't work well since CD19 expression is low on the surface of B cell lymphoma cell lines unexpectedly. To resolve this problem, 2 alternatives are proposed; 1) Establish WT/MUT CD19 overexpression stable cell lines to investigate the cytotoxic effect of V-ADC. 2) Investigate the therapeutic effect of V-ADC in a PDX model of B-cell lymphoma as proposed in the application. We anticipate that the previous findings may affect the therapeutic effect of V-ADC in vivo. If that happens, we will further evaluate whether overexpression of CD19 or wild type CD19 may help to eliminate the problem.

**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.*

We do not expect any changes on expenditures.

**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**

No significant changes

**Significant changes in use or care of vertebrate animals**

No significant changes

**Significant changes in use of biohazards and/or select agents**

No significant changes

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.*

Nothing to Report

**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".*

Kai Fu, MD, Ph.D.
PI
1.20 calendar months
Dr. Fu leads the project and its aims.

Dr. Chieko Saito, Ph.D.
Affiliate Member
6.00 calendar months
Dr. Saito had performed most of this in vitro study during past year.

Dr. Chengfeng Bi (with University of Nebraska Medical Center)
2.00 calendar months
Dr. Bi worked on both in vitro and in vivo model. He helped generating the V-ADC in this protocol.

**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.*

| |
|---|
| University of Nebraska Medical Center<br>42nd and Emile St, Omaha, NE 68198<br>Facility and collaboration |

## 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://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

N/A

**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.*

N/A