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

PRINCIPAL INVESTIGATOR: Kai Fu, MD, Ph.D.

CONTRACTING ORGANIZATION: Health Research Inc,
Roswell Park Division
Elm and Carlton Streets
Buffalo, NY 14263-0001

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14. ABSTRACT The overall aim of the project is to determine the 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 to have a potent anti-tumor effect in various cancer cells, including B cell lymphomas. Unfortunately, Volasertib was withdrawn in Phase III clinical trials due to its adverse effects, including myelosuppression. In this study, we developed an Antibody-drug conjugates (ADC), which is anti-CD19 antibody (Inebilizumab)-Volasertib conjugate (V-ADC) to increase the target specificity for B-cell lymphoma cells and minimize the toxic effects of Volasertib. We will test the efficacy of V-ADC treatment in B-cell lymphomas <i>in vitro</i> and <i>in vivo</i> . We found, unexpectedly, that V-ADC exhibited little cytostatic/cytotoxic effects in Z138, a mantle cell lymphoma (MCL) cell line. We hypothesized that the expression level of CD19 as well its gene mutation may affect the binding affinity which required for V-ADC endocytosis. In addition, lysosomal enzymatic activity might also be involved in regulating V-ADC therapeutic effect because lysosomal enzymes cleave the linker between Volasertib and Inebilizumab, which releases free Volasertib into the cells. In this study, we have shown that overexpression of wild type or mutant CD19 in Z138 cells exhibited increased cytotoxicity of Volasertib in a dose-dependent manner. Furthermore, we found V-ADC induced significant cell death after cell starvation in CD19-overexpressing Z138 cell line. Moreover, the combination of V-ADC and rapamycin which is an inhibitor of mammalian target of rapamycin complex 1 (mTORC1) induced a reduction of cell viability significantly. We are in the process of confirming whether lysosome enzymes activity might be involved in V-ADC therapeutic effect in B cell lymphoma cell lines, and we will further investigate the effects of V-ADC in xenograft models of aggressive B-cell lymphoma <i>in vivo</i> .					
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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 to have 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, including hypotension and myelosuppression. In this study, we developed an Antibody-drug conjugate (ADC), which is anti-CD19 antibody (Inebilizumab)-Volasertib conjugate (V-ADC) to increase the target specificity for B-cell lymphoma cells and to minimize the toxic effects of Volasertib. We will investigate the therapeutic efficacy of V-ADC on B-cell lymphomas and investigate the molecular mechanism of PLK-1 plays a pivotal role in B cell malignancies *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), Polo-like kinase (PLK)-1, G2/M arrest, 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 PLK-1 inhibition confers synthetic lethality to MYC-driven lymphoma.

- Completed.

Major task #4: To develop a practical method for targeting PLK-1 in B-cell lymphoma.

- 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 an 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 V-ADC in various lymphoma cell lines (Major task #4, subtask #2) and xenograft animal model of B-cell lymphomas (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.

The Antibody-drug conjugates (ADC) binds to the surface antigen on the target cells. The ADCs will then be internalized by a receptor-mediated endocytosis and transferred to late endosomes that subsequently fuse with lysosomes. Within the lysosomes, the drug will be released via cleavage of the linker by specific proteases such as Cathepsin B or degradation of the antibody (Hao Chen et.al., 2017). First, we investigated the apoptotic cell death after Volasertib or CD19 antibody-Volasertib conjugate (V-ADC) treatments in Z138, a mantle cell lymphoma (MCL) cell line. As shown in Figure 1, the percentage of late apoptotic cell were markedly increased upon 50nM Volasertib treatment, however, 100nM V-ADC have little apoptotic effect in Z138 cells. Since PLK1 inhibitor causes cell apoptosis via G2/M arrest, we further performed cell cycle analysis using flow cytometry. In Figure 2, Volasertib induced G2/M arrest, in contrast, V-ADC did not. The findings suggest that V-ADC was not working in this condition although free Volasertib was a very effective drug for B cell lymphoma cells. We hypothesized that the discrepancy seen between the free Volasertib versus V-ADC may be 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 (L174V) may prevent sufficient binding by Inebilizumab, an anti-CD19 antibody used in ADC; 3) the concentration of free drug Volasertib might be low in Z138 cells because Volasertib fails to dissociate from V-ADC with low activity of lysosomal enzymes. We first tested the CD19 expression by Flow cytometry and we found that the CD19 expression was extremely low in Z138 cells (data was shown in previous report). More recently, we have established a stable Z-138 cell line with overexpression of wild type CD19 (CD19 WT-OE) and L174V mutant CD19 (CD19 Mut-OE) to further study the cytotoxic effect of V-ADC in lymphoma cells (Figure 3 (1)). We found that V-ADC induced close to 40% reduction of the cell viability in CD19 WT and Mut OE Z138 cells; while only 10-20% reduction seen in Control Z138 cells; suggesting that overexpression of CD19 improved CD19-mediated V-ADC cytotoxic effects (Figure 3 (2)). To further investigate whether lysosome activity plays a role in CD19-mediated V-ADC effects, Z138 cells was starved overnight before exposing V-ADC since cell starvation may induce lysosome enzyme activity. As shown in figure 4 (1), starvation followed by 50nM V-ADC induced cell death in approximately 70% of CD19 WT-OE Z138 cells, however, little effects were seen in control or CD19 Mut-OE Z138 cells. During starvation, mammalian target of rapamycin complex 1 (mTORC1) activity would be inhibited, which induces the nuclear translocation of unphosphorylated transcription factor EB (TFEB). TFEB then enhances lysosomal enzyme activity including cathepsin expression and activity (Carmine Settembre et al., 2012). We then treated CD19 WT-OE Z138 cells with Rapamycin, an inhibitor of mTORC1, with or without 10nM V-ADC (Figure 4 (2)). We found both rapamycin and V-ADC reduced cell viability, however, a combination of Rapamycin and V-ADC showed a significant cytotoxic effect. We are in the process of further studying the effect of V-ADC in CD19 overexpression cell lines with regulation of lysosome activity and we plan to further investigate these effects in PDX/xenograft models of aggressive B-cell lymphoma.

Figure 1

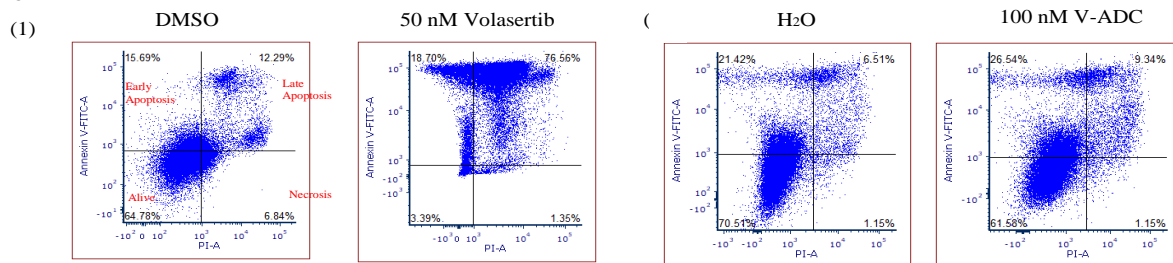


Figure 2

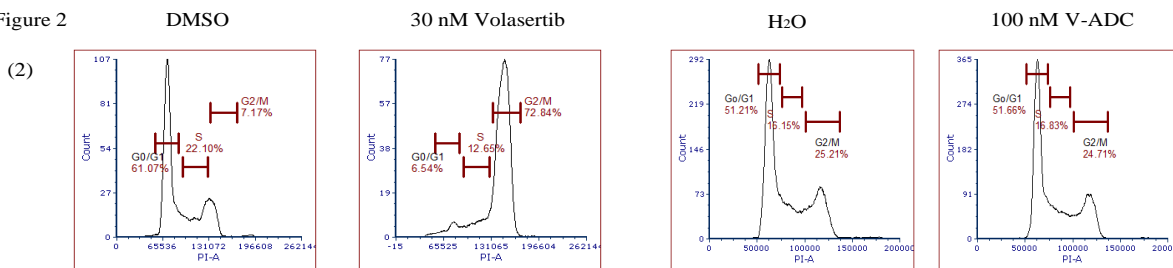


Figure 3

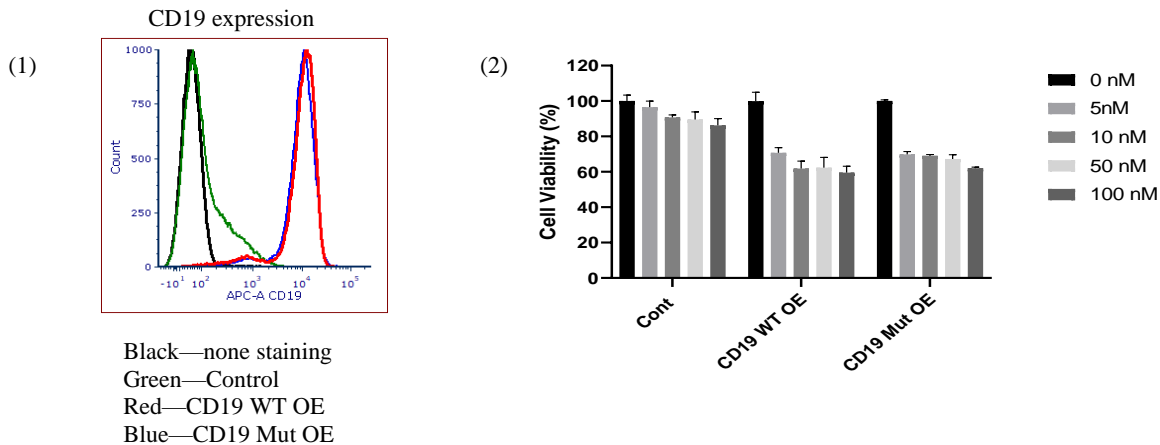
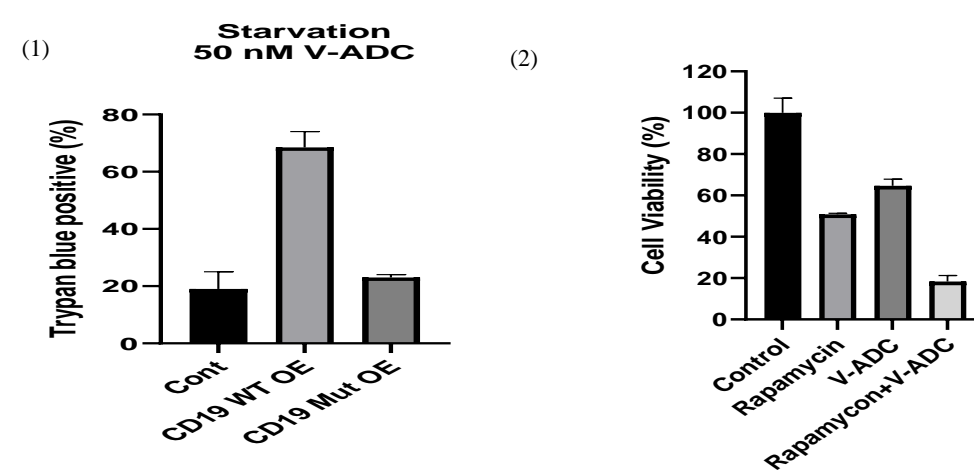


Figure 4



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 cellular and molecular biology techniques, 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 plan for next period includes: 1) Further study whether overexpression of CD19 enhance CD19-mediated V-ADC therapeutic effects in multiple lymphoma cell lines. 2) Investigate whether increased lysosome enzymatic activity would significantly enhance the therapeutic effect of V-ADC in B-cell lymphoma cells; and 3) Determine the therapeutic efficacy of V-ADC in PDX/xenograft models of B-cell lymphomas (Major task #5 subtask #2).

4. IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The findings may have significant impact in future development of clinical trials in which both CD19 expression level and mutation status as well as lysosomal enzymatic activities may affect the therapeutic effect of CD19-mediated ADC 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.

We plan to investigate the therapeutic effect of V-ADC in a PDX model of B-cell lymphoma as proposed in the application. We anticipate that the V-ADC may have potent therapeutic effect in PDX/xenograft models of B-cell lymphoma in vivo. We also plan to further evaluate whether overexpression of wild type CD19, with or without, starvation or pretreatment of rapamycin may facilitate the V-ADC therapy.

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”.

No change

Kai Fu, MD, Ph.D.
 Dr. Chieko Saito, Ph.D.
 Dr. Chengfeng Bi, MD, Ph.D.

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
 42nd and Emile St, Omaha, NE 68198
 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