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TITLE: CD24 Tumor-Initiating Cell as a Novel Therapeutic Target in Myeloma

PRINCIPAL INVESTIGATOR: Gail Bishop

CONTRACTING ORGANIZATION: University of Iowa

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UAMS, Department of Internal Medicine, Winthrop P. Rockefeller Cancer Institute, 4018 W Capitol Ave, Little Rock, AR 72205

8. PERFORMING ORGANIZATION REPORT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Multiple Myeloma (MM) is a blood cancer of the B cell lineage characterized by monoclonal plasma cells. Most patients initially respond to the therapy but majority of them relapse and become refractory to treatment. These myeloma cells, which escape current modes of therapy. We name it tumor-initiating cells (TICs) in myeloma. Understanding the nature of myeloma-TICs will provide an opportunity to cure this disease by preventing its relapse. Through a systematical screening, our studies presented here demonstrated that CD24+ myeloma cells maintain the features of self-renewal and drug resistance in myeloma. We predict that anti-CD24 antibody may eliminate myeloma tumor initiating cells resulting in cure of myeloma disease or significant extension of patient survival. This proposal focuses on validating CD38+CD45–CD24+ as TICs marker and its potential therapeutic role. Aim 1 determines the CD38+CD45–CD24+ phenotype in maintaining 'stemness' and its clinical relevance in primary myeloma samples. Aim 2 determines tumor-initiating characteristics of CD38+CD45–CD24+ cells. Aim 3 investigates the efficacy of humanized CD24 antibodies in killing myeloma tumor-initiating cells. The FY18 PRCRP Topic Area is myeloma; The FY18 PRCRP Military Relevance Focus Areas are that gaps in myeloma prevention, prognosis and treatment for extending patient survival. Myeloma is one of the common cancers seen among Veterans and each year cases will increase. Overall, this project has the potential to improve treatment outcome of all myeloma patients including veterans when we finish this project.

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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Unclassified	Unclassified	Unclassified			

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

Myeloma tumor-initiating cells (MM-TICs) characterized by increased drug-resistance and self-renewal capacity, are very likely responsible for our failure to cure myeloma in most patients. We proposed to determine how the $CD24^+$ primary MM cells ($CD38^+CD45^-$) contribute to drug resistance and to develop MM TIC-targeted therapies *in vitro* and *in vivo* in a pre-clinical mouse model. The scope of this research is to prove the clinical relevance of $CD24^+$ is a key marker for myeloma tumor-initiating cells as it is in other cancer stem cells and use humanized CD24 antibodies to kill myeloma tumor-initiating cells.

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

Multiple Myeloma, CD24, Stem cells, tumor, biomarker, drug resistance, target-therapy

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.

a. Determine the role of the $CD38^+CD45^-CD24^+$ phenotype in maintaining 'stemness' and its clinical relevance in multiple myeloma (Aim 1).

b. Determine tumor-initiating cell characteristics of $CD38^+CD45^-CD24^+$ primary myeloma cells (Aim 2).

c. Target primary myeloma tumor-initiating cells using a humanized CD24 antibody (Aim 3).

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.

Aim1. Determine the role of the $CD38^{+}CD45^{-}CD24^{+}$ phenotype in maintaining 'stemness' and its clinical relevance in multiple myeloma.

Flow Cytometry Analysis of Patients Samples to assess the association of drug response and CD24 expression in MM cells. We cultured mononuclear cells from myeloma patients' bone marrow specimens, Treated with bortezomib or CD24 antibody.

A) We continue the $CD38^{+}CD45^{-}CD24^{+}$ sub-population analysis in myeloma patients

B) The percentage of subpopulation of CD38+CD45–CD24+ cells increase in **26 of 32** primary myeloma samples post BTZ treatment.

C) The percentage of subpopulation of CD38+CD45–CD24+ cells decrease in **all 32** primary myeloma samples post CD24 antibody treatment.

D) Only 10 out of 32 patients CD38+CD45– cells decrease significantly post CD24 antibody treatment.

Our data show that CD24 antibody has the limitation of killing the bulk tumor cells even though it can cause the CD38+CD45–CD24+ cells apoptosis in all cases. We explored the combination treatment of bortezomib and CD24 antibody. We will analyze the results once we have sufficient sample size in next report.

We also seek to use CAR T-cell (CART) therapies aimed at B-cell maturation antigen (BCMA) which expressed on the myeloma cell surface. BCMA-CARTs have high response rates observed in the early stage of therapy in myeloma. We generated bispecific CAR-T cells which recognize both BCMA and CD24 antigens. We constructed a bispecific BCMA-CD24 CAR vector, with 2 complete CAR units: BCMA CAR and CD24 CAR. P2A was inserted between these two CARs. The BCMA CAR contained a safety switch in hinge region, and a CD28 co-activation domain with CD3ζ signaling domain was used in this design while the CD24 CAR contained a 4-1BB co-activation domain with CD3ζ. To eliminate the risk of severe immunological side effects, we integrated RQR8, an immunological safety switch into the hinge region. Lentivirus particles were used to transduce primary human T cells. CAR-T cells were detected on day 7 with flow cytometry using CD34 makers. We will perform co-culture killing assays, detected the T cell activation marker CD69 and measured the cytokines in the supernatant.

Aim2: **In vitro analysis of tumor initiating cells:**

We isolate CD38+CD45–CD24+ and CD38+CD45–CD24– from two MM patients. This sample did not have colonies formation. We will optimize the SOP.

Our data demonstrate that CD24 positive myeloma cells has the stem cell characteristics. How MM cells become CD24 positive and how CD24 induces IPs signaling? The answer to these questions will guide us not only better understand the disease but also provide treatment strategies. We have the following findings: **CD24 Localizes in the Nucleus in MM cells; Nuclear CD24 Depletion after BTZ Treatment (O/N); At ER: CD24 is GPI-linked**; **At the Golgi: CD24 is glycosylated and GPI-linked.**

Aim3: **Determine the therapeutic efficacy of humanized CD24 antibody *In Vivo*.**

NSG mice are radiated @ 2 Grays 4 hours before we inject 5 million mononuclear cells. We analyzed mononuclear cells from bone marrow of MM patients by flow cytometry before cell transplantation. Then we treat these mice transplanted with human MM cells with (1) Control (PBS); (2) H-CD24 Ab (100 nM); (3) bortezomib (Btz; 5nM), and (4) H-CD24 Ab + Btz for eight weeks. We analyzed the cells from bone marrow of these mice post treatment using human CD24 and CD138 antibody stain. Our results demonstrate that both CD24+CD138+ and CD138+ populations decreased in those samples treated with H-CD24 Ab or H-CD24 Ab + Btz compared to those treated with bortezomib alone or the controls.

We isolated the cells from the first transplanted mice bone marrow and transplanted to NSG mice (second transplantation). We will analyze the results of second transplantation when the control mice become sick.

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.

In the next reporting period, we will continue the flow analysis of bone marrow from untreated myeloma patients and their follow-up analysis. We will continue to study the relationship of Cd24 positive cell percentage and drug response using primary myeloma cells until we have sufficient data for statistical analysis (aim1). We will optimize the SOP for colony formation assay and validate the results using other methods (such as DiD stain) to further characterize the CD24 positive cells (aim2). We will perform the in vivo study using the PDX mouse model. The Second and Third transplantation may be performed if we can harvest enough cells from first transplantation.

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

What was the impact on other disciplines?
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

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.

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).*

Li C, Wendlandt EB, Darbro B, Xu H, Thomas GS, Tricot G, Chen F, Shaughnessy JD Jr, Zhan F. Genetic Analysis of Multiple Myeloma Identifies Cytogenetic Alterations Implicated in Disease Complexity and Progression. Cancers (Basel). 2021 Jan 29;13(3).

Li C, Xia J, Franqui-Machin R, Tricot G, Chen F, He Y, Ashby T, Teng F, Xu H, Liu D, Gai D, Johnson S, Rhee F, Janz S, Shaughnessy J, Frech I, Zhan F. TRIP13 modulates protein deubiquitination and accelerates tumor development and progression of B-cell malignancies. JCI June 1, 2021)

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".

Name:	Gail Bishop

Project Role:	Partnering Principal Investigator
Nearest person month worked:	1.1
Contribution to Project:	Provide scientific consultation on the direction of the work, evaluate the results.
Funding Support:	N/A

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*

Nothing to Report

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.*

Contact PI report will be submitted separately

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.*

https://www.jci.org/articles/view/146893

https://www.mdpi.com/2072-6694/13/3/517