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

PRINCIPAL INVESTIGATOR: Fenghuang Zhan

CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences

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6. AUTHOR(S)
Fenghuang Zhan MD, PhD

E-Mail:FZhan@uams.edu

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

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

8. PERFORMING ORGANIZATION REPORT

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

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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 within four years.

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			USAMRDC
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TABLE OF CONTENTS

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).

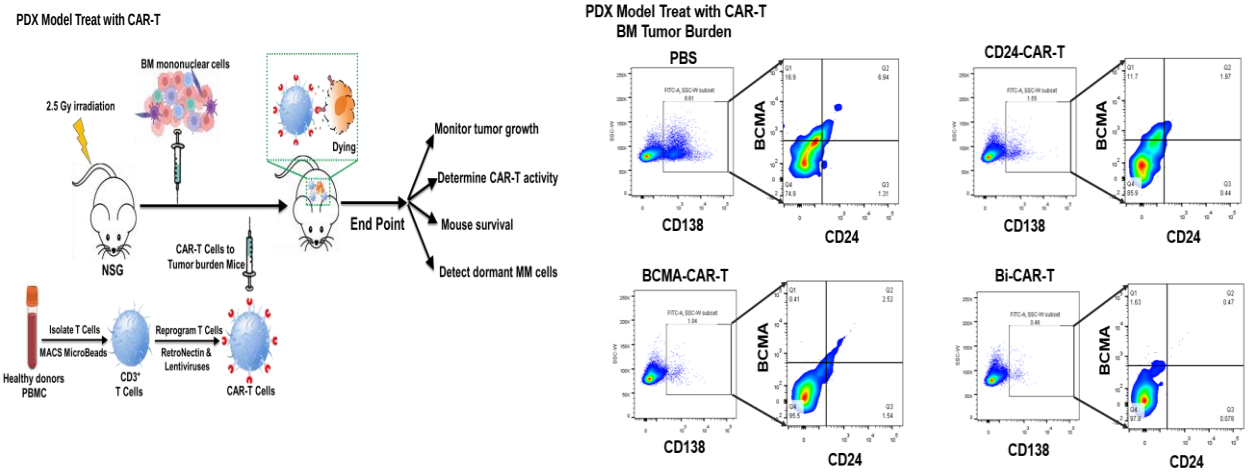
What was accomplished under these goals?

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. The percentages of CD24+ multiple myeloma (MM) cells were compared in newly diagnosed MM patients and MM patients after treatment, with samples collected for testing response to bortezomib-based therapy after two to three cycles of therapy. The mean percentage of CD138+CD38+CD24+ MM cells was increased in three different clinical data resource. We also did the surface maker detect using 22 MM cell lines. We can see most of MM cell lines high expressed BCMA, except JIM3 and MC/CAR.
For the CD24, most of MM cell lines also expressed CD24, some cell lines high expressed CD24. Like OCI-MY5 and MC/CAR.

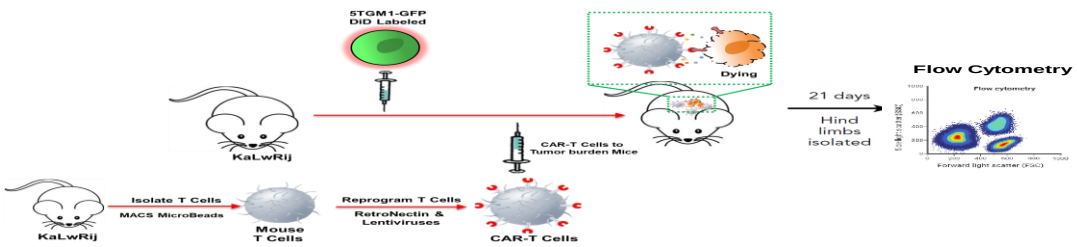
Aim2. In the immune system, CD24 is important in the regulation of cell proliferation and clonal expansion. It was recently reported that CD24 on tumor cells can act as a brake on the immune system. We used 18 multiple myeloma clinical samples from the UAMS tissue bank to detect the Siglec-10 expression in tumor-associated macrophages. We found all the tumor-associated macrophages expressed Siglec-10. The expression of Siglec-10 ranges from 5% to 30%. Further we found out the expression of Siglec-10 is correlated with the expression of CD24 not CD138. We compared the mouse BM Single-cell sequence data between normal mice and multiple myeloma burden mice. The expression of Siglec-10 changes dramatically in the tumor burden mice. There is a group of macrophages, highly expressed Siglec-10 compared to other groups. Those results suggested that tumor burden may increase the expression of Siglec-10 in tumor-associated macrophages. And CD24 on myeloma cells acted as an anti-phagocytic surface protein interacted with Siglec-10 on macrophages. This allowed $CD24^{+}$ myeloma cells to survive, which may be one of the reasons why CD24 acted as a tumor-initiating cell.

Aim3: 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. To test if our newly developed Bi CAR-T Cells are more effective than H-CD24 Ab for MM,



We treated tumor burden PDX mice with CAR-T cells. Our results demonstrated that BCMA/CD24 Bi CAR-T cells increased mice survival and most efficiently kills tumor cells. More patients' samples will be tested in the near future for both CD24 Ab and Bi CAR-T treatment. We are testing both antibody and CART cells in 5TGM1/KaLwRij model to further explore the mechanisms of immunotherapies to the microenvironment and tumor cells in the bone marrow.

In Progress: in vivo



5 Groups: 1) Normal KaLwRij w/o tumor; 2) PBS; 3) Mock CAR-T; 4) CD24 CAR-T, 5) CD24 Ab

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 be able to test more primary MM samples proposed in aim 3 and perform more second and third transplantation using the PDX mouse model. We collected mononuclear cells from different treatment group to do single cell RNA sequencing, we will analyze those data and discover the mechanisms of our immune therapies. We started to summarize our data and preparing our manuscripts.

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).*

1. Gai D, Chen JR, Stewart JP, Nookaew I, Habelhah H, Ashby C, Sun F, Cheng Y, Li C, Xu H, Peng B, Garg TK, Schinke C, Thanendrarajan S, Zangari M, Chen F, Barlogie B, van Rhee F, Tricot G, Shaughnessy JD Jr, Zhan F. CST6 suppresses osteolytic bone disease in multiple myeloma by blocking osteoclast differentiation. J Clin Invest. 2022 Jul 26;e159527. doi: 10.1172/JCI159527.

2. Cheng Y, Sun F, Thornton K, Jing X, Dong J, Yun G, Pisano M, Zhan F, Kim SH, Katzenellenbogen JA, Katzenellenbogen BS, Hari P, Janz S. FOXM1 regulates glycolysis and energy production in multiple myeloma. Oncogene. 2022 Jul 6. doi: 10.1038/s41388-022-02398-4.

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:	Fenghuang Zhan
Project Role:	Principal Investigator
Nearest person month worked:	2.3
Contribution to Project:	Clinic relevance, flow analysis, and oversee the direction of the work.
Funding Support:	N/A

Name:	Timothy C. Ashby (No Change)
Project Role:	Co-investigator
Nearest person month worked:	1.2
Contribution to Project:	Involved in data analysis
Funding Support:	N/A

Name:	John Shaughnessy (No Change)
Project Role:	Senior Scientist
Nearest person month worked:	3
Contribution to Project:	Database maintenance and primary samples process.
Funding Support:	N/A

Name:	Bailu Peng
Project Role:	Senior Scientist
Nearest person month worked:	4.8
Contribution to Project:	Experiments of in vitro and in vivo.

Funding Support:	N/A
Name:	Hongwei Xu
Project Role:	Senior Scientist
Nearest person month worked:	3.0
Contribution to Project:	Process patients' samples and flow analysis
Funding Support:	N/A

Name:	Dongzheng Gai
Project Role:	Postdoc. fellow
Nearest person month worked:	3.6
Contribution to Project:	He performs in vitro study
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.*

Partnering 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://pubmed.ncbi.nlm.nih.gov/35881476/