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

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CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences, Little Rock, AR

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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

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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**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	9	**19b. TELEPHONE NUMBER** *(include area code)*

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1. INTRODUCTION:

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:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

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?

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?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

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:**

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

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS:

Nothing to Report

Changes in approach and reasons for change

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

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:

- **Publications, conference papers, and presentations**

Journal publications.

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.

Nothing to Report

Other publications, conference papers and presentations.

Nothing to Report

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

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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: Funding Support:	Involved in data analysis 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:	2.8
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:	2.5
Contribution to Project:	Process patients' samples and flow analysis
Funding Support:	N/A

Name:	Dongzheng Gai
Project Role:	Postdoc. fellow
Nearest person month worked:	1.5
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?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Partnering PI report will be submitted separately

9. APPENDICES:

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

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