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TITLE: Targeting Metastatic Prostate Cancer with Tumor-Specific Marrow-Infiltrating Lymphocytes (MILs)

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CONTRACTING ORGANIZATION: The Johns Hopkins University
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14. ABSTRACT

Though T-cells in prostate cancer are characterized by an exhausted and suppressive state, the bone marrow represents a documented reservoir of polyclonal antigen-experienced *memory T-cells*, including clones with anti-tumor specificity, coined marrow-infiltrating lymphocytes or 'MILs'. Memory cells are naturally enriched in the bone marrow due to the unique attributes of this compartment. Thus, despite being initially counter-intuitive, the bone marrow represents a robust source of tumor-specific memory T-cells even in patients with no tumor burden in the bone marrow. Of note, memory T-cells are associated with superior engraftment, increased proliferative potential, greater persistence, and enhanced anti-tumor activity; all of which lead to improved clinical outcomes and longer survival in patients undergoing adoptive cell therapy. Therefore, our **hypothesis** is that **polyclonal tumor-specific T-cells enriched in memory phenotypes can be selectively expanded from the bone marrow of prostate cancer patients, activated ex vivo, and adoptively transferred to generate a systemic anti-tumor immune response targeting metastatic castration-resistant disease.** To evaluate this hypothesis, memory phenotypes, in addition to tumor-specific proliferative and cytotoxic responses of MILs expanded from the bone marrow of primary and metastatic prostate cancer patients will be evaluated as a function of disease progression using PBLs from the same patient as controls. Anti-tumor specificity, targeting, persistence, and efficacy will also be evaluated using *in vivo* prostate cancer models.

15. SUBJECT TERMS
Marrow-infiltrating lymphocytes (MILs), T-cells, adoptive cell therapy, prostate cancer

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

Despite the approval of several new therapies for prostate cancer (PCa), the outlook remains dismal for patients with advanced metastatic castration-resistant disease (mCRPC). Though objective tumor responses to immunotherapy have not been as robust in the mCRPC setting as those observed in other tumor types, PCa remains a logical target for the development of novel immunotherapeutic platforms. Autopsy studies have documented that PCa often begins in the 3rd and 4th decades of life, but only becomes clinically detectable decades later due to its low proliferative index. This provides ample time for the immune system to develop adaptive responses to tumor antigens. Unfortunately, this same extended timeframe ultimately leads to a tolerized microenvironment with highly anergic T-cells and population skewing towards immunosuppressive phenotypes. There is a clear association between the presence of *memory T-cells* in adoptive cell transfer infusion products and *positive clinical outcomes*, including longer overall survival as a result of increased engraftment providing long-term anti-tumor immunity. Memory cells are naturally enriched in the bone marrow due to the unique immunologic attributes of this compartment. Specifically, the bone marrow is a secondary lymphoid organ responsible for maintaining immunological memory to previously encountered antigens, including those present on cancer cells. This immunologic memory is promoted through a combination of interactions with bone marrow stromal cells and effective antigen-presentation by APCs in the context of a favorable cytokine microenvironment. Thus, counter-intuitively **the bone marrow represents a robust source of tumor-specific memory T-cells** even in patients with no tumor burden in their bone marrow. This is because circulating memory T-cells overexpress CXCR4, which mediates their trafficking to the bone marrow in response to the high concentrations of CXCL12. MILs were initially developed as the "TILs" (i.e. tumor-infiltrating lymphocytes) of hematologic malignancies by Dr. Borrello as a novel therapy for multiple myeloma. Despite both having measurable endogenous tumor specificity, MILs have several advantages over conventional TILs isolated from solid tumors. For example, though TILs have led to striking responses in a subset of patients with metastatic melanoma, this procedure requires a surgically accessible tumor and thus, is not readily applicable to many non-cutaneous solid tumor types. Furthermore, TILs have rarely shown clinical activity outside of melanoma, and even in patients with accessible tumors, TILs are frequently unable to be expanded to clinically meaningful numbers. Additionally, TIL adoptive transfer is a costly clinical procedure with estimates of ~$100,000 or more per patient due to the labor-intensive and lengthy expansion protocol requiring highly specialized facilities and equipment. In contrast, MILs have been successfully harvested from 100% of patients attempted to date via a simple bedside procedure (i.e. a bone marrow aspirate). This simple collection procedure coupled with the relatively short expansion protocol (~7-10 days vs. up to 6 weeks for TILs) results in an overall cost on the order of ~$10-15k/patient; substantially less than that required for TIL therapy. Collectively, this means the platform could be readily adapted to many leading academic centers for greater availability to patients at a reasonable cost.

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

Marrow-infiltrating lymphocytes, MILs, T-cells, adoptive cell therapy, prostate cancer

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.

Aim 1: Evaluate memory phenotypes and tumor-specific responses of MILs expanded from primary prostate cancer patients *in vitro*

Aim 2: Evaluate memory phenotypes and tumor-specific responses of MILs expanded from metastatic prostate cancer patients *in vitro*.

Aim 3: Evaluate anti-tumor responses of aMILs *in vivo* using preclinical models of prostate cancer.

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.

Major activities / Specific Objectives:

Aim 1 activities:

1. Matched peripheral and bone marrow samples were collected from 24 primary prostate cancer patients with Gleason Scores ranging from 6 to 10 (Tbl. 1); in addition to an additional 11 unmatched bone marrow and 9 unmatched peripheral blood samples.
2. Memory phenotypes (i.e. Naïve, Effector, Effector Memory, and Central Memory) assessed in CD4+ and CD8+ MILs vs. PBLs at baseline and post-expansion (Figs. 1 & 2).
3. Activation markers (i.e. CD25, CD27, CD28, and CD69) assessed in CD4+ and CD8+ MILs vs. PBLs at baseline and post-expansion (Fig. 3).
4. IFN and IL-2 expression analyzed in activated CD4+ AND CD8+ MILs vs. PBLs post-expansion (Fig. 4).
5. Immune checkpoints (i.e. PD-1, CTLA-4, Tim-3, and 4-1BB) and CXCR4 expression assessed in CD4+ and CD8+ MILs vs. PBLs at baseline and post-expansion (Fig. 5).
6. Regulatory T-cells (Tregs) defined as CD3+/CD4+/$CD25^{hi}$/$CD127^{lo}$/FoxP3+ were assed in MILs vs. PBLs at baseline and post-expansion (Fig. 6).
7. Granulocytic and Monocytic myeloid-derived suppressor cells (MDSCs) were assessed in bone marrow and peripheral blood at baseline (Fig. 7).
8. Plasma from bone marrow and peripheral blood from all patients has been collected and stored for cytokine profiling once collection has been completed.

Aim 2 activities:

1. Research coordinator identified to discuss study participation and consent with patients identified in the metastatic prostate cancer clinic by our medical oncology collaborators.
2. She has been added to the appropriate IRB-approved biobanking protocols relevant to the study.
3. A script outlining study details, risks, etc. has recently been IRB-approved for patient contact.
4. Potential study participants are now being identified to begin screening for sample collection.

Aim 3 activities:

Nothing to report for this period

Significant Results:

Sample	Gleason Score	Bone Marrow		Peripheral Blood	
		% CD3	CD4:CD8	% CD3	CD4:CD8
1	6 (3+3)	26.0	2.8	52.9	3.1
2	6 (3+3)	4.9	2.9	13.6	5.4
3	6 (3+3)	20.9	1.1	32.4	1.2
4	6 (3+3)	9.9	1.4	34.6	1.7
5	6 (3+3)	42.1	1.2	57.1	1.1
6	7 (3+4)	7.2	7.9	21.3	9.3
7	7 (3+4)	8.3	4.1	31.7	5.3
8	7 (3+4)	3.7	4.1	10.2	6.1
9	7 (3+4)	42.7	2.6	17.6	1.4
10	7 (3+4)	42.8	2.3	61.6	3.1
11	7 (4+3)	32.7	3.1	40.8	4.7
12	7 (4+3)	26.5	0.4	54.4	0.4
13	9 (4+5)	4.5	3.0	3.0	3.8
14	9 (4+5)	21.0	1.9	70.6	2.0
15	10 (5+5)	16.9	1.8	42.9	4.7
	Average	20.7	2.7	36.3	3.5
	SD	14.4	1.8	20.4	2.4
	SE	3.9	0.5	5.5	0.6

Table 1: Baseline characteristics of matched samples from primary prostate cancer patients.

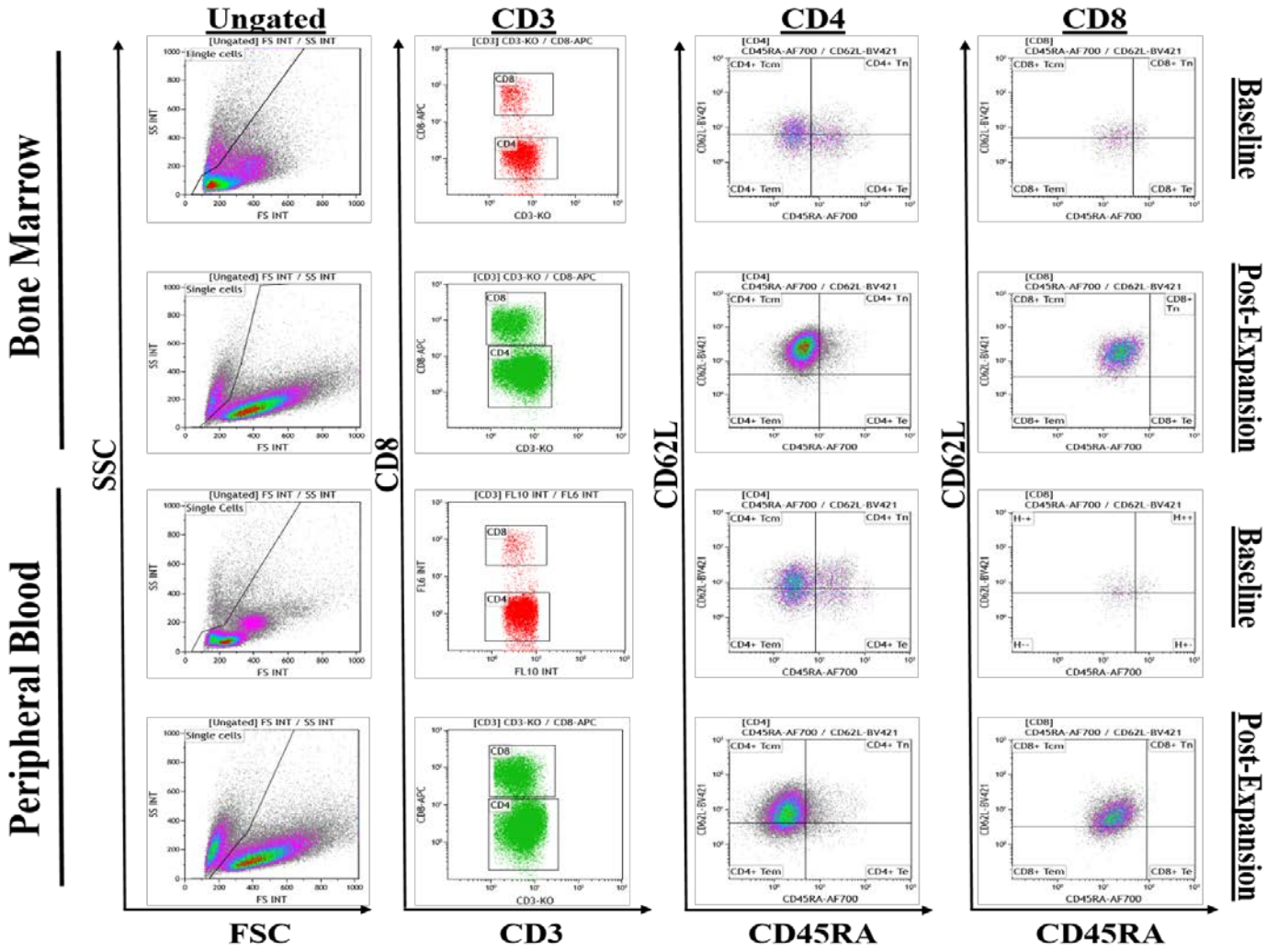

Figure 1: Representative plots and gating strategy to determine memory phenotypes in bone marrow and peripheral blood from primary prostate cancer patients at baseline and post-expansion.

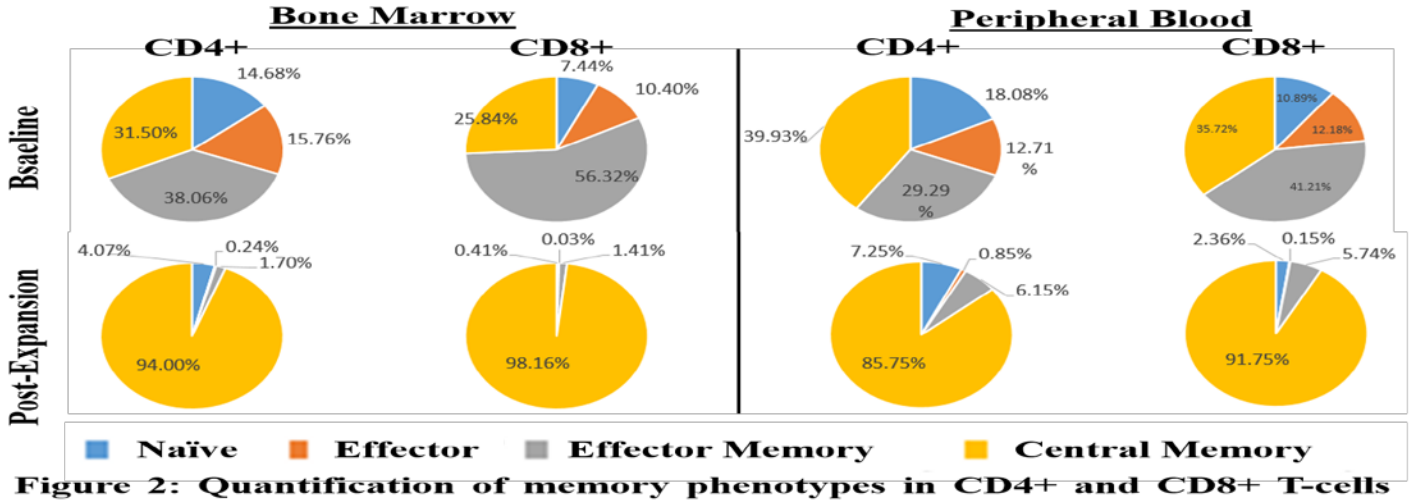

Figure 2: Quantification of memory phenotypes in CD4+ and CD8+ T-cells from bone marrow and peripheral blood from primary prostate cancer patients at baseline and post-expansion (baseline BM: n= 6; baseline PB: n = 7; post-expansion BM: n = 4; post-expansion PB: n = 3).

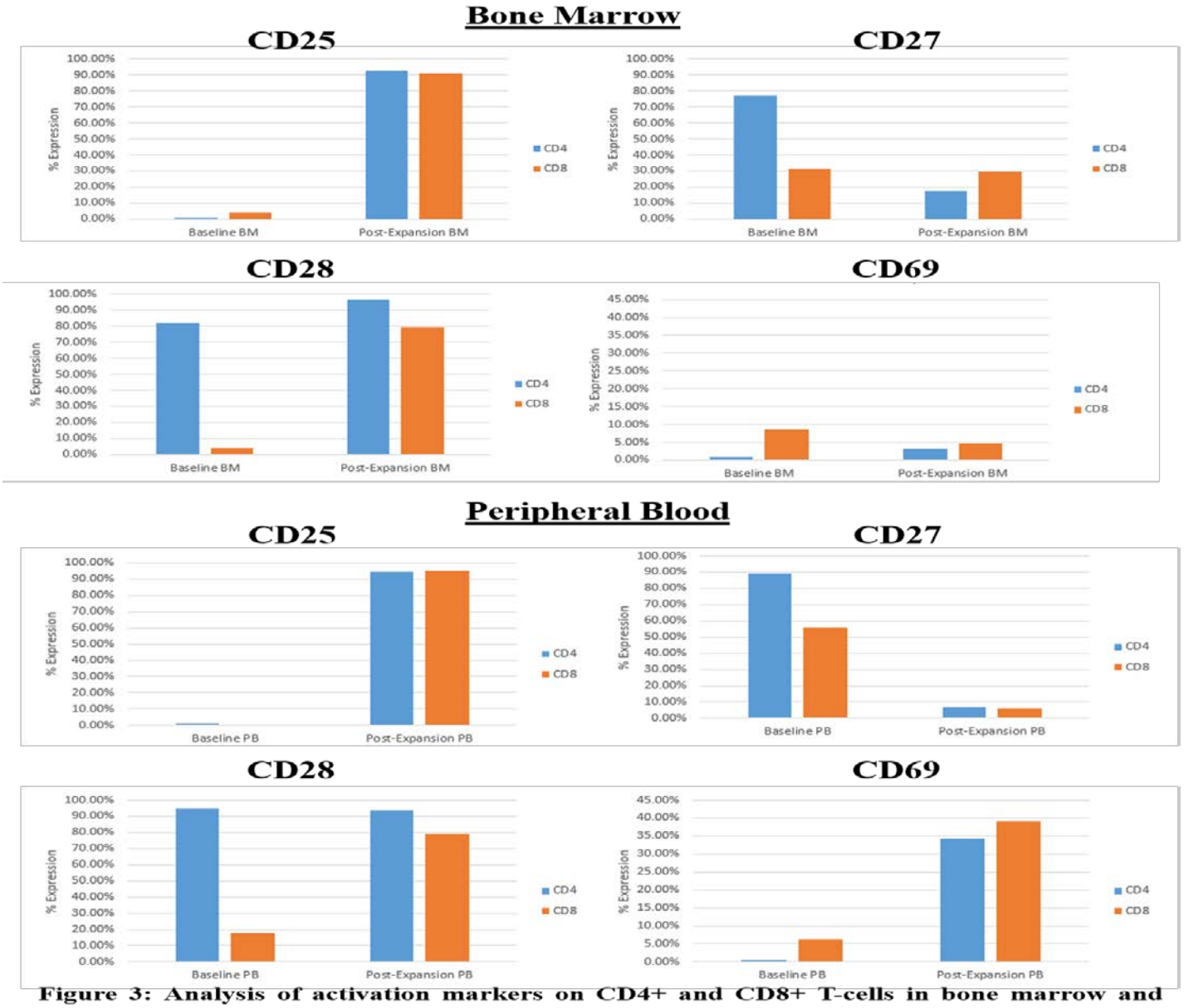

Figure 3: Analysis of activation markers on CD4+ and CD8+ T-cells in bone marrow and peripheral blood from primary prostate cancer patients at baseline and post-expansion (baseline BM: n = 4; baseline PB: n = 4; post-expansion BM: n =4; post-expansion PB: n = 3.

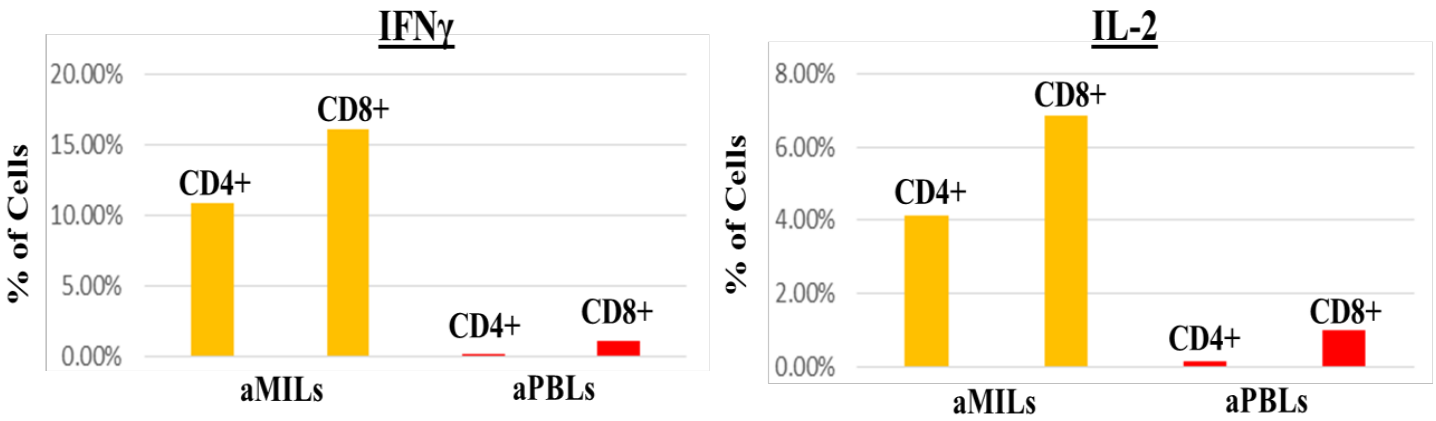

Figure 4: IFNγ and IL-2 expression in CD4+ and CD8+ activated MILs and PBLs from primary prostate cancer patients post-expansion (aMILs: n =4; aPBLs; n = 3).

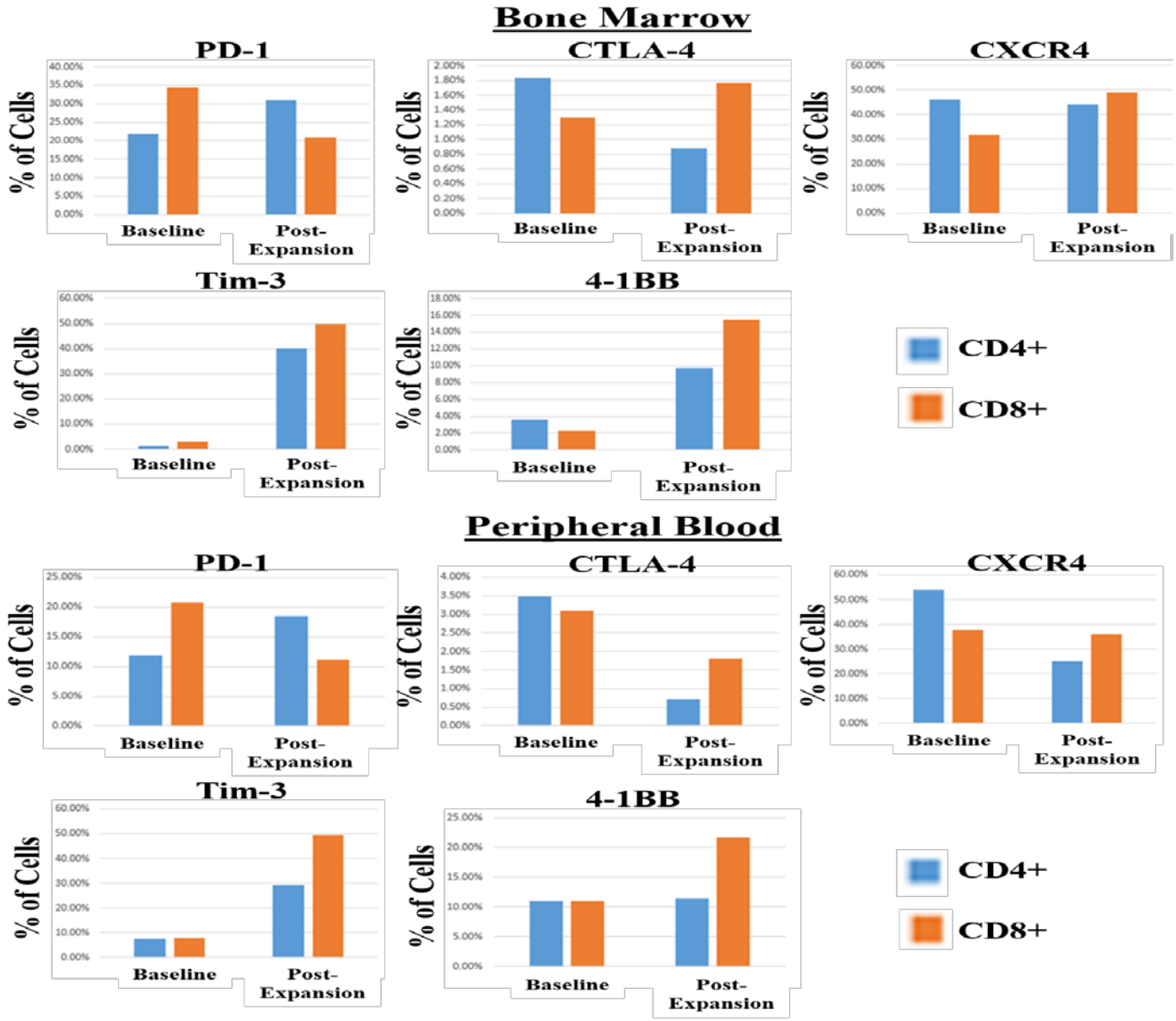

Figure 5: Immune checkpoint and CXCR4 expression in CD4+ and CD8+ T-cells from bone marrow and peripheral blood of prostate cancer patients at baseline and post-expansion (baseline BM: n = 6; baseline PB: n = 6; post-expansion BM: n = 5; post-expansion PB: n = 3).

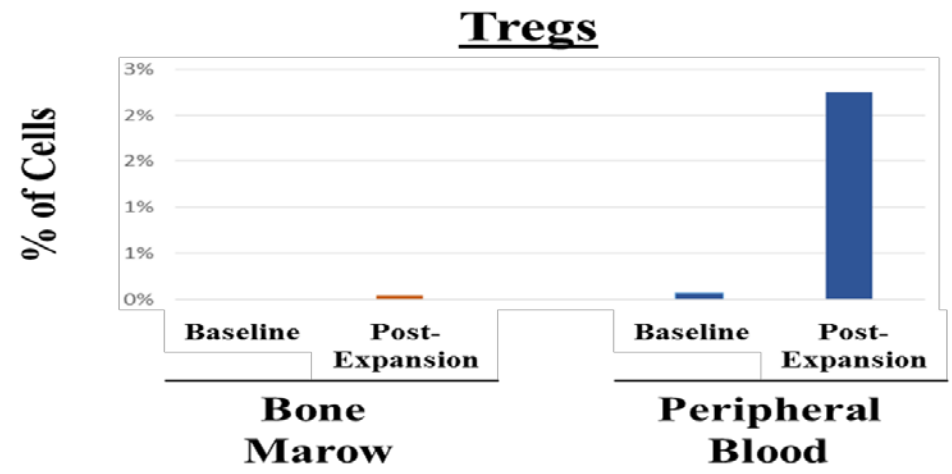

Figure 6: Percentage of Tregs in bone marrow and peripheral blood from primary prostate cancer patients at baseline and post-expansion (baseline BM: n = 4; baseline PB: n = 4; post-expansion BM: n = 3; post-expansion PB: n = 3).

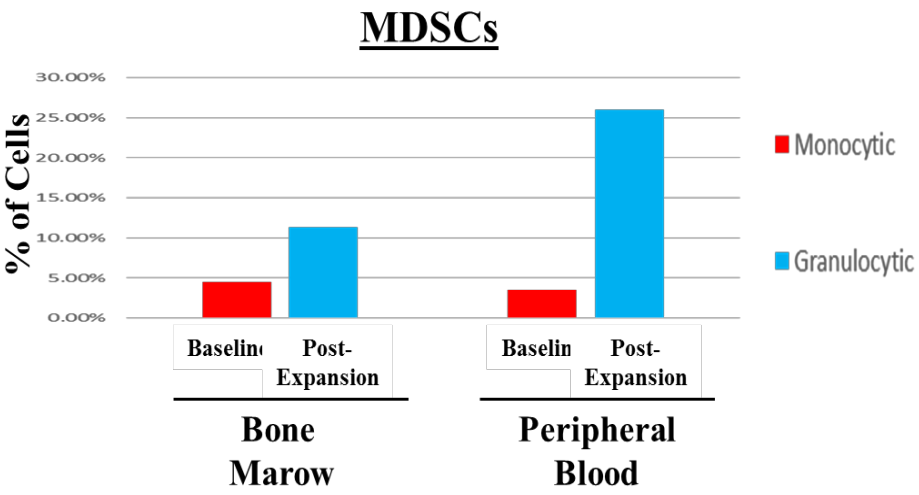

Figure 7: Percentage of granulocytic and monocytic MDSCs in bone marrow and peripheral blood from primary prostate cancer patients at baseline (baseline BM: n = 5; baseline PB: n = 6).

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 grant supported additional training of a Sr. Research Specialist in the laboratory, Mrs. Lizamma Antony, in methods relevant for the study. This data was presented by the PI at a local (12th Annual Prostate Research Day,

Baltimore, MD) and a national meeting (11th Annual Prostate Cancer SPORE Program Retreat, Ft. Lauderdale, FL) in late 2017 and early 2018, respectively.

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.

Podium presentations at the meetings described above

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.

We will continue to pursue our original objectives. We plan to collect additional samples for analysis from primary prostate cancer patients, particularly higher Gleason Scores (i.e. ≥8), in addition to initiating collection of samples from metastatic patients. Assessment of tumor-specific proliferative and cytotoxic responses will be performed on samples collected from all patient groups. Cytokine profiling will be performed once all samples are collected. We will also begin to prepare for *in vivo* experiments evaluating anti-tumor efficacy of adoptively transferred activated MILs vs. PBLs in preclinical models of prostate cancer.

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?
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:*

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.

Nothing to report

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.

Identifying a research coordinator to discuss study participation and consent with patients from the medical oncology in an effort to minimize disruption to the clinical workflow, in addition to adding her to the appropriate IRB-approved protocols and getting the dialogue script approved took longer than anticipated. However, these hurdles have been overcome, all relevant documents and individuals have been added and approved by the IRB, and identification of potential metastatic prostate cancer patients has resumed. Consequently, collection of samples needed for Aim 2 should now accelerate for completion of the studies as proposed.

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.

None

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).*

  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".

Name:	*W. Nathaniel Brennen*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9807-7433*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Brennen is the PI who has designed and oversighted all of the work described in the proposal.*
Name:	*Lizamma Antony*
Project Role:	*Sr. Research Coordinator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*4*

Contribution to Project: *Mrs. Antony isolated mononuclear cells from bone marrow and peripheral blood and performed the flow cytometric analyses pre- and post-expansion.*

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.*

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.*

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.*