

AWARD NUMBER: W81XWH-13-1-0204

TITLE: Chemokine Receptor Signatures in Allogeneic Stem Cell Transplantation

PRINCIPAL INVESTIGATOR: Ran Reshef, MD

CONTRACTING ORGANIZATION: ~~Vitro Cell Culture and Transplantation Laboratory, Walter Reed Army Institute of Research, Silver Spring, MD 20910-5012~~
~~P.O. Box 5012, Fort Detrick, MD 21722-5012~~

REPORT DATE: ~~10~~ * ~~2014~~

TYPE OF REPORT: Annual Progress Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE August 2014		2. REPORT TYPE Annual		3. DATES COVERED 01Aug2013 – 31July2014	
4. TITLE AND SUBTITLE Chemokine Receptor Signatures in Allogeneic Stem Cell Transplantation				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-13-1-0204	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Ran Reshef, MD E-Mail: ran.reshef@uphs.upenn.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Trustees of the University of Pennsylvania Philadelphia, PA 19104-6205				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this award is to define chemokine receptor signatures that contribute to lymphocyte trafficking into target organs in human graft-versus-host disease (GHVD). We use T-cell receptor deep sequencing to characterize the repertoire of effector T-cells in allogeneic hematopoietic stem-cell transplant (HSCT) recipients and identify the role of chemokine receptors in effector cell infiltration of target organs. In the recent funding period we studied blood and tissue samples from several allogeneic HSCT recipients and found that the clonal repertoire of CCR5+ T-cells was distinct from CCR5- T-cells, implying that CCR5 is a close-specific marker and not a general activation marker. We further characterized the clonal diversity and found that the asymmetry in clonal diversity of CCR5+ and CCR5- T-cells is characterized by specific Vgene usage. We are currently verifying this finding in additional patients and characterizing the presence CCR5+ clones in target organs of GVHD.					
15. SUBJECT TERMS CCR5, Chemokines, graft-versus-host disease					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (include area code)
Unclassified	Unclassified	Unclassified	Unclassified	9	USAMRMC

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Overall Project Summary.....	5
4. Key Research Accomplishments.....	7
5. Conclusion.....	7
6. Publications, Abstracts, and Presentations.....	7
7. Inventions, Patents and Licenses.....	7
8. Reportable Outcomes.....	7
9. Other Achievements.....	8
10. References.....	8
11. Appendices.....	8

Introduction

The purpose of this study is to characterize chemokine receptor signatures on effector T-cells that govern immune responses after allogeneic hematopoietic stem-cell transplantation (HSCT) in humans. Control of donor T-cells recruitment into target organs is a potentially effective strategy to reduce graft-versus-host disease (GVHD) after allogeneic HSCT without compromising the graft-versus-leukemia (GVL) effect. Using deep sequencing of the T-cell receptor beta chain (TCRB), we aim to identify the role of specific chemokine receptors in the trafficking of effector T-cells that are responsible for the GVH and GVL responses.

Keywords

Chemokine receptor

Chemokine

CCR5

Graft-versus-host disease

Allogeneic stem-cell transplantation

Overall Project Summary

Task 1. Regulatory

We completed regulatory submission and received IRB approval of a protocol named “Study of the graft-versus-host and graft-versus-tumor responses after allogeneic stem-cell transplantation”. This protocol, which was recently re-approved by the IRB, provides access to tissue biopsies and bone marrow biopsies from patients who underwent allogeneic HSCT at the University of Pennsylvania in the past 15 years.

Task 2. Specimen Selection and Clinical Database

A research coordinator was hired for my lab for the purpose of coordinating biospecimen banking, annotating their information, and selecting appropriate material for this and other project. This facilitates the identification of appropriate tissue biopsies and blood samples for TCR sequencing. This research coordinator is fully supported by our Cancer Center, recognizing the importance of biospecimen banking for this research project. We have completed the identification of appropriate patient samples for Aim 1 and Aim 2 using specific criteria, primarily referring to the availability of tissue biopsies with sufficient quantity from at least one GVHD organ in tandem with a peripheral blood mononuclear cell (PBMC) sample that was cryo-preserved. Details about sample availability for Aim 1 and Aim 2 are displayed in Table 1. We anticipate accumulating additional biopsy material for Aim 2 this year thanks to the rapid accrual to our phase II study using the CCR5 antagonist for GVHD prevention (<http://clinicaltrials.gov/show/NCT01785810>).

Table 1. Identification of appropriate biospecimens.

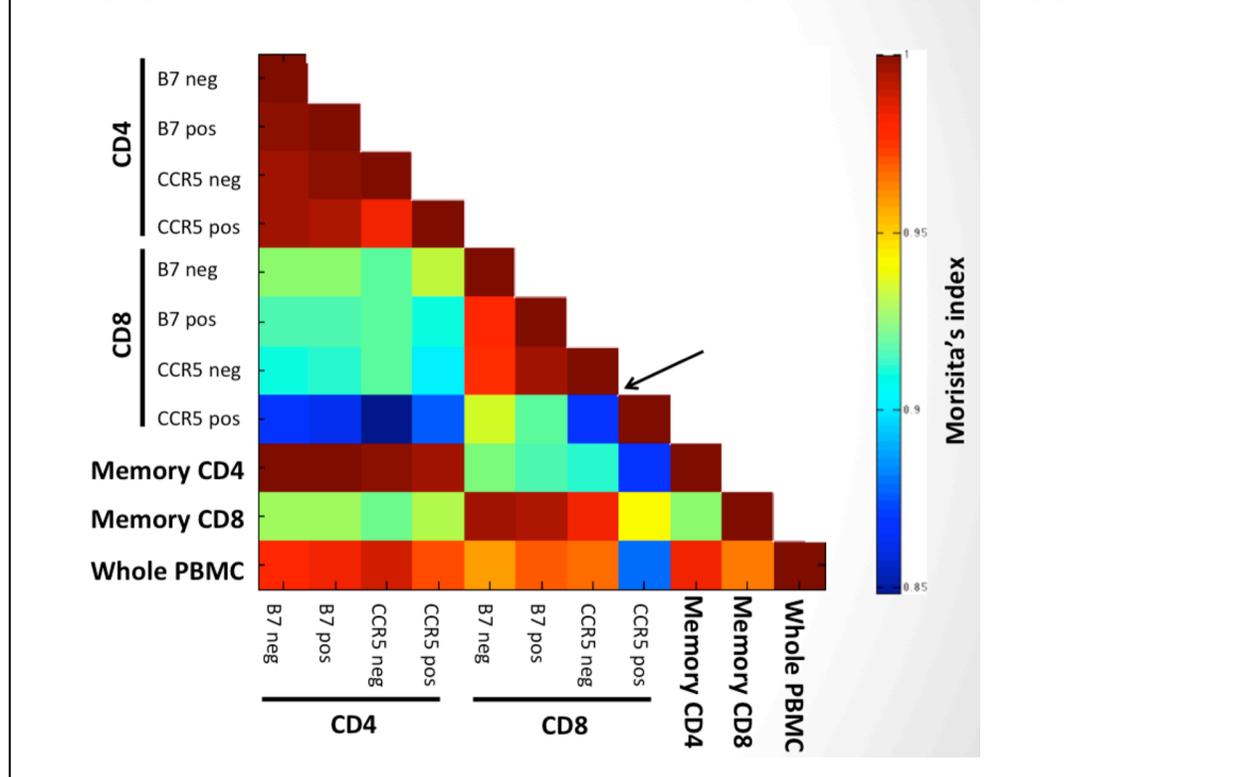
Aim	Patient cohort	PBMC + GVHD biopsy from 1 organ	PBMC + GVHD biopsy from 2 organs	PBMC + GVHD biopsy from 3 organs
Aim 1	Myeloablative HSCT	13	8	5
	Reduced-intensity HSCT	15	8	4
Aim 2	Patients treated with maraviroc on a clinical trial	5	3	2

Task 3-4. Determine the role of chemokine receptor expression in regulating the organ distribution of effector T-cells after allogeneic stem-cell transplantation (Aim 1).

To characterize the clonal diversity that correlates with specific chemokine receptor expression, we performed deep sequencing of TCRB on a PBMC sample from a patient 60 days following allogeneic HSCT. TCRB sequencing was conducted on whole PBMC and then on flow-sorted populations of memory CD4 and CD8 T-cells, CCR5-positive, CCR5-negative, integrin β 7-positive and integrin β 7-negative memory T-cells. We then compared the clonal repertoire of each subpopulation using Morisita’s Index, a statistical test that measures the overlap between diverse populations (Figure 1). A high Morisita index indicates significant similarity between populations and a low index indicates a great distance between populations. As expected, CD4 clones were different from CD8 clones and they were both similar to their parent population. However, CCR5+ and CCR5- clones had significantly less overlap within the CD8 compartment

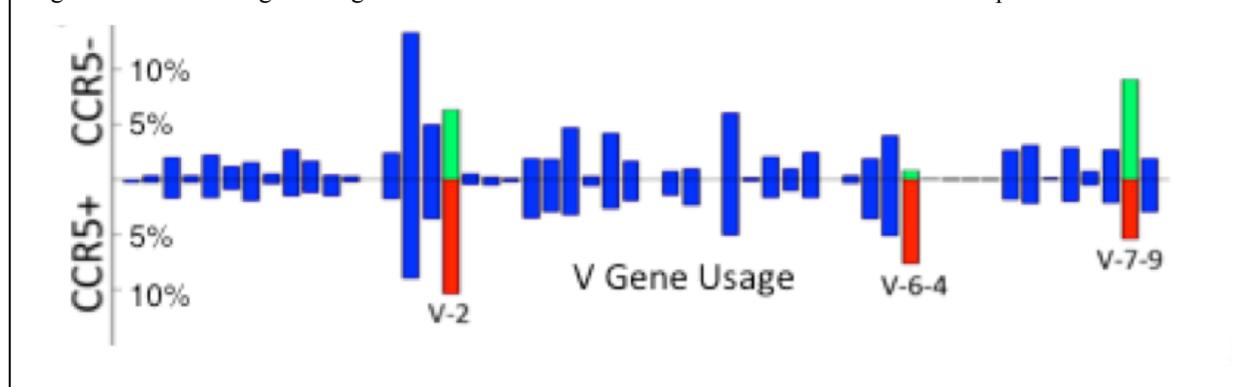
compared to the CD4 compartment (see arrow in Figure 1). In contrast, integrin $\beta 7$ -positive clones were still similar to integrin $\beta 7$ -negative clones in both the CD4 and CD8 compartments.

Figure 1. The repertoire of CD8⁺CCR5⁺ cells is uniquely biased. TCRB sequencing of flow-sorted T-cell populations demonstrate differences in the clonal diversity of CD8⁺ T-cell based on CCR5 expression. The heatmap represents the Morisita Index for comparison of the overlap between each paired cell populations.



We then analyzed the V region gene usage of T-cell clones in each population in order to identify the source for the bias in clonal diversity within the CD8⁺ cell population. We compared the frequency of V gene usage in CCR5⁺ and CCR5⁻ T-cell populations within the memory CD8 cell compartment (Figure 2). We found that the bias was driven by asymmetry in the usage of 3 V genes, not currently known to be associated with invariant T-cell populations. We are currently analyzing PBMC samples from additional allogeneic HSCT recipients in order to identify whether this clonal bias is universal and to correlate the patterns of V gene usage with clinical outcomes such as GVHD.

Figure 2. A bias in V gene usage is identified in CD8⁺ CD45R⁺ T-cells based on CCR5 expression.



Task 5-6. Determine the effect of targeted chemokine receptor blockade on trafficking patterns of T-cell clones (Aim 2).

We continue to make progress in biospecimen collection for Aim 2, primarily due to prospective collection of biospecimens on our phase II study of maraviroc for GVHD prevention. So far 22 patients were enrolled and treated on this clinical trial, which opened in April 2013. We anticipate completion of accrual of 37 patients by December 2013. Extensive biospecimen collection was built into this clinical protocol, including collection of fresh biopsy material from patients who experience symptoms of GVHD. So far 8 biopsies were collected and analyzed by flow cytometry. These samples are added to existing samples from our previously completed phase I/II study with maraviroc. TCRB sequencing will be conducted as part of the next steps of this project.

Task 7-8. Data publication and grant submission.

Preliminary data from this research project have been presented at grand rounds at Columbia University Medical Center and Memorial Sloan Kettering Cancer Center. We anticipate that data will mature into peer-reviewed publication and conference abstracts during the second year of the award.

Key Research Accomplishments

- Demonstration of differences in T-cell clonal diversity according to CCR5 expression, implying that the expression of CCR5 is linked to specific clones and not to global T-cell activation.
- Identification of specific V region genes in the TCRB gene that are biased towards CCR5 expression.

Conclusion

This study so far demonstrated that following allogeneic stem-cell transplantation, CCR5 expression on donor T-cells might be clonal, leading to a bias in clonal distribution and Vgene usage when comparing CCR5+ and CCR5- T-cell populations. This effect is seen in effector CD8 T-cells but not in effector CD4 T-cells. The next phase of this project is to identify whether CCR5-positivity is related to organ-specific trafficking and whether blocking CCR5 with a specific antagonist alters the clonal diversity of T-cells that infiltrate specific organs. Work to accomplish these goals is ongoing.

Publications, Abstracts and Presentations

Nothing to report.

Inventions, Patents and Licenses

Nothing to report.

Reportable Outcomes

Nothing to report.

Other Achievements

Nothing to report.

References

None

Appendices

Training and Professional Development

My goal during this award is to develop the knowledge and skills necessary to succeed as an independent physician-scientist. The focus of my work as a new investigator is modulation of cell trafficking to improve the outcomes of allogeneic HSCT, a logical extension to my past work that focused on GVHD. The following activities took place during the 1st year of the award:

Additional projects and collaborations. Preclinical studies revolving around chemokine receptor blockade in GVHD and immune reconstitution were initiated with my advisors Avinash Bhandoola and Taku Kambayashi, both R01-funded investigators with expertise in GvHD models and thymic function. These preliminary investigations will form the basis for my first R01 submission.

Grant Development. During the first award year I submitted 3 grant applications. Two grant applications were submitted to competitive grant mechanisms within our cancer center and the Penn Institute for Immunology. One of these grant application was funded - "The role of surface NKG2D expression by NK cells in the graft-versus-leukemia response". In addition I recently participated as a co-investigator in a R01 grant application entitled "EZH2-mediated epigenetic effects and alloimmunity", submitted by a close collaborator at Temple University. This grant is currently under review.

Training in tumor and transplant immunology. I continue to be a member of Dr. Robert Vonderheide's laboratory with increasing independence. Dr. Vonderheide and I meet weekly to review data, to discuss ongoing experiments and for didactic sessions. I currently supervise a student in the lab and provide guidance to additional post-graduate fellows. I participate in weekly meetings and other scientific activities by the Translational Research Program (TRP), led by Carl June, an internationally recognized leader in translational immunology.

Research conferences and meetings. In the past year I attended and presented abstracts at the Annual Meeting of the American Society of Clinical Oncology and the meeting of the American Society of Blood and Marrow Transplantation. In addition I attended the meeting the American Society of Hematology and presented various topics in meetings on Penn campus. I was invited to give grand rounds and scientific talks at Columbia University and Memorial Sloan Kettering Cancer Center during the past year.

Leadership roles. The Blood and Marrow Transplant Clinical Trials Network (BMT-CTN) has recently appointed me as co-chair of a multi-institutional trial that will test novel strategies in GvHD prevention (BMT-CTN 1203). During the 1st year of the award this multi-center clinical trial, which builds on findings directly related to this DOD project, was approved for funding by the NIH and is currently undergoing approval in 30 sites across the US. We anticipate that patients will be enrolled on this study starting in September 2014. My leadership position allows

me to take a leading role in the international community of SCT and GVHD experts. During the 1st award year, I expanded my leadership role with the BMT-CTN by becoming the local PI of another clinical trial related to GvHD (“BMT-CTN 1202 - Evaluation of Biomarkers Predicting Risk of Complications after Allogeneic SCT”) and by presenting a research proposal in the working committees of the Center for International Blood and Marrow Transplant Research (CIBMTR). These projects provide me with experience in global collaborative research.