AWARD NUMBER: W81XWH-15-1-0385

TITLE: Reprogramming the Metastatic Microenvironment to Combat Disease Recurrence

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REPORT DATE: October 2016

TYPE OF REPORT: Annual Report -1

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

DISTRIBUTION STATEMENT: Approved for Public Release;
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Reprogramming the Metastatic Microenvironment to Combat Disease Recurrence

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This research aims to “eliminate the mortality associated with metastatic breast cancer”. We will do so by: 1) testing a novel combination of clinically available therapeutic agents that modulate the metastatic tumor microenvironment for the prevention of metastatic disease and 2) using existing breast cancer patient samples to create a biomarker panel that combines disseminated tumor cell (DTC) classification and “immune-subtyping” to identify those patients most likely to benefit from this new treatment approach. In spite of recent advances in patient stratification and the use of targeted adjuvant therapies, 10-20% of patients with invasive tumors will eventually develop metastatic disease. These data indicate that for a significant percentage of patients, adjuvant therapies are ineffective at eliminating DTCs, which give rise to life threatening metastatic lesions. Therefore, the development of new clinical approaches that are effective at preventing metastatic breast cancer (BC) is of paramount importance. One promising approach is to “reprogram” the tissue microenvironments that provide “safe harbor” for DTCs during adjuvant therapy. Bone is one such metastatic “safe harbor.” Sixty-seven percent of metastatic patients will develop disease in the bone, which has been proposed to be a reservoir of malignant cells destined for subsequent metastatic relapse in the bone and visceral organs. Interactions between DTCs and the bone microenvironment lead to pathological bone loss, which can stimulate tumor cell outgrowth. In addition to contributing to morbidity, this ‘vicious cycle’ also protects tumor cells from the cytotoxic effects of chemotherapy. This protective microenvironment may also create an immune privileged site that prevents immune surveillance and effective immunotherapy. To better target bone metastasis with immunotherapy, we have been examining how T cell immune surveillance and antigen presentation affect metastatic relapse in the bone. In so doing, we discovered that inhibiting colony stimulating factor-1 receptor (CSF1R), which blocks pathologic activities of osteoclasts (OCs), serendipitously also increases antigen presentation by CD103+ dendritic cells (DCs).

Nothing listed

Unclassified  Unclassified  Unclassified

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1. Introduction.

This research aims to “eliminate the mortality associated with metastatic breast cancer”. We will do so by: 1) testing a novel combination of clinically available therapeutic agents that modulate the metastatic tumor microenvironment for the prevention of metastatic disease and 2) using existing breast cancer patient samples to create a biomarker panel that combines disseminated tumor cell (DTC) classification and “immune-subtyping” to identify those patients most likely to benefit from this new treatment approach.

In spite of recent advances in patient stratification and the use of targeted adjuvant therapies, 10-20% of patients with invasive tumors will eventually develop metastatic disease. These data indicate that for a significant percentage of patients, adjuvant therapies are ineffective at eliminating DTCs, which give rise to life threatening metastatic lesions. Therefore, the development of new clinical approaches that are effective at preventing metastatic breast cancer (BC) is of paramount importance. One promising approach is to “reprogram” the tissue microenvironments that provide “safe harbor” for DTCs during adjuvant therapy. Bone is one such metastatic “safe harbor.” Sixty-seven percent of metastatic patients will develop disease in the bone, which has been proposed to be a reservoir of malignant cells destined for subsequent metastatic relapse in the bone and visceral organs. Interactions between DTCs and the bone microenvironment lead to pathological bone loss, which can stimulate tumor cell outgrowth. In addition to contributing to morbidity, this ‘vicious cycle’ also protects tumor cells from the cytotoxic effects of chemotherapy. This protective microenvironment may also create an immune privileged site that prevents immune surveillance and effective immunotherapy. To better target bone metastasis with immunotherapy, we have been examining how T cell immune surveillance and antigen presentation affect metastatic relapse in the bone. In so doing, we discovered that inhibiting colony stimulating factor-1 receptor (CSF1R), which blocks pathologic activities of osteoclasts (OCs), serendipitously also increases antigen presentation by CD103+ dendritic cells (DCs). We hypothesize that thru these activities CSF1R blockade can destroy the “safe harbor” within the BM, leaving DTCs sensitive to attack by immunotherapy. We will test this using animal models and human clinical samples.

A. The tumor immune microenvironment regulates outcomes. The exact composition of immune cells in primary breast tumors is a significant predictor of chemotherapeutic responsiveness and recurrence-free survival. In particular, the presence and activation status of tumor specific T lymphocytes is strongly associated with better clinical outcomes. Unfortunately for many patients, this is not the dominant immunologic response. Instead many patients have significant numbers of tumor-infiltrating innate immune cells including macrophages and immature dendritic cells. One critical regulator of the pro-tumor activity of myeloid cells is signaling through the receptor tyrosine kinase CSF1R. In primary mammary tumors, CSF1R signaling is critical for the recruitment, survival, and pro-tumor activities of monocytes and macrophages. Previously, we demonstrated that inhibition of CSF1R improves the efficacy of neoadjuvant chemotherapy murine mammary tumor models. While CSF1R inhibition decreased the number of tumor-associated macrophages (TAMs), surprisingly it also restored anti-tumor T cell activity. This increase in CD8+ cytotoxic T lymphocytes (CTLs) was necessary for CSF1R blockade to enhance chemotherapeutic efficacy and restrain metastasis. These provocative findings led our pharmaceutical collaborators (Plexxikon, letter attached) to launch a phase I clinical trial of CSF1R inhibition in combination with chemotherapy in locally recurrent breast cancer (NCT01525602).

B. Targeting CSF1R signaling improves immunotherapeutic response and augments CD103+ DCs. Recently, in pancreatic cancer models, we showed that CSF1R blockade improves immunotherapeutic efficacy in part by increasing antigen presentation by CD11blowCD103+ DCs.
and not shown). The enhancement of CD103+ DCs by CSF1R blockade is a novel finding and of critical importance to tumor immunity. This DC subset is critical for sustaining T cell-mediated immune surveillance by antigen cross-presentation to CD8+ T cells in breast tumors.

2. Keywords:
metastatic breast cancer, immune surveillance, bone, dendritic cells

3. Accomplishments:
3A. Accomplishment on the major goals
(Please note all data figures are attached to the end of this document.)
Aim 1. Major Task 1. Determine the efficacy of CSF1R inhibition in combination with chemotherapy and immunotherapy in mouse models of established metastatic disease: Our assessments of bone metastatic models suggest that neutralization of CSF1R can modestly improve responses to chemo or checkpoint immunotherapy (Figure 1A-C). These data while suggesting that neutralizing CSF1R could be an effective approach, but also suggest other factors may compensate for CSF1R neutralization to enforce immune suppression. To further these studies we will investigate these factors as well (see below)

Aim 1. Major Task 2 Determine if CSF1R blockade improves T cell responses in bone metastases by impacting DC subsets. Our investigation in this area found that 1) mature CD24+ and SIRPα+ dendritic cells(DCs) number are dramatically down-regulated in the bone marrow of tumor bearing and bone metastatic mammary tumor models (Figure 1D-F). We have also discovered that this down-regulation of DCs is likely due to a change in the differentiation of these cells in the bone marrow. These data were demonstrated using adoptive transfer of DC-progenitor cells in tumor bearing and tumor naïve mice (Figure 1H). However, our assessment of neutralization of CSF1R effect on DC subsets determined that CSF1R signaling while increasing DC numbers in the bone marrow of tumor naïve mice, is unable alone to improve CD24+ DC numbers in tumor bearing mice (Figure 1G).

Aim 2. Major Task 1
We have collected and analyzed a total of 35 bone-marrow samples and matched blood samples from human breast cancer patients. We have also immune profiled the majority of these samples by high density FACS and compared them to samples from “healthy donors”. Our findings are as follows: 1) Several populations of dendritic cells and their pre-cursors are down regulated in cancer bearing patients compared to normal controls (Figure 2A-B). 2) The relative number of bone marrow CD141 DCs in the bone marrow of patients correlates with increased response to neo-adjuvant chemotherapy and decreased metastatic recurrence (Figure 2C). 3) Circulating numbers of DC progenitors are significantly down-regulated in breast cancer patients blood (Figure 2D). Going forward we are continuing to collect data on these cohorts and access the impact on patient outcome.

Aim 2. Major Task 2
This task is slated for year 2

3B. Dissemination of these findings.
These findings were presented as a poster at the Keystone Symposium on “Cancer Pathophysiology: Integrating the Host and Tumor Environments (C3)” in Breckenridge, Colorado March 28th. A publication submission is planned for 2017.
4. Impact
“Nothing to report.” This is year 1.

5. Changes/Problems

5A. Changes in approach, additional approaches proposed based on new data:
We are expanding Aim1A and Aim1B to include the neutralization of signaling through CSF2/CSF2R. This was originally proposed as an alternative but likely plays a significant role in DC development and immunotherapy response. We will evaluate this in addition to current CSF1R work.

No changes to human, vertebrate animal, or biohazard compliances.

6. Products
“Nothing to report.” This is year 1.

7. Participant and other collaborating organization

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8. Special Interest
“Nothing to report.”
A. Leg Bone Tumor Burden by BLI

B. Bone Tumor Burden

C. CD8+ CTLs

D. PyMT Mammary Tumor Model
Bone Marrow

E. MMTV-PyMT GEMM
Bone Marrow Analysis

F. PyMT B6 Blood

G. CSF1R blockade

H. Lin-Sca1-cKit+ Myeloid Progenitors Adoptive Transfer

Bone Marrow

Lymph Node

Spleen

Blood

Non-Tumor Bearing Host  Tumor Bearing Host

Non-Tumor Bearing

PyMT B6

MDPs

CDPs

Pre-DCs

DC1

Granulocytes

Monocytes

Granulocytes

CD103+ DCs

Resident CD8+ DCs

Inflammatory Monocytes

Granulocytes

Progenitors

Dendritic Cells

Pre-DCs

DC1s

Resident CD8+ DCs

Inflammtory Monocytes

Granulocytes
A. Human Breast Cancer Bone Marrow

- Normal
- Breast Cancer
- Pancreatic Cancer

CD45+Lin-CD135+HLADR+

Control Breast Cancer

B. Bone Marrow

CD45+Lin-CD135+HLADR+

Control

Breast Cancer

C. Breast Cancer Bone Marrow

- CD141+ DCs
- CD1c+ DCs

D. Breast Cancer and Pancreatic Cancer Blood

- Pre-DCs

No pCR

pCR

No Recurrence

Recurrence

* p=0.1426