Altered Myeloid Memory Function by BMP Signaling in Breast Cancer Bone Metastases

Claire L. Ihle[1], Desiree M. Straign[1], Johana A. Canari[2], Kathleen C. Torkko[1], Kathryn L. Zolman[1], E. Erin Smith[1], Philip Owens[1, 3]

[1]Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora CO 80045; [2]Case Western Reserve University, Cleveland OH 44106;
[3]Research Service, Department of Veterans Affairs, Denver CO Station 554;

Abstract 1448; Correspondence: claire.ihle@cuanschutz.edu

University of Colorado Anschutz Medical Campus

Abstract

Background: Metastatic breast cancer (mBC) patients exhibit a 5-year survival rate of only 29% and metastasis most commonly occurs in the bone. Myeloid progenitors in the bone marrow can undergo trained innate immune responses, resulting in long-lasting inflammatory myeloid memory. Myeloid memory has been shown to promote anti-tumorigenic myeloid functions in the tumor microenvironment (TME). The TME in bone includes unique myeloid cells which are dynamically regulated by bone morphogenetic proteins (BMPs). We investigated myeloid memory in the context of BMP signaling in the mBC bone TME. Identifying the driver of myeloid functions distinct to mBC bone metastases will advance the understanding of potential immunotherapies to overcome incurable mBC bone lesions.

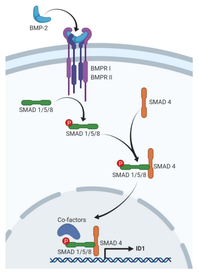
Methods: A cohort of human mBC bone metastases and matched patient primary tumors and bone metastases were assembled and profiled. Gene expression analysis was performed on patient bone metastases and matched primary tumors using nCounter immune-oncology gene expression probes. Clinical bone metastasis regional protein expression was analyzed with GeoMx Digital Spatial Profiling (DSP). Single cell protein expression and spatial analysis was measured in matched patient primary tumors and bone metastases with Akoya Polaris panels. Syngeneic mouse models of MMTV-PyMT orthotopic tumors and intratibial bone metastases were treated with beta-glucan to induce trained innate immunity and/or LDN-193189-2HCl to inhibit BMP signaling. Mouse model readouts included tumor measurements, mBC progression by histology and circulating blood analysis, and myeloid memory function by circulating blood and Akoya Polaris analysis.

Results: Differential nCounter gene expression analysis of clinical mBC bone metastases revealed a subset of patient samples with a high myeloid gene signature. High myeloid gene signature patients exhibited enhanced inflammatory myeloid genes and prolonged overall survival. Regional DSP and single cell Polaris protein expression analysis of this patient cohort showed elevated myeloid cell infiltration and heterogeneity as well as myeloid memory phenotypes. Analysis of matched patient primary tumor and bone metastases with nCounter gene expression panels exhibited enhanced BMP signaling and myeloid cell infiltration in bone samples compared to primary tumors. Syngeneic mouse models of mBC showed beta-glucan induced myeloid memory did not alter tumor growth. mBC mouse models of bone metastases treated with beta-glucan and LDN-193189-2HCl revealed increased tumor growth and decreased secondary metastasis to the lungs. Bone marrow derived macrophages treated with beta-glucan, LPS, and LDN-193189-2HCl had significantly higher gene expression of the inflammatory cytokine IL-6.

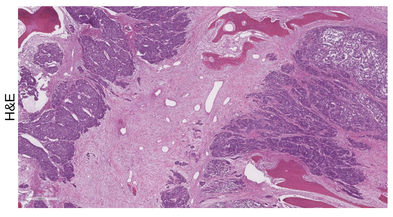
Conclusions: Distinct bone metastasis patients exhibited myeloid memory and BMP signaling which could allow for precision immunotherapy treatments to prevent myeloid suppression in mBC.

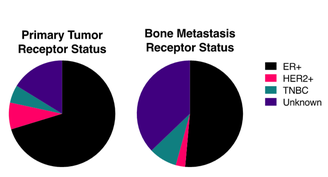
Bone Morphogenetic Protein Signaling in Myeloid Cells

- BMPs are regulators of differentiation for many cell types
  - Bone formation
  - HSC compartment
- BMPs exhibit context-dependent roles in cancer
  - Tumor promoters or suppressors
- BMPs impact myeloid phenotypes
  - BMP-2 promotes pro-inflammatory macrophages
  - BMP-4 promotes anti-inflammatory macrophages
- BMPR1a LysMCre cKO transgenic mouse model
  - BMP signaling loss restricts bone marrow myeloid progenitors
  - BMP signaling loss restricts prostate cancer flank tumor growth
  - BMPs signaling loss recruits macrophages to tumor and reprograms macrophages to be pro-inflammatory
  - For more information see: Ihle, CL et al, *Frontiers in Oncology*, 2020



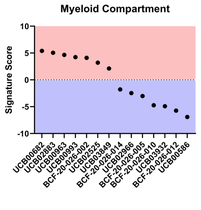
Breast Cancer Bone Metastasis Patient Cohort

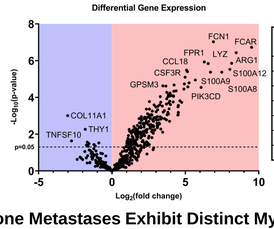




Investigating the Tumor Microenvironment in Breast Cancer Patient Bone Metastases. Hematoxylin and eosin staining of a breast cancer patient's bone metastasis demonstrates distinct features of lytic bone pathology, tumor cells, stromal cells, and immune cells. We have built a cohort of 47 metastatic bone archival FFPE samples from non-treatment naïve breast cancer patients at the University of Colorado Cancer Center. The majority of cases exhibit ER+ primary tumors and ER+ bone metastases. From this cohort, metastatic breast cancer patient bone samples were selected which had ER+ bone metastases and lytic bone pathology for analysis of their tumor microenvironment.

Myeloid Gene Expression in Bone Metastases

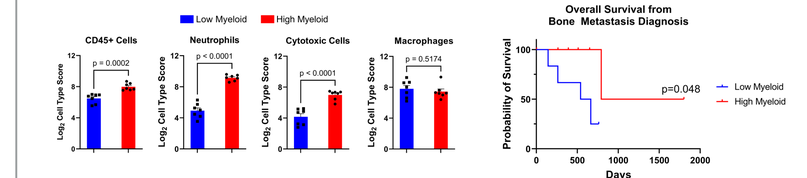




Gene Set	Global Significance Score
Epigenetic Regulation	3.397
Cytokine & Chemokine Signaling	3.333
JAK-STAT Signaling	3.173
Immune Cell Adhesion & Migration	3.043
Antigen Presentation	2.827
Metabolic Stress	2.779
TGF-beta Signaling	2.711

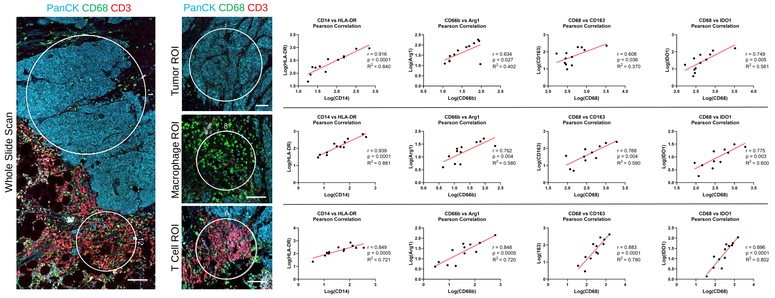
Breast Cancer Patient Bone Metastases Exhibit Distinct Myeloid Gene Signature. Gene expression was assessed from RNA isolated from 14 archival FFPE bone biopsies with the NanoString human Immune Oncology 360 gene expression panel. Distinct separation of bone samples based on high (n=7) and low (n=7) myeloid gene expression signatures was found. Differential gene expression analysis between high vs low myeloid gene signature patient samples revealed enriched myeloid functions genes in the high myeloid patient samples. Inflammatory gene pathways were upregulated in the high myeloid gene signature patient group.

Myeloid Gene Expression in Bone Metastases



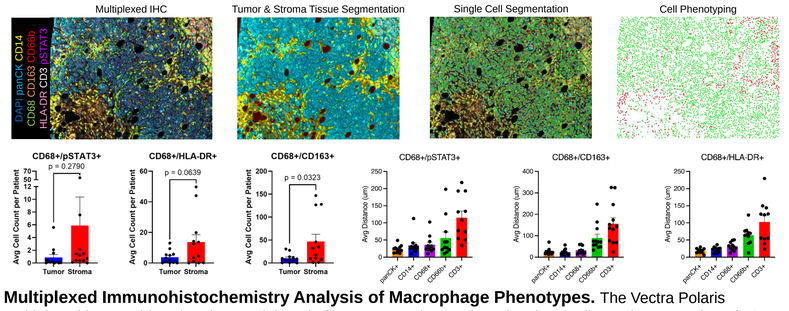
Myeloid Signature in Breast Cancer Bone Metastases Impacts Immune Phenotypes & Clinical Outcome. nCounter gene expression analysis of high vs low myeloid gene signature patients revealed altered immune cell gene signatures in the tumor microenvironment of bone metastases. High myeloid patients have significantly longer overall survival from the time of bone metastasis diagnosis.

Myeloid Cell Characterization by Digital Spatial Profiling



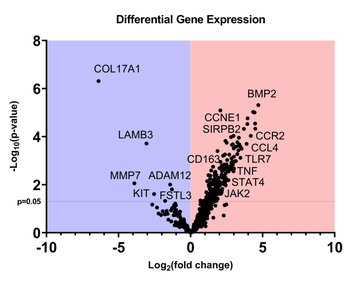
Breast Cancer Bone Metastases Have Spatially Distinct Myeloid Functions. NanoString GeoMx Digital Spatial Profiling was used to determine the distinct proteomic characteristics of the tumor, macrophage, and T cell regions of the tumor microenvironment in metastatic patient bone samples. Regions of interest (ROIs) were selected from 12 patient biopsies, with 4 tumor, macrophage, and T cell ROIs selected for each patient. Protein expression correlation analysis was performed for myeloid cell markers and functional phenotypes in the tumor cell ROIs, macrophage ROIs, and T cell ROIs.

Multiplexed Immunohistochemistry of Bone Metastases

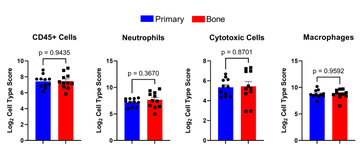


Multiplexed Immunohistochemistry Analysis of Macrophage Phenotypes. The Vectra Polaris multiplexed immunohistochemistry staining platform was used to analyze the singe cell protein expression of 12 breast cancer bone metastasis patient samples. InForm analysis allowed for tissue segmentation, single cell segmentation and cell phenotyping of the tumor microenvironment. Spatial analysis revealed macrophages are enriched in the stroma of bone metastases. Nearest neighbor analysis showed macrophages are closest to tumor cells, myeloid cells, and other macrophages, and farther from neutrophils and T cells.

Matched Primary & Bone Metastasis Gene Expression

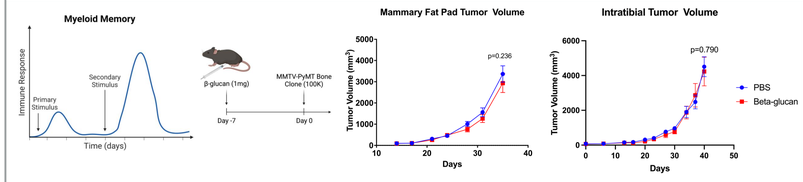


Gene Set	Global Significance Score
Cell Proliferation	2.898
DNA Damage Repair	2.801
Matrix Remodeling & Metastasis	2.637
TGF-beta Signaling	2.561
Myeloid Compartment	2.543
Angiogenesis	2.473
JAK-STAT Signaling	2.033



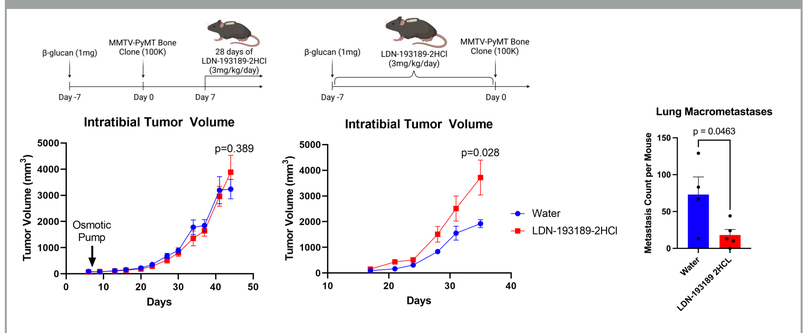
Myeloid Inflammatory and BMP Genes Enriched in Matched Bone Metastases. Gene expression was assessed from RNA isolated from 11 archival FFPE matched patient primary breast cancer tumors and bone metastases with the NanoString human Immune Oncology 360 gene expression panel. Differential gene expression analysis between primary tumor vs bone metastasis revealed enriched myeloid inflammatory function and BMP signaling genes in bone metastases. Inflammatory gene pathways were upregulated in the high myeloid gene signature patient group. Immune cell gene signature scores were unchanged in the primary vs bone metastasis gene expression analysis.

Mouse Models of Myeloid Memory & Breast Cancer



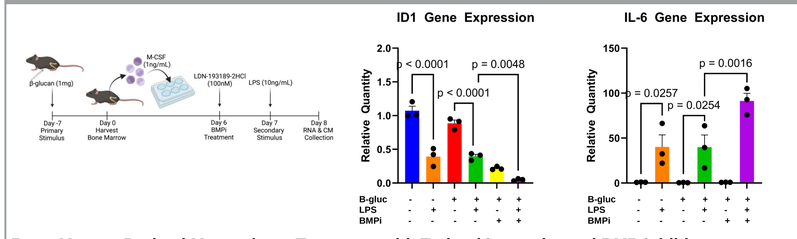
Myeloid Memory Does Not Restrict Mouse Mammary Carcinoma Primary Tumor and Bone Metastasis Growth. We investigated if myeloid memory restricts progression of a syngeneic MMTV-PyMT murine mammary carcinoma bone clone cell line in mammary fat pad primary tumors and intratibial bone metastases. Induction of trained immunity with an intraperitoneal injection of β-glucan or PBS was performed 7 days prior to orthotopic mammary fat pad or intratibial injection of syngeneic MMTV-PyMT Bone clone cells. Trained immunity treatment with β-glucan did not restrict the growth of mammary fat pad and intratibial tumors.

BMP Modulation of Myeloid Memory in Bone Metastases



BMP Inhibition During Myeloid Memory Training Promotes Tumor Growth But Restricts Secondary Metastasis. Mice underwent trained immunity with an intraperitoneal injection of β-glucan 7 days prior to receiving an intratibial injection of syngeneic MMTV-PyMT Bone clone cells. Mice were treated with the BMP inhibitor LDN-193189-2HCl or water control using osmotic pumps. Mice with 28-day delivery osmotic pumps with LDN-193189-2HCl implanted 7 days after intratibial bone metastasis formation did not have altered bone metastasis growth. Mice with 7-day delivery osmotic pumps with LDN-193189-2HCl implanted during β-glucan training had increased bone metastasis growth, but decreased secondary metastasis to the lungs.

BMP Modulation of Myeloid Memory in Bone Metastases



Bone Marrow Derived Macrophage Treatment with Trained Immunity and BMP Inhibitor. Mice underwent trained immunity with an intraperitoneal injection of β-glucan 7 days prior to collecting bone marrow and generating bone marrow derived macrophages (BMDM). 6 days after plating the BMDM, cells were treated with LDN-193189-2HCl or water control. On day 7 the cells were stimulated with LPS for 24hrs prior to collecting RNA and conditioned media. ID1 and IL-6 gene expression was analyzed by RT-qPCR.

Summary & Next Steps

Summary:

- Bone metastasis patients with inflammatory myeloid genes have longer overall survival
- Bone metastases are enriched for myeloid inflammatory genes & BMP signaling genes compared to primary tumors
- BMP inhibition during myeloid memory training promotes bone metastasis growth but limits secondary metastasis
- BMDM have decreased BMP signaling after LPS stimulation & increased IL-6 gene expression after LPS stimulation & BMP inhibition

Ongoing Experiments:

- Patient Samples: Vectra Polaris myeloid memory phenotype panel
- Mouse Models: BMPR1a LysMCre cKO, CA, & CTRL mice with trained immunity & bone metastases
- In Vitro: Trained BMDM conditioned media & cocultures with breast cancer spheres

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