Work in Progress:

Tumor-Immune Interactions in Triple Negative Breast Cancer Brain Metastases

- Triple Negative Breast Cancer
- Breast-to-Brain Metastases
  - TNBC
  - Leptomeningeal Disease (LMD)
- Proposed Project Aims
  - Rationale
  - Preliminary Data
  - Approach
- Current Work and Future Timeline

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SCIT T32 Seminar
Hayden Gephart and Plevritis Labs
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Breast Cancer

• 1 in 8 women in the U.S. will develop invasive breast cancer

• In 2018, an estimated 268,600 new cases (invasive) and 62,930 (non-invasive) breast cancer are expected to be diagnosed in women in the U.S., of which about 41,760 women are expected to die

  • In women under 45, breast cancer is most common in African-American women, and they are more likely to die of breast cancer

  • Currently more than 3.1 million women with a history of breast cancer in the U.S.

  • 85% of breast cancers occur in women who have no family history of breast cancer
Triple Negative Breast Cancer (TNBC)

- TNBC is a heterogeneous group of tumors simply defined by the absence of estrogen (ER) and progesterone (PR) hormone receptors, and lack of overexpression of epidermal growth factor receptor 2 (ErbB2/Her2) gene.

- TNBC account for **10-20%** of all invasive breast cancers.

- TNBC is associated with African-American race, younger age, higher tumor grade, and more advanced tumor stage at diagnosis.

- Chemotherapy is the **only** recommended systemic treatment, however **only 30%** of TNBC patients achieve pCR. Patients who do not have **6-fold** higher risk of relapse, and **12-fold** higher risk of death.

- Survival at 3 yrs is lower (68%) for metastatic TNBC patients compared to other metastatic breast cancer types (88%).
Triple Negative Breast Cancer (TNBC)
TNBC in African-American Women

- Women of African ancestry have a disproportionately higher frequency (up to 79%) of TNBC, compared to women of European ancestry
- TNBC frequency is consistently higher in women of African ancestry than any other racial/ethnic group

- In African-American women premenopausal status, increased parity (pregnancies), and shorter duration of breastfeeding are positively associated with increased risk of TNBC

- 5-year distant relapse-free survival is 62.8% for young black women, compared with 77% for young white women with equal access to health care (UK study)
Primary TNBC

overexpression
miR-127

reactivation of
LRIG1

TNBC Metastasis

Dissertation Research –
What molecular mechanisms and/or signaling pathways regulate TNBC cells in vitro and TNBC tumors in vivo?

Postdoctoral Research –
What molecular mechanisms drive shedding/dissemination, seeding, and outgrowth of TNBC metastases?
Breast Cancer Brain Metastasis

- Breast cancer brain metastasis (BCBM) occurs in **10-30% of metastatic breast cancer patients**
  - Second leading cause of brain metastases following lung cancer

- Incidence of BCBM continues to increase
  - Prolonged patient survival
  - Improved imaging techniques

- Median survival ranges from 2 – 25.3 months
  - **Few patients survive past 1 year**
  - Associated with serve neurological decline

![Before and After Surgical Resection](image)
- BCBM Incidence and Survival is breast cancer subtype dependent

- **Current treatment strategies:**
  - Surgical resection
  - Whole brain radiation therapy (WBRT)
  - Stereotactic Radiosurgery
  - Chemotherapy
  - Targeted therapies (HR+: Tamoxifen, HER2+: Trastuzamab)

- **Major challenge in treating BCBMs is the Blood-Brain-Barrier**

Although there are ongoing clinical trials, no FDA-approved systemic treatments for BCBM
Leptomeningeal Disease (LMD)

- LMD is defined as **tumor spread within the leptomeninges** and subarachnoid space
- 10% of patients with solid cancers present with LMD

- **Breast (TNBC), lung, and melanoma** are the most common primary tumor sites in LMD patients

- **LMD survival** is extremely poor
  - Lung: 3 - 6 months
  - Breast: 3.5 - 4.4 months
  - Melanoma: 1.7 - 2.5 months

- **Therapeutic strategies** include intrathecal therapy (spinal canal and subarachnoid space to reach CSF), systemic therapy, and radiotherapy (WBRT)

- To date, there have been only 6 randomized clinical trails specifically on treatment of LMD

- Understanding the molecular mechanisms that drive **TNBC brain/LMD metastasis (seed – primary TNBC and soil – normal brain microenvironment)** pose an unmet clinical need
“The Birth” of the Project

Angelo et al, 2014, Nature Medicine
Project Hypothesis

The spatial architecture of the tumor microenvironment reflects distinct tumor-immune interactions; these interactions prime systemic immune tolerance of disseminated tumor cells, enabling brain-specific metastases.
AIM 1: DETERMINE THE EXTENT TO WHICH THE STRUCTURED MICROENVIRONMENT CORRELATES WITH PATIENT OUTCOMES BY GENERATING A TUMOR-IMMUNE SPATIAL MAP OF TNBC BRAIN METASTASES.

RATIONALE:

1. Immune infiltration is associated with patient survival in specifically in TNBC subtype

2. Angelo Lab – Immune landscape of 41 primary TNBCs using MIBI

3. The brain was previously thought to be an “immune-privileged” space so there has been little interrogation of the immune landscape of TNBC brain metastases

Keren et al, 2018, Cell
AIM 1: DETERMINE THE EXTENT TO WHICH THE STRUCTURED MICROENVIRONMENT CORRELATES WITH PATIENT OUTCOMES BY GENERATING A TUMOR-IMMUNE SPATIAL MAP OF TNBC BRAIN METASTASES.

PRELIMINARY DATA:

1. Presence of infiltrating immune cells in a mouse model of human TNBC brain metastases

2. Astrocytes increase the production of glial fibrillary acidic protein (GFAP) in the presence of TNBC leptomeningeal disease
AIM 1: DETERMINE THE EXTENT TO WHICH THE STRUCTURED MICROENVIRONMENT CORRELATES WITH PATIENT OUTCOMES BY GENERATING A TUMOR-IMMUNE SPATIAL MAP OF TNBC BRAIN METASTASES.

APPROACH

A. Construct an in-situ subcellular protein spatial map of the TNBC brain metastases microenvironment using MIBI on archival FFPE tissue samples.

B. Quantitate the composition and spatial architecture of the tumor-immune microenvironment using a validated image analysis pipeline.

C. Assess the extent to which the composition and spatial architecture correlates with CNS disease progression, the likelihood of LMD development, and patient survival.
A. Protein **spatial map of the TNBC brain metastases microenvironment**

*Tumor-Immune Panel Keren et al, 2018, Cell*

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<th><strong>Tumor</strong></th>
<th><strong>Immune Cell Types</strong></th>
<th><strong>Antigen Presentation</strong></th>
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<tr>
<td>β-Catenin</td>
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</tr>
<tr>
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<tr>
<td>p53</td>
<td>CD16</td>
<td>MPO</td>
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| **Stroma**         |                       |                          |
| CD31               | CD138                 |                          |
| SMA                | CD16                  |                          |
| Vimentin           | CD56                  |                          |

| **Cell Status**    |                       |                          |
| dsDNA              | CD15                  |                          |
| H3K27me3           | CD133                 |                          |
| Ki-67              | CD15                  |                          |
| H3K9ac             | CD16                  |                          |
| pS6                | CD63                  |                          |

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AIM 2: IDENTIFY TUMOR-IMMUNE RECEPTOR-LIGAND PAIRS BY GENERATING A TRANSCRIPTOMIC PROFILE OF TNBC BRAIN METASTASES, AND DETERMINE IF THESE INTERACTIONS CORRELATE WITH TUMOR-IMMUNE SPATIAL ARCHITECTURE.

RATIONALE:

1. MIBI panel is highly focused – unbiased approach to identify tumor-immune interactions (receptor-ligand pairs), which can be then be assessed by MIBI or traditional IHC


PRELIMINARY DATA:

1. Assessed a few validated tumor-immune receptor-ligand pairs in GBMseq.org

![PDCD1 (PD-1) Log2 Counts Per Million](chart.png)
AIM 2: IDENTIFY TUMOR-IMMUNE RECEPTOR-LIGAND PAIRS BY GENERATING A TRANSCRIPTOMIC PROFILE OF TNBC BRAIN METASTASES, AND DETERMINE IF THESE INTERACTIONS CORRELATE WITH TUMOR-IMMUNE SPATIAL ARCHITECTURE.

**APPROACH**

A. **Build RNA expression profiles of TNBC brain metastases** (and healthy brain) using **single-cell RNA-sequencing**

B. Identify co-expression of genes that encode **receptor-ligand pairs** in tumor and immune cell populations using biocomputational approaches.

C. Assess the extent to which **receptor-ligand pairs** correlate with tumor-immune spatial architecture.
**AIM 3: DETERMINE IF TUMOR-IMMUNE INTERACTIONS IN PRIMARY TNBC PRIME TOLERANCE OF DISSEMINATED CELLS ENABLING METASTASES, AND DEFINE IF INTERACTIONS CORRELATE WITH RACE.**

**RATIONALE:**

1. Enk et al. – Altered function of dendritic cells in progressing versus regressing melanoma metastases. Hypothesized that this **tolerance was a result of dendritic cells co-opted by the tumor**, which possessed the ability to **migrate from the primary tumor to the regional lymphatic organs**.

   Suggests that the immune landscape of the primary tumor could contribute to systemic immune tolerance, enabling metastatic outgrowth
AIM 3: DETERMINE IF TUMOR-IMMUNE INTERACTIONS IN PRIMARY TNBC PRIME TOLERANCE OF DISSEMINATED CELLS ENABLING METASTASES, AND DEFINE IF INTERACTIONS CORRELATE WITH RACE.

APPROACH

A. Visualize the tumor-immune landscape in primary TNBC tumors using MIBI, and assess the extent to which it correlates with brain metastases and/or LMD development.

B. Identify tumor-immune interactions that are differentially expressed between patients of differing racial backgrounds
   **Racial disparity in primary TNBC**
   **Studies (limited) have identified differences in immune response based on patient race**

C. Measure expression of relevant targets in human cerebrospinal fluid (CSF).
   **CSF can detect changes in brain tumors**
   **Patient CSF can easily be collected/stored**
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Thank you for your attention!

Questions?