Tumor-Immune Interactions in Triple Negative Breast Cancer Brain Metastases

Work in Progress: Building Our Patient Cohort

- Triple Negative Breast Cancer
- Breast-to-Brain Metastases
  - TNBC
  - Leptomeningeal Disease (LMD)
- High-level Overview of the Project
  - Rationale
  - Patient Cohort
- Next Steps
- Future Directions

Maxine Umeh-Garcia, PhD, MSc.
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 2021, an estimated 281,550 new cases (invasive) and 42,290 (non-invasive) breast cancer are expected to be diagnosed in women in the U.S., of which about 43,600 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 3.8 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.

- **But what about socioeconomic factors?**
  - Incidence and patient outcomes have historically been ascribed to socioeconomic factors, particularly lack of access to healthcare and screenings, and distrust of medical professionals.

- 5-yr distant relapse-free survival is 62.8% for young black women, vs. 77% for young white women with equal access to health care.

- Black women exhibit a significantly higher incidence of (and mortality from) metastatic breast cancer including breast-to-brain metastasis.

- Emerging evidence suggest there may be poorly defined race/ethnicity-related factors (in the tumor microenvironment) that contribute to this disproportion.
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 severe neurological decline

Before and After Surgical Resection
Breast Cancer Brain Metastasis

- **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+: Trastuzumab)

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

- **Ongoing clinical trials…but 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 most common primary sites in LMD patients.

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

- To date, there have been 6 randomized clinical trials specifically on LMD treatment.
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.
Multiplexed Ion Beam Imaging by Time-of-Flight Mass Spectrometry (MIBI-TOF)

1. Tissue biopsies from 41 TNBC patients
2. Stain with a mixture of antibodies labeled with elemental isotopes
4. N-dimensional image

Tabulate and analyze resultant data using image segmentation with quantitative and categorical classifiers

Angelo et al, 2014, Nature Medicine

Image segmentation

Pixel A

Pixel B

Pixel C

25 µm

Isotope N

Population A

Population B

Isotope 3

Population classification

Quantitative

ERG, ERBB, P53, TNBC

Angelo et al, 2014, Nature Medicine
Project 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
   • Spatial organization associated with patient overall survival

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

4. Compartmentalized tumors (MIBI data) were less likely to be associated with recurrence than mixed tumors
**Project Approach**

**“brain-focused”**

**Question:** What features of the breast cancer brain metastases TME correlate with disease progression and patient outcomes?

**Goal:** Analyze TME (spatial and composition) of all breast cancer brain mets, correlate to patient clinical features (DFS, OS)

**“breast-focused”**

**Question:** What features of TNBC breast tumor prime immune tolerance of brain metastases?

**Goal:** Analyze TME (spatial and composition) of TNBC primary tumors in patients that develop brain mets vs. those who do not.

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**Build TWO distinct tissue microarrays (TMAs)**
Next Steps & Future Directions

Next Steps

• Request “breast-focused” FFPE blocks from Pathology (Dr. West)
  • Slide annotation

• Identify normal brain controls (epileptic patients)

• Begin construction of “breast-focused” TMA

• MIBI panel construction and optimization
## Tumor-Immune Panel Keren et al, 2018, Cell

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Immune Cell Types</th>
<th>Immune Regulation</th>
<th>Stroma</th>
<th>Cell Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Catenin</td>
<td>Lymphocytes</td>
<td>Antigen</td>
<td>CD31</td>
<td>dsDNA</td>
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<tr>
<td>EGFR</td>
<td>CD3</td>
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<td>Ki-67</td>
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</table>

**Immune Cell Types**
- Lymphocytes: CD3, CD4, CD8, CD56, FoxP3
- Monocytes: CD11b, CD11c, CD63, CD68
- Neutrophils: CD138

**Antigen Presentation**
- HLA1
- HLA-DR
- CD209

**Immune Regulation**
- Lag3
- PD1
- PD-L1
- IDO

**Stroma**
- CD31
- SMA
- Vimentin

**Cell Status**
- dsDNA
- H3K27me3
- Ki-67
- H3K9ac
- pS6
Next Steps & Future Directions

Next Steps

• Request “breast-focused” FFPE blocks from Pathology (Dr. West)
  • Slide annotation

  • Identify normal brain controls (epileptic patients)

  • Begin construction of “breast-focused” TMA

  • MIBI panel construction and optimization
Next Steps

Next Steps
• Request “breast-focused” FFPE blocks from Pathology (Dr. West)
  • Slide annotation
• Identify normal brain controls (epileptic patients)
• Begin construction of “breast-focused” TMA
• MIBI panel construction and optimization
• Preliminary IHCs

But what about African-American women?
• Dr. Victoria Seewaldt – City of Hope
  • Early molecular changes predict aggressive biology
  • TNBC in African-American women
• How do spatial and temporal changes in the TME correlate with disease onset/progression?
Acknowledgments

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Thank you for your attention! Questions?