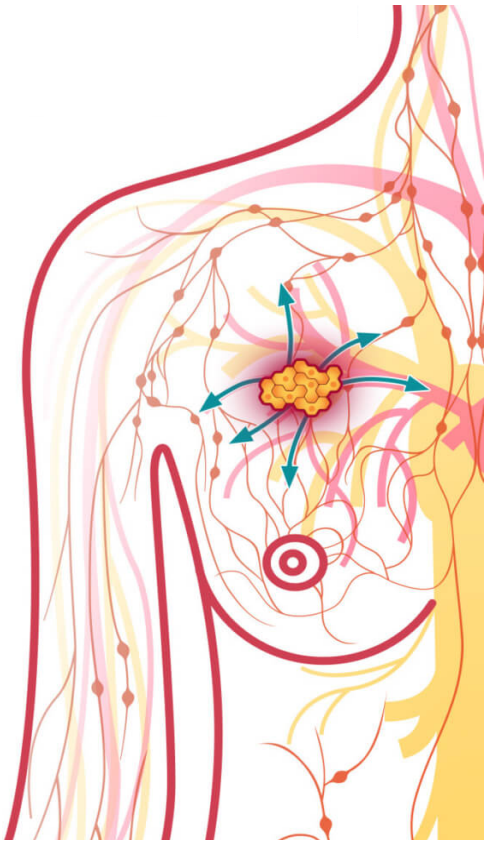
Work in Progress:

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

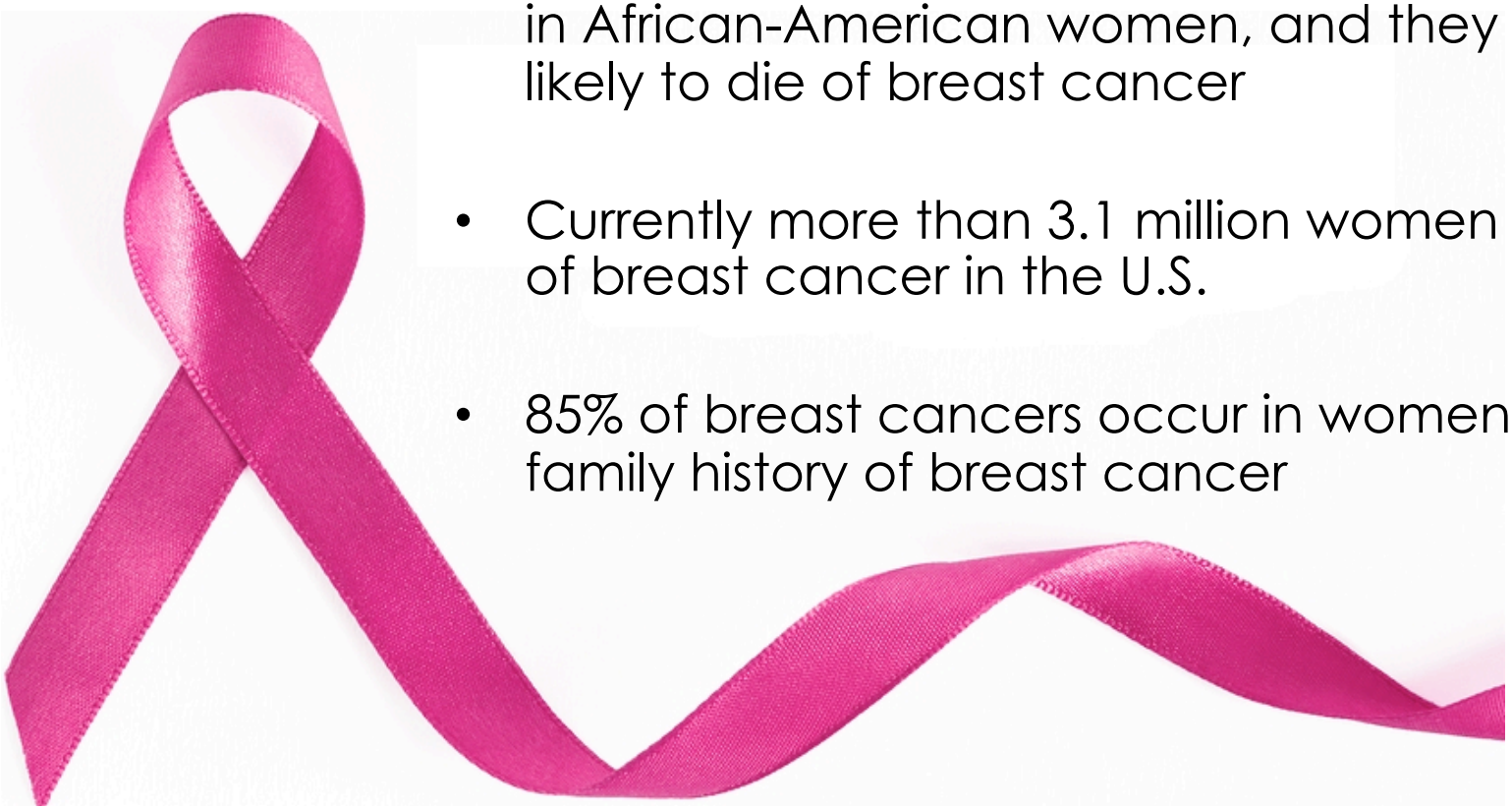
- 1 in 8 women in the U.S. will develop invasive breast cancer

- 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



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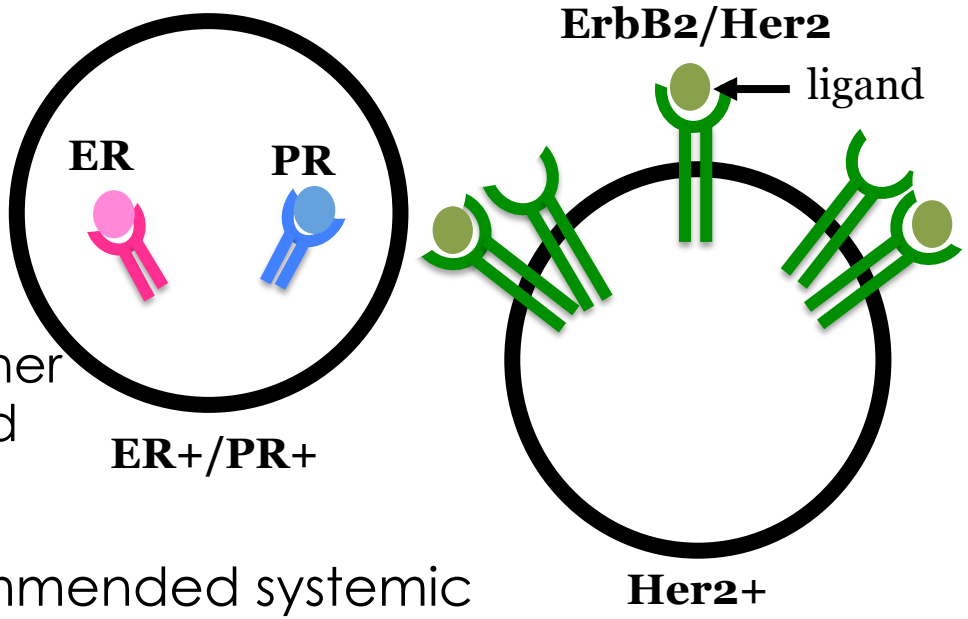
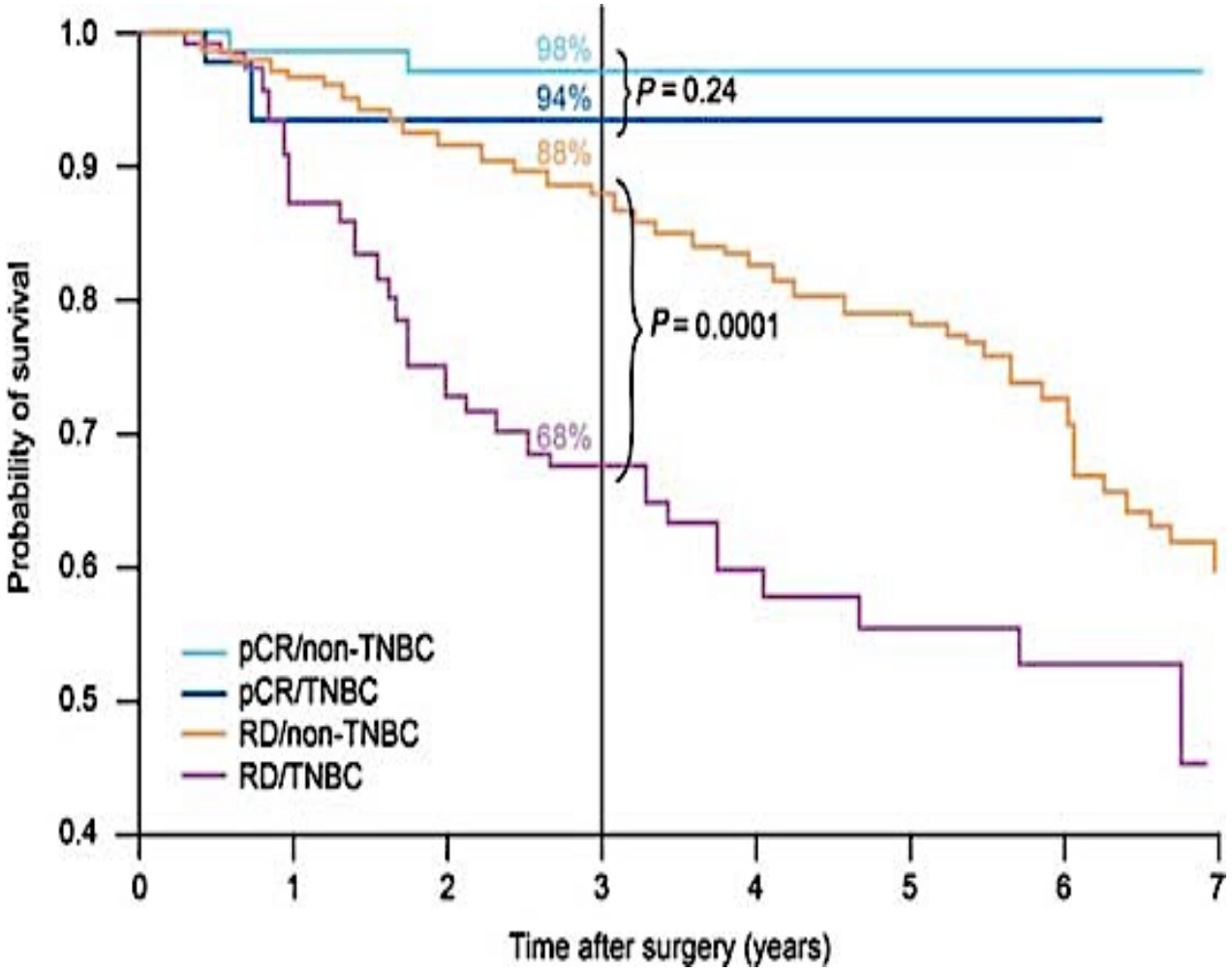
Triple Negative Breast Cancer (TNBC)

- 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)



Liedtke C, Mazouni C, Hess KR et al.

TNBC in African-American Women

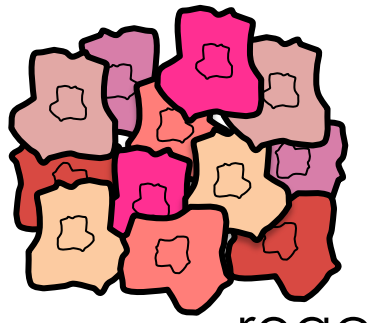
- Women of African ancestry have a disproportionately **higher frequency** (up to 79%) of TNBC, compared to women of European ancestry
  - TNBC frequency is consistently higher in women of African ancestry than **any other racial/ethnic group**

- 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)

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

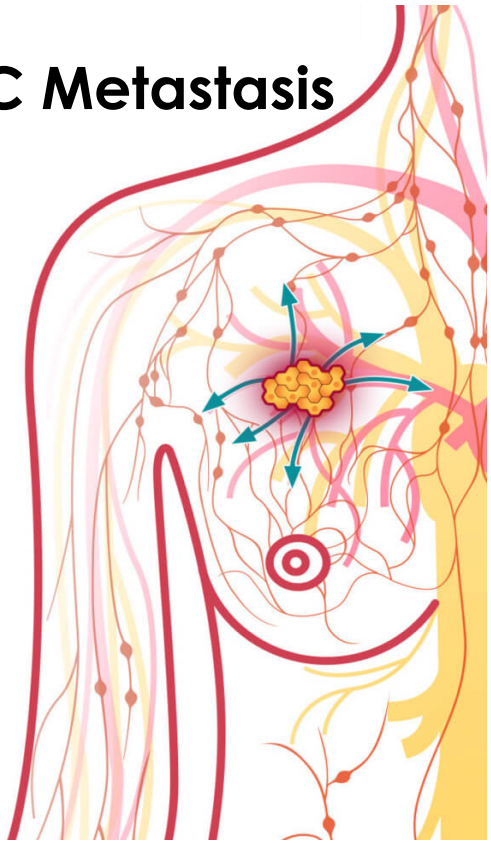
Primary TNBC



overexpression
miR-127

reactivation of
LRIG1

TNBC Metastasis



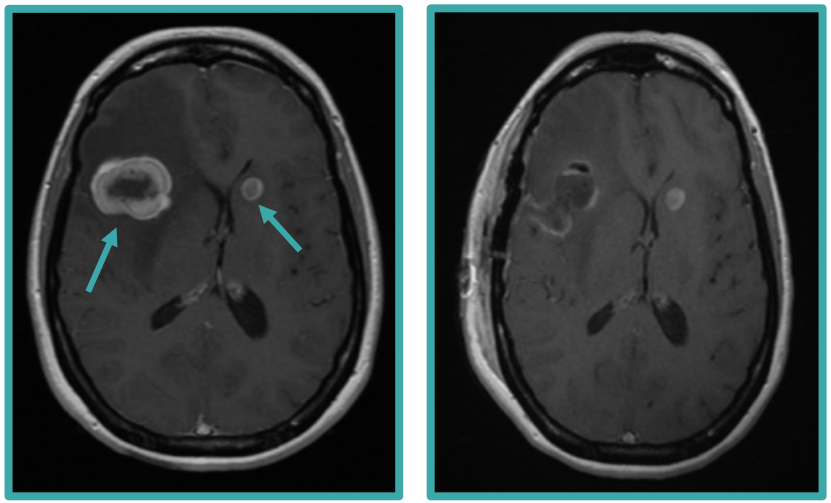
Postdoctoral Research –
What molecular mechanisms drive shedding/dissemination, seeding, and outgrowth of TNBC metastases?

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

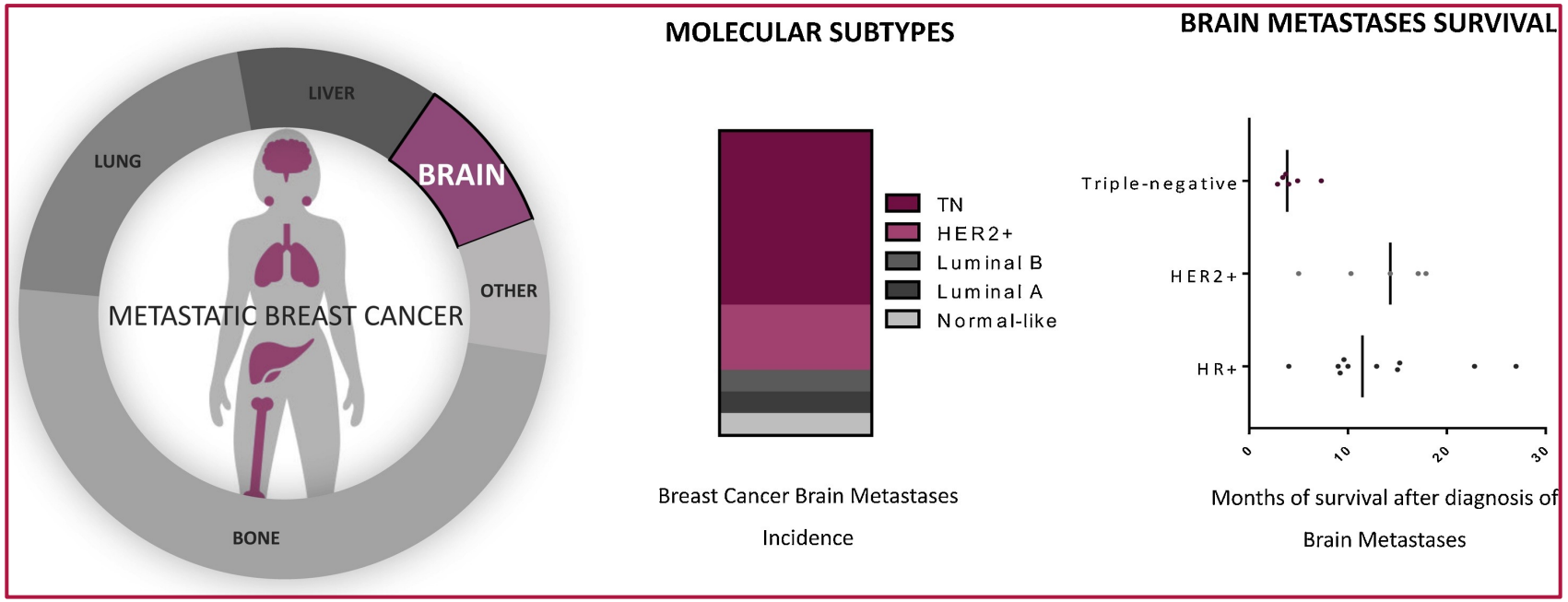
- Incidence of BCBM continues to increase
    - Prolonged patient survival
    - Improved imaging techniques

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



Before and After Surgical Resection

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+: Trastuzamab)

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

Although there are ongoing clinical trails, no FDA-approved systemic treatments for BCBM

Leptomeningeal Disease (LMD)

- LMD is defined as **tumor spread within the leptomeninges** and subarachnoid space

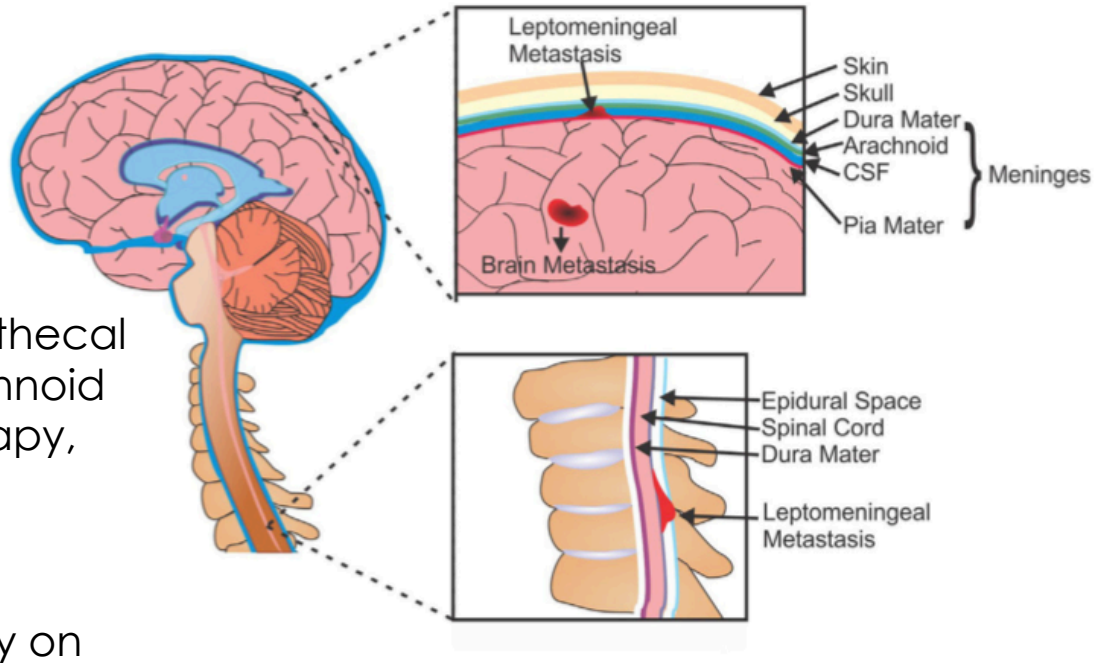
- **10% of patients** with solid cancers present with LMD

- **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

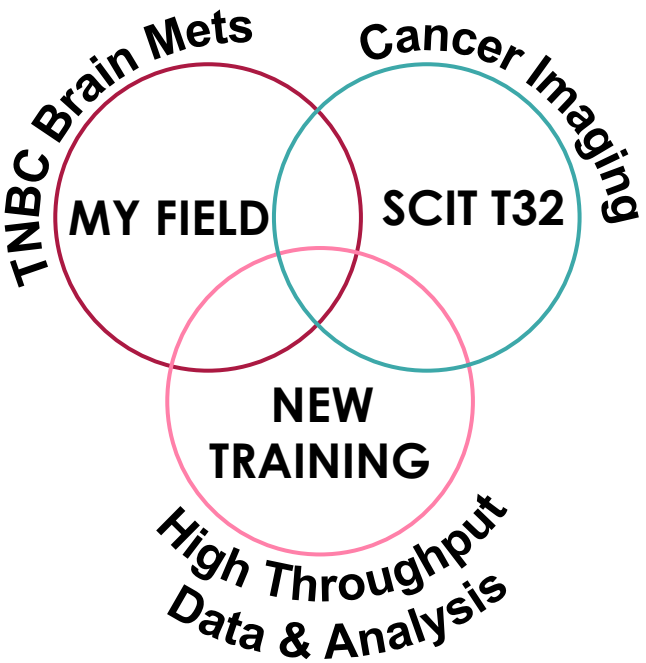
- **Therapeutic strategies** include intrathecal therapy (spinal canal and subarachnoid space to reach CSF), systemic therapy, and radiotherapy (WBRT)

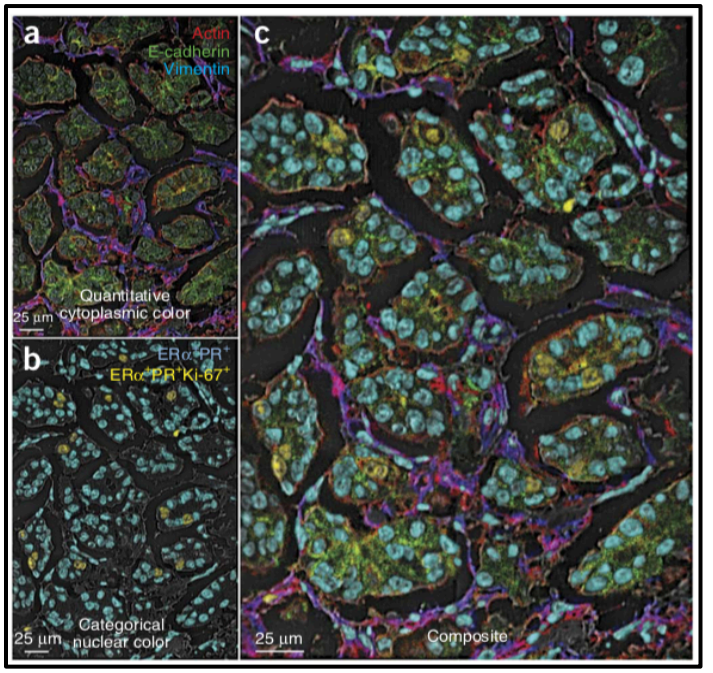
- To date, there have been only 6 randomized clinical trails specifically on treatment of LMD



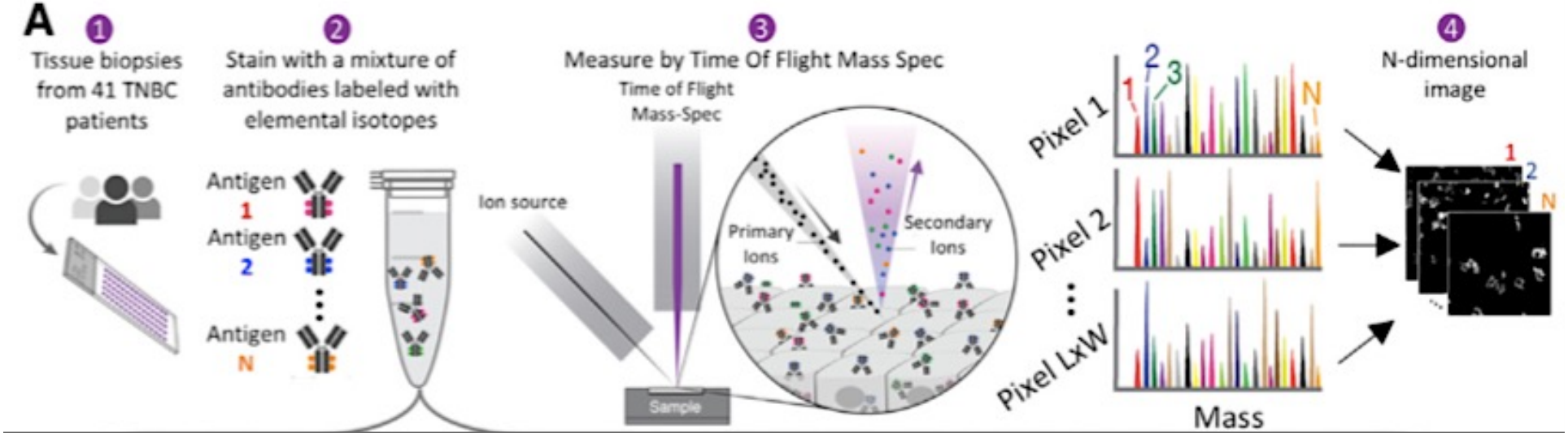
- Understanding the molecular mechanisms that drive **TNBC brain/LMD metastasis (seed – primary TNBC and soil – normal brain microenvironment**) pose an unmet clinical need

"The Birth" of the Project



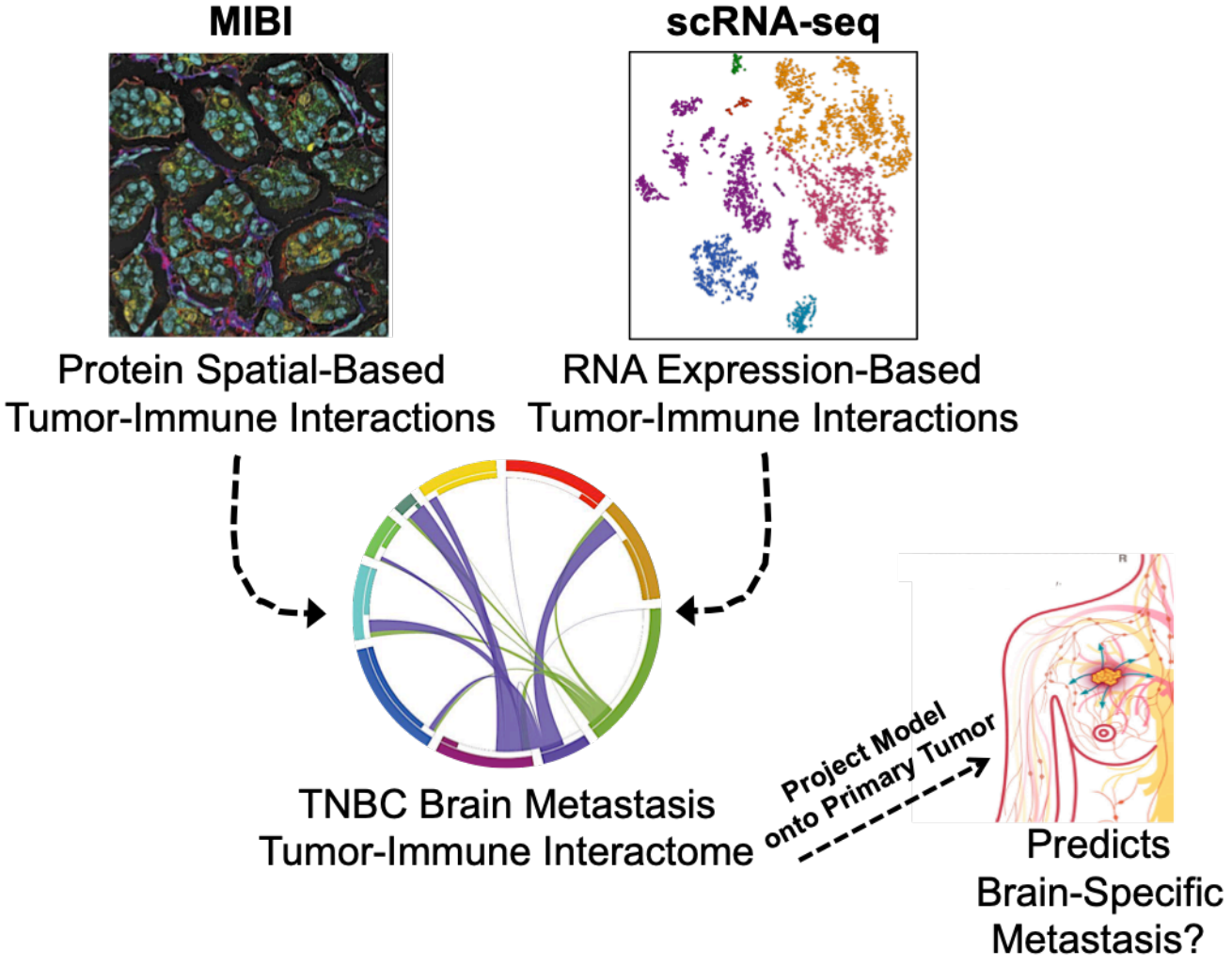


Angelo et al, 2014, Nature Medicine



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.



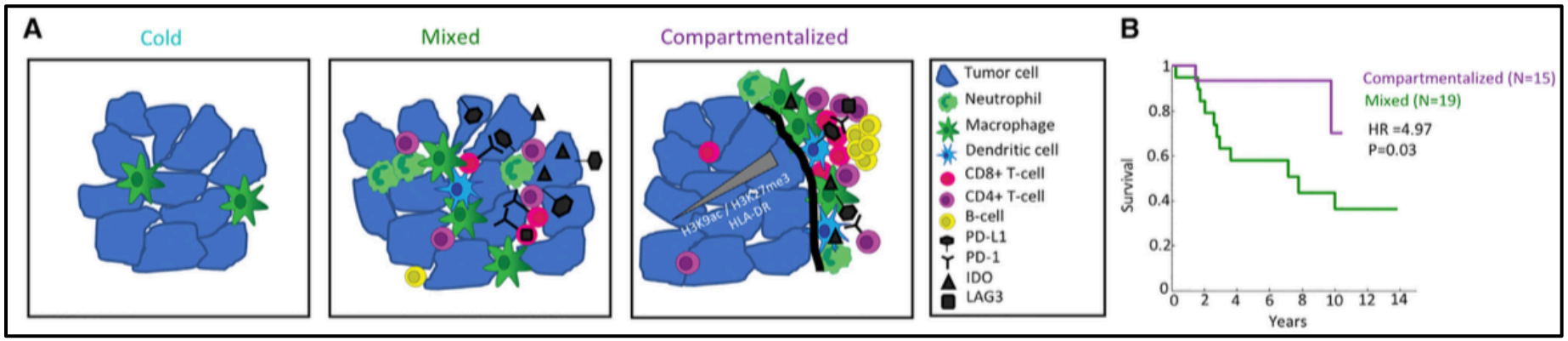
AIM 1: DETERMINE THE EXTENT TO WHICH THE STRUCTURED MICROENVIRONMENT CORRELATES WITH PATIENT OUTCOMES BY GENERATING A TUMOR-IMMUNE SPATIAL MAP OF TNBC BRAIN METASTASES.

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

Keren et al, 2018, Cell



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

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

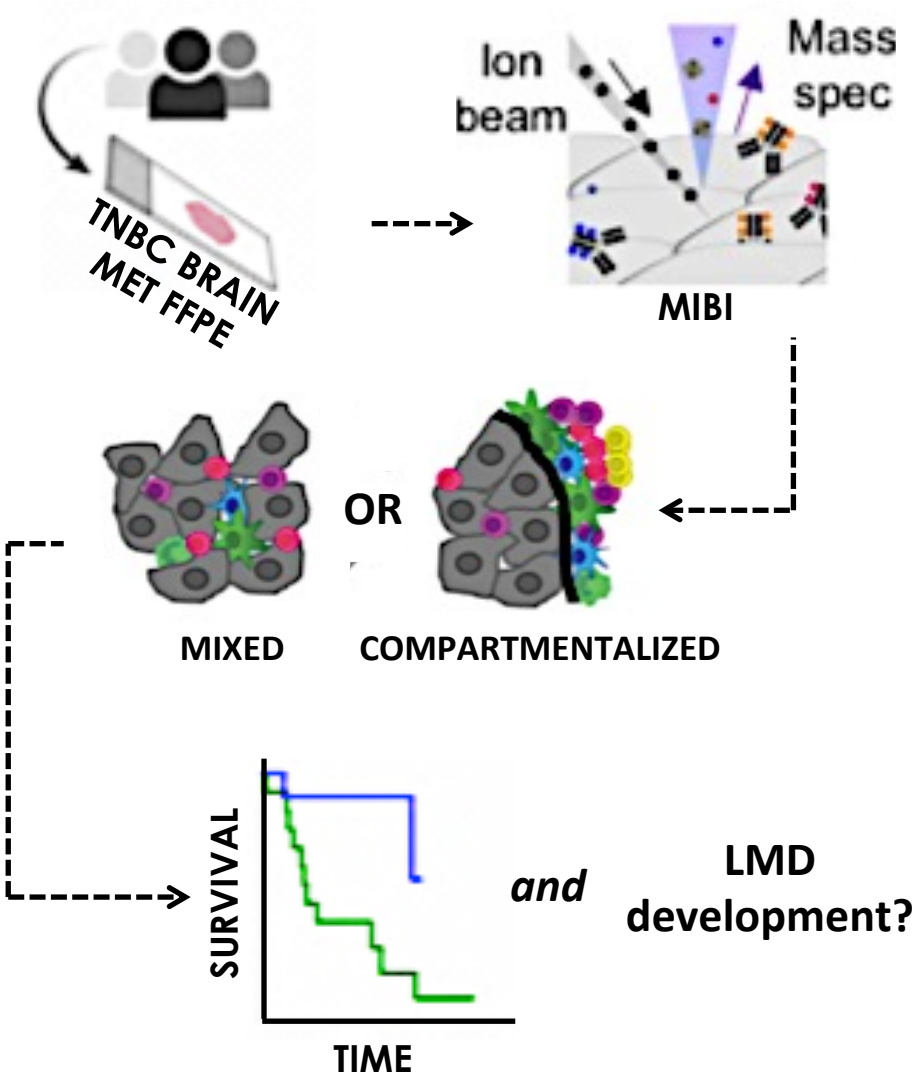
AIM 1: DETERMINE THE EXTENT TO WHICH THE STRUCTURED MICROENVIRONMENT CORRELATES WITH PATIENT OUTCOMES BY GENERATING A TUMOR-IMMUNE SPATIAL MAP OF TNBC BRAIN METASTASES.

APPROACH

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

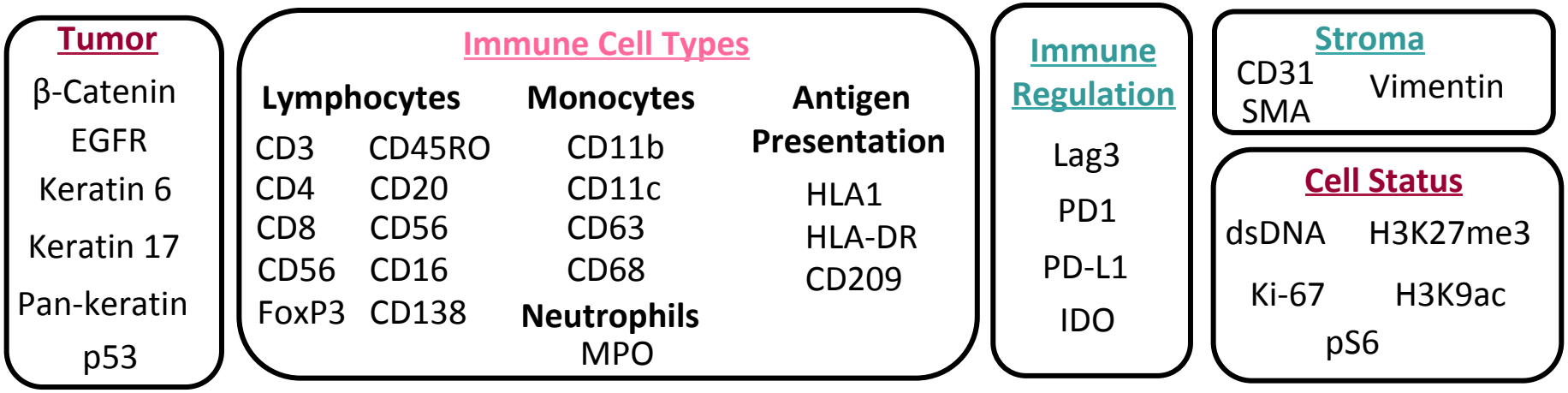
B. Quantitate the **composition and spatial architecture** of the tumor-immune microenvironment using a validated image analysis pipeline.

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



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.

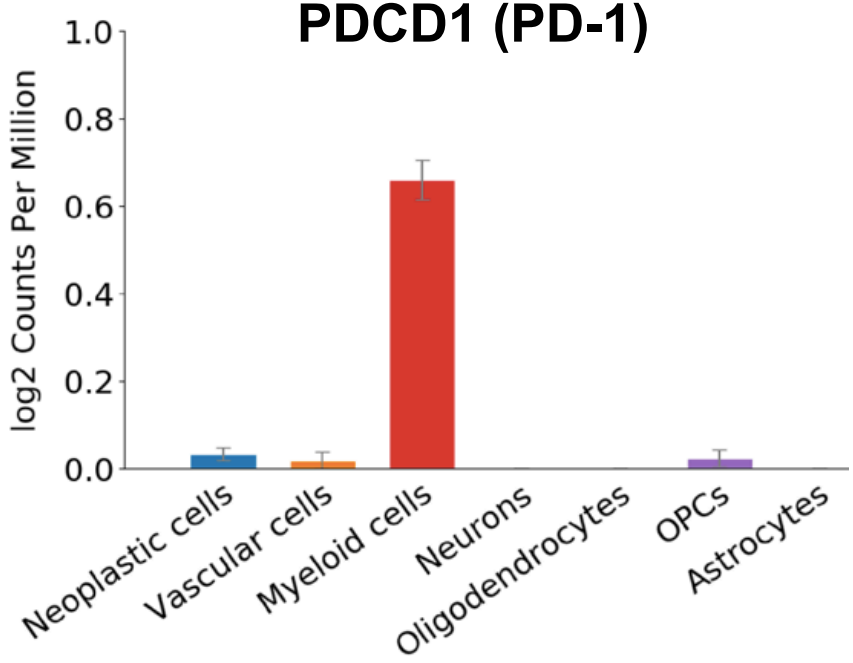
RATIONALE:

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

2. Identify novel targetable tumor-immune interactions for future therapies, beyond PD-1/PD-L1.

PRELIMINARY DATA:

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



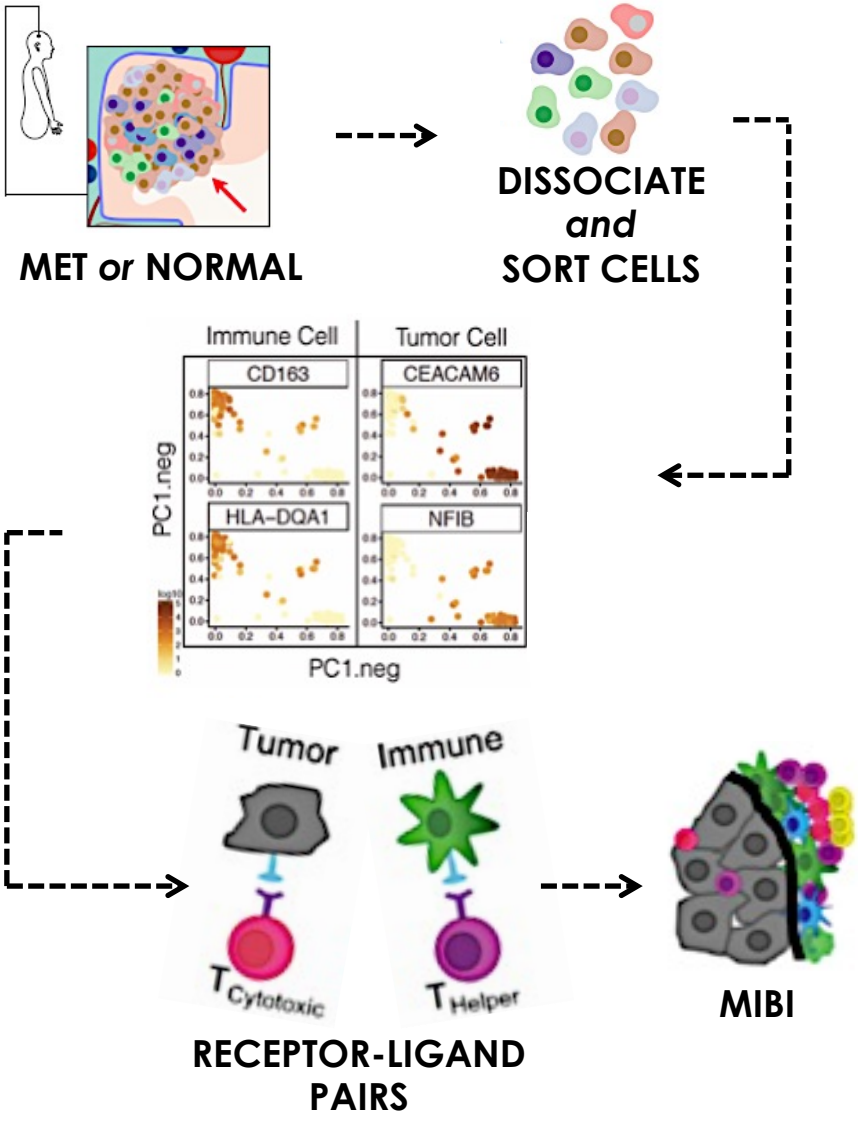
AIM 2: IDENTIFY TUMOR-IMMUNE RECEPTOR-LIGAND PAIRS BY GENERATING A TRANSCRIPTOMIC PROFILE OF TNBC BRAIN METASTASES, AND DETERMINE IF THESE INTERACTIONS CORRELATE WITH TUMOR-IMMUNE SPATIAL ARCHITECTURE.

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

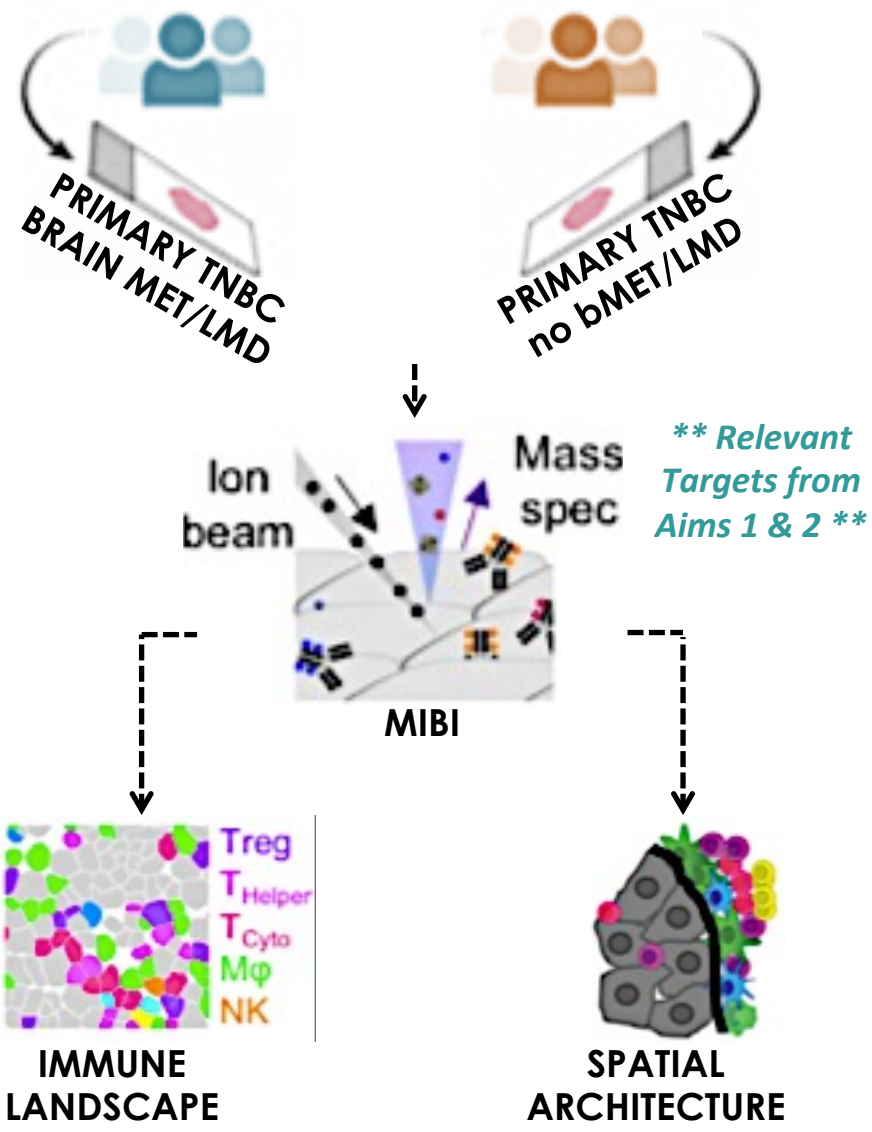
AIM 3: DETERMINE IF TUMOR-IMMUNE INTERACTIONS IN PRIMARY TNBC PRIME TOLERANCE OF DISSEMINATED CELLS ENABLING METASTASES, AND DEFINE IF INTERACTIONS CORRELATE WITH RACE.

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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- **Plevritis Lab Members**
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Thank you for your attention!
Questions?