The immune microenvironment of liver metastasis as a guide for immunotherapeutic potential in breast cancer

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Introduction

- Breast cancer is the second most common cancer in the United States. The primary tumors of hormone driven breast cancer subtypes have been treated more effectively. The 5–year survival rate for primary breast cancers is 99% [1].
- The metastatic dissemination of a primary tumor however decreases the 5-year survival rate to 23% and results in 90% of breast cancer deaths [2].
- Liver metastases are a common breast cancer metastasis site. If untreated, patients can be faced with a dismal prognosis of 3 to 15 months [3].
- Metastatic breast cancers lack a ”gold standard” treatment. There is a current need for better understanding of metastases and a more comprehensive treatment protocol.
- Immunotherapy has been an effective means to treat breast cancer but due to a lack in tumor infiltrating immune cells, it has rendered largely ineffective in metastatic cases [4].
- The immunological investigation into the primary and metastatic tumor microenvironment (TME) of breast cancers can inform physicians on key immune cell populations, biomarkers, immunogenicity profiles, and the potential use of certain treatments such as immune checkpoint inhibitors.
- In a retrospective study of 3166 patients, we discovered significant evidence pointing to higher tumor mutational burden (TMB), lower PDL-1 expression, and non-immunogenic profiles in liver metastatic sites.

Methods

- Unpaired samples were taken from primary and recurrent breast tumors, liver metastases and non-liver metastases. All were identified as breast cancers.
- Samples were compared using Fisher-exact or Chi^2 and were corrected for multiple comparison.
- Breast cancer tumors were tested using NextGen DNA sequencing (NextSeq, 592 gene panel) and whole transcriptome RNA sequencing (NovaSeq).
- The VENTANA PD-L1 (SP142) assay was used to score PD-L1 expression on immune cells.
- QuantiSeq was used to evaluate relative cell abundance in TME using transcriptome data.

Patient Characteristics

A

		Breast		Liver Met		Non-liver	
N		1268		495		1403	
Gender	Female (N/%)	1258	99.2%	494	99.8%	1258	99.2%
	Male (N/%)	10	0.8%	1	0.2%	10	0.8%
Age	Range	22-94		24-92		27-93	
	Median	60		61		58.5	

B

Metastatic sites of Non-Liver Mets	1403
Axilla, NOS	78
Bone	185
Brain	88
Chest/Chest wall	116
Connective Tissue	55
GI Organ	97
GYN Organs	31
Lung/Pleural	201
Lymph Node	356
Other	124
Skin	72

C

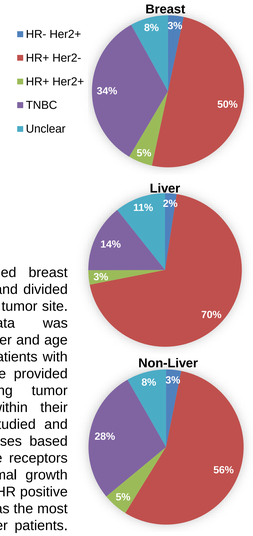

Figure 1. A) 3166 confirmed breast cancer patients were studied and divided into three categories based on tumor site. Patient characteristic data was determined by evaluating gender and age in each category. **B)** Among patients with a non-liver metastatic site, we provided further information regarding tumor location. **C)** Tumor sites within their respective categories were studied and subtyped into four major classes based on the expression of hormone receptors (HR) and the human epidermal growth factor receptor 2 (HER2). The HR positive and HER2 negative subtype was the most prevalent among breast cancer patients. Tumors lacking expression in both hormone and HER2 receptors were classified as triple negative (TNBC).

References

1. Ma & Roussos Torres et al. J Transl Med 2015; 2. Cummings et al. J Pathol 2013; 3. Adam et al Ann Surg 2006; 4. Esteva et al Lancet 2019; 5. Chen et al J Hematol Oncol 2017; 6. Christmas & Roussos Torres et al Cancer Immunol Res 2018

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Results

Figure 2: Mutational analysis reveals higher tumor mutational burden in liver metastatic sites as compared to breast tumors

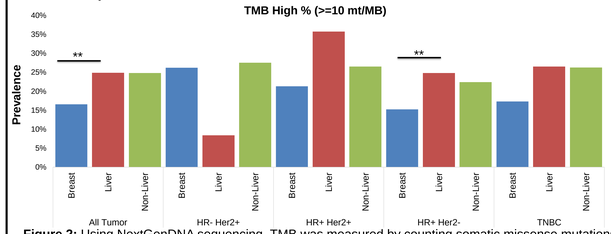


Figure 2: Using NextGenDNA sequencing, TMB was measured by counting somatic missense mutations on a 592 gene panel. Tumor sites with 10 or more mutations per Megabase were classified as high. Within all tumors sampled, liver metastatic sites had a 24.8% prevalence rate of high TMB in contrast to a 16.6% prevalence in primary breast tumors. A similar trend was observed in the HR positive and HER2 negative subtype. The TMB in liver metastatic sites was significantly elevated (**p-value<0.0001) when compared to breast tumors offering a prognostic marker for effective immunotherapy treatment.

Figure 3: PD-L1 expression was significantly lower in immune cells in liver and non-liver metastatic sites

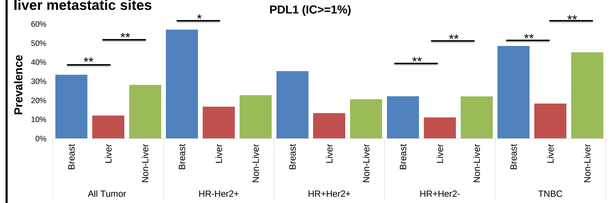


Figure 3. Using the VENTANA PD-L1 assay, PD-L1 expression was scored on immune cells. PD-L1 expression is seen in both tumor and immune cells and is thought to contribute towards immune evasion. It is currently one of the only approved biomarkers used to predict response to immune checkpoint inhibition. We found that liver metastatic patients had significantly (**p<0.0001) decreased PD-L1 expression (12%) when compared to breast cancer (34%) and non-liver metastatic patients (28%).

Figure 4: A composite biomarker of TMB and PDL-1 indicates subtype specific immunogenicity and treatment responsiveness

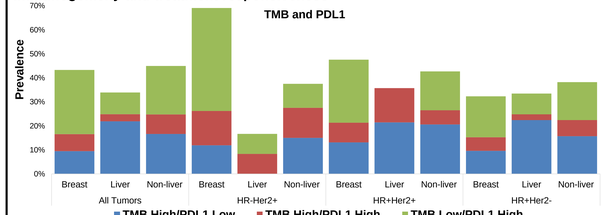
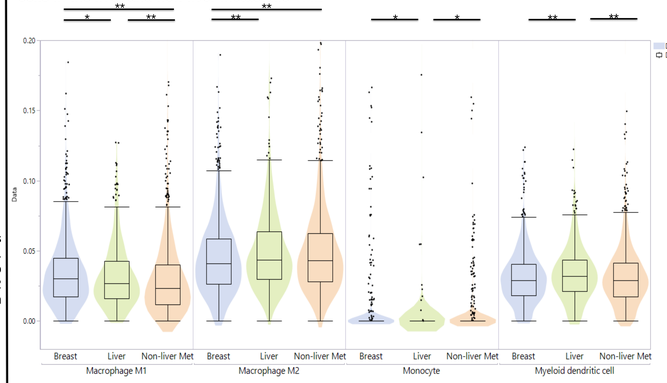


Figure 4. TMB and PDL-1 are known as traditional markers of immunogenicity and when applied together, they can act as a composite biomarker capable of predicting response to immune checkpoint inhibitors (ICIs). A high TMB and PDL-1, as displayed in liver metastatic sites among the HR+ Her2+ subtype, suggests an immunogenic TME that would be more responsive to treatment than liver metastatic sites in the HR+ Her2- subtype. Liver metastatic sites in all tumors displayed a composite biomarker that indicated a low immunogenic TME profile.

Figure 5. Transcriptome data demonstrates suppressive nature of myeloid derived immune cells in the liver and non-liver metastatic TME



** : p<0.0005
* : p<0.05 but >0.005

Figure 5: Myeloid derived cells stem from progenitor cells in the bone marrow. They are critical for the body's ability to mount effective immune responses. However, the role of myeloid derived cells, especially tumor associated macrophages (TAMS), continue to be studied as potential contributors towards tumorigenesis and poor clinical outcomes [5]. M1 cells are critical in mediating anti-tumor phagocytosis while M2 macrophages have been found to release anti-inflammatory cytokines and are active in promoting metastasis [6]. As such, we found there to be significantly fewer M1 cells, and significantly more M2 macrophages in liver and non-liver metastatic sites as compared to primary breast tumors, supportive of a more suppressed TME in all metastatic sites. Monocyte and myeloid dendritic cells were both found to be increased in liver metastatic sites as compared to primary sites.

Figure 6. Immune cells of T cell lineage are lower in the TME of liver metastatic sites

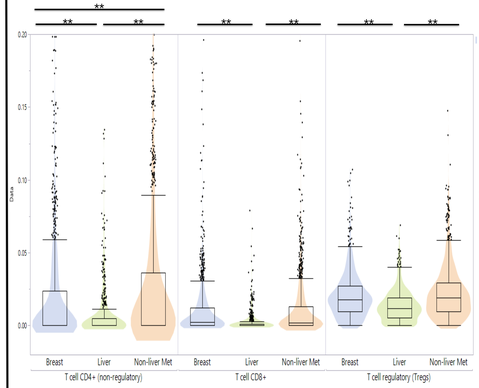


Figure 6: Therapy resistant breast cancers have been linked to a lack of T-cell infiltration within the TME through immunosuppressive mechanisms involving myeloid derived suppressor cells (MDSCs). ICIs have shown promise in re-sensitizing the TME to T-cell infiltration and, resultingly, the propagation of cytotoxic signaling pathways [5]. We found there to be significantly lower CD4+, CD8+ T cells, and T-regulatory cells among liver metastatic TMEs when compared to breast and non-liver metastatic sites. Our findings indicate another metric of immunosuppression by which liver metastatic breast cancers may function in evading immune responses and ICIs.

Figure 7. Metastatic sites show reduced recruitment of lymphocytes of the innate and adaptive immune system

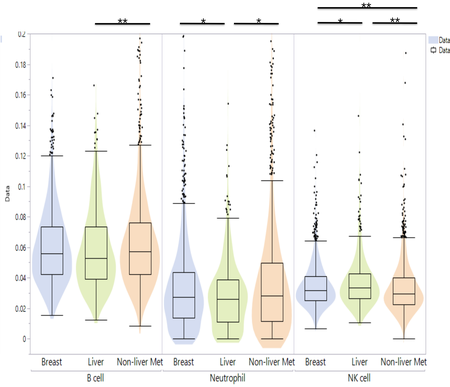


Figure 7: Using transcriptome data analysis, neutrophil populations were found to be significantly lower in liver metastatic sites as compared to both breast and non-liver tumor sites. B-cell populations were significantly decreased in liver metastasis as compared to non-liver metastatic sites while natural killer (NK) cells were significantly decreased in non-liver metastatic sites when compared to breast and liver sites. These findings suggest a reduction in the NK and B cells ability to promote apoptotic pathways and produce necessary antibodies, respectively. Without these necessary immune functions, liver and non-liver metastatic sites are likely prone to unregulated tumor growth and increased metastatic potential.

Summary

- In our patient cohort, immune cells in the TME of liver metastatic sites were less abundant.
- The paucity of immune cells suggests breast cancer metastases to the liver harbors a less immunogenic niche.
- Increased TMB in all metastatic sites uncovers the heterogeneity among different tumor sites.
- Immunosuppressive cells of myeloid and lymphoid origin prevent further immune cell infiltration in the TME.
- Lack of PD-L1 expression among liver metastatic sites suggests another possible mechanism explaining the lack of response to ICIs in certain patients.
- The characterization of the liver metastatic TME through TMB, PD-L1 expression, and the relative population of immune cells suggests these metrics could serve as a composite biomarker when considering immunotherapeutic treatment options.
- Our current findings will be supplemented by further characterization of the genetic and molecular alterations in breast cancer, liver and non-liver metastatic TMEs. The identification of additional immune cells that act as biomarkers of immunogenicity will help determine their role in tumor immune response to ICIs.