Mimicking the breast metastatic microenvironment: characterization of a syngeneic model of HER2 positive breast cancer

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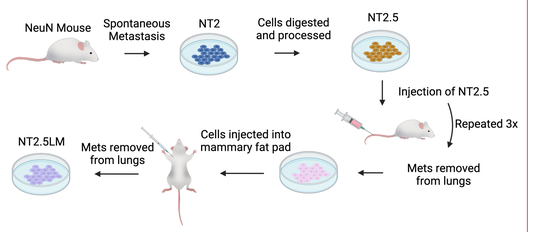
INTRODUCTION

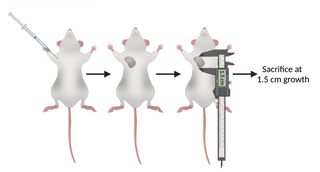
Breast cancer remains a leading cause of global cancer incidence in women, causing >685,000 deaths yearly, with most breast cancer deaths resulting from metastatic burden. The survival rate for triple-negative breast cancer and HER2-overexpressing breast cancer is the lowest. Immune checkpoint inhibition has revolutionized cancer treatment, providing durable response to patients with advanced disease. These findings have been made possible through the utilization of mouse models, which allows us to understand tumor biology in an immune system close to humans. However, current models of breast cancer are limited in the ability to thoroughly study the metastatic tumor microenvironments: the NT2.5 model allows for spontaneous metastatic growth, but occurs with low penetrance; the 4T1 model has rapid mammary tumor growth, which necessitates euthanasia of mice before adequate growth of metastases. **Thus, we aimed to develop the NT2.5-LM syngeneic model of spontaneous metastatic disease with increased penetrance of metastasis without reaching the end point too quickly.**

METHODS

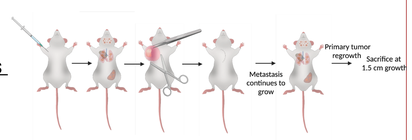
Derivation of the NT2.5-LM Cell Line



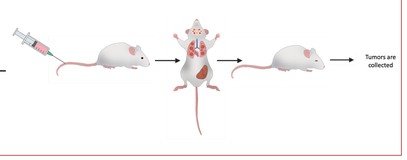
NT2.5 Spontaneous Model



NT2.5-LM Spontaneous Model

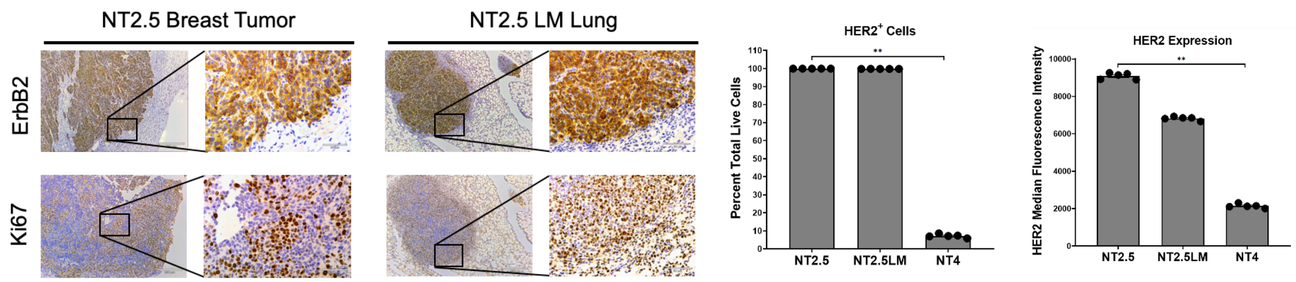


NT2.5-LM Tail Vein Model



RESULTS

Figure 1 : Histological and Flow Cytometry Comparison of NT2.5 and NT2.5-LM



- Histological and flow cytometry analysis of NT2.5-LM cell lines compared to NT2.5 showed there to be positive stains for both Erbb2 and Ki67
- Flow data comparing NT2.5, NT2.5-LM and NT4 showed NT2.5 and NT2.5-LM to have more cells stained with HER2 markers

Figure 2 : Breast Tumors Growth Rate and Lung Metastases

- *In vivo* experiments showed differences in survival , tumor growth rate, lung metastasis area ratios, and the number of lung metastases
- NT2.5-LM tail vein model shows decreased survival, increased ratio of lung metastasis area to total lung tissue area, and increased number of lung metastases

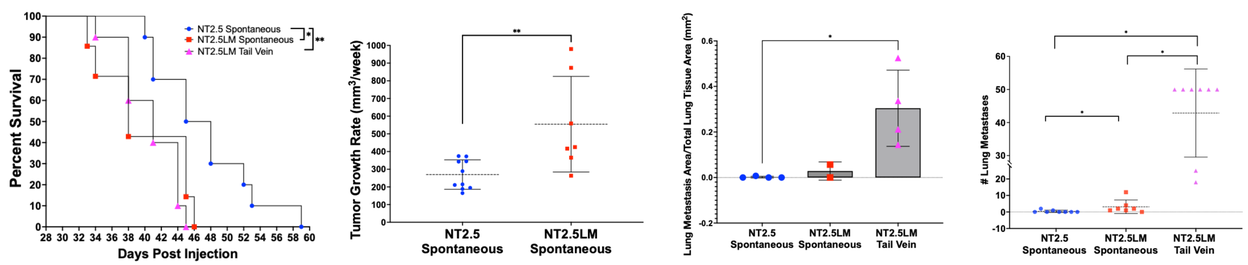
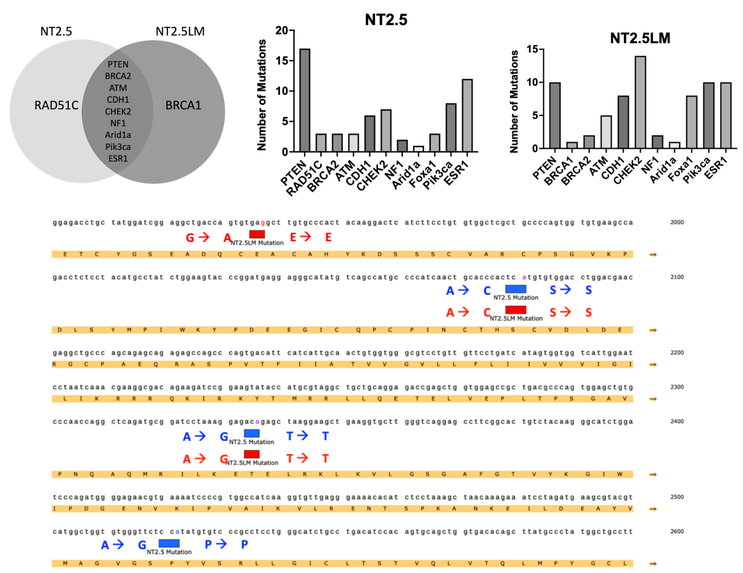


Figure 3 : Whole Exome Sequencing of NT2.5 and NT2.5-LM

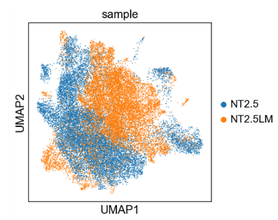
Common breast cancer mutations found in NT2.5 and NT2.5-LM



- Whole exome sequencing shows no differences between NT2.5 and NT2.5-LM

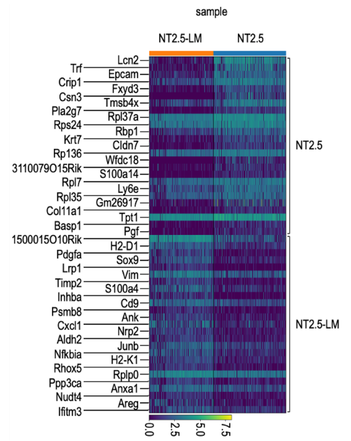
Figure 4 : ScRNA-Sequencing of NT2.5 and NT2.5-LM

UMAP of NT2.5 & NT2.5-LM Tumors

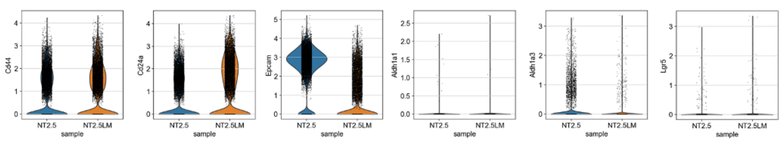


- In NT2.5-LM tumors, scRNA-seq showed upregulation of genes associated with increased cellular proliferation (Pdgfa, Sox9), invasion and migration (Lrp1, Cd9, Cxcl1, Anxa1, Areg, Ifitm3), epithelial-to-mesenchymal transition (Vim, Inhba) and downregulation of genes associated with epithelial-to-mesenchymal transition (Epcam)

Top 25 up- and down-regulated genes



Violin plots of breast cancer stemness genes



CONCLUSIONS

- Here, we characterized a syngeneic murine breast tumor cell line, NT2.5-LM that provides a model of spontaneously metastatic neu-expressing breast cancer
- NT2.5-LM is HER2+ and shows metastatic potential shown by flow data in comparison to NT2.5 and NT4, Ki67 markers in IHC, and murine experiments.
- NT2.5-LM cells shows up-regulation of epithelial-to-mesenchymal transition, expressing increased levels of Vimentin and a highly invasive and aggressive phenotype compared to NT2.5
- NT2.5-LM can be utilized to further understand different therapies, metastatic progression, and the characteristics of the metastatic tumor microenvironment.

ACKNOWLEDGEMENTS

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