Entinostat enhances the efficacy of checkpoint inhibition in breast-to-lung metastases and is associated with alterations in the phenotype and function of myeloid cell populations

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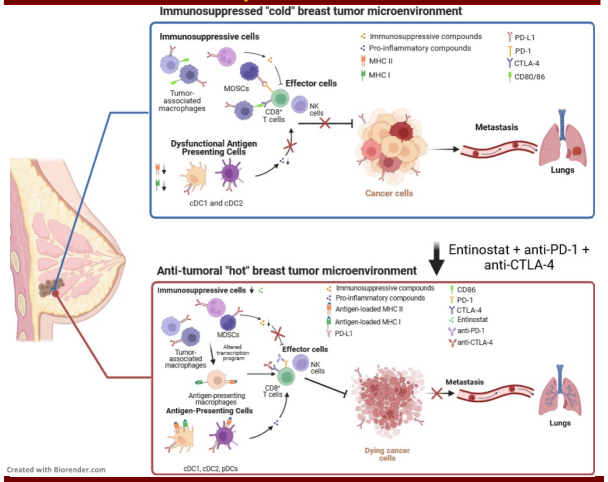
Abstract #3256

Graphical Abstract



Introduction

Despite the success of immune checkpoint inhibitors (ICIs) in various types of cancers, ICIs are largely ineffective against breast cancer[1]. The presence of dysfunctional antigen presenting cells and high infiltration of breast tumors by immunosuppressive myeloid cell populations, such as monocytic myeloid-derived suppressive cells (M-MDSCs), granulocytic myeloid-derived suppressive cells (G-MDSCs), and macrophages, could account for the lack of success[2]. Entinostat, a histone deacetylase inhibitor, has been used successfully in combination with the ICIs anti-PD-1 and anti-CTLA-4 to limit breast cancer metastasis in the 4T1 model of triple-negative breast cancer (TNBC) and was associated with a decrease in immunosuppression[3]. Our group has shown that entinostat + anti-PD-1 + anti-CTLA-4 also limits tumor growth in the NT2.5 model of HER2$^+$ breast cancer and decreases immunosuppression by MDSCs[4]. In this study, we used the NT2.5LM model of breast-to-lung metastasis along with single-cell RNA-sequencing (scRNA-seq) and flow cytometry to uncover the mechanism behind an observed increase in survival in mice treated with entinostat + anti-PD-1 + anti-CTLA-4, which was associated with upregulation of genes linked to antigen presentation and downregulation of genes linked to immunosuppression.

Results

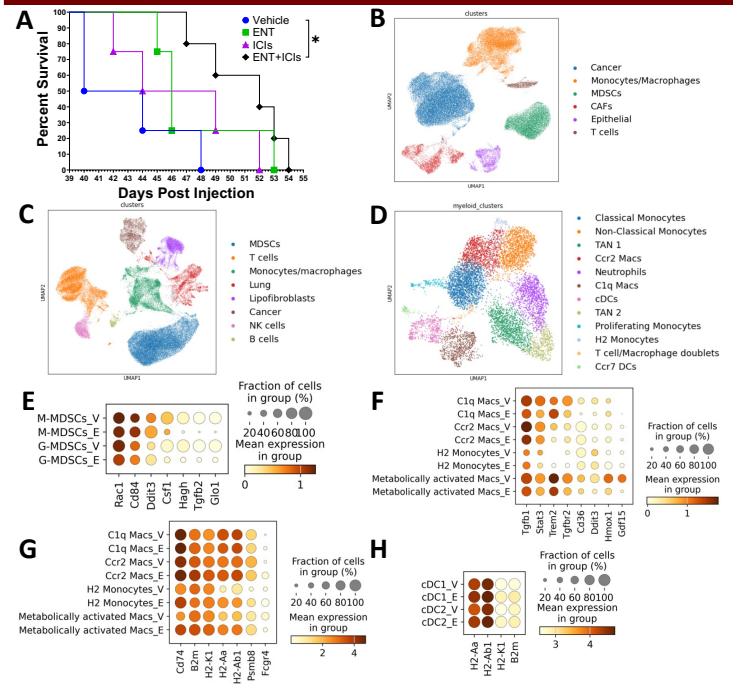


Figure 1. Entinostat + ICIs increases survival and is associated with anti-tumoral changes in transcription using the NT2.5LM model of breast-to-lung metastasis. A. NeuN mice (n=4-5/group) treated with entinostat (ENT) + α-PD-1 + α-CTLA-4 (ICIs) demonstrated significantly increased survival (Log-Rank test) using an experimental model of breast-to-lung metastasis. B-C. UMAPs obtained from scRNA-seq data showing similar composition of higher order clusters in treated NT2.5 mammary tumors (B) and NT2.5LM lung metastases (C) using the spontaneous metastasis model. D. UMAP showing mature myeloid clusters in NT2.5LM lung metastases. E-H. Dot plots showing entinostat-driven downregulation of genes associated with immunosuppression (E-F) in MDSC (E) and myeloid cell subtypes (F) and upregulation of genes associated with antigen presentation (G-H) in monocytes/macrophages (G) and cDCs (H).

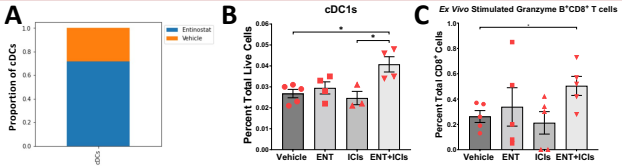
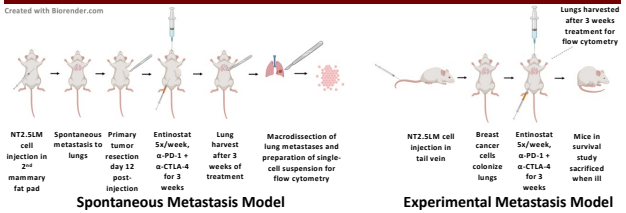


Figure 2. Entinostat increases the frequency of cDCs and granzyme B-producing CD8$^+$ T cells in NT2.5LM breast-to-lung metastases. A. Bar graph demonstrating an increased proportion of cDCs with entinostat in NT2.5LM lung metastases using scRNA-seq as in Figure 1. B-C. Flow cytometry results showing increased cDC1 (experimental metastasis model) and granzyme B-producing CD8$^+$ T cells (spontaneous metastasis model) in NT2.5LM lung metastases with entinostat.

Materials and Methods



NT2.5LM, a HER2$^+$ breast cancer cell line that spontaneously metastasizes to the lungs following mammary fat pad implantation, was used to study the effects of entinostat, α-PD-1, and α-CTLA-4 treatment on breast-to-lung metastases. An experimental metastasis model involving tail vein injection of NT2.5LM was used for the survival study and flow cytometry data for cDC1s described. A spontaneous metastasis model was used to produce breast-to-lung metastases that were analyzed via scRNA-seq. Flow cytometry panels described in Sidiropoulos *et al.*[5] were used to analyze immune cell infiltration in breast-to-lung metastases.

Conclusions

Entinostat combined with anti-PD-1 and anti-CTLA-4 has proven effective in limiting metastasis in mouse models of TNBC and HER2$^+$ breast cancer, and this benefit was recapitulated in a phase I clinical trial with HER2$^-$ breast cancer patients[6]. Preliminary scRNA-seq and flow cytometry analyses of immune cell changes in the TME of breast-to-lung metastases revealed an upregulation of antigen presentation genes and a downregulation of genes associated with suppression in myeloid cells. These results lay the groundwork for targeting specific myeloid cell populations in breast-to-lung metastases to further increase the efficacy of ICIs in metastatic breast cancer.

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Link to lab website: