Introduction/Rationale

- Heme oxygenase-1 (HMOX1/HO-1) expression and activity are elevated in triple-negative breast cancer (TNBC) specimens (A).
- HO-1 metabolizes heme to bilirubin, a metabolite known to impact macrophage function in autoimmune diseases. (Lu et al. J Immunol. 2008)
- Even though HO-1 expression predicts increased abundance of suppressive immune cells (B) and decreased overall survival (C), however, the impact of the bilirubin on the TNBC microenvironment had not been studied.

Fig 1. Tumor cell-HO-1 alters immune suppressive and efferocytosis macrophage genes via secreted bilirubin.

- We hypothesized that tumor cell-HO-1 activity and subsequent bilirubin secretion enhance triple-negative breast cancer (TNBC) metastasis by supporting immune suppressive, pro-tumor macrophage function.

Methods

- I assessed immune suppressive and efferocytosis genes in RAW264.7 mouse macrophages via qRT-PCR after direct treatment with bilirubin or treatment with conditioned medium (CM) from HO-1 inhibited mammary carcinoma cells.
- Macrophage PD-L1 expression and efferocytosis capacity, dead tumor cell engulfment, were observed by flow cytometry and Incucyte live cell imaging (Essen Bio).
- HO-1 was depleted in 66Cl4 mammary carcinoma cells using shRNA. 66Cl4 shHO1 cells were injected orthotopically into immune-competent hosts and I assessed primary tumor growth, lung metastatic capacity, and macrophage status via flow cytometry.

Conclusions/Future Directions

- HO-1 expression and activity is increased in TNBC specimens, and it predicts poor overall survival, a suppressed microenvironment, and decreased response to immunotherapy.
- Tumor produced bilirubin increased macrophage expression of PD-L1 but decreased efferocytic capacity by MerTK and Tyro3.
- In metastatic models, inhibition of tumor cell-HO-1 decreased macrophage expression of suppressive markers at the primary site and decreased lung metastatic capacity.
- Future studies will focus on the impact of HO-1 inhibition (shRNA or SnMP) on T cell cytotoxicity and macrophage immune suppression and function in the metastatic lung and liver.
- Summary: HO-1 targeting with FDA approved SnMP may limit macrophage immune suppression via bilirubin depletion, resulting in decreased TNBC metastasis and enhanced sensitivity to immunotherapy.

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Hypothsis

We hypothesized that tumor cell-HO-1 activity and subsequent bilirubin secretion enhance triple-negative breast cancer (TNBC) metastasis by supporting immune suppressive, pro-tumor macrophage function.

Fig 2. Bilirubin enhances PD-L1 expression in human-derived and primary mouse macrophages.

Fig 4. HO-1 expression and activity is increased in TNBC specimens, and it predicts poor overall survival, a suppressed microenvironment, and decreased response to immunotherapy.

Fig 5. HO-1 supports macrophage expression of suppressive markers and immunoreceptor resistance.