Immune checkpoint blockade (ICB) therapies have shown promising results in treating a variety of cancer types. However, the median overall survival of triple negative breast cancer (TNBC) remains very low.

A new protein B7 homolog 3 (B7H3), or CD276, was found to be overexpressed in many cancer cells and have little to no expression on normal cells.

It initially was characterized as a costimulatory molecule promoting the activation of T cells but is now understood to act mainly as a coinhibitory molecule of the immune system.

B7H3’s mechanism of action and receptor remain uncharacterized, but its preference for malignant cells offers hope for a new therapeutic approach with less toxicity and more efficiency.

### Introduction

- To determine the expression of B7H3 in primary TNBC tumors and measure the effect of novel anti-B7-H3 antibodies in the activation of NK cells and T lymphocytes against TNBC

### Methods

- We performed immunohistochemistry to investigate B7H3 expression in TNBC primary tumors (n=49) and adjacent normal mammary tissue (n=30). The data was acquired and quantitated using Vectra-Polaris®, multi-spectral imaging system
- Natural killer (NK) cells and T lymphocytes were isolated fromuffy coats of healthy donors by Ficoll density gradient centrifugation.
- RFP-tagged B7H3+/− TNBC cell lines were co-cultured with primary NK cells or T cells at different immune cell to target cells ratios. The cells were simultaneously incubated with different concentrations of novel anti-B7H3 monoclonal antibodies (mAb) or chimeric antibodies (chAb) against a control antibody
- Immune cell-mediated apoptosis and antibody-dependent cytotoxicity was measured by IncuCyte® live cell imaging system

### Results

#### Fig. 1
IHC assay shows higher B7H3 expression in tumor tissue than normal mammary tissue

- Normal mammary tissue (N=25)
- Tumor tissue (11-20%) (N = 2)
- Tumor tissue (21%) (N = 43)

#### Fig. 2
Addition of anti-B7H3 monoclonal antibodies promotes NK cell-mediated apoptosis in HCC38 RFP cells. More apoptosis can be seen with treatment of NK:TNBC cell ratio 8:1 + 10ug/ml mAb (yellow) versus control IgG (green) at 8 hours (b)

- Fig. 3
Addition of anti-B7H3 chimeric antibodies promote ADCC in MCF7 RFP cells. A decrease in surviving cells can be seen with treatment of NK:TNBC cell ratio of 2:1 + 5ug/ml chAb (yellow) versus rituximab control (green) in 24 hours (b)

- Fig. 4
Addition of anti-B7H3 monoclonal antibodies promote apoptosis by T lymphocytes in HCC38 RFP cells. An increase in killing can be seen with treatment of T cells: TNBC cell ratio of 10:1 + 10ug/ml mAb (yellow) versus IgG control (green) in 6 hours (b).

### Conclusions

- B7H3 is overexpressed in TNBC cells compared to healthy donors, validating it as a potential therapeutic target in patients
- Activation of NK cells is evident in figure 2 as seen by the increased killing with our novel mAb compared to IgG control antibody. The antibody also enhanced T cell killing by blocking B7H3 as shown in figure 4
- Figure 3 demonstrates the decreasing amount of live MCF7RFP cells as a result of treatment with the chimeric anti-B7H3 antibody co-cultured with NK cells. This required a much lower dose of NK cells than the experiment with monoclonal antibodies. This is due to the activation of the ADCC mechanism.
- The data suggests that our novel monoclonal and chimeric B7H3 blocking antibodies inhibit its immunomodulatory function and activate immune cells against TNBC cells

### Acknowledgements

I would like to thank the Department of Leukemia and all members of the Battula Lab. Thank you to the donors at the University of Notre Dame for making this research possible.

### References