Application of immunohistochemistry to the analysis of CAR-T cell therapy in triple negative breast cancer

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Introduction

- CAR (Chimeric Antigen Receptor)-T cells are genetically modified immune cells able to initiate an immune response in response to a selected protein
- While CAR-T cell therapy is effective in B cell lymphomas by targeting the CD19 antigen, solid tumors pose a greater challenge on account of the immunosuppressive tumor microenvironment (TME) [1]
- Analysis of the spatiotemporal distribution of T cells, other immune cells and tumor via immunohistochemistry (IHC) during the administration of CAR-T cell therapy will enable design of immunotherapies capable of clearing a solid tumor

Objectives

1. Optimize IHC staining protocol to reliably and selectively stain cell populations of interest in tumor samples for use in *in vivo* CAR-T cell and TME studies
2. Refine fluorescent microscopy methods to informatively measure and visualize IHC stained tissue
3. Infer impact of CAR-T cell therapy on TME and intercellular interactions involved in immunosurpression

Materials and Methods

- Tumors were taken from mice inoculated with CD19+ 4T1 mammary carcinoma [2] responsive and unresponsive to CAR-T cell treatment, frozen, and fixed to microscopy slides
- A standard IHC protocol (see fig. 1) was utilized to treat and stain tissue sections for selected markers and DNA for cell counting with DAPI
- Primary antibodies were selected for recognition of CAR construct (Thy1.1), tumor cells (CD19), T cells and subtypes (CD3, CD4, CD8, CD25), and other immune cell populations (CD14, MPO, CD56)
- A Zeiss fluorescence microscope was used to measure and process images of stained tissue
- Multiple concentrations of individual primary antibodies, combinations thereof, types of Alexa Fluor conjugated secondary antibodies, and microscope settings were attempted to find informative staining techniques

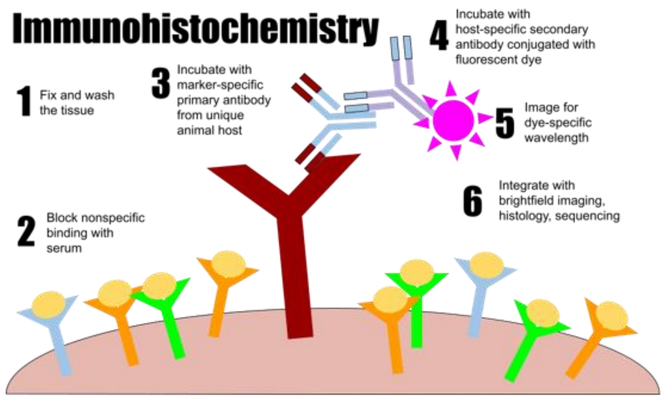

Figure 1: Overview of immunohistochemistry protocol utilized in study (Sodicoff, 2021)

Results

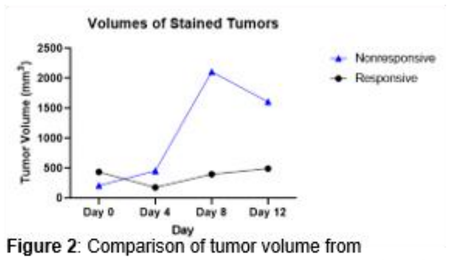

Figure 2: Comparison of tumor volume from responsive and nonresponsive samples in treatment group, implying active reduction in tumor proliferation in responsive group.

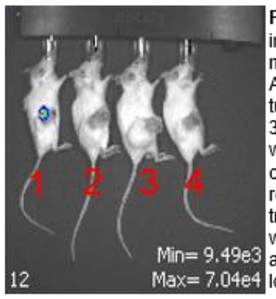

Figure 3: Bio-layer inferometry of live mice at t = 12 days. All mice have visible tumors, mice 1,2, and 3 received treatment while 4 acted as a control. Mouse 1 was responsive to treatment whereas 2 was not, likely on account of antigen loss in the tumor.

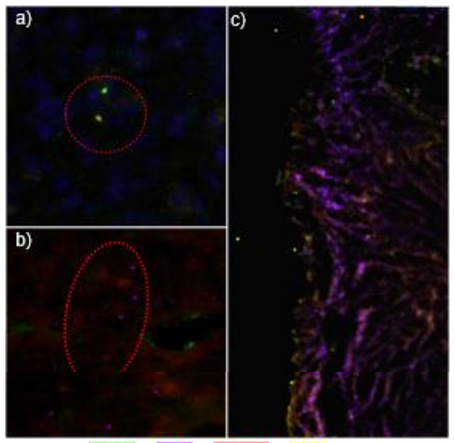

Figure 4: CD25 + CD8 + Thy1.1 + CD4 pan-T cell staining protocol reveals a) CD4+ and activated CAR T cells, b) cytotoxic T cells and c) the overal distribution of T cell subtypes on the periphery of tumor at low zoom

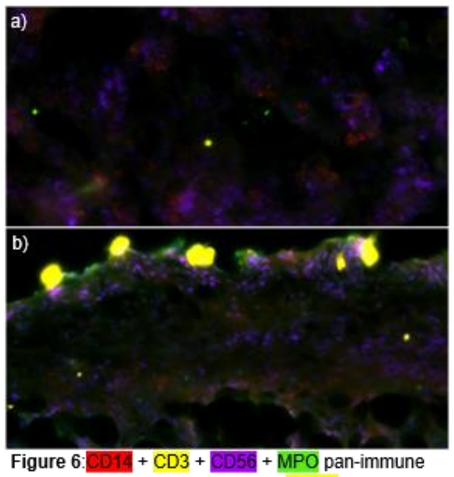

Figure 6: CD14 + CD3 + CD56 + MPO pan-immune staining shows a) the presence of T cells, inflammatory neutrophils, macrophages, and NK cells and b) the localization of large number of T cells and NK cells to the tumor periphery

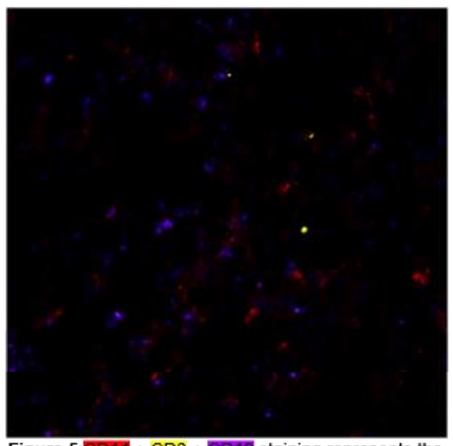

Figure 5: CD14 + CD3 + CD19 staining represents the high proportion of macrophages present deep within the tumor highly colocalized with 4T1 tumor cells, acting as a proof of concept for the use of genetically engineered macrophages in T cell recruitment in a related study for which this staining protocol will be used

- Staining and imaging for CAR-T cell killing (CD19 + Thy1.1 + CD3), T cell subtypes (CD25 + CD8 + Thy1.1 + CD4) , T cell-macrophage interaction (CD14 + CD3 + CD19), and immune cell populations (CD14 + CD3 + CD56 + MPO) was successfully completed in both responsive and nonresponsive tumors
- Results revealed differences in prescence of CAR-T cells, distribution and abundance of immune cell types, and supported theorized antigen loss in nonresponsive sample.
- Though some stains were somewhat nonselective (Thy1.1, CD3), optimization of imaging post-processing allowed for differentiation of true and false positive cells

[Poster Code ###]

Discussion and Conclusion

Discussion

- CAR T cell staining reflects the known cytotoxic effect of the therapy while demonstrating current limitations of the therapy related to tumor infiltration
- Pan-T cell staining allows for the analysis of tumor infiltration, abundance, and interaction of subpopulations
- Pan-immune staining highlights the need for further study of NK cell response to the TME and suggests macrophage localization deep within the tumor

Conclusions

- IHC staining for immune markers successfully reproduces known features of the 4T1 mammary carcinoma model
- The protocols developed for this project have broad uses in *in vivo* cancer immunological studies, though expansion of stain combinations would likely require production of new primary antibodies or multiplexing

Future Directions

- Single cell sequencing of treated and negative control tumors for quantitative comparison of cell type abundances and phenotypes
- Expansion of primary antibodies to include key metabolic markers to study metabolic reprogramming of cancer and immune cells[4]
- Histology with H&E and DAB stains for impact on cancer morphology
- Application of automated image analysis for high throughput determination of cell phenotypes and locations for further analysis

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References

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