Examining the Effects of SARS-CoV-2 on Metastatic Breast Cancer in a Murine Model

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Background and Aims

- SARS-CoV-2 has led to 771 million infections and ~7 million deaths (Nov 2, 2023)[1]
- Estimated that at least 12% of Canadians and 39% of Americans have been infected (likely a significant underestimation)
- Infection and vaccination seen to interfere with clinical FDG-PET scans[2]
- Fluoro-2-deoxy-D-glucose (FDG) is preferentially taken up by cells with increased glucose metabolism[3,4]
  - COVID-19 inflammation mimics cancer FDG uptake patterns
- COVID-19 is a CL3 pathogen → Expensive and difficult to work with
  - VSVΔG S expresses the spike protein of COVID-19 to facilitate early research

Goal: Characterize imaging and biological features of infection with VSV expressing SARS-CoV-2 spike in breast cancer model. Distinguish FDG uptake mediated by tumour and infection.

Methods

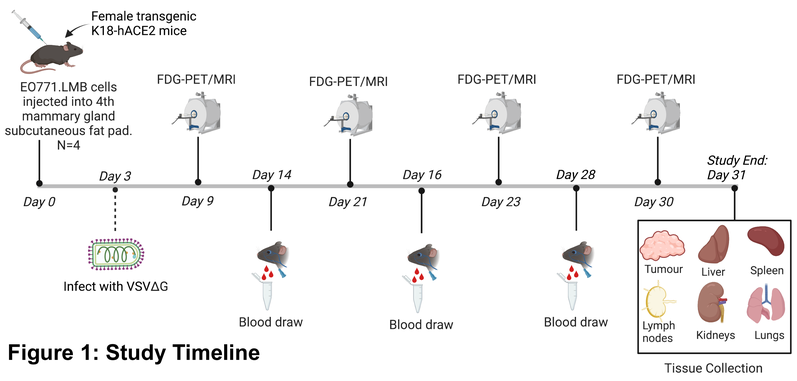
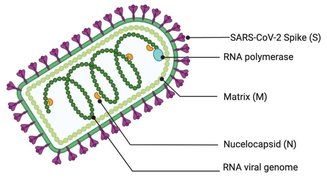
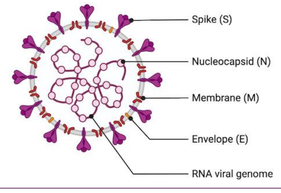


Figure 1: Study Timeline

Experimental Design

- **Mouse model:**
  - K-18-hACE2 mice (C57BL/6 background)
  - Express human ACE2 under control of the K18 promotor
  - Directed expression to epithelial tissues, mimics human ACE2 distribution
- **Biological analysis:**
  - Samples: weekly and terminal blood, terminal organ collection
  - Flow cytometry for immune phenotyping
  - Level of GFP+ cells as marker of infection level
- **Groups:**
  - E0771.lmb implant only $5X10^4$ cells in 100µl HBSS/Matrigel
  - E0771.lmb implant + VSVΔG S (SARS-CoV-2 omicron) infection $1X10^6$ PFU/mL
- **FDG-PET/MRI:**
  - ~350µCi injection 1 hour prior to scan
  - Simultaneous PET/MRI
- **Virus model:**
  - VSVΔG S (GFP+)
  - Pseudotype virus expressing the S protein of COVID-19 Omicron
  - Intranasal administration to mimic human route of infection





Results

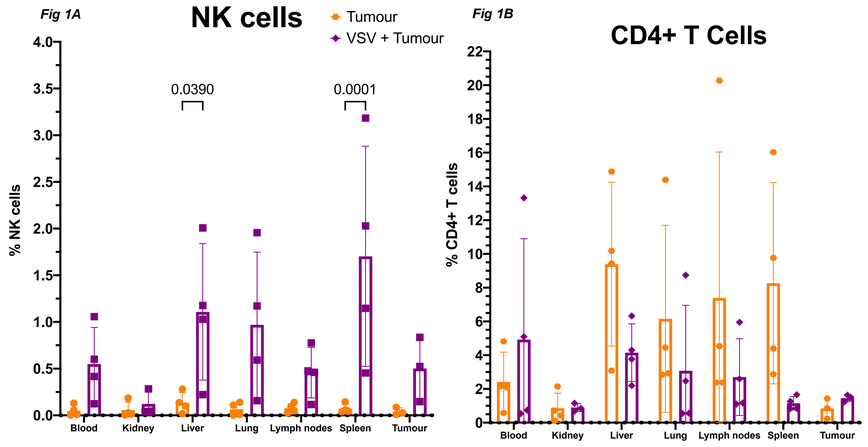
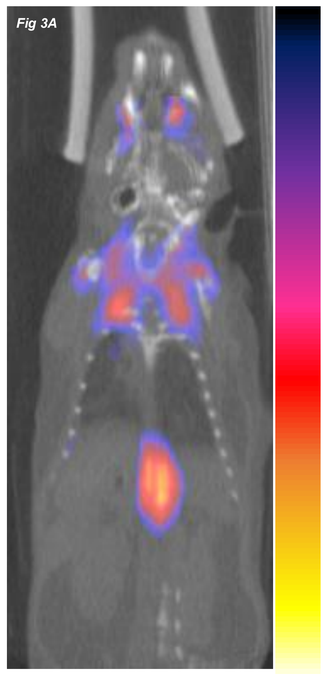


Figure 1 Immune cell changes between experimental groups. A) *Combination group had increased numbers of NK cells in all organs compared to tumour alone. This was most pronounced in the spleen and liver.* ***B)*** *CD4+ T cell levels were increased overall for tumour only mice, particularly in the liver and spleen.*



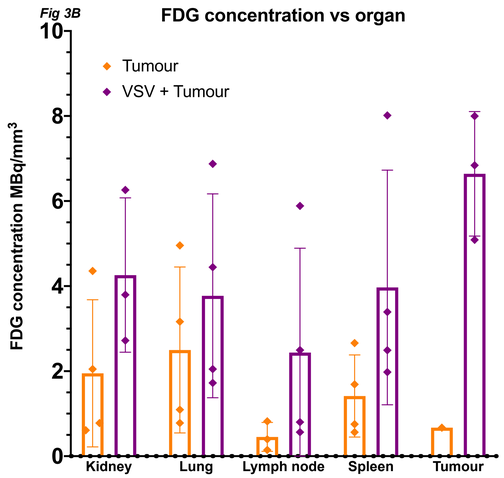
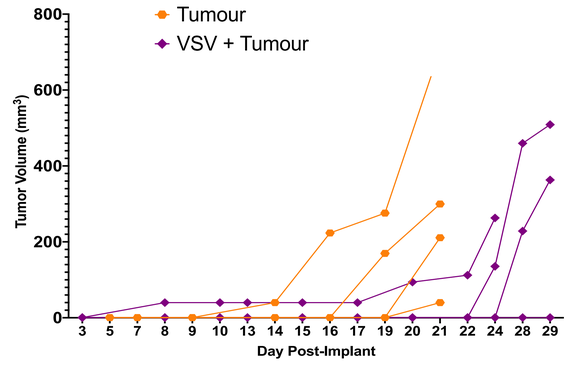
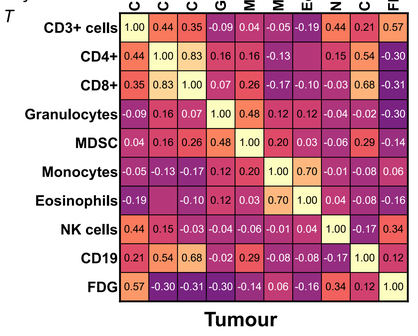
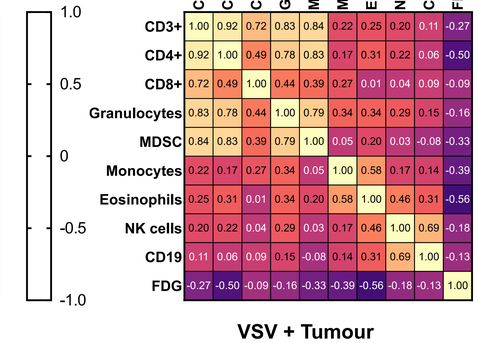


Figure 3 Increased FDG uptake in combination group
A) *Representative FDG-PET/MRI scan of mouse with E0771.lmb tumour and VSVΔG S (SARS-CoV-2 omicron) infection. Increased FDG uptake can be seen in the tumour, lungs, kidneys, and spleen.* ***B)*** *FDG concentration vs organ determined from FDG-PET/MRI imaging. Values have been standardized against muscle tissue, FDG dose, and mouse weight. Overall, the combination group had increased FDG concentration, with a P-value of 0.0467 between groups (ANOVA).*

Figure 2: Tumour volume changes over time. *Volumes were calculated using caliper measurements and MRI data. Tumour volumes were initially monitored by caliper; however, MRI data was needed to determine internal tumour volume. The combination group showed slower initial tumour growth than those with EO771.lmb tumours only.*







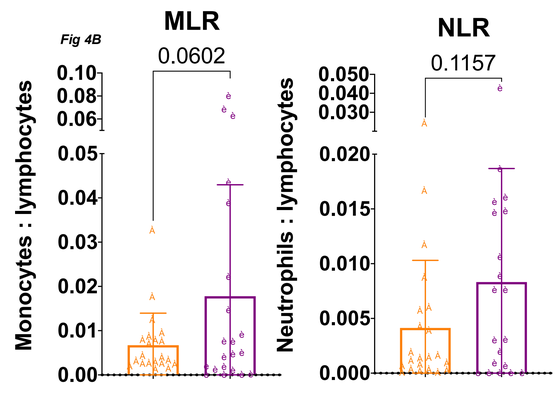


Figure 4 Immune cell changes between experimental groups.
A) *Pearson correlation matrix for both experimental groups showing the correlation between cell types. The combination group showed an increased correlation between granulocytes and T cells*
B) *Neutrophil: lymphocyte and monocyte: lymphocyte ratios were increased for the combination group. These ratios are indicators of systemic inflammation and are often reported in cancer and infections, suggesting increased inflammation in combination groups.*

Conclusions & Future Directions

CONCLUSIONS

- Tumour growth was delayed in combination group
- Increased FDG concentration in combination group – possible overall inflammatory state?
- Combination group has increased monocytes but decreased lymphocytes
- Increased levels of NK cells in combination

FUTURE WORK

- Compare to VSVΔG "empty" as a mock control and to mice infected only with VSVΔG omicron
- Test different VSV infection times relative to tumour implantation
- Perform tumour resection to extend survival time to observe long term changes
- Test different variants of SARS-CoV-2
- Test with full SARS-CoV-2 in a CL3 setting

References and Acknowledgements

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