While BRAF is among the most well-established oncogenes in human cancers, more recently it has garnered attention for its role in suppressing antitumor immunity, especially in melanoma. Because tumor-infiltrating lymphocyte (TIL) density is strongly prognostic in colorectal cancer (CRC)\(^1\), we decided to investigate the connection between TIL density and the BRAF-activating V600E mutation in CRC.

CoAD samples with the BRAF-V600E mutation have higher levels of immune cells than those without the mutation (\(p = 0.05\), Figure 1). The MSS subtype of CRC is characterized by epigenetic silencing of DNA mismatch-repair genes and higher mutational load, neoaigen expression and TIL density. Since it has been previously established that BRAF mutation is highly correlated with MSS subtype\(^2\), we performed an ANOVA to assess if BRAF mutation status predicts ESTIMATE score independently of MSS subtype. While the BRAF term was not significant in this multivariate linear model, we included an interaction term that was significant (\(p < 0.005\)), suggesting that both variables contribute to ESTIMATE score. To determine the effect of the interaction, we grouped samples into MSI-H and MSS groups and compared the wild-type and BRAF V600E subgroups within them. In the MSS group only, immune scores were higher in the BRAF-V600E subgroup (\(p = 0.05\), Figure 2).

Next, we sought to reproduce our results in an independent cohort of CRC tumor samples from the BROAD institute that have been assigned immune scores based on immunostaining\(^3\). We grouped samples into TIL-positive and TIL-negative groups and compared the numbers of BRAF-V600E and BRAF-WT samples within each group. We observed a significant correlation between BRAF mutation and TIL+ immune score within the MSS group (\(p = 0.01352\), odds ratio 5.76, Figure 3). Notably, the MSS group is the same group from the TCGA cohort in which we observed a significant correlation between BRAF mutation and ESTIMATE score.

### Discussion

The analysis we present here reveals a statistically significant correlation between the BRAF-V600E mutation and increased immune infiltrate in MSS tumors across two independent colon cancer datasets. Because V600E is an activating mutation, this finding suggests an immunity-stimulating role for BRAF in CRC, in contrast to BRAF’s role in melanoma, which appears largely immunosuppressive.

### References