Background While immune checkpoint blockade (ICB) treatments such as programmed death-1 (αPD-1) and cytotoxic T lymphocyte antigen-4 blockade (αCTLA-4) shifted the paradigm of cancer treatment, this ICB combination produces objective response rates of only 35.9% for patients with non-small cell lung cancer,1 and 58% for melanoma patients.2 Tumors are able to suppress the anti-tumor T cells that drive ICB through the reduction extracellular tumor pH,3–5 and by promoting hypoxia in the tumor microenvironment,6 both of which are functions inherent to tumor cells due to the Warburg effect. We thus hypothesize that the magnitude of tumor acidosis and hypoxia modulation influences ICB response.

Methods To interrogate modulation of acidity in combination with ICB, we screened a panel of six inhibitors that interfere with tumor cell mechanisms that reduce pH, termed pH sensitizers, using Seahorse, cell viability assay, and T cell suppression assay. We probed the effect of our pH sensitizer candidate in combination with αPD-1 and αCTLA-4 blockade for changes in immunogenicity, tumor control, and correlation between acidity and tumor response or immune cell infiltrate. Tumor pH is measured using a modified Chemical Exchange Saturation Transfer Magnetic Resonance Imaging technique, or acidoCEST MRI, which tracks tumor pH non-invasively. To interrogate the modulation of hypoxia, we measured the correlation between oxygen saturation and immune cell infiltrate and the tumor growth kinetics of myoinositol trispyrophosphate (ITPP) in combination with αPD-1 and αCTLA-4 blockade. Oxygen saturation is monitored non-invasively using Multispectral Optoacoustic Tomography, or MSOT.

Results Out of the pH sensitizers, we found that esomeprazole significantly reduced proton efflux rate without inducing cytotoxicity or T cell suppression. Furthermore, esomeprazole significantly reduced tumor burden when given one day prior to starting αPD-1 and αCTLA-4 treatment (figure 1), an effect mediated by decreasing the frequencies of tumoral Ly6C+ myeloid cells. We also observed that post-esomeprazole pH correlated with tumor mass (figure 2) and the frequencies of TCF-1+ effector CD4 and CD8 T cells in the tumors following αPD-1 and αCTLA-4 treatment. Lastly, we found that ITPP is able to significantly delay tumor growth, and that tumor immunogenicity increases in correlation with oxygen saturation induced by ITPP.

Conclusions AcidoCEST MRI can predict tumor control in the combination treatment of esomeprazole followed by αPD-1 and αCTLA-4 one day afterwards, with TCF-1+ effector CD4 and CD8 T cells being possible biomarkers for treatment success as well. MSOT may also indicate αPD-1 and αCTLA-4 tumor control by determining changes in tumor oxygenation by ITPP.
Abstract 4 Figure 2  Post-esomeprazole pH predicts treatment efficacy
A) AcidoCEST MRI images depicting tumor pH distribution after esomeprazole treatment in a responsive (left) and unresponsive (right) 4T1 tumor. B) Average pH from acidoCEST MRI after esomeprazole treatment compared to tumor mass after three treatments of ICB in 4T1-bearing mice, n = 12