Background The recent evidence shows that Programmed Death Ligand 1 (PD-L1) presentation on the plasma membrane is dynamically modulated by the delivery via the secretory pathway and withdrawal, recycling and degradation, which may involve endolysosomal pathway. PD-L1 is delivered to the plasma membrane via the secretory pathway which may involve lysosomal activity. Whereas the significance of this step is not completely understood, it provides a rationale for testing lysosomal inhibition as a means to regulate PD-L1. Limited, but intriguing, prior attempts yielded mixed results, suggesting that regulation of this process is complex. We hypothesized that inhibition of endolysosomal traffic would suppress PD-L1 plasma membrane presentation and synergize with anti-PD-1 immune checkpoint blockade.

Methods PD-L1 plasma membrane presentation was measured via flow cytometry using lysosomotropic drugs, apilimod or hydroxychloroquine (HCQ). Lysosomal exocytosis was measured using beta-hexosaminidase activity. PIKfyve knock down cells were created using CRISPR-Cas9 technique. We designed an in vitro assay to co-culture peripheral blood CD8+ T cells with human cancer cell lines. C57/Bl (4-week-old) mice were implanted subcutaneously with MOC-1 or UPCI: M4Tu lines on each flank and randomized. Treatments were administered two times a week intraperitoneally. The volume of the tumors was measured three times a week.

Results We find that in vitro, treatment with the PIKfyve inhibitor apilimod or suppresses plasma membrane presentation of PD-L1. Similarly, treatment with the lysosomal inhibitor hydroxychloroquine (HCQ) also reduced PD-L1 presentation, confirming endolysosomal involvement in PD-L1 handling. Using syngeneic mouse models of head and neck cancers, we find that HCQ synergizes with anti-PD-1 therapy, causing tumor growth inhibition of 80% and dramatically increased survival (p<0.001, log-rank test). Treatment with HCQ promoted in vitro cancer cell cytotoxicity, suggesting that HCQ may directly promote CD8+ T cell activity.

Conclusions HCQ is a potent adjuvant for anti-PD-1 therapy, by mediating PD-L1 plasma membrane presentation on cancer cells and possibly mediating CD8+ T cell activation.

Ethics Approval The animals were handled and euthanized in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. Human blood samples were obtained from the University of Pittsburgh Medical Center in accordance with established University of Pittsburgh IRB guidelines. Written informed consent was obtained from all the patients before inclusion in the study.