Methods To activate antigen-specific CD4+ T cells in vivo, we utilized our nucleic acid platform, UNITE (UNiversal Intracellular Targeted Expression), which fuses a tumor-associated antigen with lysosomal-associated membrane protein 1 (LAMP1). This lysosomal targeting technology results in enhanced antigen presentation and a balanced T cell response. LTS220A, encoding a mutated form of MCPyV-LT that abrogates its pro-oncogenic properties, was introduced into the UNITE platform. LTS220A-UNITE, known as ITI-3000, was administered to female C57BL/6 mice intradermally in the ear with electroporation. Results ITI-3000 promoted a potent, antigen-specific CD4+ T cell response to MCPyV-LT. Vaccination with ITI-3000 significantly delayed and slowed growth of B16F10 tumors expressing LTS220A in prophylactic and therapeutic settings, respectively. ITI-3000 induced a favorable tumor microenvironment (TME), including significantly enhanced numbers of CD4+ T cells, CD8+ T cells, NK cells, and NKT cells. Tumor-infiltrating myeloid cells were reduced in frequency in vaccinated mice and polarized towards an anti-tumor phenotype. Cytokine analysis of the TME showed significantly enhanced levels of cytokines associated with anti-tumor immune responses in ITI-3000-vaccinated mice, including IFNγ, TNFα, IL-2, and IL-1β. Additionally, ITI-3000 synergized with PD-1 blockade, further reducing tumor burden and enhancing survival in mice receiving combination therapy. Conclusions We find that DNA vaccination with ITI-3000 using the UNITE platform enhances CD4+ T cell responses to MCPyV-LT and results in anti-tumor immune responses in a mouse model of Merkel cell carcinoma.

Ethics Approval This study was approved by Immunomic Therapeutics’ Institutional Animal Care and Use Committee, protocol number 16-11-002.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0857

The present study examined the expression of HPK-1 and SLP76 in tumor-infiltrating lymphocytes (TILs) obtained from renal cell carcinoma tissues, in relation with the expression of PD-1 and other immune checkpoint receptors by performing flow cytometry analysis. In addition, we examined if inhibition of the kinase activity of HPK1 by CMPD0914, that is a potent, selective and orally available HPK1 inhibitor, enhanced effector functions of tumor-infiltrating CD8+ T cells in the presence of anti-PD-1 blocking antibodies.

Results First, we found that HPK1 and SLP76 are expressed in both CD8+ and CD4+ T cells including Foxp3+ regulatory T cells irrespective of PD-1 expression. Intriguingly, the expression levels of HPK1 and SLP76 were significantly higher in the PD-1bright population compared to the PD-1- or PD-1dim populations. Further characterization revealed that HPK1 and SLP76 were highly expressed in CD8+ T-cell populations expressing TOX, a transcription regulator of T-cell exhaustion,