Background: RP1 is a novel, enhanced potency, oncolytic version of HSV-1 engineered to express human GM-CSF and GALV-GP R-. RP1 + anti-PD1 therapy combination has resulted in deep and durable responses, including in melanoma patients who have previously failed anti-PD1 therapy.

Methods: Tumor biopsies were taken pre-treatment and at 43 days after the first dose of RP1. The tumor immune microenvironment (TIME) was analyzed using IHC for CD8 (SP57 clone, Ventana) and PD-L1 (PD-L1 IHC 28-8 pharmDx by Agilent) and by gene expression analysis using the NanoString IO360 panel. The tumor inflammation signature score (TIS) was also calculated using an 18 gene signature. Systemic anti-tumor immunity was assessed using PBMCs by sequencing the CDR3 regions of human TCRβ chains using the immunoSEQ assay. Correlation analysis of baseline tumor PD-L1 and CD8 status versus clinical response was also performed.

Results: A consistent increase in CD8 and PD-L1 expression in the tumor was observed in most of the tested biopsies (30/44), which generally appeared to be co-located. These increases were observed both in superficial lesions and visceral tumors, including in the liver. A notable reversal of CD8+ T cell exclusion was observed in a melanoma patient who failed prior ipilimumab and nivolumab treatment. Clinical responses were independent of baseline CD8 T cell infiltration, PD-L1 expression levels, and prior anti-PD1 therapy. Gene expression analyses of tumor biopsies (n=11) demonstrated significant increases in the expression levels of genes associated with innate and adaptive immune activation and genes previously reported to be associated with responsiveness to anti-PD1 therapy, particularly CD8, CXCL9, CD27, and TIGIT, as well as consistent increases in TIS. TCR sequencing of PBMCs revealed expansion of pre-existing T cell clones and the appearance of new clones with 20-80% of these changes being newly detected clones. Expansion of new clones (n=170) was observed in a melanoma patient who had a complete response.

Conclusions: The biomarker data indicate broad immune activation by RP1 + nivo. Clinical responses are independent of baseline PD-L1 expression and associated with increases in gene signatures associated with cytotoxic T, NK, and Th1 cells. The data indicate the potential for broad utility of RP1 in a range of tumor types, including in patients with primary or acquired resistance to immune checkpoint blockade.

Trial Registration: NCT03767348