Background Globally more than 600,000 cases of HPV-associated cancers occur annually. Approximately 15-20% of cases respond to PD-(L)1 inhibitors, and approximately 30%, including 10% of immune checkpoint inhibitor (ICI) refractory patients, respond to bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1. Thus, for most patients who are ICI refractory, there is no effective therapy. Preclinical studies have shown that the triple combination of bintrafusp alfa, M9241, a tumor-targeting IL-12 immunocytokine, and PDS0101, a therapeutic vaccine targeting HPV-16, resulted in maximal tumor reduction. A phase II trial (NCT04287868) evaluating this triple therapy has shown a manageable safety profile and preliminary evidence of clinical activity in ICI refractory HPV-associated cancers, with 45% of patients having disease reduction, including 27% with objective responses.

Methods Peripheral blood from patients with ICI refractory HPV-associated malignancies (n=27) treated with the triple therapy was analyzed prior to and 2 weeks post first treatment (a timepoint prior to restaging) for multiple serum cytokines and soluble factors, complete blood counts, and 158 immune cell subsets. HPV-16 specific T-cells were assessed before and during treatment in a subset of patients (n=14). Immune parameters were evaluated for changes with therapy and compared between patients deriving clinical benefit (with a best overall response of stable disease, partial response, or complete response) versus those with progressive disease (PD).

Results The triple therapy promoted a pro-inflammatory serum cytokine and factor milieu, and significantly increased NK cells (p=0.002) and decreased conventional dendritic cells (cDCs, p=0.001), plasmacytoid DCs (p=0.011), CD4+ T-cells (p=0.008), CD8+ T-cells (p=0.004), T-regulatory-cells (p=0.002), and B-cells (p=0.042). HPV-16 specific T-cells were increased >2 fold after therapy in 11/14 patients evaluated. Before therapy, patients developing clinical benefit from the triple therapy had significantly higher levels of CD8+ naïve T-cells (p=0.037), trends of higher CD8:MDSC ratios (p=0.098), and significantly lower levels of cDCs (p=0.019) and classical monocytes (p=0.049), than patients developing PD. A greater early increase (2 weeks after one treatment cycle) in soluble granzyme B (p=0.004), TNFa (p=0.013), and monocytes (p=0.025), and less of a decrease in cDCs (p=0.006) associated positively with clinical benefit, while trends of an increase in the neutrophil to lymphocyte ratio (p=0.073) associated inversely.

Conclusions These studies interrogating the peripheral immunome add insight into the combined mechanism of action of bintrafusp alfa, M9241, and PDS0101 in patients with HPV-associated cancers, and provide valuable information to identify ICI refractory patients potentially more likely to benefit from immunotherapy.

Ethics Approval All patients gave written informed consent for participation. This study was approved by the National Cancer Institute’s Institutional Review Board. The trial registration number is NCT04287868.