Background Merkel cell carcinoma (MCC) is an aggressive skin cancer often associated with clonal integration of Merkel cell polyomavirus (MCPyV) and expression of T antigen oncoproteins in 80% of cases with the remainder of cases caused by UV mutations.\(^1,2\) PD-1 blockade is effective in treating both etiologies of MCC patients, however, only ~60% of patients respond.\(^3\) MCPyV-specific T cells are implicated in immunotherapeutic responses\(^4,\) yet further qualitative and quantitative analysis of those cells among responders and non-responders is needed.

**Methods** We analyzed MCPyV-specific T cells in blood from responders (CR, \(n=13\)) and non-responders (\(n=6\) for partial responses, PR; \(n=5\) for stable and progressive disease, SD/PD) before, during and after anti-PD-1 immunotherapy (from trial NCT02267603) using a mass cytometry (CyTOF)-based multiplexed peptide-MHC tetramer staining approach. This allowed us to simultaneously assess 76 MCPyV epitopes and 34 control epitopes derived from viruses such as CMV, EBV, Flu, and HSV, in parallel with 34 cellular phenotyping markers of differentiation, trafficking, and exhaustion. We also performed deep TCR sequencing of sorted T cell populations from the blood samples to track T cell clonotypes between the periphery and tumor.

**Results** Combinatorial tetramer staining with CyTOF allowed us to detect T cells specific for 11 previously reported and 5 novel MCPyV-specific epitopes. Frequencies of MCPyV-specific cells were higher in patients with CR (mean = 0.103%; \(P=0.007\)) and SD/PD (mean = 0.003%; \(P<0.0001\)) at the baseline, and decreased over the course of immunotherapy in patients with CR. Strikingly, the phenotypes of MCPyV-specific CD8\(^+\) T cells were enriched for an activated/exhausted (CD71\(^+\)PD-1\(^+\)CD39\(^+\)) phenotype. Those cells also highly expressed cutaneous lymphocyte-associated antigen (CLA), a marker of skin trafficking, and CD103, a marker of tissue-recirculating cells. Informed by these phenotypes, high-dimensional profiling of bulk CD8\(^+\) T cells revealed correlations of the frequencies of CD39\(^+\) cells co-expressing CLA or CD103 with both the baseline tumor burden and magnitude of clinical outcome. TCR sequencing was used to assess clonal sharing between tumor infiltrating T cells and these subpopulations of CD8\(^+\) T cells from blood.

**Conclusions** Although these findings need to be confirmed in an independent cohort, our high-dimensional analysis and immune profiling of MCC patients suggest that MCPyV-specific cells and CD39\(^+\) cells co-expressing CLA or CD103 in blood are enriched for tumor-reactive TCRs and potentially useful as blood-based biomarkers of response to immunotherapy or for novel cellular therapeutic strategies.

**Trial Registration** ClinicalTrials.gov NCT02267603

**REFERENCES**


**Ethics Approval** The samples were provided by Cancer Immunotherapy Trials Network (trial registration: ClinicalTrials.gov NCT02267603) and the analysis was performed according to the IRB file/approval number IR File #10686.