Background PD-1 pathway blockade has revolutionized oncology, though most patients do not derive durable benefit. Accurate prediction of response is not currently possible. Cancer-specific CD8 T cells can mediate tumor regression, however, identifying these cells is difficult because many tumor antigens are patient-specific. Merkel cell carcinoma (MCC), driven by Merkel cell polyomavirus (MCPyV) oncoproteins in ~80% of cases, is an attractive cancer for studying tumor-specific T cells due to shared, viral antigens and a low tumor mutational burden. Using samples from a recent trial of neoadjuvant nivolumab in MCC (NCT02488759), we studied anti-PD-1 resistance by interrogating MCPyV-specific CD8 T cells before and during therapy.

Methods MCPyV-specific CD8 T cells were identified using an expanded panel of 16 MCPyV-specific HLA class I multimers. PBMC from 21 patients with suitable multimers (among 35 patients assessed) collected before, 2 and 4 weeks after initiating anti-PD-1 were stained with MCPyV-multimers in 26-plex flow cytometry. Intratumoral MCPyV-specific T cell frequency was calculated using a combination of 1) HLA-I multimers and paired T cell receptor (TCR)-seq of phytohemagglutinin-expanded tumor infiltrating lymphocytes to identify virus-specific TCRs and 2) beta-TCRseq of formalin-fixed tumors to determine T cell clone frequency. To study phenotypic differences between cancer-specific CD8 T cells found in tumors versus blood, single cell RNAseq and paired TCRseq were performed on a separate cohort of 7 MCC patients.

Results Patients without detectable circulating MCPyV-specific CD8 T cells before treatment had shorter recurrence-free survival (RFS; figure 1; median RFS=12 months; n=7) than patients with detectable MCPyV-specific cells (median RFS not reached; n=11; p=0.0078). In contrast, response was not associated with intratumoral, MCPyV-specific CD8 T cells (13% [mean] of intratumoral T cells in patients with pathological complete response versus 23% in those without response; p=NS). T cells were considered ‘dysfunctional/exhausted’ if they fell within single cell RNAseq clusters characterized by TOX, PD-1, and LAG-3 transcripts. MCPyV-specific T cells were significantly more likely to be dysfunctional/exhausted if they were intratumoral (>90% dysfunctional) versus in blood (0–50%; p=0.002).

Conclusions MCC-specific CD8 T cells in blood were less dysfunctional than their intratumoral counterparts. The frequency of pre-existing MCC-specific CD8 T cells in blood strongly correlated with anti-PD-1 response, while their frequency within tumors was unrelated to response. These results suggest that approaches to increase the number of circulating, less exhausted, cancer-specific T cells may benefit patients with anti-PD-(L)1-refractory MCC, and the frequency of these cells may be a predictive marker of anti-PD-(L)1 response.

Trial Registration NCT02488759

Ethics Approval This study was approved by the Fred Hutchinson Cancer Center’s Institutional Review Board, approval number 6585. All patients represented here participated with written informed consent.