Background PD-(L)1 blockade has transformed treatment for advanced Merkel cell carcinoma (MCC), with over 50% of patients initially responding to therapy. However, the need to understand the underlying biology of responses and explore alternative treatments for non-responders remains unmet. MCC is a highly immunogenic skin cancer that is caused by the Merkel cell polyomavirus in ~80% of cases with remaining cases due to UV exposure. Both etiologies result in high immunogenicity due to viral antigens or UV-neoantigens. Prior studies of MCC immuno-biology have mainly focused on CD8 T-cells and their exhaustion status. However, the role of innate immunity, particularly myeloid cells in MCC immune evasion, has not been well studied. We sought to investigate the types of myeloid cells in MCC and their association with outcomes to PD-(L)1 blockade.

Methods Myeloid cell profiling in MCC tumors was conducted using single-cell RNA sequencing (scRNAseq) of associated tumors from 9 patients. MCC tumors collected from 54 patients prior to anti-PD-(L)1 treatment were then used to identify myeloid cells via multiplex-IHC and evaluate their association with response.

Results scRNAseq analysis showed that tumor-associated macrophages (TAMs) are the main myeloid component within MCC tumors. TAMs in MCC express an immunosuppressive gene signature characteristic of monocytic myeloid derived suppressor cells and express targetable immune checkpoint molecules, including PD-L1 and LILRB receptors, that are not present on tumor cells. Analysis of 54 pre-immunotherapy tumor samples showed that a subset of TAMs (CD163+, CD14+, S100A8+) correlated with CD8 T-cell infiltration, despite their presumed immunosuppressive role. Stratifying tumor samples based on CD8 T-cell levels and measuring the TAMs-to-CD8 T-cell ratio (TAM/CD8) revealed a suppressive relationship between TAMs and CD8 T-cells. A higher TAM/CD8 ratio among high CD8 tumors was associated with resistance to PD-(L)1 blockade treatment, and the ratio was a far better predictor of initial response than CD8 T-cell level alone (ROC analysis: TAM/CD8 ratio AUC = 0.96, p=0.008; CD8% AUC = 0.60, p=0.544). Progression free survival analysis of the 54-patient validation cohort also showed that a higher TAM/CD8 ratio predicted earlier disease progression in PD-(L)1-treated patients (HR: 1.24 per 2-fold increase, p = 0.0074).

Conclusions Immunosuppressive activity of TAMs in MCC tumors may counteract the benefit of intratumoral CD8 T-cells, reducing the likelihood of response to treatment. This study suggests that immunotherapy-refractory MCC may be an appropriate setting in which to explore myeloid-targeted therapies in clinical trials.

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