**Background**

T lymphocytes reactive to melanoma antigens can cause regression of advanced melanoma and mediate long-term immunologic memory. However, immune control of melanoma depends on the ability of T cells to infiltrate sites of melanoma deposits. The objective of this study was to determine whether 6 synthetic melanoma helper peptides (6MHP) vaccine plus pembrolizumab increased infiltration of vaccine-induced T cells into tumor metastases compared to 6MHP vaccine alone.

**Methods**

Patients in Mel64 (NCT02515227) received 6MHP vaccines on days 1/8/15/43/64/85. Pembrolizumab was administered intravenously every three weeks, beginning on day 1. Patients also provided blood samples, and sentinel immunized node (SINs) biopsies if available, at specific time points for immune analyses. Tumor biopsies were collected on days 1 (pre-treatment) and 22. Patients in a prior trial, Mel41 (NCT00089219), served as controls, who received 6MHP vaccines on days 1/8/15/29/36/43. Tumor biopsies were collected pre-vaccination and post-vaccination at time of tumor recurrence (figure 1). Across both trials, DNA was extracted from peripheral blood mononuclear cells (PBMCs) pre-treatment, and PBMCs/SINs at time of peak T cell response to 6MHP. These were submitted for high throughput T cell receptor (TCR) sequencing to assess for T cell clonotypes that were significantly increased in number post-treatment or novel clonotypes evident de novo post-treatment. These sequences were cross referenced against those in PBMCs/SINs for those that overlapped and were present in tumor post-treatment, but not pre-treatment.

**Results**

Patients across both trials (Mel41 n = 5; Mel64 n = 4) had expanded circulating vaccine-induced T lymphocytes in PBMCs/SINs post-treatment compared to pre-treatment (figure 2). All patients had novel T cell clonotypes in tumor post-treatment compared to pre-treatment (figure 3A). The fraction of vaccine-induced tumor infiltrating lymphocytes (VITILs) of total tumor infiltrating lymphocytes (TILs) was significantly increased in Mel64 patients compared to that in Mel41 patients (p = 0.0159) (figure 3B).

**Conclusions**

These findings suggest that adding pembrolizumab may enhance infiltration of melanoma metastases by vaccine-induced T lymphocytes compared to 6MHP vaccine alone. This treatment combination holds promise in the management of advanced melanoma, and additional interventions may further enhance tumor infiltration of vaccine-induced T cells.

**Trial Registration**

These clinical trials were conducted at the University of Virginia and registered with Clinicaltrials.gov (Mel41 NCT00089219, Mel64 NCT02515227).

**Ethics Approval**

These clinical trials were performed with the University of Virginia Institutional Review Board (IRB), previously known as the Human Investigations Committee (HIC), approval (Mel41 HIC #10464, Mel64 IRB #18174). All participants provided their informed consent before taking part in these clinical trials.