Background Immune-related adverse events (irAE) are major barriers of clinical management and further development of cancer immunotherapy. In this study, we used mass cytometry to characterize the immune system before and during presentation of severe (grade 3+ that required clinical intervention) irAE to identify cell signatures correlated with irAE development.

Methods We performed analysis on banked peripheral blood mononuclear cells (PBMCs) from 29 patients with melanoma undergoing checkpoint inhibitor (CPI) therapy, of which 18 developed severe irAEs (3 gastrointestinal, 5 endocrine, 4 cutaneous, 9 hepatobiliary, 1 pulmonary irAEs and 1 acquired lipodystrophy) while 11 did not. Samples were taken at initiation of CPI (pre-treatment), before onset of irAE (pre-irAE) and at presentation of irAE (irAE max). The median number of CPI cycles per patient was 5 for patients with severe irAEs and 11 for patients without severe irAEs. We used a 44-marker mass cytometry panel designed to capture cell subsets and activation states across the innate and adaptive immune system, with both supervised analysis of manually gated immune cell subpopulations and unsupervised analysis by Clustering LARge Applications (CLARA) clustering.

Results At the peak of irAE presentation, patients with severe irAE demonstrated significantly more CD38+ (marker of immune activation) CD4+ central memory T cells (TCM), CD39+ (marker of tumor antigen-specificity) and HLA-DR+ (marker of activation) CD8 TCM cells, and CCR7+ (marker of migration) CD4+ T cells compared to patients with no or non-severe irAEs. Conversely, patients with severe irAEs had significantly fewer CD16+ NK cells and CD161+ (marker of inhibition) CD4+ T cells. Interestingly, patients with severe irAEs already had fewer CD16+ NK cells and CD161+ CD4+ T cells pre-treatment, and more CCR7+ CD4+ T cells pre-irAE. Presentation of severe irAEs was also correlated with significantly lower levels of TIGIT+ regulatory T (Treg) cells pre-treatment, and more CD4+ T naïve cells at all three timepoints.

Conclusions Development of severe irAE in melanoma could be the result of reduced inhibitory immune capacity pre-treatment, marked by fewer regulatory TIGIT+ Treg cells and CD161+ CD4+ T cells, leading to dysregulated increase in migratory CCR7+ CD4+ T, activated CD38+ and tumor-reactive CD39+ TCM cells, and activated CD16+ NK cells. This study demonstrates that high-dimensional immune profiling can detect novel blood-based immune signatures associated with presentation of severe irAEs.

Ethics Approval The study was approved by Huntsman Cancer Institute’s Ethics board, approval number IRB_00010924. Participants gave informed consent before taking part in the study.