Decreased frequencies of suppressive regulatory T cells and higher frequencies of naïve CD4⁺ T cells in peripheral blood at baseline are associated with severe immune-related adverse events and clinical benefit in checkpoint inhibitor-treated melanoma.

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Summary

- Immune-related adverse events (irAEs) are major barriers to clinical management and further development of cancer immunotherapy[1]. We used mass cytometry to characterize the immune system before and during presentation of severe irAEs (defined as grade 2+ irAEs that required clinical intervention) to identify cell features associated with irAE development.
- Patients with severe irAE had more TIGIT⁺ Tregs at baseline, and fewer CD16⁺ NK cells and more CD4⁺ naïve T cells before and during treatment. irAE presentation was also associated with more activated T cells. Similar immune features were associated with clinical benefit.
- This study demonstrates that high-dimensional immune profiling can reveal novel blood-based immune features associated with risk and mechanisms of severe irAEs.

Patient cohort and methods

Figure 1. Melanoma patient cohort

The cohort used peripheral blood mononuclear cell (PBMC) samples from 28 patients with melanoma across 29 lines of immunotherapy. In the 29 lines of therapy, 18 resulted in severe irAE and 11 did not.

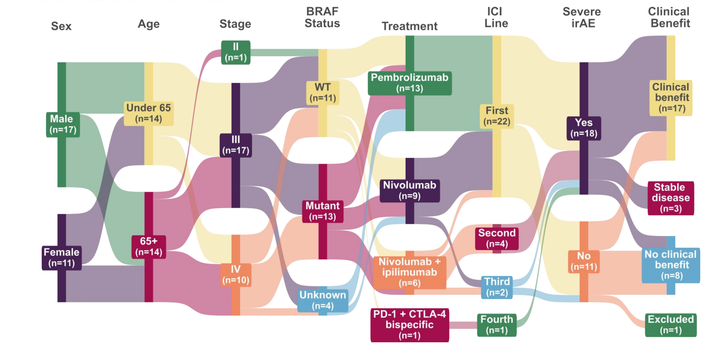
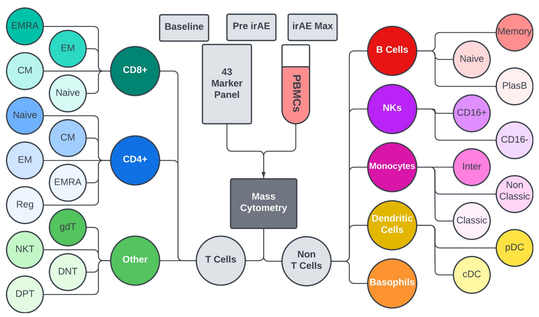


Figure 2. Mass cytometry methodology



PBMCs were collected before treatment (baseline), before the onset of irAEs (pre-irAE) and at the peak of irAE (irAE-max) and stained with a 43-marker panel. Statistical comparisons were performed using Significance Analysis of Microarrays (SAM). * fdr < 0.05; ** fdr < 0.01.

Acknowledgements

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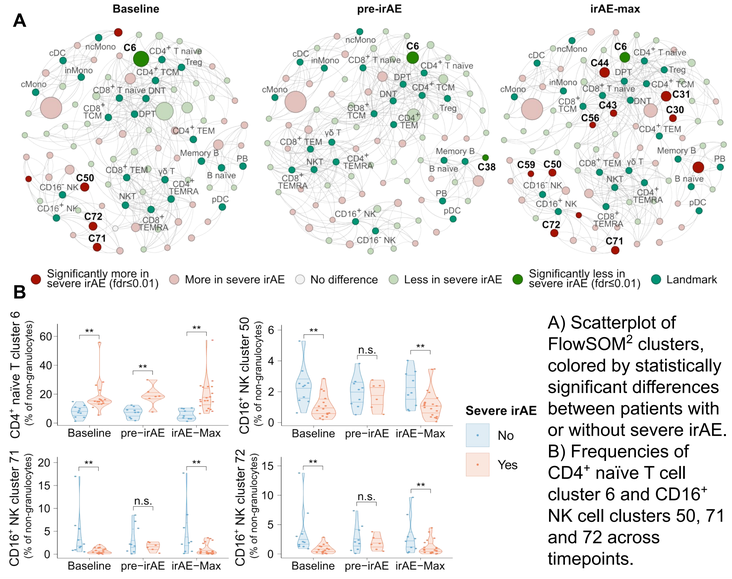
Unsupervised clustering shows more CD4⁺ naïve T cells but fewer cytotoxic CD16⁺ NK cells in patients with severe irAE

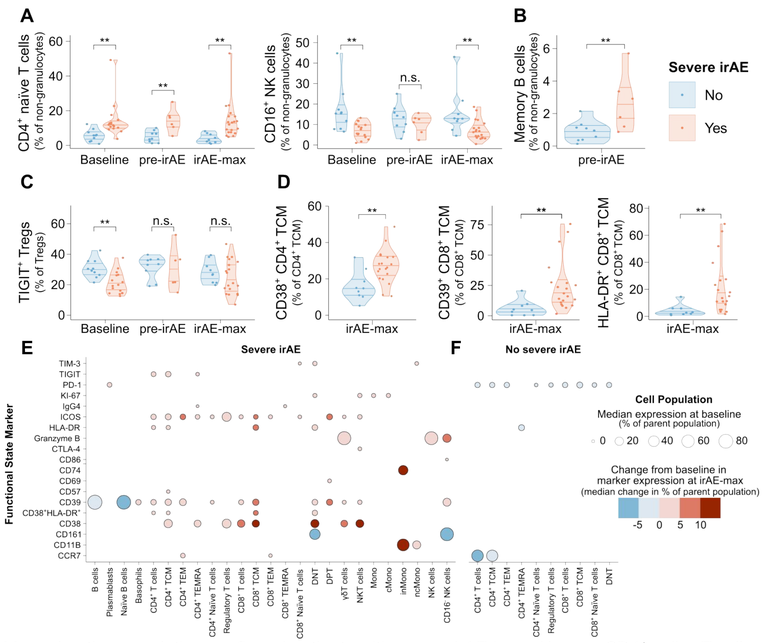
Figure 3. FlowSOM Unsupervised Clustering Analysis



A) Scatterplot of FlowSOM[2] clusters, colored by statistically significant differences between patients with or without severe irAE. B) Frequencies of CD4⁺ naïve T cell cluster 6 and CD16⁺ NK cell clusters 50, 71 and 72 across timepoints.

Manual gating shows severe irAEs are associated with fewer TIGIT⁺ Treg and more activated central memory T cells

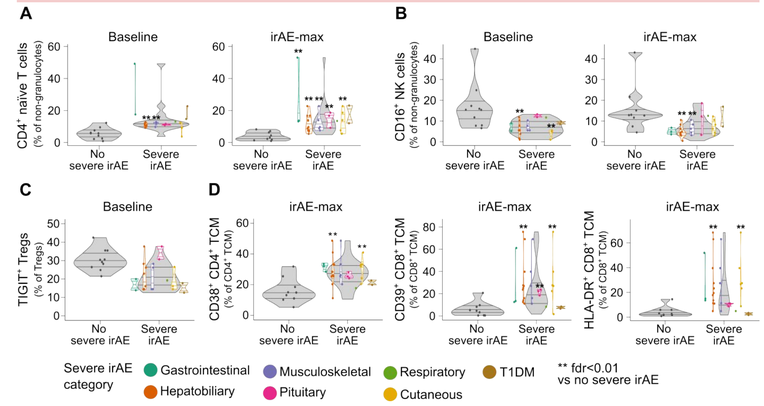
Figure 4. Manually Gated Populations in Patients with Severe irAE



Manual gating population confirmed patients with severe irAE have A) more CD4⁺ naïve T cells and fewer CD16⁺ NK cells across timepoints, but also showed severe irAE were associated with B) more memory B cells pre-irAE, C) fewer suppressive TIGIT⁺ Treg[3] at baseline and D) more activated CD38⁺ CD4⁺ central memory (CM) T cells[4], CD39⁺ and HLA-DR⁺ CD8⁺ TCM[5-6] at irAE-max. E-F) Median fold change from baseline in state marker expression at irAE-max.

Some, but not all, previously identified immune features are associated with specific subcategories of severe irAE

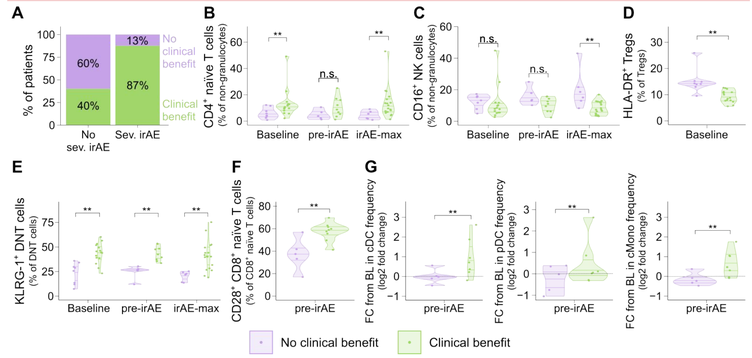
Figure 5. Contribution of Individual irAE Categories



Contribution of individual irAE categories to the immune features associated with severe irAE, specifically A) CD4⁺ naïve T cells; B) CD16⁺ NK cells; C) TIGIT⁺ Treg; D) CD38⁺ CD4⁺ TCM and CD39⁺ and HLA-DR⁺ CD8⁺ TCM. Test: SAM of individual irAE vs no severe irAE.

CD4⁺ naïve T cells, CD16⁺ NK cells and suppressive Treg are associated with clinical benefit

Figure 6. Manually Gated Populations in Patients with Clinical Benefit



Clinical benefit was defined as partial or complete response to palliative immunotherapy or no relapsed disease with adjuvant therapy. Stable disease was excluded from analysis. A) Patients with severe irAE were more likely to experience clinical benefit (chi-square p-value=0.04). Patients with clinical benefit had B) more CD4⁺ naïve T cells; C) fewer CD16⁺ NK cells and D) suppressive HLA-DR⁺ Treg[7]; E) more KLRG-1⁺ double-negative T cells (DNT); F) more CD28⁺ CD8⁺ naïve T cells; and G) a greater increase (represented as fold change from baseline (FC from BL)) in classical dendritic cells (cDC), monocytes (cMono) and plasmacytoid DC (pDC).

Conclusions

- Using mass cytometry, we found various markers associated with severe irAE at baseline and before onset of severe irAE. Some of these markers are also associated with clinical benefit, such as CD4⁺ naïve T cells, CD16⁺ NK cells and TIGIT⁺ or HLA-DR⁺ suppressive Treg cells.
- Peak of irAE presentation is associated with higher frequencies of activated CD4⁺ and CD8⁺ TCM, as characterized by expression of activation molecules CD38, CD39 and HLA-DR.
- Although development of severe irAE and clinical benefit to immunotherapy both require immune system activation, different mechanisms likely apply. For example, increases in myeloid cells during the course of treatment was only seen in association with clinical benefit.
- We plan to validate these findings and identify additional markers of severe irAE and clinical benefit with immune profiling in a larger patient cohort from the same institution.