Immune features of irAEs and aPD1 response in urothelial cancer patients of the RADIOHEAD study, as detected in blood by mass cytometry immune profiling

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Abstract Presentation #2138

Summary

- Previous reports have established a correlation between occurrence of immune-related adverse events (irAE) and better checkpoint immunotherapy patient outcomes, yet these correlations and their underlying biological mechanisms are poorly understood.
- To identify cell signatures associated with response and irAE development, we used mass cytometry to characterize the immune system in patients with urothelial cancer receiving aPD-1 therapy.
- We found several immune features early on treatment associated with survival, including activated CD8 T cells, Treg and TIM-3+ classical monocytes (cMono).
- Similarly, Treg, TIM-3+ cMono and CD16+ NK cells were associated with response and irAE presentation.
- While some immune features were only associated with response, others were also associated with irAE.

Patient cohort and methods

Figure 1. Urothelial cancer (UC) patient cohort from RADIOHEAD study

To better understand the immunological mechanisms behind the correlation of irAE presentation and response, we performed immune profiling on 75 PBMC samples from 40 UC patients receiving aPD-1 as part of the Parker Institute for Cancer Immunotherapy RADIOHEAD study[1]. For clinical outcome analyses, we defined responders as patients who had overall survival greater than 12 months.

N	% Female	Median Age	% Concurrent Chemo	Median rwOS (mo)	% irAE Patients	% Responders
40	30	74.5	2.5	6.1	20	67.5

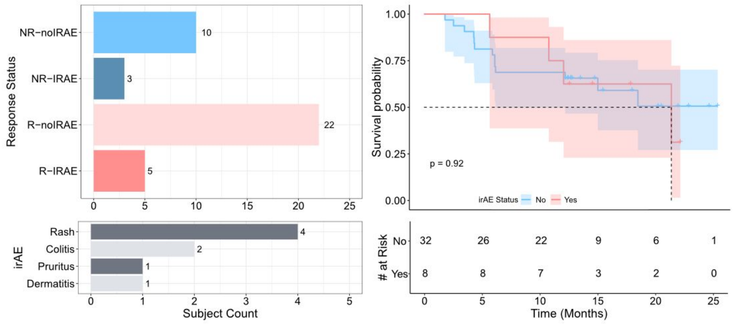
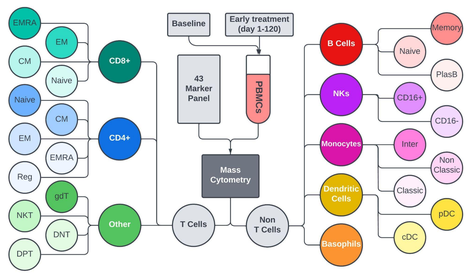


Figure 2. Mass cytometry methodology



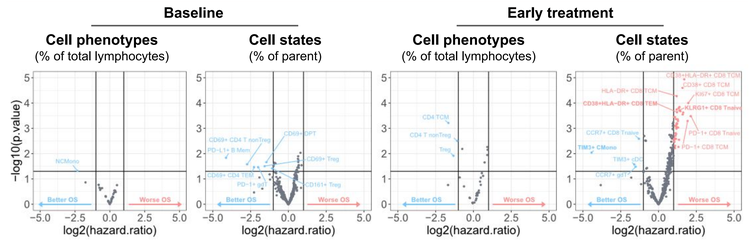
A 44-marker mass cytometry panel was used to profile pretreatment and early on-treatment PBMCs to capture cell subsets and functional states across innate and adaptive immune cells.
We performed analysis on gated immune cell populations and through unsupervised clustering. Group comparisons were made by adjusted Kruskal-Wallis test, whereas survival correlates were assessed through univariate Cox regression analysis.

References

1. Lucas et al. SITC 2022, abstract #1256
2. Wolf et al. *Nat Rev Immunol* 2019
3. Jacobs et al. *Lancet Oncology* 2012
4. Hemdler-Brandstetter et al. *Immunity* 2018
5. Piedra-Quintero et al. *Front Immunol* 2020
6. Lee et al. *Cancers* 2021
7. Faure-André et al. *Science* 2008

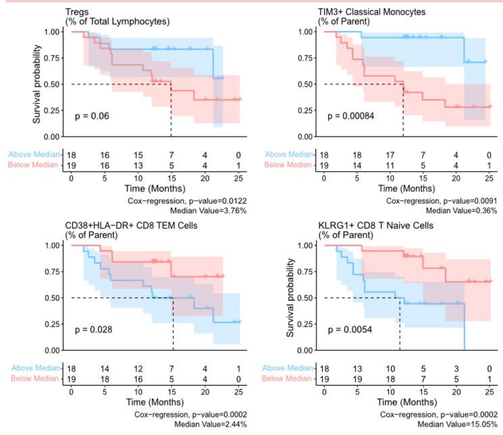
Overall survival is associated with peripheral immune populations at baseline and early on treatment

Figure 3. Univariate Cox regression analysis of overall survival correlates



Several immune features at baseline and early on treatment were significantly associated with better or worse overall survival, including Treg, CD38+ HLA-DR+ CD8 populations, and others.

Figure 4. Kaplan Meier overall survival curves for early on treatment populations

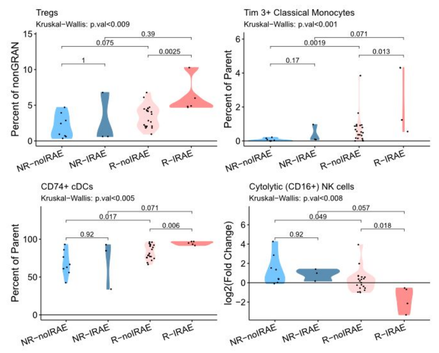


Immune populations found significantly associated with survival through Cox regression analysis at early treatment were assessed with Kaplan Meier curves. Patients were divided into having cell subset frequencies above (blue) or below (red) the median of the patient population. Higher frequencies of several regulatory cell subsets, like TIM-3+ cMono[2] and Treg[3] were associated with better overall survival. Conversely, higher frequencies of various activated cell subsets like KLRG1+ CD8 T naive cells[4] and CD38+ HLA-DR+ CD8 T effector memory (TEM) cells[5] were associated with worse overall survival over the follow-up time period.

Gated analysis on PBMCs confirms immune features associated with response and irAE early on treatment

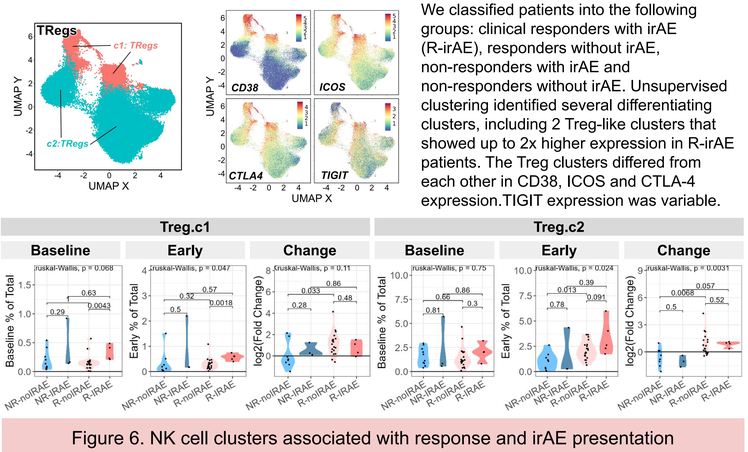
Figure 7. Immune features associated with response and irAE through gated analysis

We classified patients into the same groups as described above and assessed immune features associated with response and irAE through Kruskal-Wallis analysis of manually gated populations. Several immune features found through unsupervised clustering were confirmed through this analysis. For example, at early treatment, R-irAE patients had up to 2-fold more Treg cells and had a greater fold decrease in CD16+ NK cells from baseline compared to other patients. R-irAE patients also had up to 4-fold more TIM-3+ classical monocytes, as well as up to 30% more non-migratory CD74+ cDCs at early treatment[7].



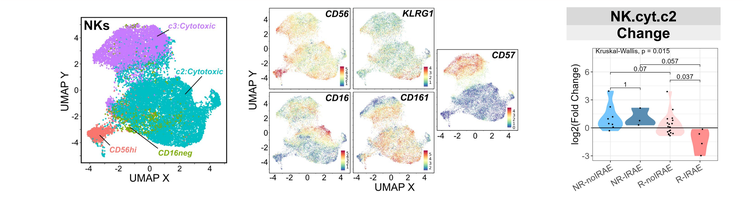
Peripheral Tregs and CD16+ NK cell clusters are associated with response and irAE

Figure 5. Peripheral Treg clusters associated with response and irAE presentation



We classified patients into the following groups: clinical responders with irAE (R-irAE), responders without irAE, non-responders with irAE and non-responders without irAE. Unsupervised clustering identified several differentiating clusters, including 2 Treg-like clusters that showed up to 2x higher expression in R-irAE patients. The Treg clusters differed from each other in CD38, ICOS and CTLA-4 expression.TIGIT expression was variable.

Figure 6. NK cell clusters associated with response and irAE presentation



Unsupervised clustering also found a differentiating NK cell cluster. NK.cyt.c2 is marked by high expression of CD16 and CD161 and low expression of KLRG-1 and CD57, suggesting an activated, cytotoxic (cyt) phenotype[6]. R-irAE patients have the greatest decrease in NK.cyt.c2 from baseline (2-3 fold) across patient groups. Absolute frequencies of NK.cyt.c2 at baseline or early treatment were not differentiating across groups.

Conclusions

- We demonstrate that high-dimensional immune profiling by mass cytometry can detect novel blood-based biomarkers associated with clinical outcomes.
- Based on our findings, there are markers associated with responders broadly, as well as immune features specific to responders who present with irAE.
- Early on treatment, clinical response (including survival) and irAE presentation are associated with higher frequencies of immune cells with regulatory phenotypes and lower frequencies or greater decreases of cells with an activated phenotype.
- Although checkpoint immunotherapy response and irAE incidence both require activation of a patient's immune system, different mechanisms likely apply.
- We plan to validate these findings and identify additional markers and mechanisms of response and irAE with immune profiling on more RADIOHEAD indications.

Acknowledgements

RADIOHEAD sample collection was co-funded by PICI, JDRF and the Helmsley Trust. Teiko Bio and PICI also thank the patients who participated in the RADIOHEAD study and their families.

JDRF

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