Spatial immunophenotyping of longitudinal metastatic melanoma specimens to identify biomarkers of response and resistance to combination anti-LAG-3 + anti-PD-1-based immunotherapies

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Background

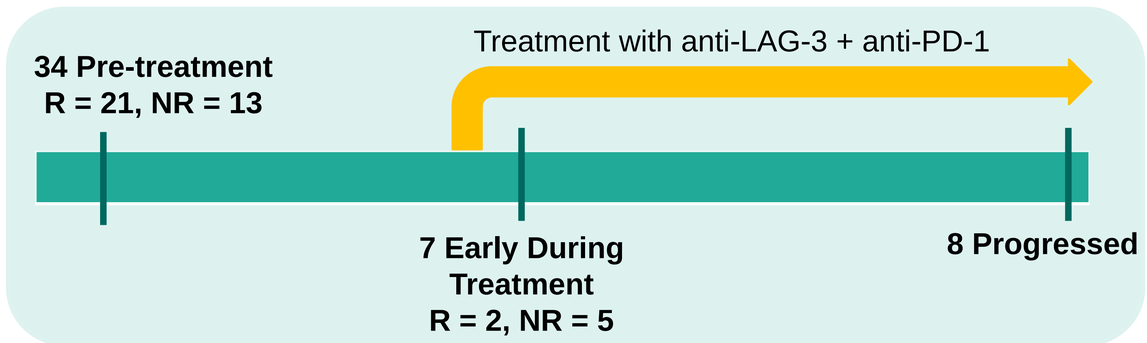
- Lymphocyte-activation gene-3 (LAG-3) is an immune checkpoint receptor which negatively regulates the function of T-cells and promotes tumor immune escape[1].
- Dual inhibition of the LAG-3 and programmed cell death 1 (PD-1) receptors has significantly improved clinical outcomes in patients with metastatic melanoma compared to treatment with anti-PD-1 monotherapy[2].
- However, the role of the spatial immune contexture in determining response to this combination therapy remains unknown.

Objectives

- To assess the spatial immune profiles associated with response and resistance to combined anti-LAG-3 and anti-PD-1-based immunotherapy in patients with metastatic melanoma
- To evaluate the changes in immune populations between baseline, early during treatment and progression tumour biopsies

Methods

- Multiplex immunofluorescent staining was performed on 49 formalin-fixed paraffin-embedded metastatic melanoma specimens from patients treated with combined anti-LAG-3 and anti-PD-1-based immunotherapy

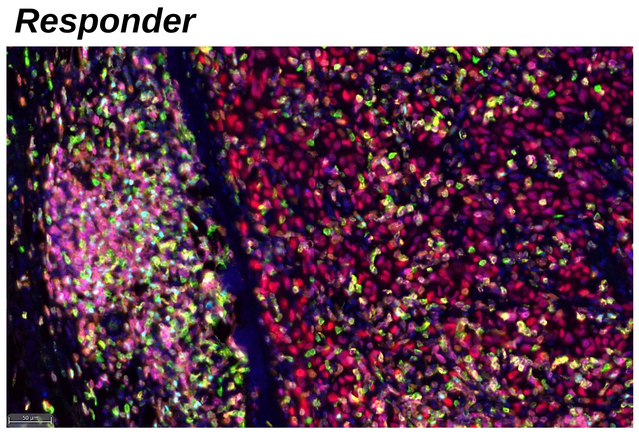


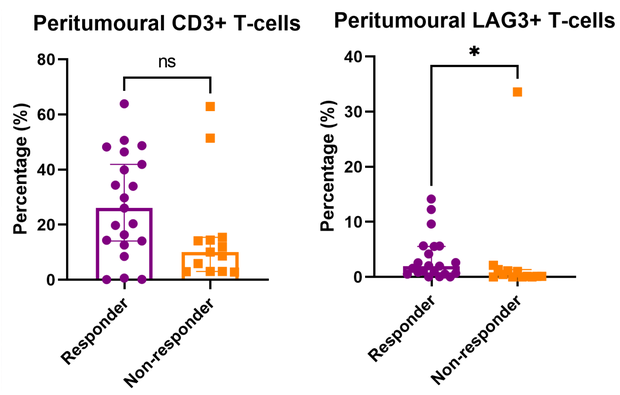
- Patients were categorised as responders (R; complete/partial response), or non-responders (NR; stable/progressive disease) based on RECIST
- Slides were imaged using the Vectra 3.0.5 microscope (Akoya Biosciences), and quantitative analysis was performed using HALO v.3.4 (Indica Labs)

Results

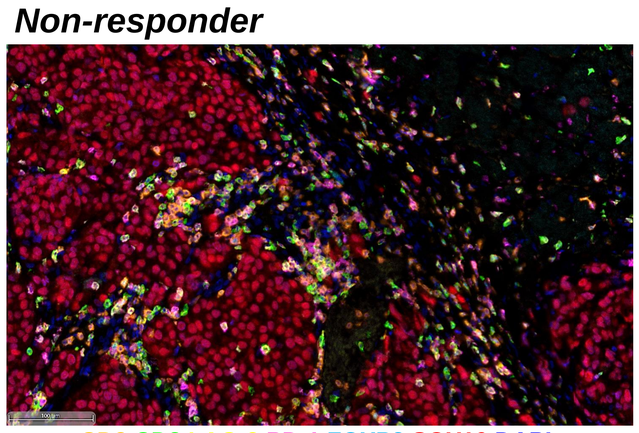
1. Baseline LAG-3+ and PD-1+ T-cells correlate with response

Responder





Non-responder



CD3 CD8 LAG-3 PD-1 FOXP3 SOX10 DAPI

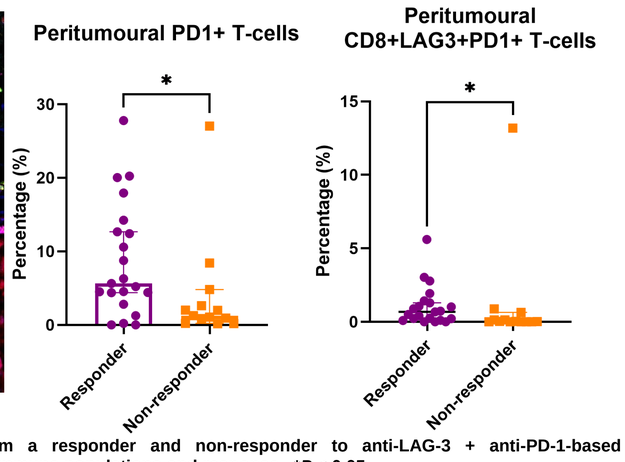
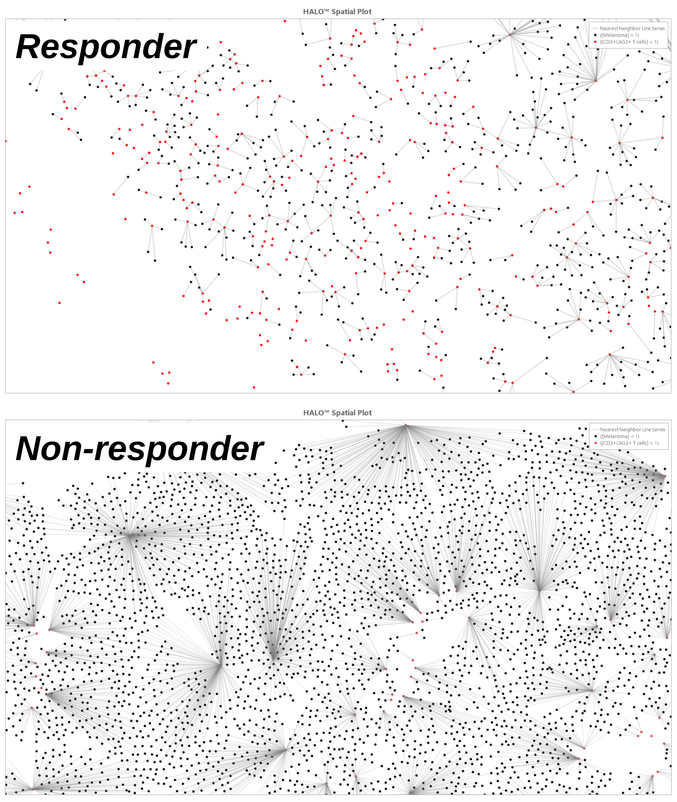


Figure 1. Representative multiplex immunofluorescence images from a responder and non-responder to anti-LAG-3 + anti-PD-1-based immunotherapy. Bar graphs showing associations between peritumoural immune populations and response. *$P < 0.05$

2. Proximity of LAG-3+ cells to melanoma correlates with response



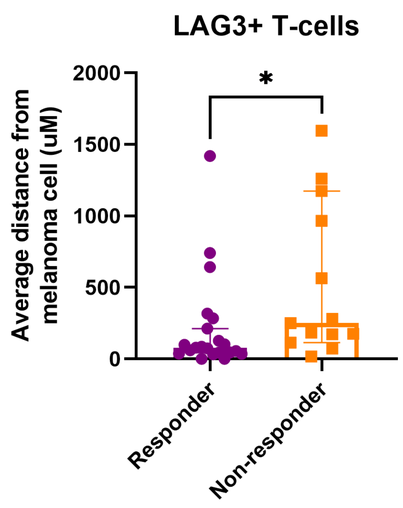


Figure 2. Spatial plots illustrating nearest neighbour distances between CD3+LAG3+ T-cells and melanoma cells. Bar graph showing the average distance between LAG-3+ T-cells and melanoma cells. *$P < 0.05$

3. LAG-3 expression is associated with PFS

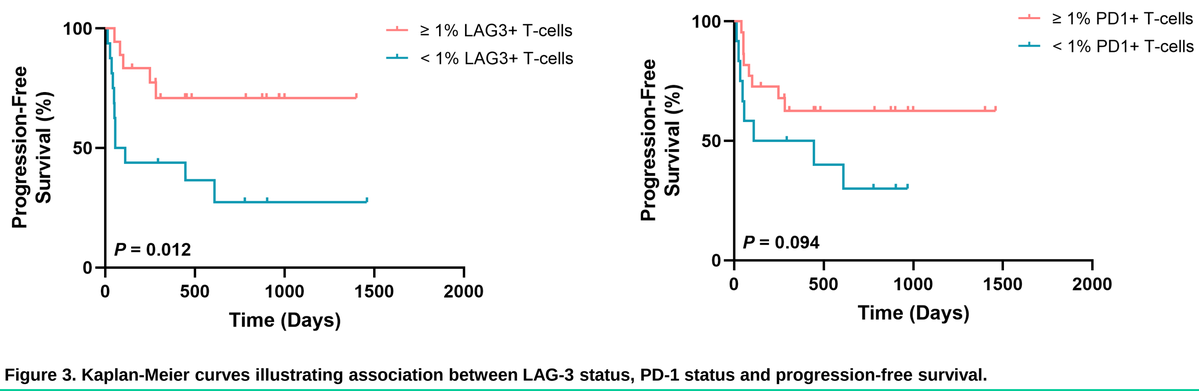


Figure 3. Kaplan-Meier curves illustrating association between LAG-3 status, PD-1 status and progression-free survival.

4. Comparison between R and NR early during treatment

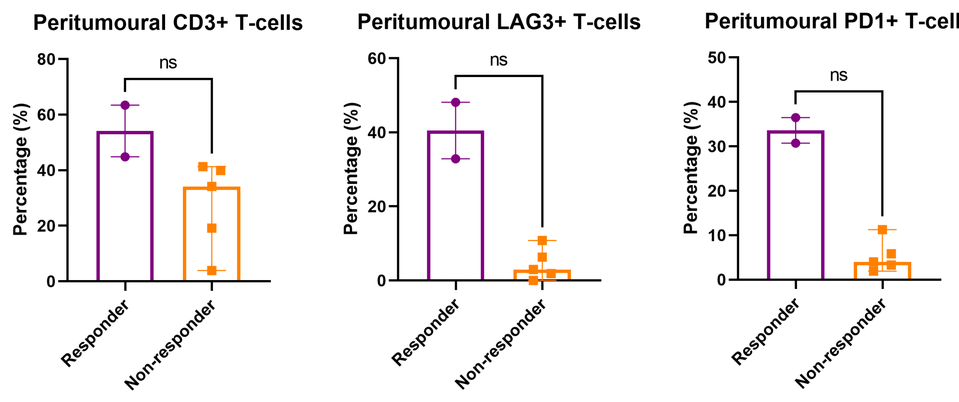
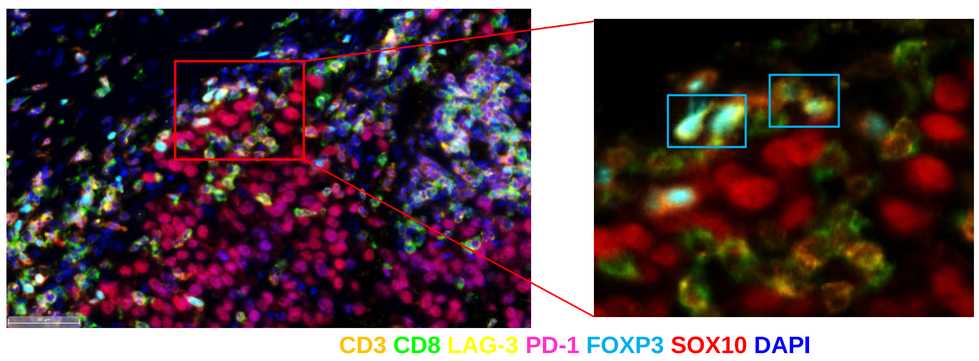


Figure 4. Bar graphs comparing peritumoural immune cell populations between responders (R) and non-responders (NR) early during treatment.

5. CD8-LAG-3+FOXP3+ cells increase during progression

PROG



CD3 CD8 LAG-3 PD-1 FOXP3 SOX10 DAPI

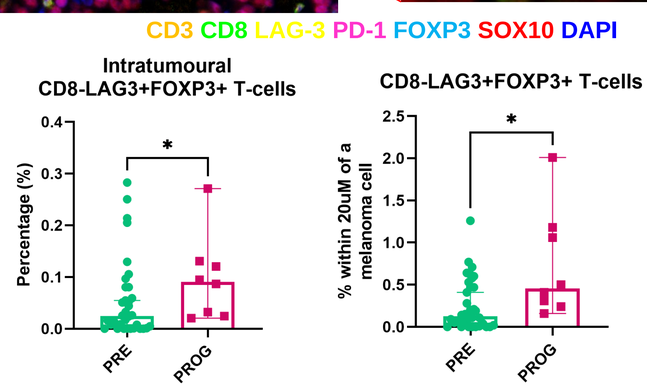


Figure 5. Representative multiplex images showing CD8-LAG3+FOXP3+ T-cells (illustrated by blue boxes) in a progression sample. Comparison of the percentage of CD8-LAG3+FOXP3+ T-cells between PRE and PROG. *$P < 0.05$

Conclusions

- Baseline peritumoural LAG-3 and PD-1 expression is associated with response to combination anti-LAG-3 + anti-PD-1-based immunotherapy
- LAG-3+ T-cells are located closer in proximity to melanoma cells in responders compared to non-responding patients
- Patients with LAG-3-positive tumours have significantly longer PFS compared to those with LAG-3-negative tumours
- Intratumoural CD8-LAG-3+FOXP3+ regulatory T-cells increase post-progression, with a significantly higher proportion located within 20µM of a melanoma cell in PROG biopsies

References

1. Andrews et al. LAG3 (CD223) as a cancer immunotherapy target. Immunological reviews 2017;276:80-96
2. Tawbi et al. Relatlimab and Nivolumab versus Nivolumab in Untreated Advanced Melanoma. N Engl J Med 2022;386:24-34

Acknowledgements

- All patients and their families
- Melanoma Institute Australia
- Cancer Institute NSW