Abstracts

82 SPATIAL IMMUNOPHENOTYPING OF LONGITUDINAL METASTATIC MELANOMA SPECIMENS TO IDENTIFY BIOMARKERS OF RESPONSE AND RESISTANCE TO COMBINATION ANTI-LAG-3 + ANTI-PD-1-BASED IMMUNOTHERAPIES

Background Dual inhibition of the lymphocyte activation gene 3 (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. However, the role of the spatial immune contexture in determining response to this combination therapy remains unknown. This study sought 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.

Methods Multiplex immunofluorescent staining, including the markers LAG-3, CD3, CD8, PD-1, FOXP3 and SOX10, was performed on 34 pre-treatment (PRE; n=21 responders and n=13 non-responders), 7 early during treatment (EDT; n=2 responders, n=5 non-responders), and 8 progression (PROG) formalin-fixed paraffin-embedded (FFPE) metastatic melanoma specimens from patients treated with combined anti-LAG-3 and anti-PD-1-based immunotherapy. Patients were categorized as responders (complete/partial response), or non-responders (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 Median age of the cohort was 68 years and 62% were male. Seventy-four percent presented with stage M1C/D, 32% had elevated LDH, and 24% had received prior anti-PD-1-based therapy. Analysis of PRE biopsies revealed significantly higher proportions of peritumoral LAG-3+ and PD-1+ CD3+ T-cells in responders compared to non-responders (P=0.032 and P=0.022, respectively). LAG-3+ CD3+ T-cells were located closer in proximity to melanoma cells in responders compared to non-responding patients (P=0.022). Responders also displayed higher levels of peritumoral CD8+LAG-3+PD-1+ T-cells compared to non-responders (P=0.016). There were no differences in immune cell populations between subcutaneous or lymph node metastases (P>0.05). Progression-free survival (PFS) was significantly longer in patients with ≥1% of total CD3+LAG-3+ T-cells at baseline compared to those with <1% expression (median PFS: not reached vs 2.7 months, respectively; P=0.012). In contrast, PFS did not differ significantly between patients with ≥1% and <1% total CD3+PD-1+ T-cell expression (P=0.094). There were no significant differences in immune cell populations between responders and non-responders in EDT biopsies. Comparison of PRE and PROG biopsies revealed higher intratumoral CD8-LAG-3+FOX3+ regulatory T-cells (P=0.047) at progression. Furthermore, a significantly higher proportion of these regulatory T-cells were located within 20μM of a melanoma cell in PROG biopsies compared to PRE (P=0.013).

Conclusions These findings highlight the potential role of the proportions and spatial locations of LAG-3+ immune cell subsets within the tumor microenvironment as predictors of response and resistance to combined anti-LAG-3 and anti-PD-1-based immunotherapy in metastatic melanoma.

Ethics Approval This study was approved by the New South Wales Department of Health Human Research Ethics Committee (Protocol no. X15–0454) and conducted in accordance with the Declaration of Helsinki. Samples were acquired with consent from the Melanoma Biospecimen Tissue Bank (HREC/11/RPAH/444).