Development of severe immune related adverse events associated with early B cell changes and altered gut microbial community stability

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Background

Immune related adverse events (irAEs) are a major hurdle to the success of immune checkpoint inhibitor (ICI) immunotherapy.

These autoimmune or autoinflammatory manifestations often cause severe morbidities and limit both the use and the effectiveness of ICI immunotherapy [1]

There is increasing evidence that the gut microbiota influences irAE development, however the microbe-immune crosstalk underpinning this remains cryptic [2].

Aims

1. To assess longitudinal microbiome-immune dynamics during treatment with ICI immunotherapy
2. To determine the relationship between the gut microbiome and peripheral immune populations in the context of irAE development

Methods

Cohort: Australian and Dutch melanoma patients treated on trial with neo-adjuvant combination anti-PD1/anti-CTLA4 immunotherapy (OpACIN-neo/PRADO)[3].

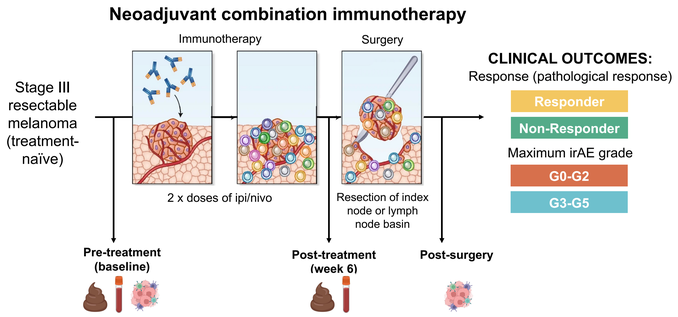

Figure 1- Schematic overview of trial and sample collection (stool, blood, tumour)

Microbiome analysis: Paired pre- & post-treatment (baseline/week 6) stool samples (n=129), 16S rRNA gene amplicon sequencing

Immune analysis: Matched longitudinal PBMCs at both timepoints, analysed using mass cytometry (CyTOF) (n=71, AUS patients).

Results

Stratification based on baseline gut microbiome profiles

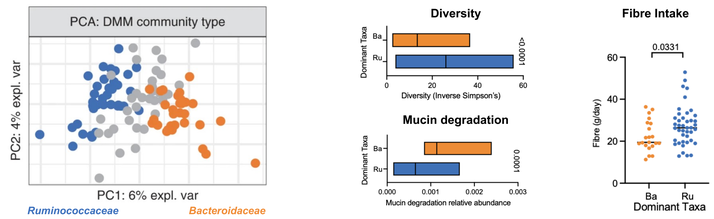

Figure 2- Dirichlet multinomial mixtures used to stratify patients based on microbiota profiles (n=103); simplified to ratio between *Ruminococcaceae* and *Bacteroidaceae* family relative abundance. Diversity, mucin degradation potential and fibre intake was assessed by community type (Simpson et al, 2022)[3].

Bacteroidaceae-dominated gut microbiomes are associated with higher risk of early severe irAEs

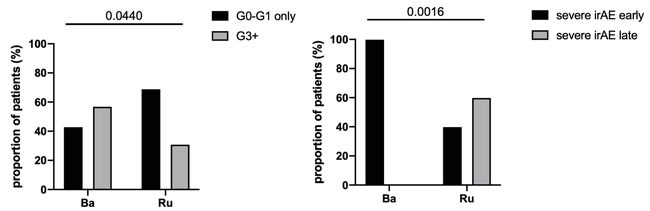

Figure 3- Rates of severe irAEs (G0-G1 vs G3+) by microbial community type and rates of patients that experienced severe (G3+) irAEs prior to week 6 (early) compared to after surgery (late).

Enhanced oralisation of the gut microbiome was observed with treatment

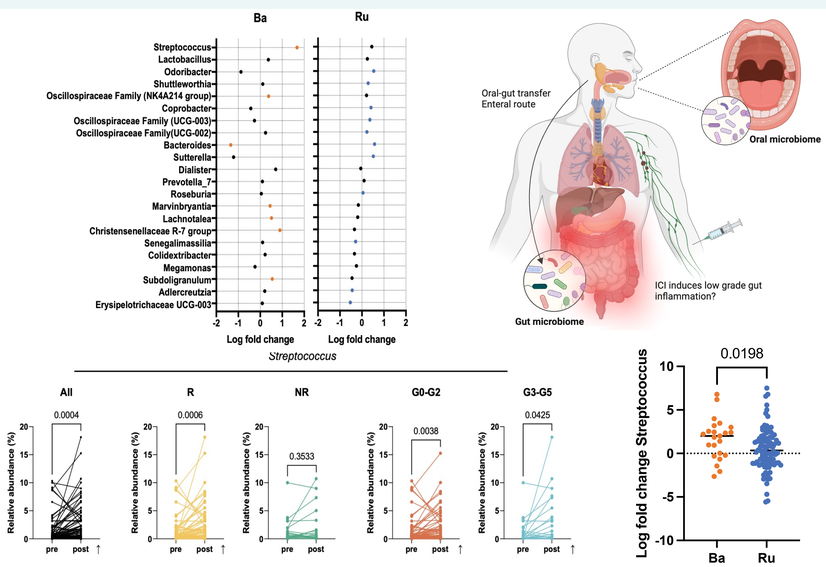

Figure 4- ICI immunotherapy resulted in changes in the relative abundance of specific bacterial groups in the faecal microbiota, however the microbial community as a whole remained relatively stable. Average log fold change of genus level taxa split by microbial community type was assessed. Larger fluctuations in the gut microbiota between timepoints was associated with irAE development and observed in Ba-dominated patients. An increase in *Streptococcus* relative abundance with ICI-immunotherapy treatment was particularly evident in Ba-dominated patients.

Distinct post treatment peripheral immune profile associated with severe irAE development

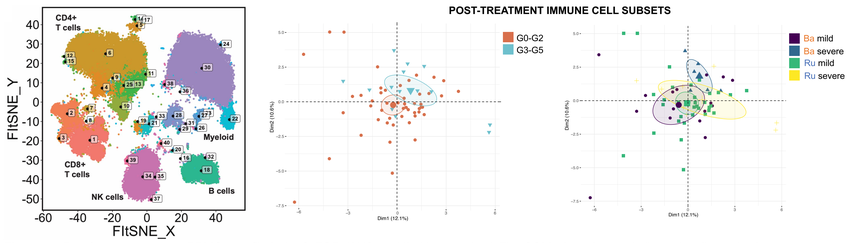

Figure 5- Pre & post treatment PBMCs profiled via CyTOF (AUS n=71). Unsupervised FlowSOM clustering and dimensionality reduction to generate 40 metaclusters spanning T cell, B cell and myeloid populations. PCA plots of post treatment peripheral immune repertoire coloured by irAE grade and microbial community type.

Changes in peripheral B cell subsets associated with irAE development

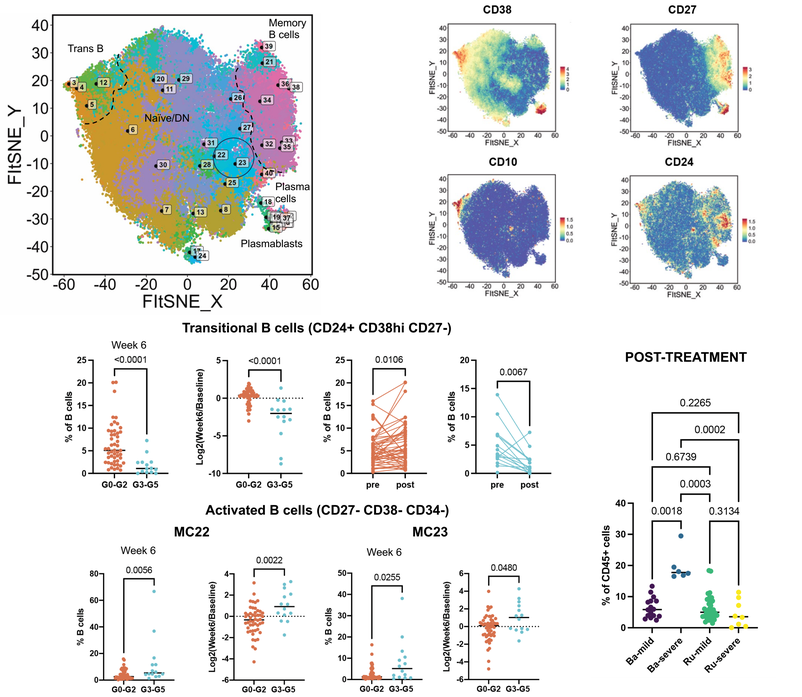

Figure 6- Unsupervised FlowSOM clustering and dimensionality reduction was performed on CD19+ cells. A reduction in transitional B cells (CD24+ CD38hi CD27-) and an increase in activated B cells subsets (CD27- CD38- CD24- populations) were observed between baseline and week 6 in patients who developed or went on to develop severe irAEs. Higher overall frequencies of post-treatment B cells in Ba patients who developed early, severe irAEs.

- The changes in B cells subsets observed in severe irAE patients during ICI immunotherapy suggest that B cells may be involved in irAE development
- *Bacteroidaceae*-dominated patients who developed early, severe irAEs had higher B cell frequencies post treatment

Conclusions

- Higher risk of early, severe irAEs were associated with *Bacteroidaceae*-dominated gut microbiomes, reduced microbial community stability, and increases in activated circulating B cells during treatment.
- We postulate that differing baseline microbial community assemblages are associated with altered pre-established intestinal homeostasis influencing the likelihood of irAE development.
- Together, the data highlights a potential relationship between B cells, the gut microbiota and irAE development which may point to early steps in the induction of irAEs during ICI immunotherapy.

References

1. Larkin et al *N Engl J Med* (2015)
2. Simpson et al *Nature Rev Clin Onc* (2023)
3. Simpson et al *Nature Medicine* (2022)

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