Mechanisms of response to anti-PD1 (PD1) combined with Ipilimumab (IPI) in patients (pts) with PD1-resistant metastatic melanoma (MM).

Ines Pires da Silva[1,2,3], Jordan W. Conway[1,2], Jarem Edwards[1,2], Felix Marsh-Wakefield[2], Camelia Quek[1,2], Angela Fergusson[2], Peter Johnansson[4], Matteo S. Carlino[1,2,3], Alexander A. Menzies[1,2,5], James Wilmott, Umaimainthan Palendira[2], Richard Scolyer[1,2,6], Georgina V. Long[1,2,5]

[1]Melanoma Institute Australia, Sydney, Australia; [2]The University of Sydney, Sydney, Australia; [3]Crown Princess Mary Cancer Centre Westmead & Blacktown Hospital, Sydney, Australia; [4]QIMR Berghofer Medical Research Institute, Brisbane, Australia; [5]Royal North Shore and Mater Hospitals, Sydney, Australia; [6]Royal Prince Alfred Hospital, Sydney, Australia.

Background

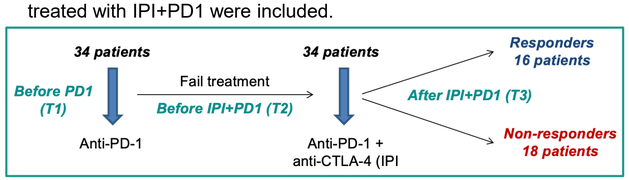
- IPI+PD1 is associated with better objective response (ORR), progression-free survival (PFS) and overall survival (OS) compared to PD1 monotherapy in patients with advanced melanoma[1,2,3].
- Approximately 1 in 3 patients with PD1-resistant metastatic melanoma will benefit from IPI+PD1[4,5].
- The role of adding IPI in this context is yet to be clarified.

Objectives

- We sought to: (a) identify biomarkers and (b) determine mechanisms of response to IPI+PD1 in PD1 resistant patients.

Methods

- 34 patients with PD1-resistant metastatic melanoma subsequently treated with IPI+PD1 were included.



- We performed:
  - On melanoma samples
    - RNA sequencing (RNAseq)
    - 3 multiplex immunofluorescence (mIF) panels:
      - CD8, CD103, CD39, TCF7, FOXP3, SOX10
      - CD8, Tbet, ICOS, TCF7, CD3, SOX10
      - CD16, CD68, HLA-ABC, MAGE, SOX10
    - Imaging mass cytometry (IMC)
  - On peripheral blood mononuclear cells (PBMCs)
    - Cytometry by time of flight (CYTOF)

from 3 timepoints: before PD1 (T1), before IPI+PD1 (T2), and after IPI+PD1 (T3).

Results

Table 1. Summary of patient characteristics at start of IPI+PD1, stratified by response to IPI+PD1.

Characteristics	Responders (n=16)	Non-responders (n=18)	p-value
Age (median, range)	65 (37 - 86)	70 (41 - 79)	0.7524
Sex (male)	11 (69%)	8 (42%)	0.1854
BRAF V600	3 (19%)	3 (17%)	>0.9999
ECOG PS	2 (13%)	4 (22%)	0.6602
LDH	5 (31%)	6 (33%)	>0.9999
Stage M1c/d	7 (44%)	11 (61%)	0.4921
Liver metastases	5 (31%)	7 (39%)	0.7289
Brain metastases	3 (19%)	4 (22%)	>0.9999
Innate resistance to PD1	13 (81%)	13 (72%)	0.6933

Results

1. Higher expression of cancer testis antigens (CTA) in responders versus. non responders (RNAseq and mIF) & the impact of PD1 and IPI+PD1 in the levels of MAGE-A3-specific CD8 T cells (CyTOF) in the blood of advanced melanoma patients resistant to PD1.

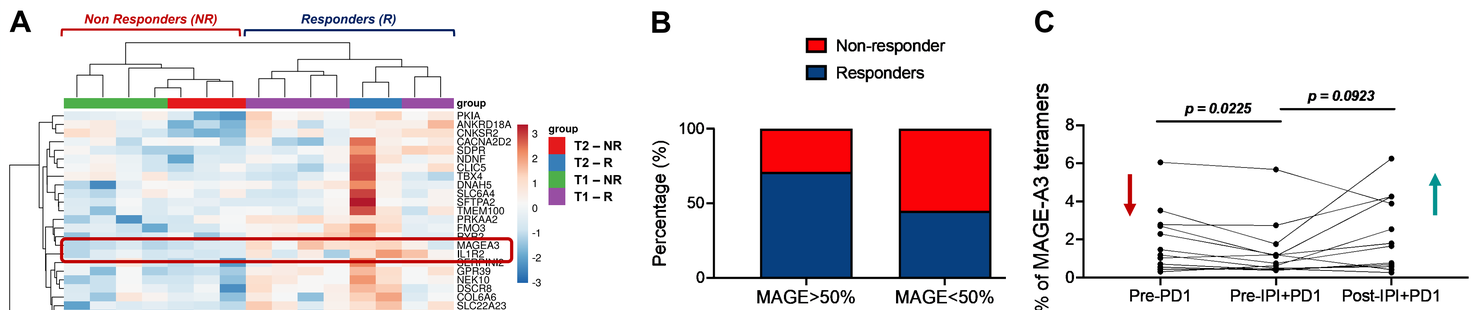


FIGURE 1. Expression of CTA (e.g. MAGE family) in responders versus. non responders and longitudinal levels of MAGE-A3+ tetramers in the peripheral blood. (A) Gene expression analysis (RNAseq) on T1 and T2 tumor samples revealed higher expression of CTA (MAGEA3 and MAGEC1; adjusted p<0.05) and immune regulatory genes (ILR2; adjusted p<0.2), but lower expression of fibrosis regulation genes (FABP3 and MMP7; adjusted p<0.2) in responders versus. non-responders. **(B)** Higher proportion of responders (*red*) versus. non responders (*blue*) in tumours with MAGE expression in >= 50% compared to <50% of tumour cells (mIF). **(C)** CyTOF analysis of MAGE-A3 tetramers on PBMCs showed a decrease in peripheral MAGE-A3-specific CD8+ T cells with PD1 (T1 to T2; p=0.0225); and expansion of this T-cell subset with subsequent IPI+PD1 (T2 to T3) was seen in 7/12 HLA-A2+ patients.

2. Characterisation of the TME immune infiltrate (mIF) at baseline (T1) and after PD1 (T2) in responders vs. non-responders to IPI+PD1

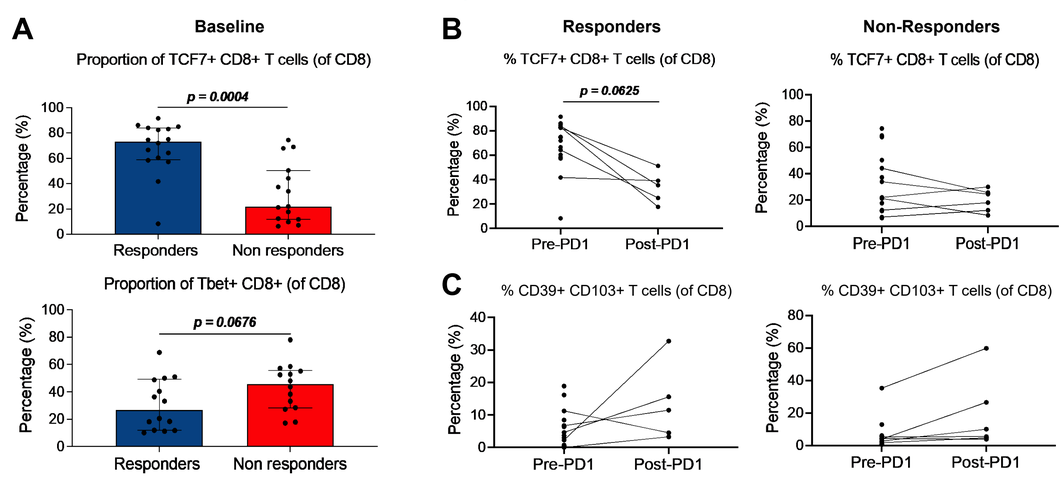


FIGURE 2. Multiplex immunofluorescence (mIF) to characterize the tumour microenvironment (TME) on baseline (T1) and after PD1 (T2) samples. (A) IPI+PD1 responders had a higher % of TCF7+ CD8+ T cells (p=0.0004), but lower % of Tbet+ CD8+ T cells (p=0.07), compared with non-responders. **(B)** There was a decrease in the % of stem cell-like TCF7+ CD8+ T cells (mainly seen in responders; p=0.06) but **(C)** an increase in the % of effector CD39+ CD103+ CD8+ T cells with PD1 (T1 to T2; p>0.05). There were only 3 T2 to T3 IHC paired samples, precluding further analysis.

3. Effect of IPI+PD1 on the peripheral immune profile (CyTOF) of patients resistant to PD1

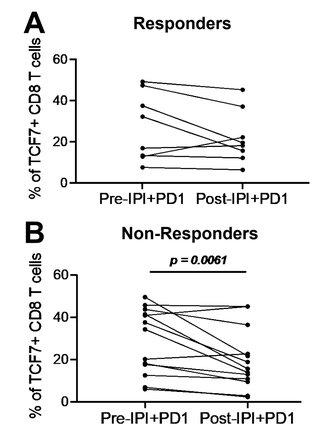


FIGURE 3. Analysis on PBMC longitudinal samples. Decrease in % of TCF7+ CD8+ T cells in non-responders (p=0.0061) with the addition of IPI (T2 to T3), but not in responders.

4. Imaging mass cytometry (IMC) on baseline (T1) samples from responders vs. non-responders to IPI+PD1

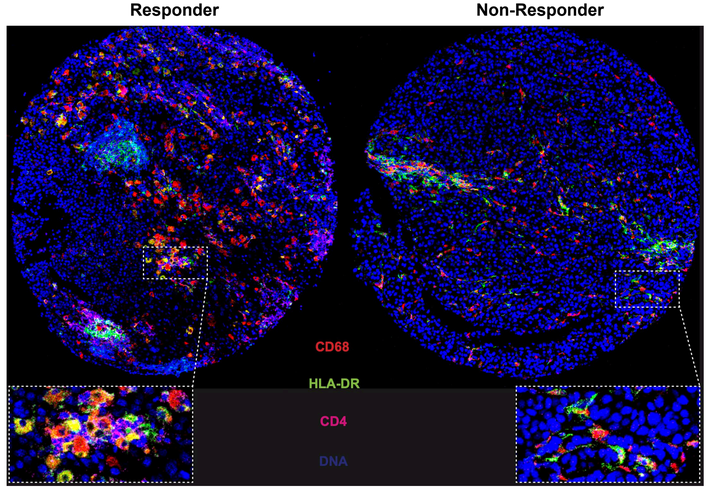


FIGURE 4. IMC analysis on baseline samples from responders vs. non-responders to characterize the interaction between immune cells in the tumour microenvironment (TME).

Conclusions

- We identified potential mechanisms of response to IPI+PD1 in patients with advanced melanoma resistant to PD1.
- Our data suggests that response to IPI+PD1, after progression on PD1:
  - Requires baseline stem cell-like TCF7+ T cells with the potential to differentiate to an effector phase (CD39+ CD103+ CD8+ T cells).
  - IPI facilitated expansion of antigen-specific T cells in a CTA-expression context, that PD1 alone is unable to do.

References

1. Larkin J, *et al*. NEJM 2019
2. Wolchok JD, *et al*. NEJM 2017
3. Larkin J, *et al*. NEJM 2019
4. Pires da Silva I, *et al*. Lancet Onc 2021
5. Olson D, *et al*. JCO 2021

Acknowledgements

All patients and their families
Melanoma Institute Australia
Tour de Cure

COIs

- Travel support: BMS and MSD
- Speaker fees: Roche, BMS, MSD, Novartis, and Pierre Fabre
- Advisory board: MSD

Ines.silva@melanoma.org.au