EGFR as a Predictive Biomarker for anti-CTLA4/anti-PD1 Sensitivity in BRAFi/MEKi resistant Melanoma

Riyaben P. Patel [1,2], Reem Saleh [1,2], Emily J. Lelliott [3], Anna Trigos [1,2], Nicole Haynes [1], Grant A McArthur [1,2], Karen E. Sheppard [1,2]

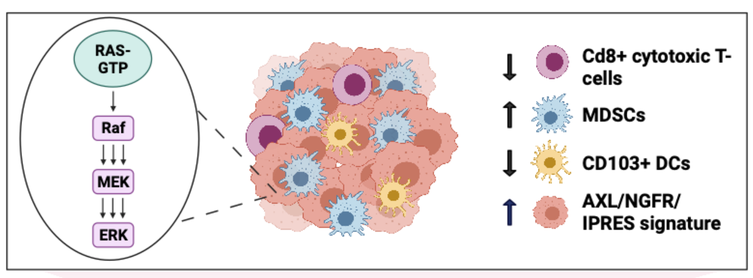
[1] Research division, Peter McCallum Cancer Centre, Melbourne ,VIC, AUS [2] Sir Peter MacCallum Department of Oncology, Melbourne, VIC, AUS. [3] Research division, Olivia Newton John Cancer Research Institute, Heidelberg VIC AUS

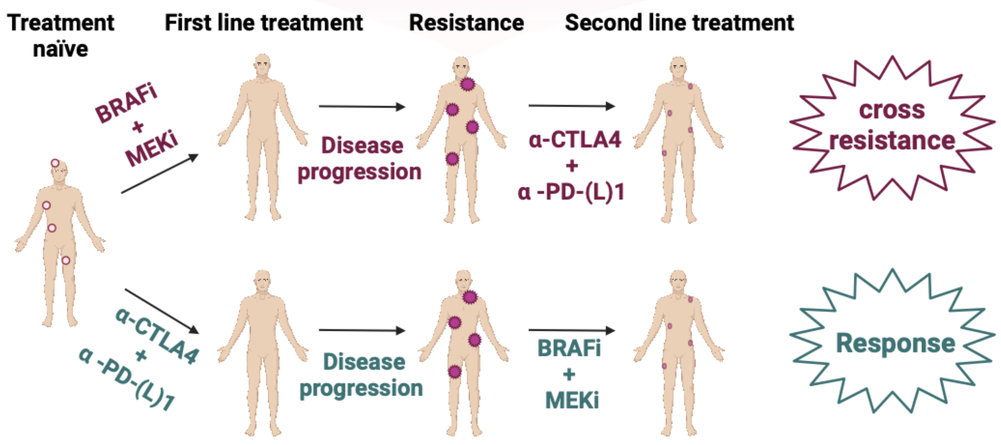
Cutaneous Melanoma

- 3rd most commonly diagnosed cancer in 2021[1]
- **Targeted therapy (TT)** – BRAFi (Dabrafenib - Dab) + MEKi (Trametinib - Tram) – induces a remarkable initial response, however resistance develops[2]
- **Immunotherapy (ICI)** – anti-CTLA4 + anti-PD1 – induces a host immune response with durable response however resistance develops[3]

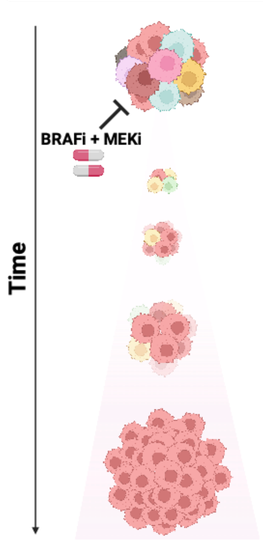
Cross-resistance of second-line Immunotherapy

- **DreamSeq[4]** and **Secombit[5]** clinical trials have revealed cross-resistance of ICI in patients that acquire resistant to first line BRAFi + MEKi
- In ~70% of patients, acquired resistance with TT is associated with **reactivation of the MAPK pathway[6]**
- Reactivation of MAPK pathway induces an immune suppressive microenvironment which leads to **cross-resistance with second-line immunotherapy[7]**





***In-vitro* AR model**



Rationale

A distinct subset of patients who have developed resistance to TT remain responsiveness to second-line immunotherapy

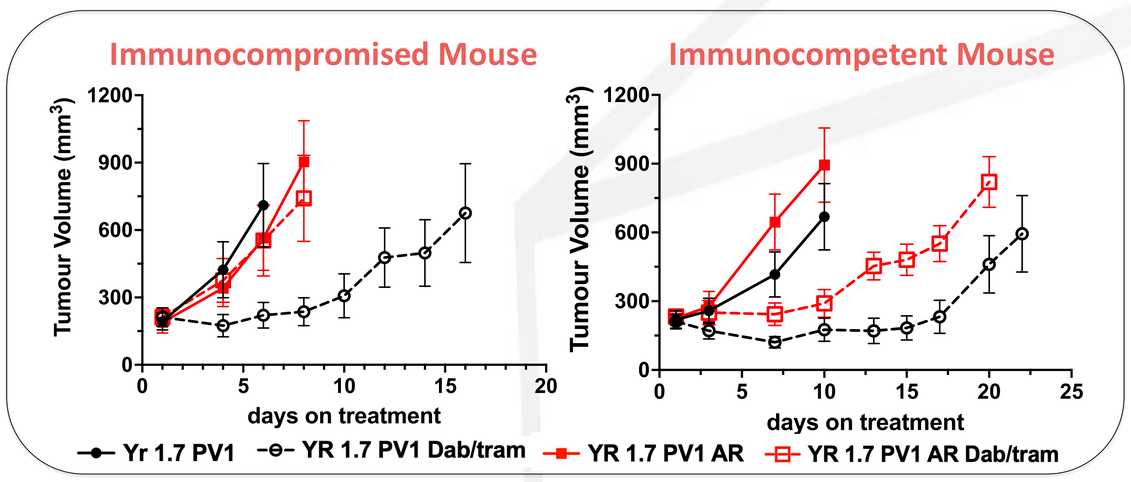
It is important to understand alternative mechanism of resistance to TT that may or may not lead to cross – resistance to ICI

Methodology

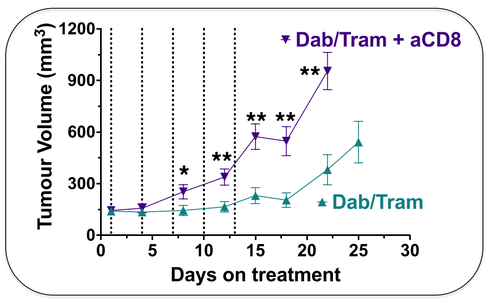
Melanoma Disease model : yummer 1.7 PV1 AR

The acquired resistant model was developed through *in vitro* passaging under drug pressure. The mechanism of resistance was uncovered by analysing transcriptomic data. To assess the tumour's immune microenvironment, flow cytometry was employed. This analysis was instrumental in determining the sensitivity of the acquired resistant (AR) model to ICI, helping to establish whether the tumours exhibited cross-resistance to ICI.

Yummer 1.7 PV1 AR – response to TT *in vivo*



Depleting CD8+ T-cells in AR tumours



pERK levels in treated P vs AR cells

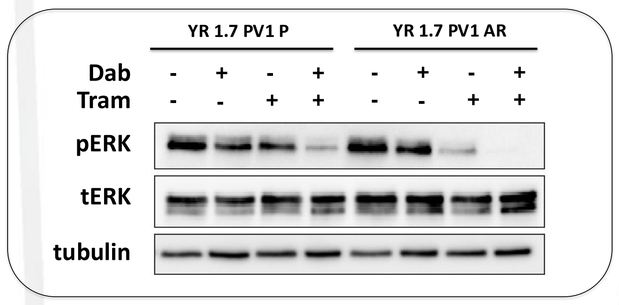


Figure 1 : (Top) Yummer 1.7 PV1 parental and AR cells were subcutaneously implanted in NSG mice (left) and C57BL/6 male mice (right). The graphs illustrate that while AR cells exhibited resistance in an immunocompromised setting, they showed partial sensitivity in immunocompetent mice. **(Bottom left)** Depleting CD8+ T cells in the AR tumors resulted in reduced sensitivity to D/T treatment in the AR cells. (**Bottom right)**, To determine the mechanism of resistance, MAPK pathway activity was assessed. Notably, Dab/Tram decreases p-ERK levels in AR cells. The error bars in the graph represent mean tumor volume ± SEM, and the statistical significance is indicated by P-values (*<0.05, **<0.005).

References:

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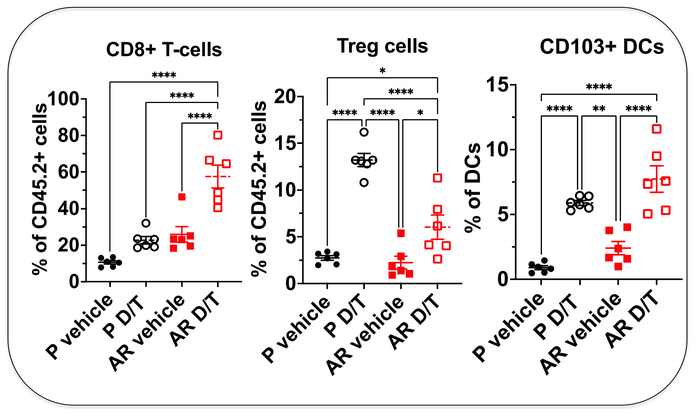
Immune microenvironment



Immunotherapy response

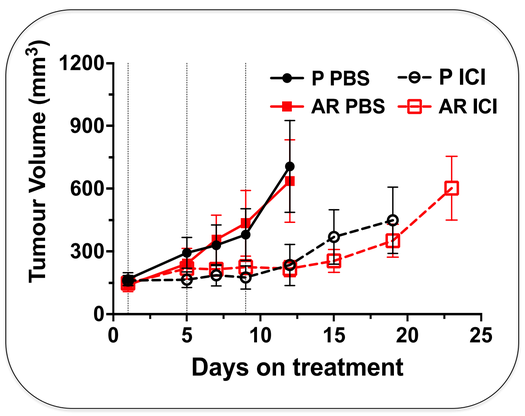


Figure 2 : (Left) Yummer 1.7 PV1 Parental (P) and AR tumours were harvested 5 days after Dab(D) and Tram(T) treatment. CD8+ T effectors were significantly upregulated in AR-treated tumours compared to Parental-treated tumours. Similarly, T regulatory (reg) cells were significantly downregulated in AR-treated tumours compared to P-treated tumours. Although no difference was found in the percentage of CD103+ dendritic cells (DCs) between P and AR-treated tumours, they were upregulated upon treatment. **(Right)** Evaluating and comparing the sensitivity of AR and P tumours to immunotherapy (anti-CTLA4 + anti-PD1), they were equally responsive, indicating no cross-resistance. Error bars show mean tumour volume ±SEM. P-values, *<0.05, **<0.005, ***<0.0001, ****<0.00001

Upregulation of EGFR-stat3/stat5 axis in AR cells

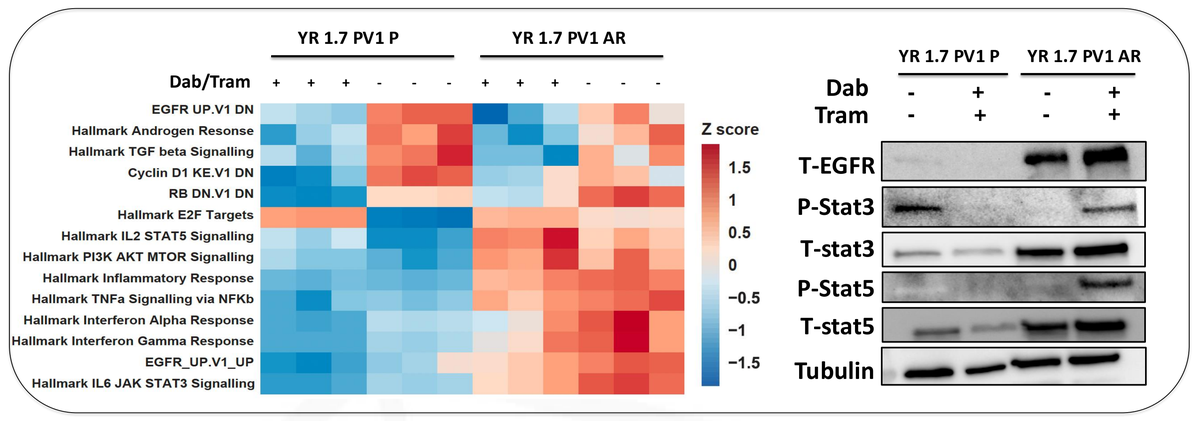
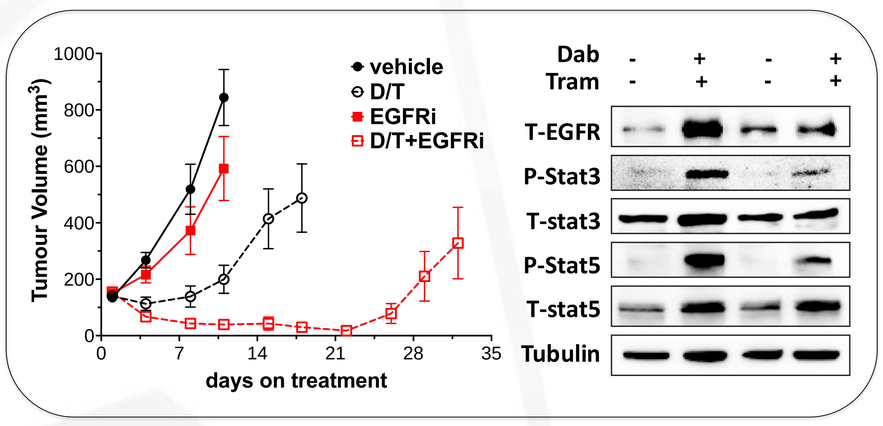


Figure 3 : (left) To understand the resistance mechanism, we conducted RNAseq analysis on parental and AR cells 24 hours post treatment *in-vitro*. Gene set variation analysis (GSVA) revealed increased immune signatures in AR cells which is found to be further enhanced by treatment. Additionally, Estrogen responses and IL6/STAT3 showed upregulation in AR treated cells. **(right)** Western blot analysis of the EGFR-STAT3/STAT5 axis was performed, confirming increased EGFR ,STAT3 & STAT5 protein and increased phosphorylation of STAT3 and STAT5 in AR treated cells upon D/T treatment.

EGFRi combined with BRAFi/MEKi in AR cells



Patient data

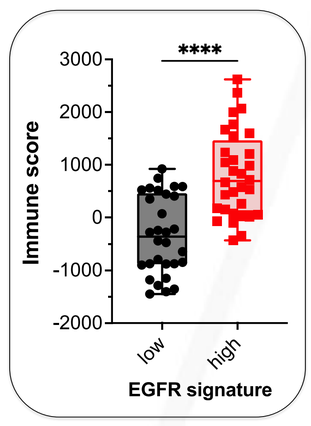


Figure 4 : (left) Tumour response curves of Yummer1.7PV1AR cells reveals re-sensitivity of BRAFi/MEKi with an addition of an EGFRi. This suggests that these inhibitors were able to reduce the proliferation of AR cells in the presence of Dab/Tram. **(Middle)** Western blot analysis indicates reduction of all three proteins; EGFR, pstat3 and pstat5, with the quadruple therapy. **(Right)** RNAseq analysis of patient samples post progression with BRAFi/MEKi corroborated our findings where high EGFR signature led to higher immune score, indicative of sensitivity to ICI.

Mutant NRAS^{G12D} drives resistance in immunocompetent mice

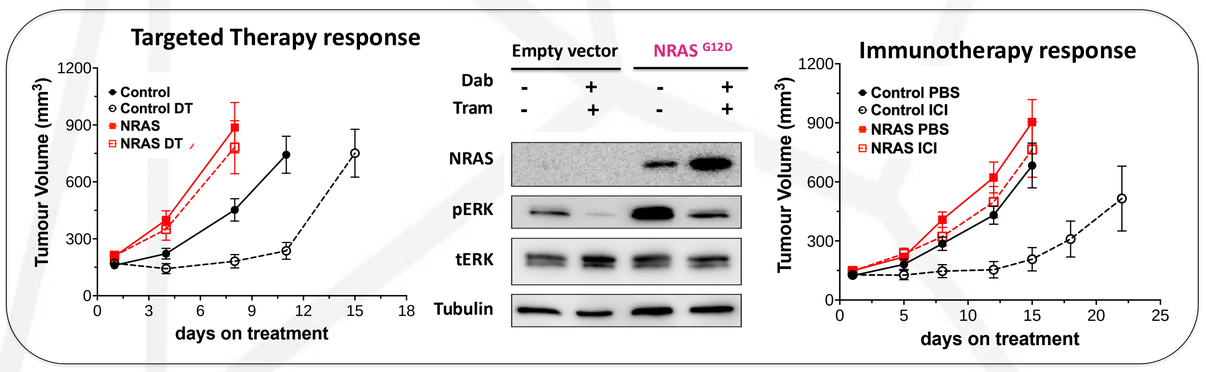


Figure 5 : To validate previous findings, we introduced mutant NRAS^{G12D} into Yummer 1.7 PV1 cells to assess if heightened MAPK pathway activity leads to ICI cross-resistance. **(left)** *In vivo* tumour response curves of Yummer 1.7 PV1 NRAS^{G12D} cells demonstrated complete resistance to Dab/Tram. To determine the MAPK pathway activity, we investigated pERK levels in treated NRAS cells compared to their counterpart, empty vector cells. As seen **(middle)** in the western blot analysis, increased pERK levels were observed in NRAS treated cells compared to control treated cells, suggesting these cells acquire resistance through overactivation of the MAPK pathway. (**Right)** Assessing the ICI response revealed cross resistance to immune checkpoint inhibitors, highlighting the unique phenomena in our model

Major Findings

- Yummer 1.7 PV1AR cells, acquire resistance through increased EGFR expression and EGFR-pstat3/pstat5 signalling upon TT.
- In the AR cells, there is an immunostimulatory microenvironment present which leads to anti-tumour immunity and sustained sensitivity to immunotherapy.
- Inhibiting the EGFR-axis re-sensitises the AR cells to TT.
- EGFR upregulation could serve as a biomarker to identify targeted therapy resistant patients who would respond favourably to immune checkpoint inhibitors

Conclusion - These findings provide valuable insights into the interplay between TT resistance, immune response modulation, and biomarker-driven patient stratification in the context of melanoma treatment