Australia has the highest incidence of melanoma in the world. In NSW, Melanoma disproportionately impacts the most productive years and is the commonest cancer in those aged 15-50 years (men) and 15-35 years (women). Rationally designed targeted therapies are changing the landscape of treatment options for those with the poorest prognosis (stage 4 or metastatic melanoma). Specifically, BRAF inhibitors and combined targeted therapies have significant activity, however emerging limitations are duration of response. The goals of this program of research are to explore the mechanisms of resistance to targeted therapies in melanoma, with the ultimate aim to develop effective therapies to prevent (adjuvant) and cure metastatic melanoma.
**Project Aims/ Objectives:**  
*Please state the aims and objectives and how they were achieved.*

This research program aims to refine the mechanisms of response and resistance (primary and acquired) in human melanoma patients receiving targeted therapies to ultimately effect change in treatment and patient outcome.

The aims of this program of research are outlined in 1-4 below.

Specifically, the RACP fellowship supported aim 3:

1. Generate and analyze melanoma tumors and cell clones that are resistant to kinase inhibitors including from patients receiving single and combination therapies (including, but not restricted to, BRAF inhibitors, MEK inhibitors, BRAF + MEK inhibitors in combination, CKIT inhibitors, dual PI3K and mTOR inhibitors, and ERK inhibitors)
2. Perform a genome-wide loss-of-function lentiviral siRNA library screen to identify novel molecular components that are required for sensitivity to BRAF & MEK inhibition; validate candidates using tumor samples from pts on inhibitor trials.
3. Perform genome-wide exomic deep sequencing, detailed morphologic, RNA expression and protein expression analysis in multiple samples acquired from metastatic melanoma patients with primary and acquired resistance to drugs targeting the MAP kinase pathway.
4. Investigate the mechanisms of enhancing apoptosis in melanomas treated with BRAF inhibitors.

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**Research conducted to date:**  
*Please provide a brief summary of*

The RACP funds were used to contribute to the to exome sequencing part of the research program outlined above. Human melanoma tissue collected from patients at baseline (prior to treatment) and on progression with targeted drug therapies that were given as part of clinical trials (BRAF inhibitors or combined...
**Methodology, Trials, Experimental Procedures, etc.**

BRAF inhibitor with MEK inhibitor) was examined for melanoma content, dissected, and DNA and RNA was extracted. The RNA and DNA was analysed for known resistance mechanisms, and the RNA was analysed for the expression profile (49 000 genes) and exomes within the DNA were sequenced.

**Significance and Outcomes:**

Through this research we have identified several new mechanisms of resistance which have implications for treatment choice for patients. Three have been published:

i) BRAF amplification (published 2012, see below Shi et al)
ii) MEK mutation (published 2012, see below Shi et al)
iii) AKT mutation (submitted for publication 2013)

We have also identified other mechanisms of resistance, including mechanisms in COMBINATION targeted therapies, and will publish these in 2013. Some of this work was supported using the RACP grant 2012.

The identification of these resistance mechanisms is the first step to designing therapies to prevent resistance and improve clinical outcomes for patients with metastatic melanoma, including progression-free and overall survival.

We are continuing the research program as outlined in Aims and Objectives above, and have planned additional experiments and investigator-led trials to build on our early success. Two such trials include a study of neoadjuvant targeted therapies in 1) patients with resectable brain metastases and 2) patients with bulky stage 3 (lymon node) melanoma.

**Academic Output:**

The specific publication relating to exome sequencing that this grant funded will be published in 2013. It was presented at the Society for Melanoma Research 2012;


Below are a list of my publications since 2012, excluding 20 conference presentations and 13 invited speaker engagements for 2012 and 2013 (see next section below). This indicates my level of research productivity:

1. Long GV, Wilmott JS (shared first authorship), Haydu L, Chatfield M, Tembe V, Sharma R, Thompson JF, Howle J, Scolyer RA, Kefferd RF. ‘Affects of Potent BRAF inhibitors Dabrafenib and Vemurafenib on Human Melanoma Tissue Before Treatment, During Treatment and on Progression’. Accepted for publication Pig Cell Mel Res 2013. IF 5.059
2. Carlino MS, Gowrishankar K, Saunders CAB, Snoyman S,


Additional Advice and Comments

Please state items of interest which have arisen as a result of the project, such presentations or other outcomes.

Below are a list of conference abstracts/presentations form 2012 (x14) and invited speaker engagements (x12).

1. ASCO 2013 x 6 abstracts – acceptance/rejection due March 2013.


1. Invited Speaker, European Cancer Conference, Amsterdam, Netherlands, September 2013
   ‘Combination modality treatment for brain metastases’
2. Invited Speaker, German Skin Cancer Meeting, Essen, Germany, September 2013
   ‘Systemic Treatment of metastatic melanoma. Current status and perspectives’
3. Invited Speaker, Medical Oncology Group of Australia, Brisbane, Australia, August 2013
   ‘MAPK inhibitors and melanoma, where are we now’
4. Invited Speaker, 8th World Congress for Melanoma, Hamburg, Germany, July 2013
   ‘Kinase Inhibitors - Updates form ASCO 2013’
5. Invited Speaker, ASCO Education Session, Chicago, IL, USA, June 2013
   ‘Management of Melanoma Brain Metastases’
6. Invited Speaker, Annual Scientific Meeting of the Australasian College of Dermatologist, Sydney Australia, May 2013
   ‘Systemic Therapies for Melanoma; a rapidly changing landscape, and a poster-child for targeted therapies and immunotherapy’
7. Invited Speaker, Joint Meeting for the Asian Society for Pigment Cell Research (ASPCR) and the Australasian Society for Dermatology Research (ASDR), Sydney Australia, May 2013
   ‘Melanoma Heterogeneity, Resistance and Implications for Systemic Therapy’
8. Invited discussant and Chair, Society for Melanoma Research Conference, Hollywood, CA USA, November 2012
   ‘Discussant for Late Breaking Clinical Trials’
9. Invited Speaker, Perspectives in Melanoma XVI, Valencia, Spain, September 2012
   ‘Update on prognostic significant of tumor BRAF and other mutations’
   ‘Combination of BRAF and MEK inhibitors for BRAF mutant metastatic melanoma’
10. Invited Speaker, American Association for Cancer Research, Chicago, USA, April 2012
    ‘Building translation into clinical practice: Lessons from melanoma and targeted therapies’
11. Invited Speaker, Global launch vemurafenib, Paris, April 2012
    ‘Managing metastatic melanoma, now and in the future’
    ‘Targeted therapies, what lessons have we learnt’

Acknowledgements
Helen Rizos, Richard Scolyer, Richard Kefford, John Thompson, Robyn Saw, Jonathan Stretch, Andrew Spillane, Kerwin Shannon, Jessica Hyman, Giulietta Pupo, Valerie Jakrot
Award Recipient Signature:

I certify that the information supplied in this report is true and correct. I understand that the Royal Australasian College of Physicians may wish to verify this information with any institution or individual. I consent to such inquiries.

Signature: [Signature] 28/3/2013

Chief Investigator/Supervisor Signature:

I, [Name], (Supervisor) of the [Institution] have read this report and believe it to be true and correct version of the research undertaken during this period.

Signature: [Signature]

Please submit completed and signed report to the Executive Officer via email – foundation@racp.edu.au.