

ASX Announcement

Zantrene shows impressive synergy with BRAF & MEK inhibitors in treating melanoma

- Zantrene in combination with BRAF inhibitor vemurafenib or MEK inhibitors binimetinib and cobimetinib improves the killing of human melanoma cells
- Zantrene synergizes with vemurafenib to better kill melanoma organoid tumours
- Zantrene in combination with vemurafenib better targets human melanoma tumours in a mouse xenograft model
- Results are supportive of future clinical trials using Zantrene in combination with BRAF and MEK inhibitors to potentially improve melanoma patient outcomes.

28 June 2022 – Race Oncology Limited (“Race”) is pleased to share the final results from our preclinical melanoma research program in collaboration with the University of Newcastle (ASX announcement: 19 March 2021). This program aimed to explore the use of Zantrene® (bisantrene dihydrochloride) in novel drug combinations for the treatment of both immunotherapy and drug resistant melanomas using cell and mouse models.

In previous research, Zantrene as a single agent was found to be highly effective at killing a diverse range of melanoma cell subtypes, showing an association between FTO expression levels and melanoma cell sensitivity to Zantrene (ASX announcement: 30 September 2021).

Zantrene in combination with BRAF and MEK protein kinase inhibitors has been found to improve the killing of human melanoma cells and to better target melanoma in organoid and animal tumour models. These discoveries offer potential non-immunotherapeutic pathways for the use of Zantrene in melanoma treatment.

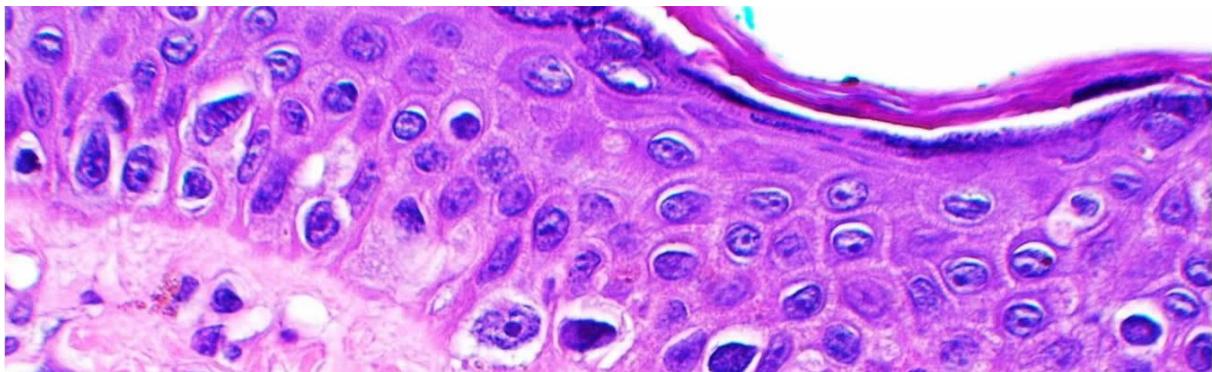


Figure 1. Human malignant melanoma cells *in situ*.

Race Chief Scientific Officer, Dr Daniel Tillett said: *“These exciting preclinical results offer an alternative path to use Zantrene in the clinic to help advanced melanoma patients who are unable to tolerate, or are unresponsive to immunotherapy. The synergy that has now been*

observed with a diverse range of kinase inhibitors suggests possibilities for the clinical use of Zantrene in combination with kinase inhibitors far beyond melanoma.”

Race Chief Executive Officer, Mr Phillip Lynch said: "This research adds to the growing body of research we have on Zantrene and its various areas of potential. While there is a lot of combination work being completed around the world with checkpoint inhibitors, there is much less competition from combinations with BRAF/MEK inhibitors. This therefore presents an interesting commercial angle for Race to explore as we assess how to best show Zantrene's value to potential partners, regulators and patients."

Study Background

Melanoma Treatment

Melanoma can be treated very effectively by surgical removal if identified at an early stage, but once the melanoma has spread into the dermis or beyond, treatment becomes more difficult. While some melanomas respond to conventional cancer treatment options such as chemotherapy and radiation, many are resistant to such treatments, especially those that are advanced.¹

Recent breakthroughs in immunotherapy, including anti-PD-1 and PD-L1 checkpoint inhibitor therapies, have benefitted a growing number of melanoma patients.² Despite these advances, more than half of these patients do not show a durable response to immunotherapy.³ Multiple mechanisms, such as driver mutations, epigenetic changes, tumor plasticity, and immunosuppression, all mediate resistance to immunotherapy.⁴ In addition, a significant number of patients that do respond to immunotherapy suffer serious side effects, which limit their further treatment with immune modulating drugs.^{3,4}

Newer targeted treatments such as the BRAF and MEK inhibitors have proven effective in melanoma, however, the majority of the patients treated with an inhibitor of mutant BRAF kinases eventually suffer relapse, treatment resistance, and disease progression.⁵

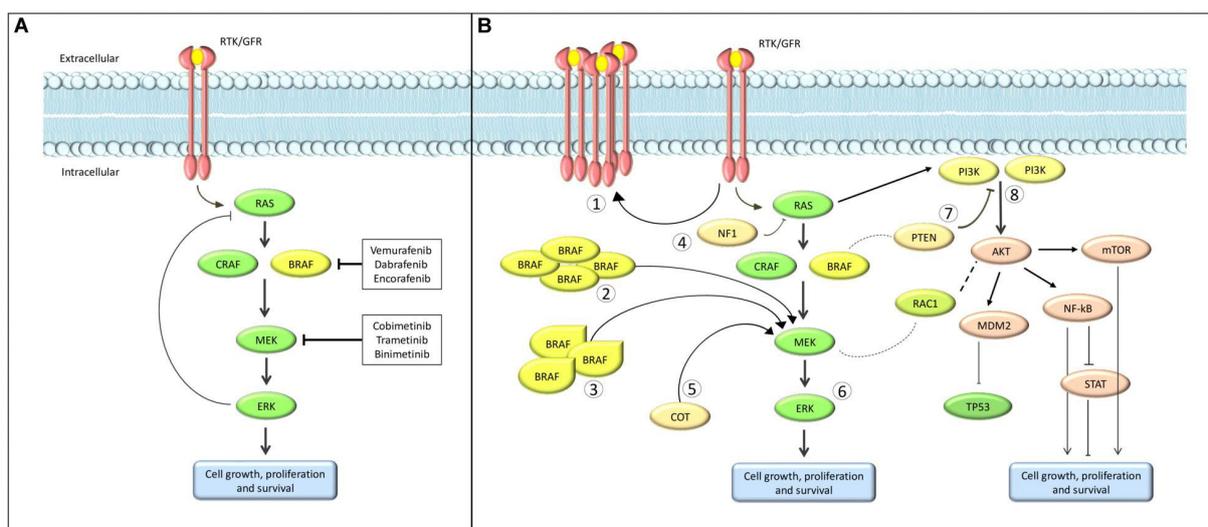


Figure 2. Overview of the Mitogen-Activated Protein Kinase (MAPK) pathway. (A) Normal pathway. **(B)** The most common resistance mechanisms. (1) Upregulation of RTK. (2) BRAF amplification. (3) BRAF alternative splicing. (4) Loss of NF1. (5) COT overexpression. (6) ERK activation. (7) Loss of PTEN. (8) Alternative pathways activation. Reproduced from Tanda *et al.* 2020⁵

Mitogen-Activated Protein Kinase Pathway

Melanoma has one of the highest mutational rates of all solid tumours, reflecting the skin's continued exposure to damaging UV light. These mutations can involve specific oncogenes, causing changes in proliferation, apoptosis and cell cycle regulation. One of the most frequently observed changes is in the Mitogen-Activated Protein Kinase (MAPK) pathway. This molecular pathway is composed of the RAS, RAF, MEK and ERK proteins (Figure 2). The binding between a growth factor and the Tyrosine Kinase Region (TKR) leads to a phosphorylation cascade, resulting in the activation of ERK which regulates the expression of many genes involved in cell proliferation and survival.⁶ The mutation of any gene coding for one of these proteins can activate the whole MAPK pathway leading to cancer.

BRAF Mutations

Activating BRAF mutation occurs in approximately 50% of cutaneous melanomas.⁷ The identification and characterization of BRAF mutations led to the development of highly specific drugs that radically changed the therapeutic landscape of melanoma. Indeed, mutant BRAF targeted inhibitor therapies substantially improved survival in patients with advanced or metastatic melanoma from a median of 6 months with chemotherapy⁸ to a median of 25.9–33.6 months.^{9,10}

BRAF Inhibitors

Four BRAF inhibitors are currently FDA licensed for the treatment of metastatic melanoma with BRAF mutations: atezolizumab, vemurafenib, dabrafenib and encorafenib. All target the protein kinase domain of BRAF and show similar improvements in progression free survival and overall survival.⁵

MEK inhibitors

Preclinical studies suggested that the addition of a MEK inhibitor to a BRAF inhibitor regimen could reduce the BRAF inhibitor induced side effects, delay the development of resistance, and generate a synergistic improvement in efficacy outcomes (King et al., 2013). Subsequent clinical trials have supported this observation and the use of MEK inhibitors such as cobimetinib and binimetinib in combination with the BRAF inhibitor vemurafenib has become common clinical practice, improving patient quality of life and treatment efficacy and longevity.^{5,13}

BRAF Inhibitor Resistance

The emergence of drug resistance in melanoma patients receiving BRAF-targeting drugs is a primary contributor to treatment failure and disease progression, with approximately 50% of patients developing acquired resistance after a median of 6–8 months.^{5,11} Options for secondary treatments following BRAF-inhibitor resistance remain limited, creating a great need for new agents able to effectively kill melanoma cells following treatment failure.

Previous preclinical studies by Race have identified improved cell killing (synergy) when Zantrene was used in combination with the protein kinases lenvatinib, cabozantinib and

pazopanib in kidney cancer cells (ASX announcement: 10 March 2022). We reasoned that Zantrene may offer improved synergy when used in combination with BRAF and MEK inhibitors, contributing to improved treatment outcomes and a delay in the occurrence of BRAF and MEK resistance for melanoma patients.

Study Highlights

1. Zantrene synergizes with both BRAF and MEK inhibitors to better kill human melanoma cells

The sensitivity of human melanoma cells to Zantrene was previously found to not correlate with BRAF or NRAS mutational status (Table 1), but correlated instead to FTO expression levels (Table 2) (ASX announcement: 30 September 2021). The results of this prior work are summarised in Tables 1 and 2.

Table 1. Effect of Zantrene on BRAF and NRAS mutant cell lines.

Cell Line	IC ₅₀ (nM)	BRAF	NRAS
HEMn-MP	1,403	Wild	Wild
SK-Mel-28	515	Mutant	Wild
Mel-JD	335	Wild	Mutant
Mel-RM	178	Wild	Mutant
Mel-RMu	167	Mutant	Wild
Mel-CV	115	Mutant	Wild
Mel-FH	102	Wild	Wild
A375	87	Mutant	Wild
ME1007	78	Wild	Wild
MM200	96	Mutant	Wild
IgR3	72	Mutant	Wild
ME4405	53	Wild	Mutant

Table 2. IC₅₀ values for Zantrene and FTO protein levels normalized to the FTO level of the normal human melanocyte cell line HEMn-MP.

Cell Line	IC ₅₀ (nM)	FTO Level
HEMm-MP	1,403	1.0
Mel-BP	1,003	2.2
SK-MEL-110	1,002	5.0
SK-Mel-28	515	1.0
Mel-JD	335	1.3
MM426	312	2.8
Mel-RM	178	1.6
Mel-RMu	167	1.4
Mel-CV	115	1.4
Mel-FH	102	2.6
MM200	96	1.1
70W	87	1.9

A375	87	2.7
MM170-5	85	3.5
MM283	79	2.4
ME1007	78	1.3
SK-MEL-37	75	2.4
IGR3	72	2.7
SK-MEL-13	67	4.4
ME4405	53	2.0
Mel-BE	39	1.7
MV3	39	5.8
Mel-EH	37	2.2
Mel-JR	30	3.6
Mel-KD	28	2.5
MM962	23	5.0

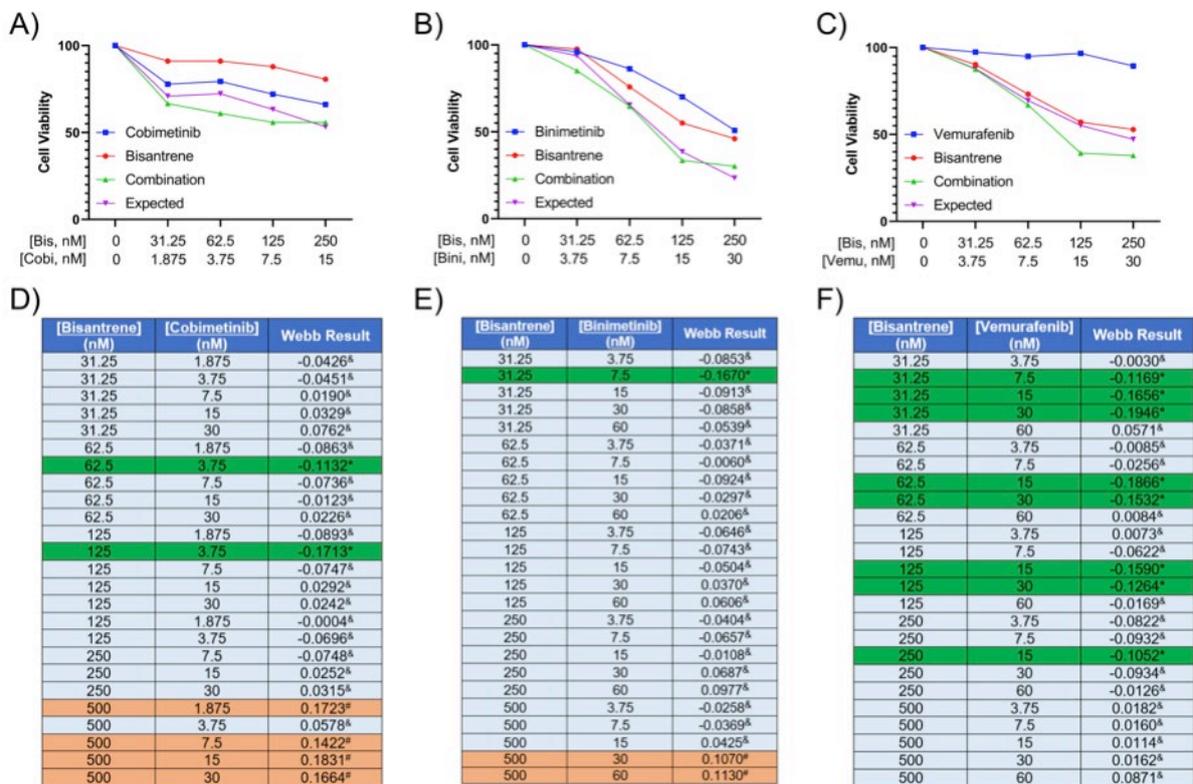


Figure 3. Webb synergy analyses of Zantrene + cobimetinib, Zantrene + binimetinib or Zantrene + vemurafenib drug combinations in Mel-RMu cells. Cell viability in response to different dose ranges of: (A) cobimetinib; (B) binimetinib; or (C) vemurafenib in combination with Zantrene, as indicated. Experimental data is shown for each drug alone and the combinations. The 'Expected value' is calculated using the method of Webb and shows the expected value if the drug combination was additive. Therefore, any experimental values below this line are considered synergistic. At or near the line is additive; and above the line is antagonistic. Webb analysis for: (D) cobimetinib; (E) binimetinib; or (F) vemurafenib in combination with Zantrene where a result of <-0.1 indicates synergy (*); between -0.1 to 0.1 is additive (&); and >0.1 is antagonistic (#).

In light of this Zantrene FTO specific sensitivity data, mutant BRAF melanoma cell lines IgR3, A375, and Mel-RMu primary cells, were plated in a 96-well plate at a concentration of 1×10^4 cells/well in triplicate. 2D synergy modeling was performed by treating cells with increasing doses of Zantrene (0 nM to 250 nM) and/or increasing doses of the MEK inhibitors cobimetinib or binimetinib, or the BRAF inhibitor vemurafenib (0 nM to 30 nM). Cell viability was assessed 72 h post-treatment using the VisionBlue™ Quick Cell Viability Assay kit with observed values normalized to the vehicle controls followed by Webb analysis.¹³

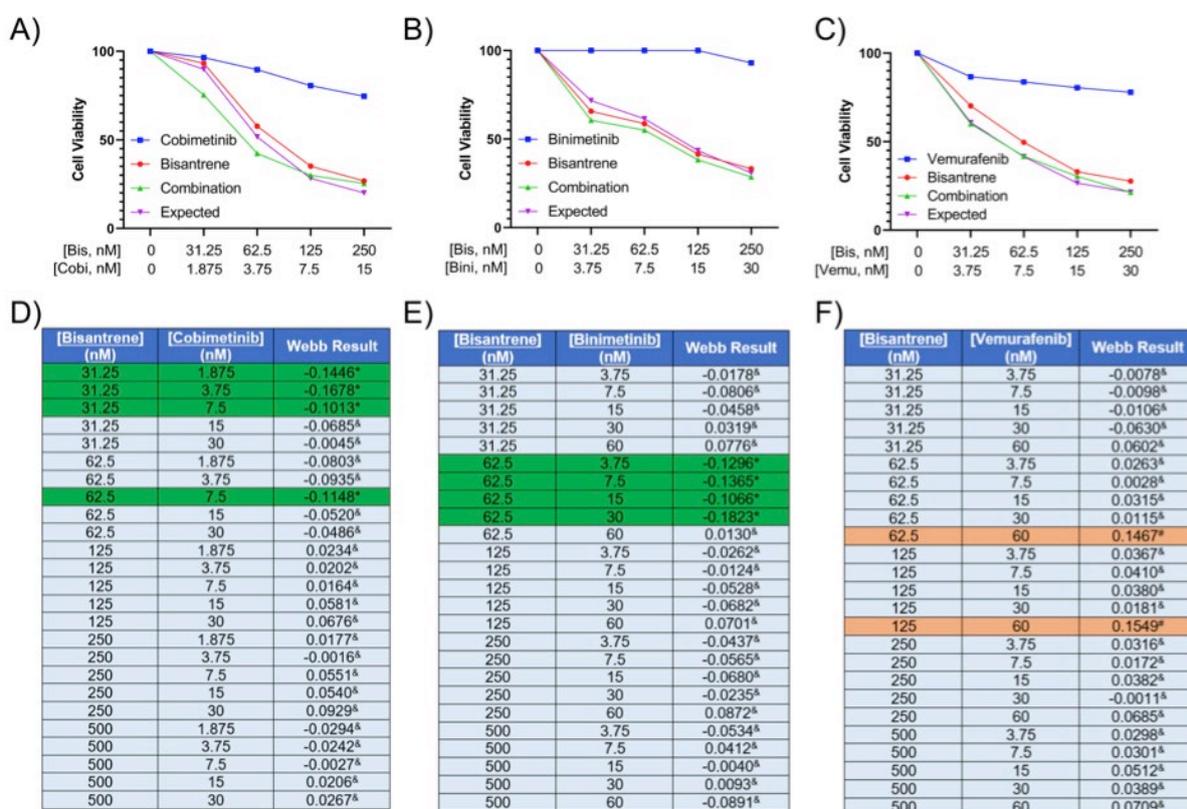


Figure 4. Webb synergy analyses of Zantrene + cobimetinib, Zantrene + binimetinib or Zantrene + vemurafenib drug combinations in IgR3 melanoma cells. Cell viability in response to different dose ranges of: (A) cobimetinib; (B) binimetinib; or (C) vemurafenib in combination with Zantrene, as indicated. Experimental data is shown for each drug alone and the combinations. The 'Expected value' is calculated using the method of Webb and shows the expected value if the drug combination was additive. Therefore any experimental values below this line are considered synergistic. At or near the line is additive; and above the line is antagonistic. Webb analysis for: (D) cobimetinib; (E) binimetinib; or (F) vemurafenib in combination with Zantrene, where a result of < -0.1 indicates synergy (*); between -0.1 to 0.1 is additive (&); and > 0.1 is antagonistic (#).

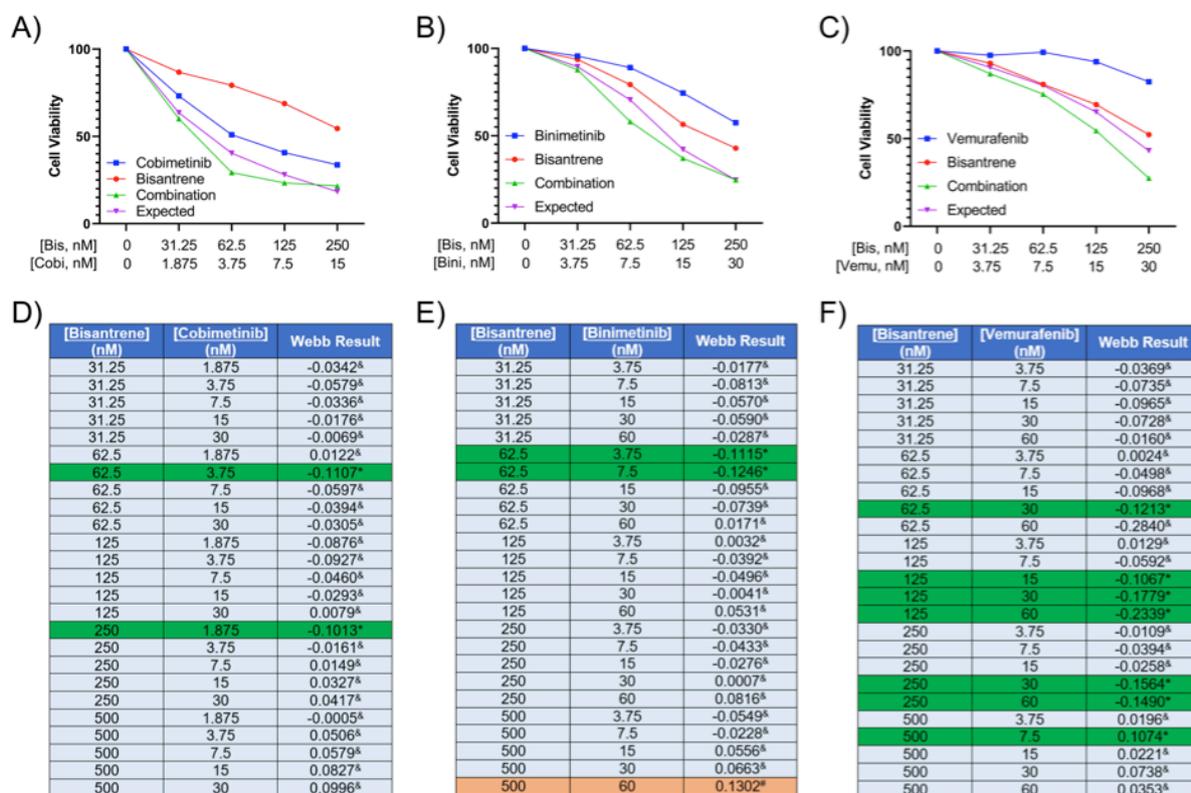


Figure 5. Webb synergy analyses of Zantrene + cobimetinib, Zantrene + binimetinib or Zantrene + vemurafenib drug combinations in A375 melanoma cells. Cell viability in response to different dose ranges of: (A) cobimetinib; (B) binimetinib; or (C) vemurafenib in combination with Zantrene, as indicated. Experimental data is shown for each drug alone and the combinations. The 'Expected value' is calculated using the method of Webb and shows the expected value if the drug combination was additive. Therefore any experimental values below this line are considered synergistic. At or near the line is additive; and above the line is antagonistic. Webb analysis for: (D) cobimetinib; (E) binimetinib; or (F) vemurafenib in combination with Zantrene where a result of <-0.1 indicates synergy (*); between -0.1 to 0.1 is additive (&); and >0.1 is antagonistic (#).

Webb synergy analysis revealed synergy across multiple drug doses for Zantrene and MEK inhibitors cobimetinib, binimetinib and the BRAF inhibitor vemurafenib (Figures 3-5). **The greatest synergy was observed for Zantrene in combination with cobimetinib, with synergy also seen with binimetinib and vemurafenib** in two of the three cell lines tested. In particular, **high dose combinations of Zantrene and vemurafenib produced robust killing in both A375 and Mel-RMu cells.**

2. Zantrene synergizes with the BRAF inhibitor vemurafenib to kill melanoma organoid tumours

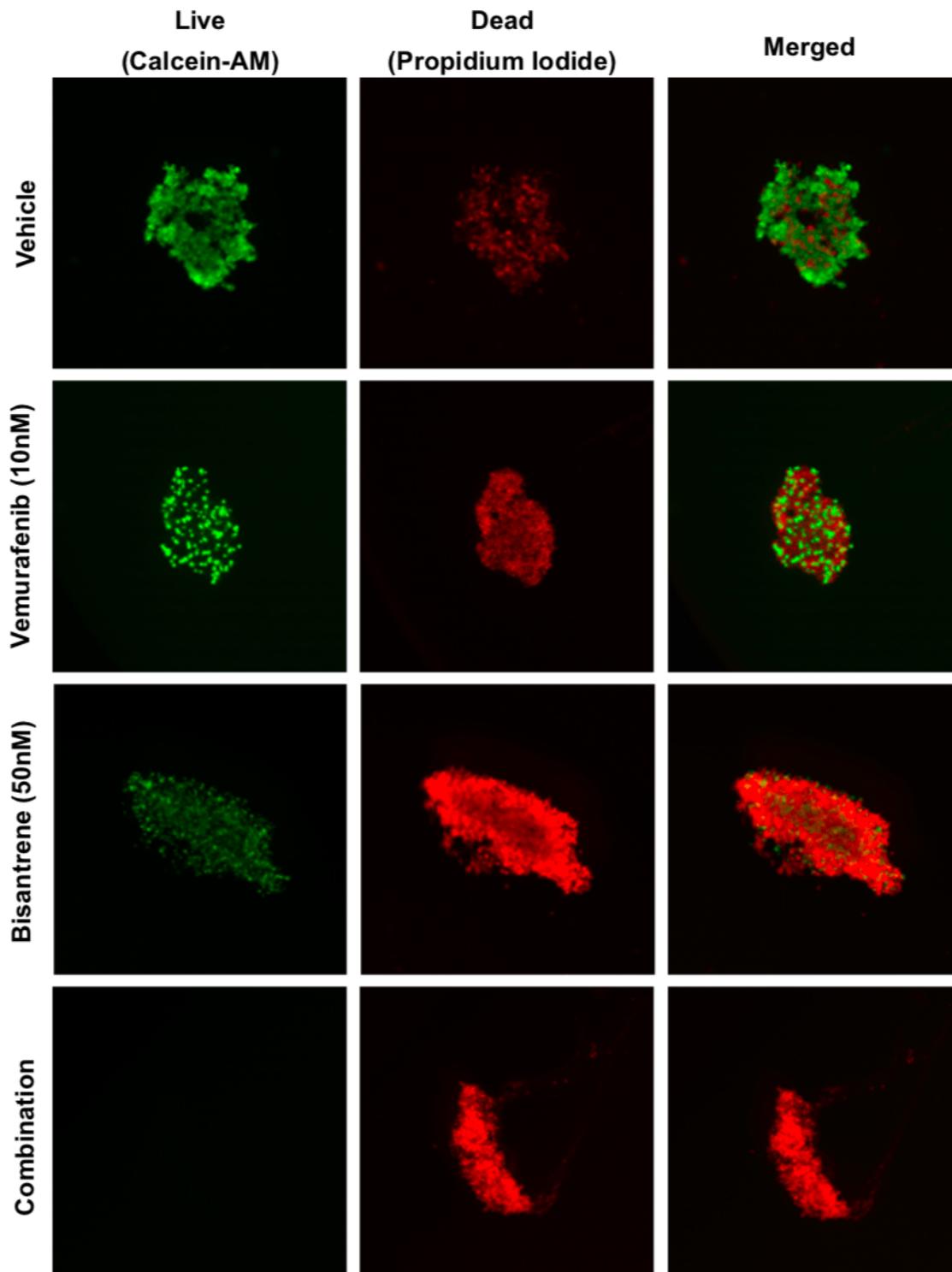


Figure 6. Treatment of IgR3 (BRAF mutant) melanoma organoids with Zantrene, vemurafenib or Zantrene + vemurafenib. After treatment at the indicated drug concentrations for 72 h, the organoids were stained with calcein-AM or propidium iodide to identify the live (green) and dead (red) cells under fluorescent microscopy.

Cancer organoids are 3-dimensional cell clusters of tumour grown *in vitro* (in tissue culture) that better mimic the true *in vivo* (in the body) tumour environment. Numerous studies have found cancer organoids more closely resemble real tumours in regard to response and sensitivity to anti-cancer agents.¹⁴

While both vemurafenib and Zantrene were able to kill IgR3 melanoma cells in organoids clusters, the combination of Zantrene + vemurafenib was more effective (Figure 6).

3. Zantrene synergizes with the BRAF inhibitor vemurafenib to better target human melanoma tumours in a xenograft mouse model

In order to assess the efficacy of Zantrene/BRAF inhibitor combinations on melanoma tumours, Zantrene was combined with the BRAF inhibitor vemurafenib in NOD/SCID mice engrafted with human BRAF mutated IgR3 melanoma cells.

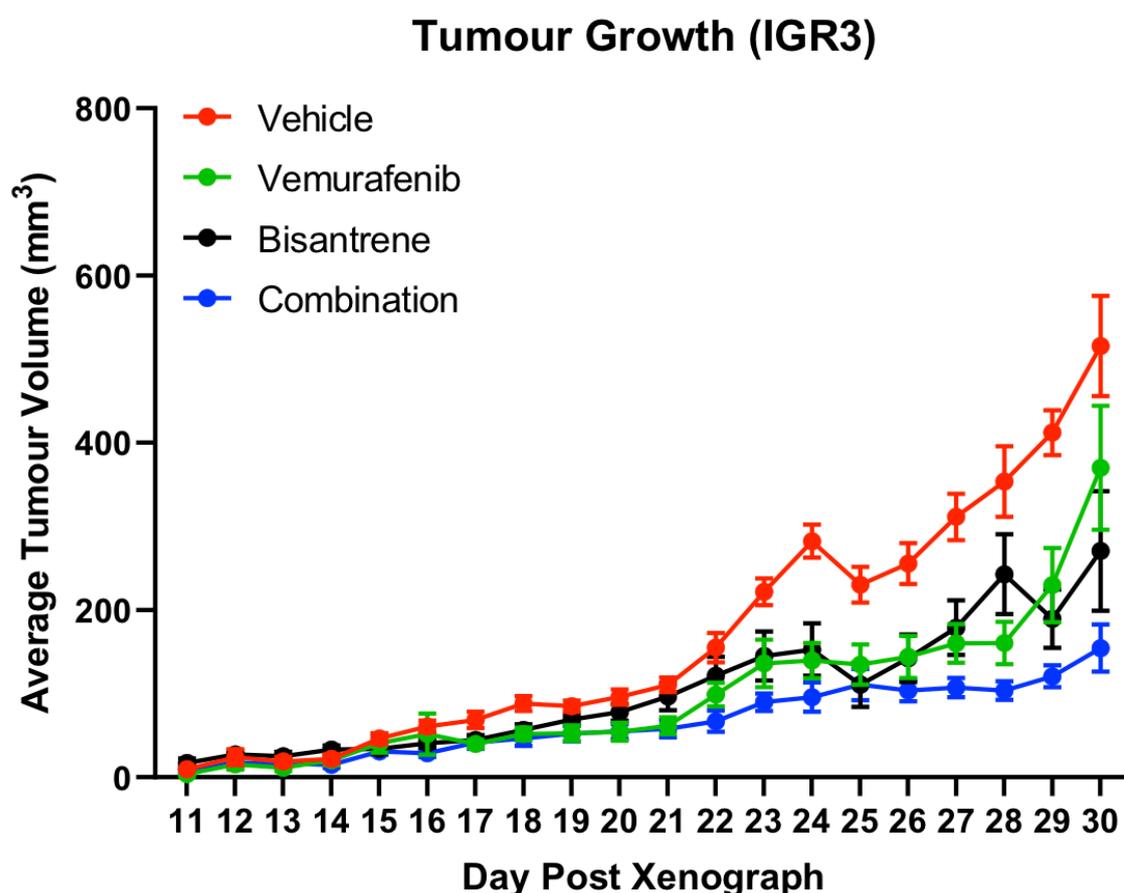


Figure 7. Treatment of IGR3 human melanoma tumours in NOD/SCID mice with vehicle control (red) vemurafenib (green), Zantrene (black), or vemurafenib plus low dose Zantrene (blue). Mice were dosed with 30 mg/kg vemurafenib 6 out of 7 days, or with 15 mg/kg of Zantrene every 2 days starting on Study Day 11. n=8 per group at Day 11. Error bars = SEM.

Groups of eight NOD/SCID mice were acclimated for 7 days before the commencement of experiments. Mice were xenografted with the human BRAF-mutant melanoma cell line IgR3 (1x10⁶ cells in 200 µL) supplemented with 20% Matrigel™. After the development of tumors (11 days post xenografting), the mice were treated with the BRAF inhibitor

vemurafenib 6 days per week (30 mg/kg by oral gavage) and/or Zantrene every second day (15 mg/kg, intravenous injection). Mouse body weight and tumor growth were monitored daily using Vernier calipers. Mice were euthanised on study day 30, or when their tumor burden exceeded a volume of 2000 mm³. Significant differences between groups were calculated using the 2-way ANOVA with Tukey's test for multiple comparisons tool in GraphPad PRISM v9.

The combination of 15 mg/kg Zantrene every second day, with 30 mg/kg vemurafenib six days out of every seven produced a significant slowing in the growth of the engrafted tumors relative to either matched dose Zantrene or vemurafenib-alone from Day 22 until study endpoint (Figure 7). Significant decreases in tumor volume were also seen from Day 24 until study endpoint for vemurafenib, Zantrene and the Zantrene + vemurafenib combination when compared to vehicle treated animals.

At study end (Day 30), tumours were excised from the euthanised mice and photographed (Figure 8). The tumours exposed to the combination of Zantrene and vemurafenib were significantly smaller than those excised from the other treatments.

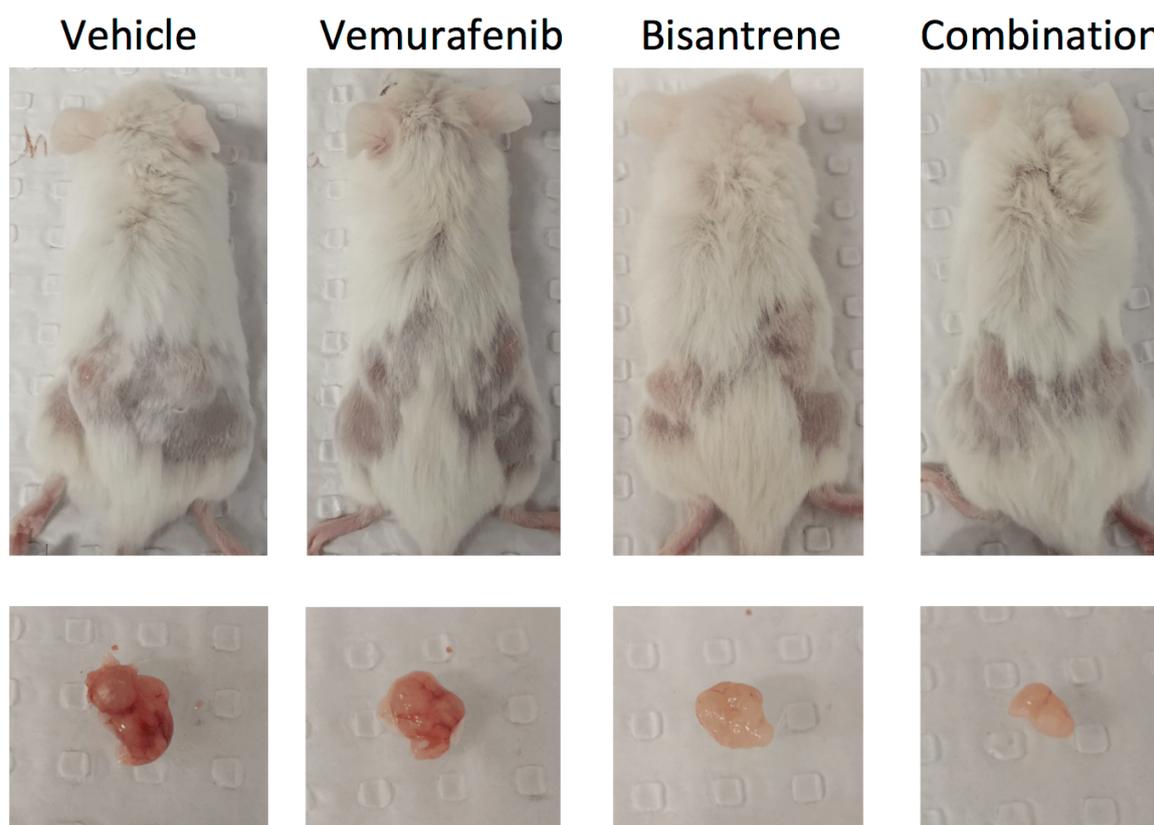


Figure 8. IgR3 human melanoma tumours treated with vehicle control, vemurafenib, Zantrene, or Zantrene + vemurafenib combination. Tumours were excised from mice euthanased at Day 30 and photographed.

These results demonstrate that Zantrene is able to significantly increase the efficacy of mutant BRAF kinase targeted therapy in animals as well as cells and tumour organoids.

Conclusions

- Zantrene improves the killing of melanoma cells when used with standard of care BRAF and MEK inhibitors.
- Zantrene better kills melanoma organoid tumours when used in combination with the BRAF inhibitor vemurafenib. Tumour organoids are closer mimics of patient tumours than cells grown in culture.
- Zantrene in combination with vemurafenib significantly slows the grow of human melanoma tumours in mice.
- These synergistic combinations have high clinical relevance and potential for rapid translation into the clinic.
- A new patent protecting these findings has been submitted. If granted, the patent would be valid until 2042.

Next Steps

- Optimisation of the dosage and drug combinations to identify the best clinical treatment opportunity.
- Further preclinical studies using other BRAF and MEK kinase inhibitors to explore the mechanism of action of the Zantrene synergies that have been discovered.
- Publication of results in a high quality, peer reviewed journal.
- Consultation with key opinion leaders to explore initiating a clinical study utilising Zantrene in combination with BRAF and MEK inhibitors to improve the treatment of advanced melanoma patients.

Q&A

Is this a good result?

Yes. Finding that Zantrene synergies with the widely used BRAF and MEK protein kinase inhibitors offers the potential for a rapid pathway to clinical development. The more ways that Zantrene can be used in the clinic and the larger the potential market and the more opportunities we have to rapidly advance Zantrene towards approval.

Which approach would be easier to translate to the clinic - Zantrene + immunotherapy or Zantrene + BRAF/MEK inhibitors?

Zantrene + BRAF/MEK inhibitor. Melanoma is a highly competitive clinical space with more than 600 clinical trials recruiting around the world.¹⁵ The vast majority of these trials are aimed at enhancing immunotherapy. For example, in Australia there are 49 melanoma trials currently recruiting, the vast majority in the immunotherapy area with only four targeted at improving BRAF/MEK inhibition treatments. With patient recruitment becoming the limiting factor in clinical cancer research, focusing on less crowded opportunities can accelerate a drug's development.

What additional preclinical work do you need to do?

While we can potentially move to clinical trials from these results, it is wise to explore the use of Zantrene with other BRAF and MEK inhibitors before doing so. In addition, it is still unknown if Zantrene can help overcome BRAF inhibitor resistance in melanoma.

What do these results mean for the use of Zantrene beyond melanoma?

Like immunotherapies, protein kinase inhibitor drugs are widely used across many different cancer types. Zantrene has now shown cell killing synergy with six different kinase inhibitors across both melanoma and kidney cancer. There is a good chance that such synergy will be seen both in other cancer types and with other kinase inhibitors, but additional preclinical studies will be required to explore this opportunity.

How soon could you start a human trial of Zantrene using a BRAF/MEK inhibitor in melanoma patients?

2023. The answer to this question ultimately depends on the additional preclinical work that is underway to optimise the dose levels of the combination of Zantrene and BRAF/MEK inhibitors and if Zantrene improves the BRAF/MEK response in resistant patients.

What is the market potential of this discovery?

As outlined at the 2021 Race Annual General Meeting, melanoma has significant commercial potential with the existing melanoma treatment market estimated to exceed US\$20 billion per year.

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About Race Oncology (ASX: RAC)

Race Oncology is an ASX listed precision oncology company with a Phase 2/3 cancer drug called Zantrene®.

Zantrene is a potent inhibitor of the Fatso/Fat mass and obesity associated (FTO) protein. Overexpression of FTO has been shown to be the genetic driver of a diverse range of

cancers. Race is exploring the use of Zantrene as a new therapy for melanoma and clear cell renal cell carcinoma, which are both frequent FTO over-expressing cancers.

In breakthrough preclinical research, Race has also discovered that Zantrene protects from anthracycline-induced heart damage, while in tandem acting with anthracyclines and proteasome inhibitors to improve their ability to target breast cancer. Race is evaluating this discovery.

The Company also has compelling clinical data for Zantrene as a chemotherapeutic agent and is in clinical trial in Acute Myeloid Leukaemia (AML).

Race is pursuing outsized commercial returns for shareholders via its 'Three Pillar' strategy for the clinical development of Zantrene.

Learn more at www.raceoncology.com

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