

## ASX Announcement

### Zantrene<sup>®</sup> kills melanoma cancer cells that overproduce FTO

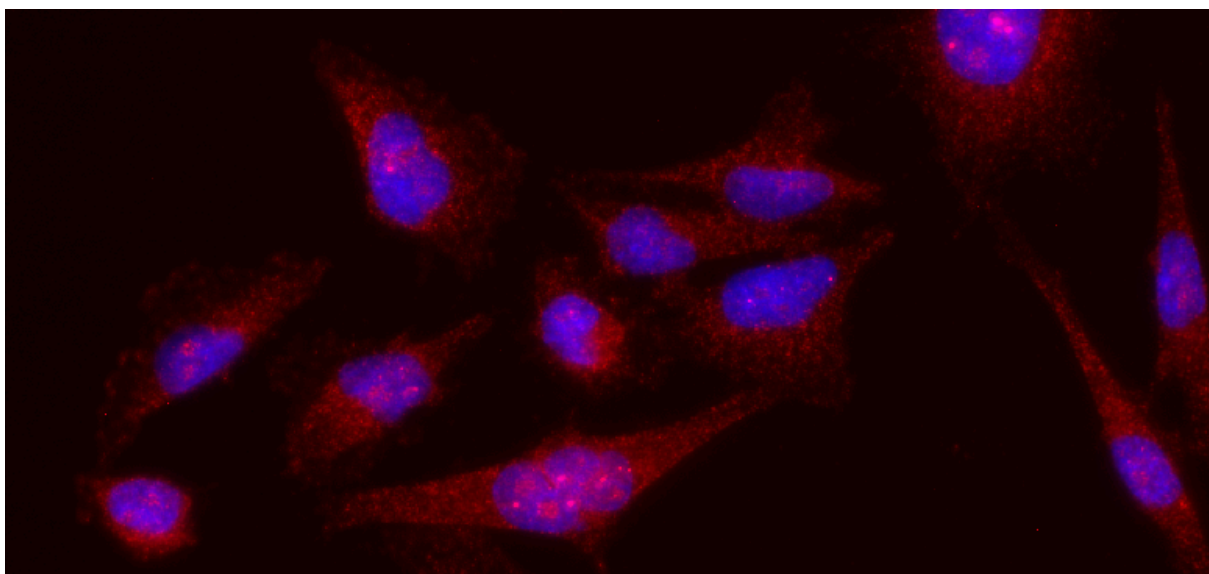
- Zantrene<sup>®</sup> at low concentrations kills high FTO producing melanoma cancer cells
- Sensitivity to Zantrene<sup>®</sup> correlates with FTO levels, where high FTO producing cells show up to 60x greater sensitivity than low FTO producing cells
- Results are highly supportive of future clinical trials in melanoma using Zantrene<sup>®</sup> in combination with standard of care treatments.

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**30 September 2021** – Race Oncology Limited (“Race”) is pleased to share interim results from our collaborative preclinical melanoma research program with the University of Newcastle (ASX Announcement: 19 Mar 2021). Eminent melanoma researchers, Professor Xu Dong Zhang and Associate Professor Lei Jin, are leading the project.

This program is exploring the use of Zantrene<sup>®</sup> (bisantrone dihydrochloride) as a novel potential treatment for melanoma using cellular and mouse models. The aim is to identify drug combinations and melanoma subtypes that show improved treatment responses, with a focus on treatment-resistant melanomas.

**These interim results showed Zantrene to be highly effective at killing a diverse range of high FTO producing melanoma cell subtypes. Data from the expression of the Fat Mass and Obesity-associated protein (FTO) showed an association between FTO expression level and sensitivity to Zantrene.**



**Figure 1.** Melanoma cells stained with the BAK1 (red) and Dapi (blue).

Zantrene has been identified as a potent targeted inhibitor of the Fat Mass and Obesity associated protein (FTO).<sup>1</sup> Previous studies have observed that FTO is over-produced in approximately 50% of metastatic melanomas<sup>2</sup> and that inhibition of FTO can overcome PD-1 immune checkpoint resistance in mouse melanoma models.<sup>2,3</sup> PD-1 immune checkpoint inhibitors have emerged as a front-line treatment for many types of cancer, including melanoma. While there have been major advances in melanoma treatments in recent decades, the five-year survival rate for advanced melanoma remains low.<sup>4</sup>

**Race CSO Dr Daniel Tillett said:** *“These interim results are highly encouraging and support our clinical plans for Zantrene, with the correlation between FTO overexpression and sensitivity to Zantrene suggesting a strong anti-FTO therapeutic opportunity. The high sensitivity of many of the melanoma cell lines to Zantrene as a single agent at concentrations well below chemotherapeutic doses is unexpected and may offer new treatment options for melanoma patients.”*

**Race CEO & MD Phillip Lynch said:** *“While challenged by COVID 19 related shutdowns we appreciate the encouraging and continued work from the team at the University of Newcastle. Zantrene continues to positively surprise us – we are very pleased with these early results. Melanoma remains a difficult cancer to treat, and one that’s of particular relevance to the Australian community, so as we continue with this work, we look forward to learning more about our potential to offer new treatment options to patients.”*

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## **Study Background**

Melanoma is unresponsive to existing anthracyclines, yet Zantrene showed significant historical *in vitro* activity against fresh human melanoma samples taken from patients in human tumor cloning assays.<sup>5,6</sup> In a subsequent Phase I trial of Zantrene administered weekly, a patient with metastatic melanoma achieved a complete response lasting 6 months.<sup>7</sup> This weekly dosing schedule would likely have resulted in sustained inhibition of FTO due to the long time Zantrene remains in the human body.

Despite these early successes, four subsequent Phase 2 studies of Zantrene in 100 melanoma patients used much longer dosing intervals of once every three or 4 weeks and did not achieve the same levels of clinical response, possibly due to limited, transient inhibition of FTO.<sup>8-11</sup> Seventeen patients (1/16, 2/16, 0/17, 14/51) achieved disease stabilization, but no further complete responses were observed.

In light of the recent discovery that Zantrene is a potent inhibitor of the m<sup>6</sup>A RNA demethylase FTO<sup>1</sup> and that FTO is frequently overexpressed in metastatic melanoma<sup>2</sup>, Race sought to explore the use of Zantrene for treating melanoma, both as a single agent and in combination with other standard of care drugs. This research will also help inform the dose regimen to be explored in future clinical trials.

## **Materials and Methods**

Twenty five melanoma cancer cell lines were screened for their sensitivity to Zantrene<sup>®</sup>. The cell lines were selected from a wide range of primary and metastatic melanomas to

cover the most common sub-types carrying a range of BRAF and NRAS mutations (Table 1).

**Table 1.** Cell lines used in study.

<b>Cell Line</b>	<b>Origin</b>
HEMm-MP	Normal Melanocytes (Medium Pigment)
MEL-BP	Malignant Melanoma
SK-MEL-110	Malignant Melanoma
SK-Mel-28	Malignant Melanoma 51yr ♂ (skin)
Mel-JD	Malignant Melanoma
MM426	Malignant Melanoma (skin)
Mel-RM	Malignant Melanoma
Mel-RMu	Malignant Melanoma
Mel-CV	Malignant Melanoma
Mel-FH	Malignant Melanoma
MM200	Primary Melanoma 43yr ♀ (skin)
A375	Malignant Melanoma 54yr old ♀
MM170-5	Malignant Melanoma (skin)
MM283	Malignant Melanoma (skin)
ME1007	Primary Melanoma 70yr ♂ (leg)
SK-MEL-37	Continuous Melanoma Cell Line
IgR3	Metastatic Melanoma 60yr ♂ (nodule stage IV cutaneous)
SK-MEL-13	Malignant Melanoma 29yr ♂
ME4405	Primary Melanoma 83yr ♀ (head)
MEL-BE	Malignant Melanoma
MV3	Metastatic Melanoma
MEL-EH	Malignant Melanoma
MEL-JR	Malignant Melanoma
MEL-KD	Malignant Melanoma
MM962	Malignant Melanoma (skin)

Cell viability was determined using the resazurin metabolic assay and confirmed by visual inspection under light microscopy.

IC<sub>50</sub> values (i.e. the drug concentration that resulted in 50% cell death after 72 hrs) were determined using Prism 8 software with Nonlinear Regression analysis (variable slope, four parameters).

FTO protein expression was determined by western blotting, normalised to GAPDH expression and normal untransformed melanocytes.

All experiments were replicated a minimum of three times.

## Study Highlights

### 1. Zantrene<sup>®</sup> is highly effective in killing melanoma cells at sub-chemotherapeutic levels.

Zantrene proved to be highly effective at killing melanoma cell lines, with 60% (15 of 25) displaying IC<sub>50</sub> values below 100 nM concentrations (Table 2). This was seen with cell lines derived from both primary and metastatic melanoma patients. Interestingly, six of the 25 cell lines showed extreme sensitivity to Zantrene (IC<sub>50</sub> values under 40 nM), suggesting that Zantrene may provide an effective single agent treatment for some patients.

The untransformed melanocyte cell line (normal) was highly resistant to cell killing by Zantrene, as were some of the melanoma cell lines (Table 2). The molecular mechanisms underlying this resistance to Zantrene remain to be determined.

**Table 2.** IC<sub>50</sub> values for Zantrene<sup>®</sup>.

Cell Line	IC <sub>50</sub> (nM)
HEMm-MP	1403
MEL-BP	1003
SK-MEL-110	1002
SK-Mel-28	515
Mel-JD	335
MM426	312
Mel-RM	178
Mel-RMu	167
Mel-CV	115
Mel-FH	102
MM200	96
A375	87
MM170-5	85
MM283	79
ME1007	78
SK-MEL-37	75
IgR3	72
SK-MEL-13	67
ME4405	53
MEL-BE	39
MV3	39
MEL-EH	37
MEL-JR	30
MEL-KD	28
MM962	23

## 2. Sensitivity to Zantrene<sup>®</sup> is independent of BRAF and NRAS mutational status.

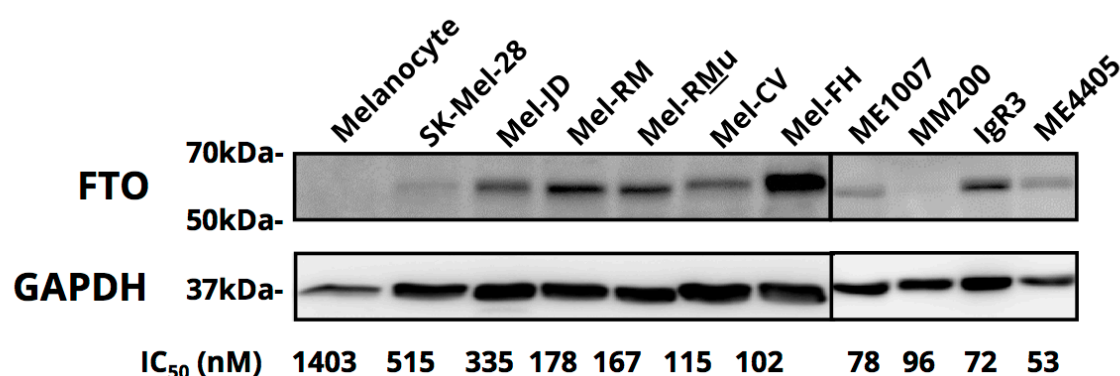
Zantrene sensitivity did not show any correlation to either BRAF or NRAS mutational status, with individual mutant and wild type cell lines displaying a wide range of IC<sub>50</sub> values. This result suggests that Zantrene<sup>®</sup> may show utility in patients resistant to BRAF and NRAS inhibitors.

**Table 3.** Effect of Zantrene<sup>®</sup> on BRAF and NRAS mutant cell lines.

Cell Line	IC <sub>50</sub> (nM)	BRAF	NRAS
HEMn-MP	1403	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
ME1007	78	Wild	Wild
MM200	96	Mutant	Wild
IgR3	72	Mutant	Wild
ME4405	53	Wild	Mutant

## 3. Sensitivity to Zantrene<sup>®</sup> correlates with FTO expression levels

Zantrene sensitivity was correlated with FTO protein overexpression levels (Figure 2 and Table 4).



**Figure 2.** FTO protein expression determined by western blot.

The eight most Zantrene-resistant cell lines (i.e. those with an IC<sub>50</sub> values greater than 100 nM) had a median FTO expression level 1.4 fold higher that of the untransformed (normal) melanocyte cell line, HEMn-MP.

In contrast, the 15 most sensitive melanoma cell lines had a median increase in FTO protein levels of 2.5 fold. The five most sensitive cell lines (i.e. those with IC<sub>50</sub> values below 40 nM) had an average increase in FTO protein levels 3.8 fold higher (Table 4).

**Table 4.** FTO protein levels normalized to the FTO level of the normal human melanocyte cell line HEMn-MP.

<b>Cell Line</b>	<b>FTO Level</b>
HEMm-MP	1.0
Mel-BP	2.2
SK-MEL-110	5.0
SK-Mel-28	1.0
Mel-JD	1.3
MM426	2.8
Mel-RM	1.6
Mel-RMu	1.4
Mel-CV	1.4
Mel-FH	2.6
MM200	1.1
70W	1.9
MM170-5	3.5
MM283	2.4
ME1007	1.3
SK-MEL-37	2.4
IGR3	2.7
SK-MEL-13	4.4
ME4405	2.0
Mel-BE	1.7
MV3	5.8
Mel-EH	2.2
Mel-JR	3.6
Mel-KD	2.5
MM962	5.0

The SK-MEL-10 was an exception to the trend of high FTO overexpression being associated with high sensitivity to Zantrene. This cell line had an IC<sub>50</sub> value over 1000 nM while displaying an FTO expression level 5 fold that of HEMn-MP. The reason for this resistance is unknown, but may be linked to high expression of drug efflux pumps like MDR1 that are known to reduce the intracellular concentration of Zantrene.

## Conclusions

- Zantrene showed unexpectedly effective killing of melanoma cell lines at concentrations well below 100 nM (sub-chemotherapeutic), with a number of cell lines displaying very high sensitivity (less than 40 nM)
- Zantrene proved effective at killing melanoma cell lines with BRAF or NRAS mutations
- Sensitivity to Zantrene was correlated with overproduction of the FTO protein, supporting Race's clinical plans for using Zantrene in combination with standard of care drugs for the treatment of melanoma patients.

## Next Steps

- Further preclinical studies of Zantrene in combination with standard of care melanoma drugs to identify synergistic combinations
- Animal studies exploring the potential of Zantrene to overcome immune checkpoint inhibitor anti-PD-1(L) resistance.

## References

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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. The Company also has compelling clinical data for the use of Bisantrene as a chemotherapeutic agent with reduced cardiotoxicity in Acute Myeloid Leukaemia (AML), breast and ovarian cancers and is investigating its use in these areas.

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](http://www.raceoncology.com).

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