

## ASX Announcement

### Zantrene Protects Mouse Hearts from Chemotherapy Damage

- Zantrene protects the hearts of mice from the permanent damage caused by the chemotherapeutic anthracycline doxorubicin
- Heart protection was achieved at higher levels of chemotherapy treatment with no additional general toxicity or myelosuppression
- Results are supportive of future clinical trials using Zantrene in combination with anthracyclines to potentially improve cancer patient treatment and quality of life.

**30 June 2022** – Race Oncology Limited (“Race”) is pleased to share further interim results from our preclinical cardioprotection program in collaboration with researchers from the University of Newcastle (ASX announcement: 28 April 2021). This program aimed at exploring the use of Zantrene® (bisantrene dihydrochloride) as a cardioprotective agent which offered synergy with anti-cancer treatments.

Zantrene was found to protect the hearts of mice from the damaging effects of anthracyclines (specifically doxorubicin) even when the chemotherapeutic dose was increased without significant additional toxicity or bone marrow suppression.

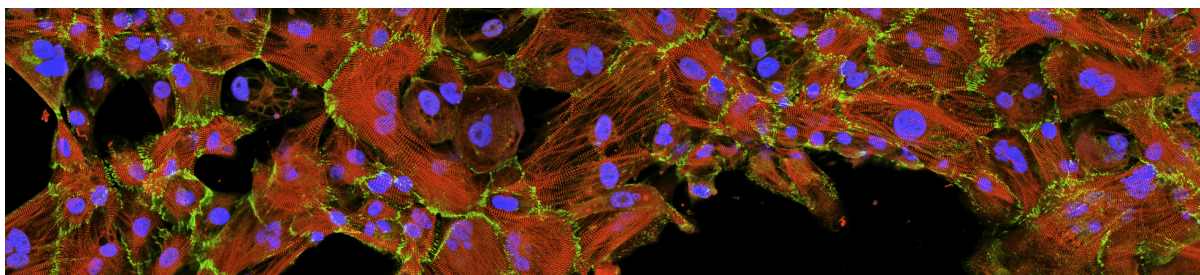


Figure 1. Human cardiomyocytes stained for EH-myomesin (red), beta-catenin (green) and DNA (purple). Image courtesy of Christian Zuppinger, University Hospital of Bern–Inselspital.

**Chief Scientific Officer, Dr Daniel Tillett said:** *“Extending the initial cardioprotection study from cells to hearts is a major step forward for Race. We now know Zantrene is not only able to protect human heart muscle cells from anthracycline induced death, but that this also applies to hearts in animals. When combined with the historical clinical data around Zantrene heart safety, we believe Zantrene may offer millions of patients a unique combination of cardioprotection with enhanced anti-cancer efficacy. Such opportunities are rare in oncology.”*

**Associate Professor Aaron Sverdlov said:** *“To date, there are no widely used or well-established strategies to protect the heart against chemotherapy-induced damage. Furthermore, the concept of potential cancer therapy that is not only non-cardiotoxic, but cardioprotective has not been evaluated or even entertained. These results suggest that Zantrene, an effective anti-cancer agent, can concomitantly provide protection against toxic*

*effects on the heart from one of the most used chemotherapy agents, doxorubicin. This is the first evidence of its kind to demonstrate that there is a therapy that both targets the cancer and protects the heart! This has the potential to improve health outcomes for countless cancer patients and survivors by both improving their cancer treatment while preventing development of cardiovascular disease."*

**Chief Executive Officer, Mr Phillip Lynch said,** *"It's pleasing to see our strategic ambitions for cardioprotection independently validated at an animal level which enables us to prioritise clinical translation. We are committed to producing further preclinical data that will continue to prove the case for this opportunity for Zantrene. It's certainly a large commercial opportunity and one that's got significant potential to improve modern chemotherapy."*

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## **Study Background**

### ***Anthracycline Chemotherapeutics***

Anthracyclines are one of the most effective anti-cancer treatments developed and are used in more cancer settings than any other class of chemotherapeutic agent.<sup>1</sup> These drugs are used to treat millions of cancer patients every year, including those with leukemias, lymphomas, neuroblastoma, kidney, liver, stomach, uterine, thyroid, ovarian, sarcomas, bladder, lung and breast cancers. The clinically most important anthracyclines are doxorubicin, daunorubicin, epirubicin and idarubicin.<sup>2</sup>

The anti-cancer efficacy of anthracyclines is known to correlate to dose with higher doses providing greater efficacy, but at the expense of higher rates of serious side-effects.<sup>1</sup> In clinical practice, anthracycline chemotherapeutics such as doxorubicin are given at a level close to the patient's maximal tolerated dose (MTD) in order to maximise the anticancer efficacy. Common dose limiting toxic side-effects of anthracyclines include: cardiotoxicity, suppression of the immune system and red blood cell production (myelosuppression), hair loss (alopecia), and gastrointestinal toxicity that can cause serious nausea and weight loss.<sup>1,2</sup>

### ***Anthracycline Cardiotoxicity***

While highly effective anti-cancer drugs, the anthracyclines can cause serious and permanent damage to the heart in many patients. Some studies have estimated that over half of patients exposed to anthracyclines will develop some form of heart disease within 6 years of treatment.<sup>5</sup>

Anthracyclines, such as doxorubicin and epirubicin, can lead to either acute or late onset cardiotoxicity. Acute toxicity is associated with increased inflammation and can lead to a pericarditis-myocarditis syndrome. Flaccid dilative cardiomyopathy is the predominant form of late onset anthracycline cardiotoxicity and can occur months to years after anthracycline exposure.<sup>6</sup>

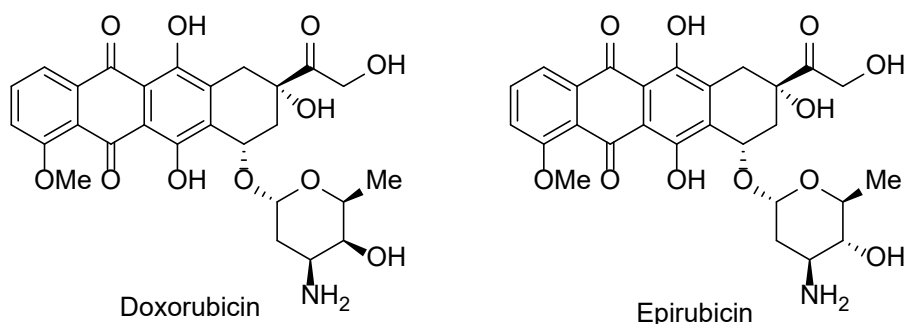


Figure 2. Chemical structures of the anthracyclines doxorubicin and epirubicin.

Although the mechanism of early and late onset anthracycline cardiotoxicity remains unclear, risk factors include increased cumulative anthracycline dose, concurrent mediastinal radiation, extremes of age, female gender, and pre-existing heart disease.<sup>7</sup>

### ***Prevention of Anthracycline Cardiotoxicity***

A number of potential cardioprotective techniques and therapies have been explored over the years, ranging from modified anthracycline preparations, anti-oxidants, free radical scavengers, renin-angiotensin-system antagonists, cardio-selective beta-blockers to statins. While many showed promise in animal studies, clinical studies have often had mixed results with many agents offering little or no cardio-protective benefit and/or compromising the cancer treatment.<sup>7</sup> None have offered the combined features of cardioprotection and improved anti-cancer efficacy.

As a consequence, cardioprotective treatments are not routinely used in clinical oncology practice. Indeed, there remains clear demand for treatment options that prevent today's cancer patient from becoming tomorrow's cardiac patient, all while extending anti-cancer effect.

### ***Anthracyclines & Breast Cancer***

Breast cancer accounts for 30% of all new cancer diagnoses in women.<sup>8</sup> It is estimated in the US that during 2021 there will be 281,550 new breast cancer diagnoses and more than 43,600 deaths.<sup>9</sup> The vast majority (94%) of new breast cancer patients present with early-stage disease.<sup>8</sup>

Anthracycline agents such as doxorubicin and epirubicin, are routinely used for the management of breast cancer with follow-up taxane-based therapy.

Surprisingly, women diagnosed with early-stage breast cancer were found to be more likely to die from cardiovascular disease than from breast cancer. Cardiovascular disease is also the leading cause of death in women over 75 diagnosed with stage II breast cancer.<sup>10</sup>

The risk of cardiotoxic damage is so elevated in breast cancer patients that many oncologists have moved in recent years to using anthracycline-free treatment regimens or limiting patient dosages, despite the well-established efficacy of anthracyclines.<sup>11</sup>

## Zantrene

Zantrene was originally developed as a heart safer alternative to the anthracyclines particularly with respect to preservation of heart muscle.<sup>3</sup>

In the late 1980s, Zantrene was the subject of a US based Phase 3 single agent clinical trial in advanced breast cancer patients. This Phase 3 trial showed that Zantrene had comparable efficacy to standard of care treatment, doxorubicin, but was associated with significantly less damage to the patients' hearts. Approximately 24% of patients who received doxorubicin suffered serious heart failure compared to just 6.6% with Zantrene.<sup>12</sup>

Zantrene was previously found to protect human heart cardiomyocytes (heart muscle cells) grown in cell culture from doxorubicin-induced cell death (ASX Announcement: 22 November 2021). Race sponsored research has now independently replicated these findings at a specialised cardiology contract research organisation (CRO) which has extended the study to mice and explored if Zantrene is able to protect mouse hearts from chemotherapy induced damage.

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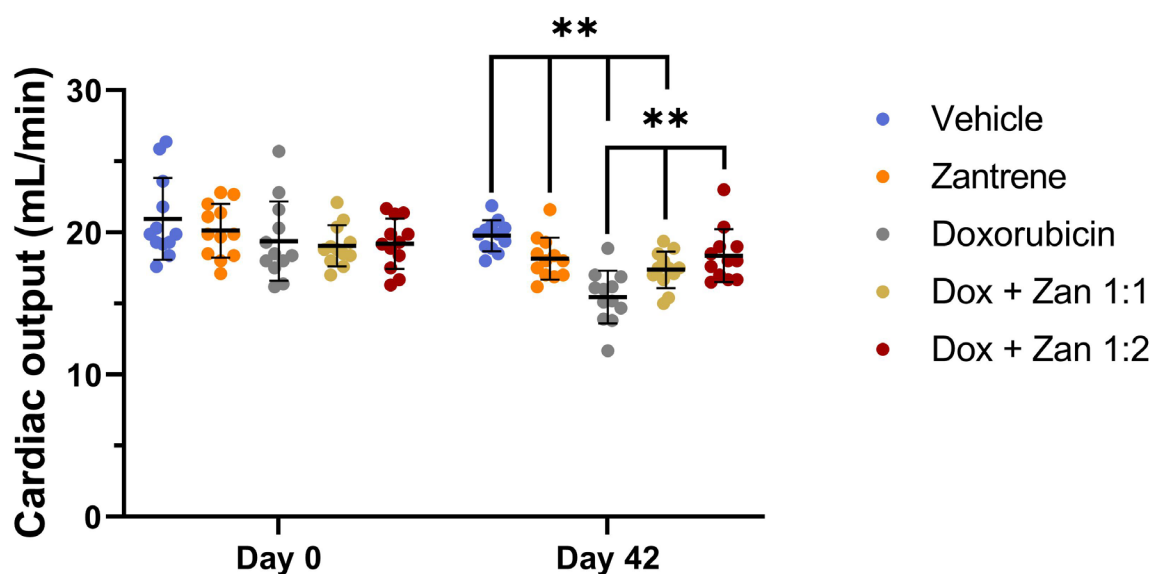
## Study Highlights

### *1. Zantrene protects mouse hearts from doxorubicin induced damage*

Groups of 12 mice were dosed every 7 days (Days 0, 7, 14, & 21) with either vehicle control, Zantrene alone at 7.33 mg/kg, doxorubicin alone at 5 mg/kg, a 1:1 molar ratio of doxorubicin + Zantrene (5 mg/kg + 3.67 mg/kg), or 1:2 molar ratio doxorubicin + Zantrene (5 mg/kg + 7.33 mg/kg). Cardiac output (blood pumped by the heart) was measured by echocardiography at Day 42.

**In this model of anthracycline-induced cardiac damage, Zantrene not only led to significantly less heart muscle damage in comparison to doxorubicin, but in a landmark finding was shown to significantly reduce doxorubicin-induced heart damage when combined with doxorubicin at either 1:1 or 1:2 molar ratios (Figure 3).**

Importantly, doxorubicin + Zantrene at both 1:1 molar ratio (5 mg/kg: 3.67 mg/kg) and 1:2 molar ratio (5mg/kg: 7.33 mg/kg) provided significant cardioprotection. This suggests that the desired cardioprotective effect does not demand a precise drug ratio, so making it more practicable to achieve combined cardioprotective and anticancer outcomes.



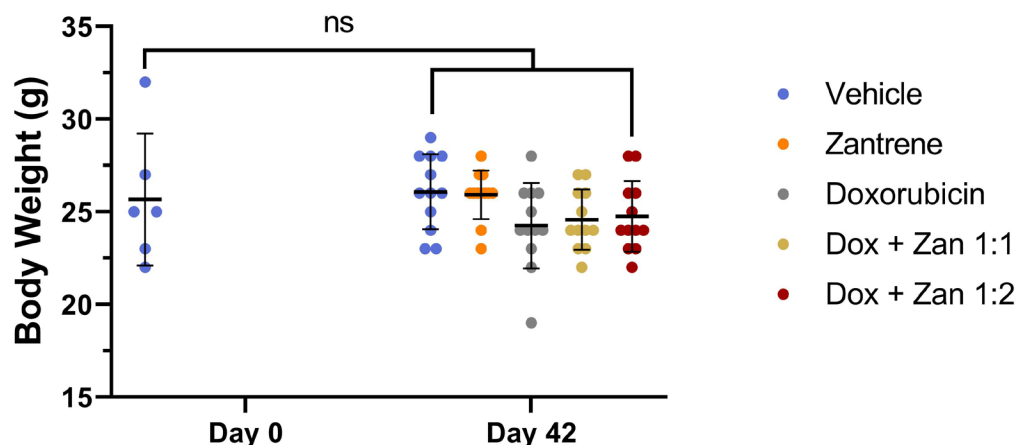
**Figure 3. Cardiac output of C57BL/6 mice treated with either vehicle control (blue), Zantrene alone (orange), doxorubicin alone (grey), 1:1 molar ratio doxorubicin + Zantrene (yellow), or 1:2 molar ratio doxorubicin + Zantrene (red) at Day 0 and Day 42.** All mice were dosed intravenously weekly with either: vehicle control, 7.33 mg/kg Zantrene, 5 mg/kg of doxorubicin, 5 mg/kg of doxorubicin + 3.67 mg/kg of Zantrene, 5 mg/kg of doxorubicin + 7.33 mg/kg of Zantrene. n=12 per group. Error bars = SEM. \*\*p < 0.01.

## ***2. Zantrene allows higher chemotherapeutic doses to be used without additional general toxicity or myelosuppression.***

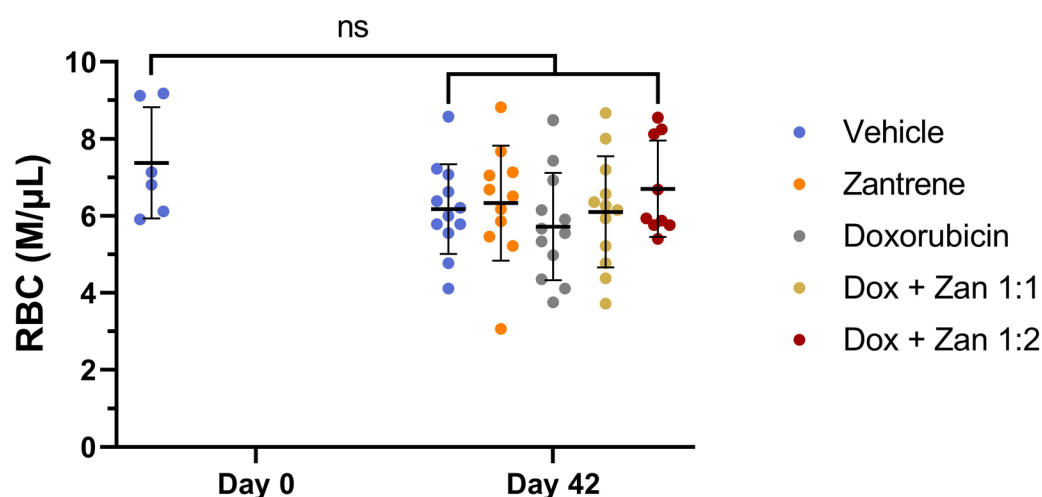
Zantrene at 320mg/m<sup>2</sup> has previously been associated with equivalent levels of clinical myelosuppression as 60mg/m<sup>2</sup> of doxorubicin.<sup>12</sup> Assuming a similar toxicity profile in mice, a 1:1 molar ratio mix of Zantrene and doxorubicin is equivalent to a combined cytotoxicity dosage of 5.94 mg/kg doxorubicin (i.e. a 18% higher overall cytotoxic dose), and a 1:2 molar ratio is equivalent to 6.88 mg/kg (a 36% higher cytotoxic dose).

When exposed to excessive levels of toxins such as anthracyclines, mice rapidly lose body weight. Despite the mice being exposed to greater levels of cytotoxicity through the addition of Zantrene to the 5 mg/kg doxorubicin dose, the body weight of the combination-treated mice was not significantly different to the untreated vehicle control group (Figure 4). Interestingly, there was a non-significant trend of weight loss in mice exposed to cardioprotective combinations of doxorubicin and Zantrene compared to those exposed to doxorubicin alone.

There was no statistically significant change in any of the blood and immune cell counts over the course of treatment for any group indicating little or no myelosuppression. For example, red blood cell counts showed no significant changes over the study, however, as was observed with the body weight there was a non-significant trend of high red blood cell counts in those mice exposed to cardioprotective combinations of doxorubicin and Zantrene verses those exposed to doxorubicin alone (Figure 5).



**Figure 4. Mouse body weight of C57BL/6 mice treated with either vehicle control (blue), Zantrene alone (orange), doxorubicin alone (grey), 1:1 molar ratio doxorubicin + Zantrene (yellow), or 1:2 molar ratio doxorubicin + Zantrene (red) at Day 0 and Day 42.** All mice were dosed intravenously weekly with either: vehicle control, 7.33 mg/kg Zantrene, 5 mg/kg of doxorubicin, 5 mg/kg of doxorubicin + 3.67 mg/kg of Zantrene, 5 mg/kg of doxorubicin + 7.33 mg/kg of Zantrene. n = 12 per group. Error bars = SEM. ns = not significant.



**Figure 5. Mouse red blood cell counts of C57BL/6 mice treated with either vehicle control (blue), Zantrene alone (orange), doxorubicin alone (grey), 1:1 molar ratio doxorubicin + Zantrene (yellow), or 1:2 molar ratio doxorubicin + Zantrene (red) at Day 0 and Day 42.** All mice were dosed intravenously weekly with either: vehicle control, 7.33 mg/kg Zantrene, 5 mg/kg of doxorubicin, 5 mg/kg of doxorubicin + 3.67 mg/kg of Zantrene, 5 mg/kg of doxorubicin + 7.33 mg/kg of Zantrene. n = 12 per group. Error bars = SEM. ns = not significant.



## Conclusions

- Zantrene protects mouse hearts from the irreversible damage caused by the anthracycline doxorubicin. This extends upon earlier reported results observed in human heart muscle cells.
- Combination of Zantrene with chemotherapeutic doses of doxorubicin does not cause significant increases in general toxicity or myelosuppression.
- Race has submitted an updated patent application addressing the combination of Zantrene with an anthracycline for the protection of the hearts of patients with increased anti-cancer efficacy. This patent (if granted) would provide protection through to 2042.
- Advanced discussions are underway with clinicians in Australia to run a Phase 2b clinical trial in breast cancer patients at serious risk of anthracycline-induced heart damage.
- This discovery causes Race to identify novel market opportunities for Zantrene, similar in their clinical and commercial magnitude to the discovery that Zantrene is a potent FTO inhibitor.

## Next Steps

- Additional cell and animal studies to determine optimal anthracycline-to-Zantrene doses for both cardioprotection and anti-cancer efficacy.
- Further studies to determine the molecular mechanism of Zantrene's cardioprotective activity. This may allow identification of additional protective functions of Zantrene at the cellular level.
- Development of new and optimised drug combination formulations with improved clinical and commercial value.
- Initiation of a Phase 2b breast cancer clinical trial.

## Q&A

### *Is this an outstanding result?*

**Yes.** While the earlier human cell work was most encouraging, it was possible that the cardioprotection effect of Zantrene seen in human heart muscle cells would not extend to whole animals and ultimately humans.

### *What additional preclinical studies do you need to do?*

We have a range of preclinical studies underway exploring the optimal doxorubicin/Zantrene ratio for anti-cancer efficacy, the minimum amount of Zantrene required for the cardioprotective effect, and to determine Zantrene's cardioprotective mechanism of action. The results of these studies will be reported as they are completed and associated IP is filed.

### *What is the chance that the same cardioprotective effect will be seen in humans?*

**Good.** Mouse and human hearts show a similar cardiotoxic effect when they are exposed to high levels of anthracyclines<sup>1,2</sup>. The mouse cardiotoxicity model is well established over many decades as being predicative of human cardiotoxicity. In addition, it is well known from more than 40 historical human clinical trials that Zantrene on its own is considerably less cardiotoxic than canonical anthracyclines.<sup>4</sup>

### *Why is this release shorter than other Race releases?*

Our collaborators believe this work is of the highest scientific and clinical interest. To the best of our understanding, no cardioprotective drug has been described that is also an anti-cancer agent. The highest impact journals have a policy of not publishing papers where the data has been previously released in any form. If we were to include all the results (as we normally do) then it would block our collaborators from publishing in these prestigious journals where the work will be most likely to be seen by both key opinion leaders and potential industry partners.

### *When can we expect the full details of this research to be published?*

The scientific paper is being written now and we hope it will be released before the end of 2022, but this timeline depends on the review process which can be unpredictable. We will announce to the market when the paper is published.

### *How soon could you start a human trial of Zantrene as a cardioprotective agent?*

**Late 2022/early 2023.** While we now have all the preclinical data we need to move into humans, its appropriate to do further preclinical research to identify the right balance between doxorubicin and Zantrene that maximises cardioprotection and anti-cancer efficacy while minimising unwanted side-effects. Planning for a trial is advanced and we hope to be able to update the market on progress in Q3 2022.



## References

1. Weiss RB. The anthracyclines: will we ever find a better doxorubicin? *Semin Oncol.* (1992) 9(6):670-86.
2. Venkatesh P, Kasi A. *Anthracyclines.* (2021) In: StatPearls. Treasure Island (FL): StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/books/NBK538187/>
3. Citarella, R. V. et al. Activity of a novel anthracenyl bishydrazone, 9,10-anthracenedicarboxyaldehydeBis[(4,5-dihydro-1H-imidazol-2-yl)hydrazone] dihydrochloride, against experimental tumors in mice. (1982) *Cancer Res* 42, 440-4.
4. Rothman, J. *The Rediscovery of Bisantrene: A Review of the Literature.* (2017) *Int J Cancer Res Ther* 2, 1-10.
5. Swain SM, Whaley FS, Ewer MS. *Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials.* (2003) *Cancer* 97:2869-79.
6. Cai, F. et al. *Anthracycline-induced cardiotoxicity in the chemotherapy treatment of breast cancer: Preventive strategies and treatment.* (2019) *Mol Clin Oncol* 11, 15-23.
7. McGowan, J. V. et al. *Anthracycline Chemotherapy and Cardiotoxicity.* (2017) *Cardiovascular drugs and therapy* 31, 63-75.
8. Kimmick, G., Dent, S. & Klem, I. *Risk of Cardiomyopathy in Breast Cancer: How Can We Attenuate the Risk of Heart Failure from Anthracyclines and Anti-HER2 Therapies?* (2019) *Curr Treat Options Cardiovasc Medicine* 21, 30.
9. [https://www.breastcancer.org/symptoms/understand\\_bc/statistics](https://www.breastcancer.org/symptoms/understand_bc/statistics).
10. Waks AG, Winer EP. *Breast cancer treatment: a review.* (2019) *JAMA.* 321(3):288-300.
11. Ding, W. et al. *Anthracycline versus nonanthracycline adjuvant therapy for early breast cancer: A systematic review and meta-analysis.* (2018) *Medicine* 97, e12908.
12. Cowan, J. D. et al. *Randomized Trial of Doxorubicin, Bisantrene, and Mitoxantrone in Advanced Breast Cancer: A Southwest Oncology Group Study.* (1991) *J National Cancer Inst* 83, 1077-1084.

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

**Release authorised by:**

Phil Lynch, CEO/MD on behalf  
of the Race Board of Directors  
[phillip.lynch@raceoncology.com](mailto:phillip.lynch@raceoncology.com)

**Media contact:**

Jane Lowe  
+61 411 117 774  
[jane.lowe@irdepartment.com.au](mailto:jane.lowe@irdepartment.com.au)