# ASX Announcement

## Breakthrough Chemotherapy Heart Protection Discovery for Zantrene

- **Anthracyclines are highly effective anti-cancer drugs, but their use in patients comes with the serious risk of permanent damage to the heart**
- **Zantrene has been shown in preclinical studies to protect human heart muscle cells from anthracycline-chemotherapy induced cell death**
- **Zantrene synergises with existing anthracyclines to better kill breast cancer cells**
- **Discovery to be fast-tracked to the clinic with a Phase 2b trial planned for 2022 in breast cancer patients at high risk of anthracycline-caused heart damage**
- **Offers the potential of outsized clinical and commercial returns from new Zantrene/anthracycline formulations and combinations**
- **Cardio-protection is a novel use of Zantrene independent of its ability to target FTO in cancer.**

**22 November 2021** – Race Oncology Limited ("Race") is pleased to share interim results from the Zantrene® preclinical heart safety research program led by eminent cardiotoxicity researchers, Associate Professors Aaron Sverdlov and Doan Ngo, in collaboration with cancer scientist Associate Professor Nikki Verrills, at The University of Newcastle (ASX announcement: 28 April 2021).

**This research has found that Zantrene is able to protect heart muscle cells from anthracycline (specifically doxorubicin) induced cell death while improving the killing of breast cancer cells.**

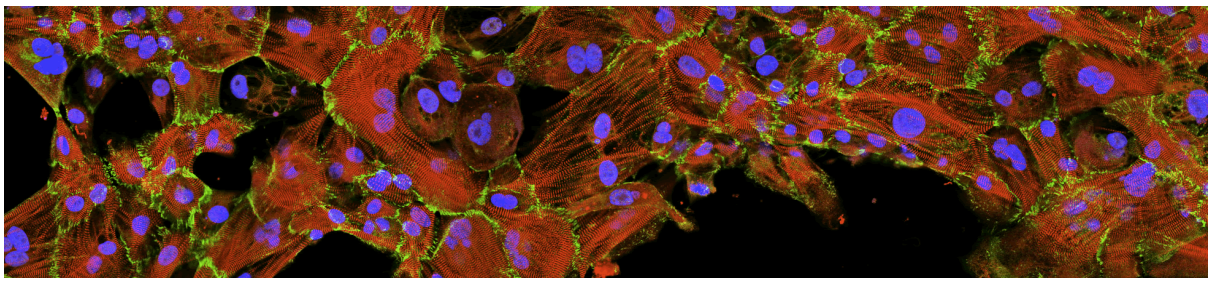

**Figure 1. Human cardiomyocytes stained for EH-myomesin (red), beta-catenin (green) and DNA (purple).** Image courtesy of Christian Zuppinger, University Hospital of Bern – Inselspital.

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[1]. 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[2].

While highly effective anti-cancer drugs, the anthracyclines cause serious and permanent damage to the heart in many patients.

Zantrene® (bisantrene dihydrochloride) was originally developed as a heart-safer alternative to the anthracyclines particularly with respect to preservation of heart muscle[3]. While Zantrene's improved heart safety was proven in more than 50 clinical trials[4], , the question as to whether Zantrene could help *prevent* the heart damage caused by anthracyclines has never been addressed.

**Associate Professor Aaron Sverdlov said:** "*To date, the concept of potential cancer therapies that are not only non-cardiotoxic but, in fact, cardio-protective has not been evaluated or even entertained, largely due to 'disease-specific' approaches in healthcare. Our results suggest that Zantrene, an effective anti-cancer medication, can concomitantly provide protection against toxic effects on the heart from one of the most commonly 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 Scientific Officer, Dr Daniel Tillett said:** *"After the discovery that Zantrene is a potent FTO inhibitor, this is a second 'once in a lifetime' discovery. To find that Zantrene can protect the heart from chemotherapy while also killing cancers better is an extraordinary 'best of both worlds' outcome. Given anthracyclines are used in millions of cancer patients every year, it is hard to overstate the clinical and commercial potential of this breakthrough!"*

**Chief Executive Officer, Mr Phillip Lynch said,** *"Zantrene's cardio toxic properties were always of interest, but this novel cardio protective insight is far more significant to the drug's potential application as an adjunctive to anthracycline backbone chemotherapy. I look forward to the team quickly advancing these investigations so we might assess and progress this opportunity as a matter of substantial priority."*

## Study Background

### *Anthracycline Cardiotoxicity*

Anthracyclines are highly effective anticancer agents, but their use in patients comes with a significant risk of permanent damage to the heart. Some studies have estimated that over half of patients exposed to anthracyclines will develop some form of heart disease within 6 years of treatment[5].

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[6].

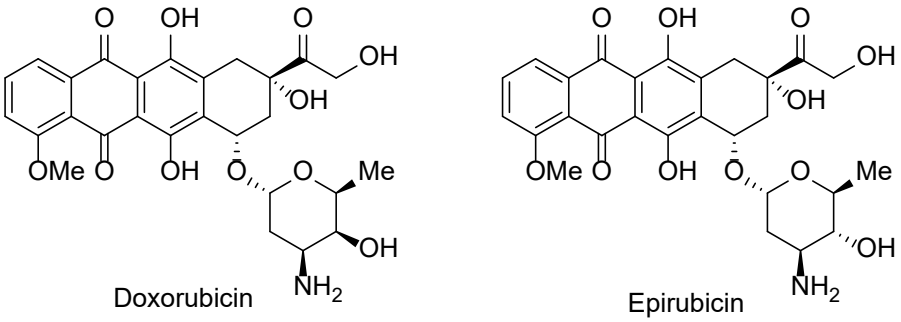


**Figure 2. Chemical structures of the anthracyclines doxorubicin and epirubicin.**

Although the mechanism for 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[7].

### *Prevention of Anthracycline Cardiotoxicity*

A number of potential cardio-protective 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 rendered mixed results with many agents offering little or no cardio-protective benefit and/or compromising the cancer treatment[7].

As a consequence, cardio-protective treatments are not routinely used in clinical oncology practice, and there remains a great demand for new treatment options that can prevent today's cancer patient from becoming tomorrow's cardiac patient.

### *Anthracyclines & Breast Cancer*

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

Anthracyclines, 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[10].

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[11]. As a result, many patients may receive sub-optimal cancer treatment, so potentially increasing their risk of cancer relapse and mortality.

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. Some 23% of patients who received doxorubicin suffered serious heart failure compared to just 4% with Zantrene[12].

## Study Highlights

### *1. Zantrene protects cardiomyocytes from doxorubicin-induced cell death*

The laboratory study found Zantrene was able to protect or rescue primary human heart muscle (cardiomyocyte) cells from doxorubicin-induced cell death. Incubation of cardiomyocytes in the presence of 1000 nM doxorubicin for 72 hours resulted in 80% cell death (Fig. 3). Protection from cardiomyocyte cell death was observed upon the inclusion of 250 nM Zantrene and increased further with higher concentrations, reaching 60% survival (3-fold increase) at 5000 nM. At 1000 nM of Zantrene, cardiomyocyte survival was greater than 48%, a concentation that is less than 15% of the *in vivo* maximium achieved and tolerated dose in humans[13].

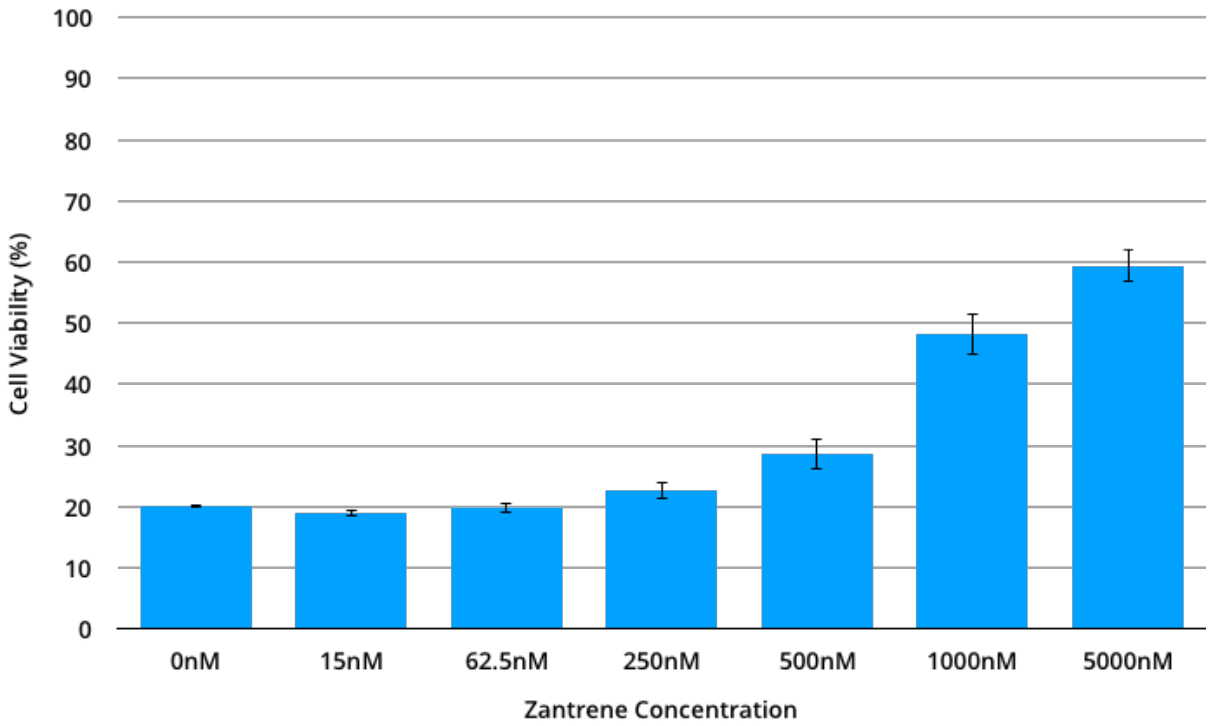


**Figure 3. Primary human cardiomyocyte cell viability when cultured in the presence of 0-5000 nM of Zantrene and 1000 nM doxorubicin for 72 hours.** Data is the average of eight full replicates, each performed in triplicate. Bars show the standard error.

### *2. Zantrene synergises with doxorubicin and epirubicin to better kill breast cancer cells*

Previous studies have found Zantrene synergises with the breast cancer chemotherapy drug, cyclophosphamide (ASX Announcement: 9 March 2021). This study was extended to examine synergy between Zantrene and doxorubicin or Zantrene and epirubicin. Inclusion of even lower levels of Zantrene (sub-125 nM) resulted in significant breast cancer cell death in the presence of doxorubicin or epirubicin (Table 1). This synergy was not limited to MB-231 as similar results were seen using MCF7 breast cancer cells which showed high levels of resistance to doxorubicin and epirubicin (ASX Announcement: 9 March 2021).

**Table 1. MB-231 breast cancer cell viability (%) when cultivated in the presence of increasing concentration of Zantrene and doxorubicin (upper) or Zantrene and epirubicin (lower).** Red shows drug combinations which result in high cell killing.

| | Doxorubicin (nM) | | | | | |
|---|---|---|---|---|---|---|
| **Zantrene (nM)** | **0** | **4** | **8** | **15** | **31** | **62.5** |
| **0** | 100.00 | 87.89 | 77.94 | 60.76 | 48.26 | 39.86 |
| **15** | 86.49 | 74.15 | 63.49 | 50.40 | 42.95 | 38.00 |
| **31** | 66.58 | 57.29 | 54.44 | 46.37 | 40.61 | 34.61 |
| **62.5** | 43.50 | 39.39 | 38.70 | 34.94 | 30.75 | 24.59 |
| **125** | 17.73 | 16.68 | 17.32 | 14.72 | 12.69 | 13.21 |
| **250** | 11.98 | 9.59 | 10.31 | 8.25 | 7.23 | 6.70 |

| | Epirubicin (nM) | | | | | |
|---|---|---|---|---|---|---|
| **Zantrene (nM)** | **0** | **4** | **8** | **15** | **31** | **62.5** |
| **0** | 100.00 | 86.10 | 76.47 | 55.97 | 45.39 | 38.97 |
| **15** | 91.63 | 74.19 | 62.01 | 52.12 | 38.31 | 37.11 |
| **31** | 62.57 | 51.21 | 48.08 | 39.19 | 34.44 | 31.76 |
| **62.5** | 43.21 | 35.12 | 32.56 | 28.58 | 24.81 | 21.28 |
| **125** | 15.96 | 13.77 | 13.11 | 11.29 | 9.86 | 7.99 |
| **250** | 12.01 | 9.15 | 8.25 | 8.06 | 5.56 | 5.64 |

Bliss Synergy Analysis[17] showed a synergistic effect for both Zantrene and doxorubicin and Zantrene and epirubicin combinations in MB-231 breast cancer cells (Figs. 4 & 5). Similar results were also seen with MCF7 breast cancer cells (data not shown).

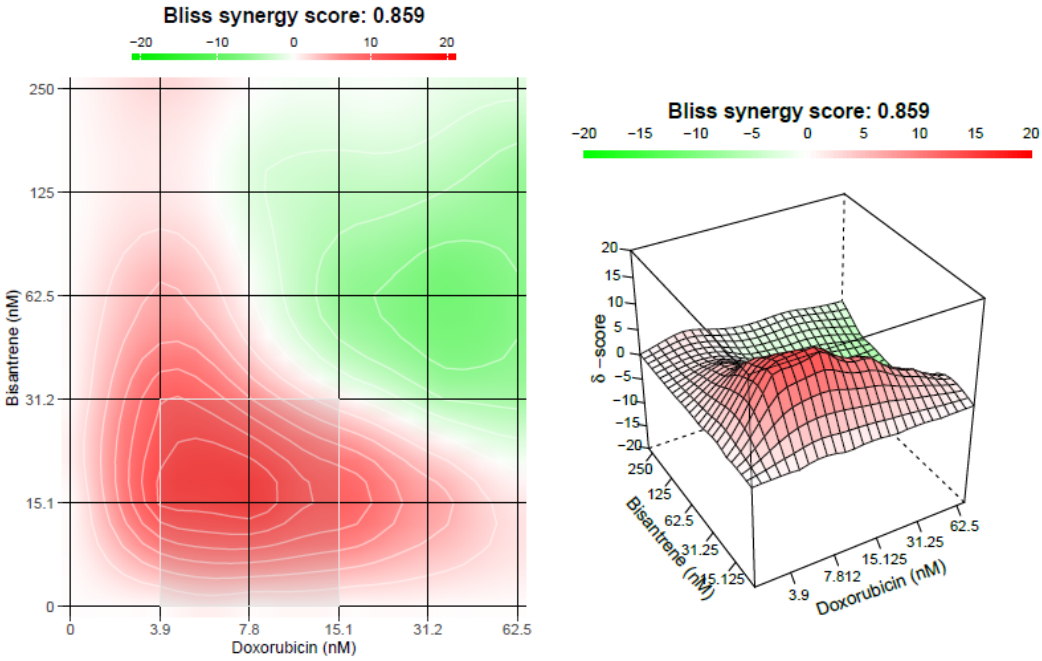


**Figure 4. Bliss Synergy Analysis for MDA-MB-231 breast cancer cells treated with Zantrene and doxorubicin.** Red to green scale indicating areas of synergy to antagonism. Values >10 are synergistic (red); values below -10 are antagonistic; values between -10 to 10 are additive. The most synergistic 2x2 area is indicated with a white box.

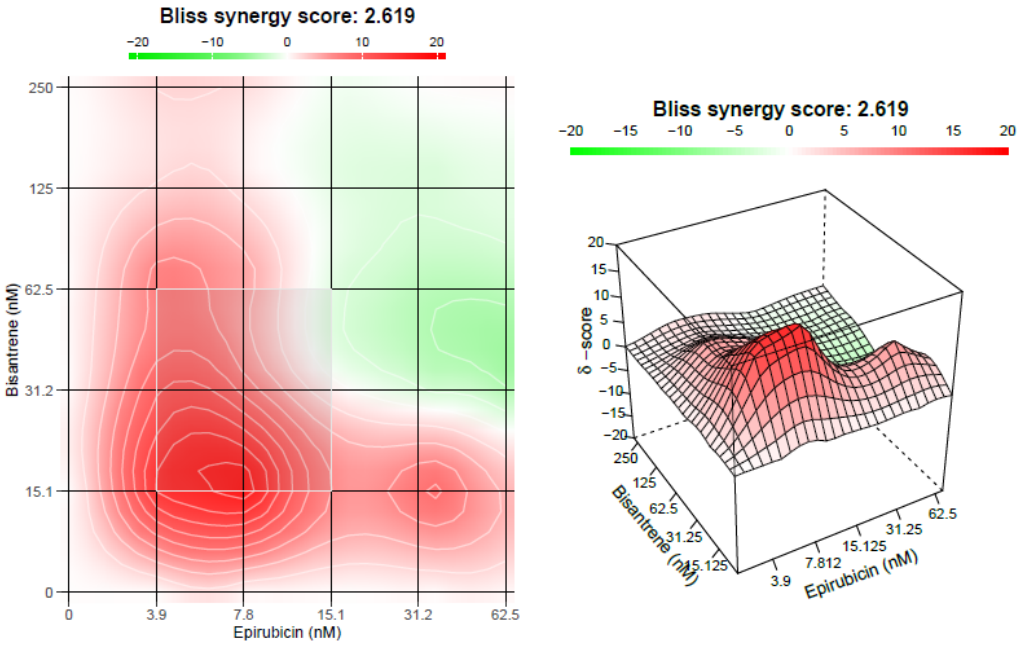


**Figure 5. Bliss Synergy Analysis for MDA-MB-231 breast cancer cells treated with Zantrene and epirubicin.** Red to green scale indicating areas of synergy to antagonism. Values >10 are synergistic (red); values below -10 are antagonistic; values between -10 to 10 are additive. The most synergistic 2x2 area is indicated with a white box.

Webb analysis[16] showed additive synergy between Zantrene and doxorubicin and Zantrene and epirubicin over three different dose ranges (Fig. 6). At all drug concentrations and ratios, the combinations of Zantrene and doxorubicin or Zantrene and epirubicin were superior to either drug on its own.

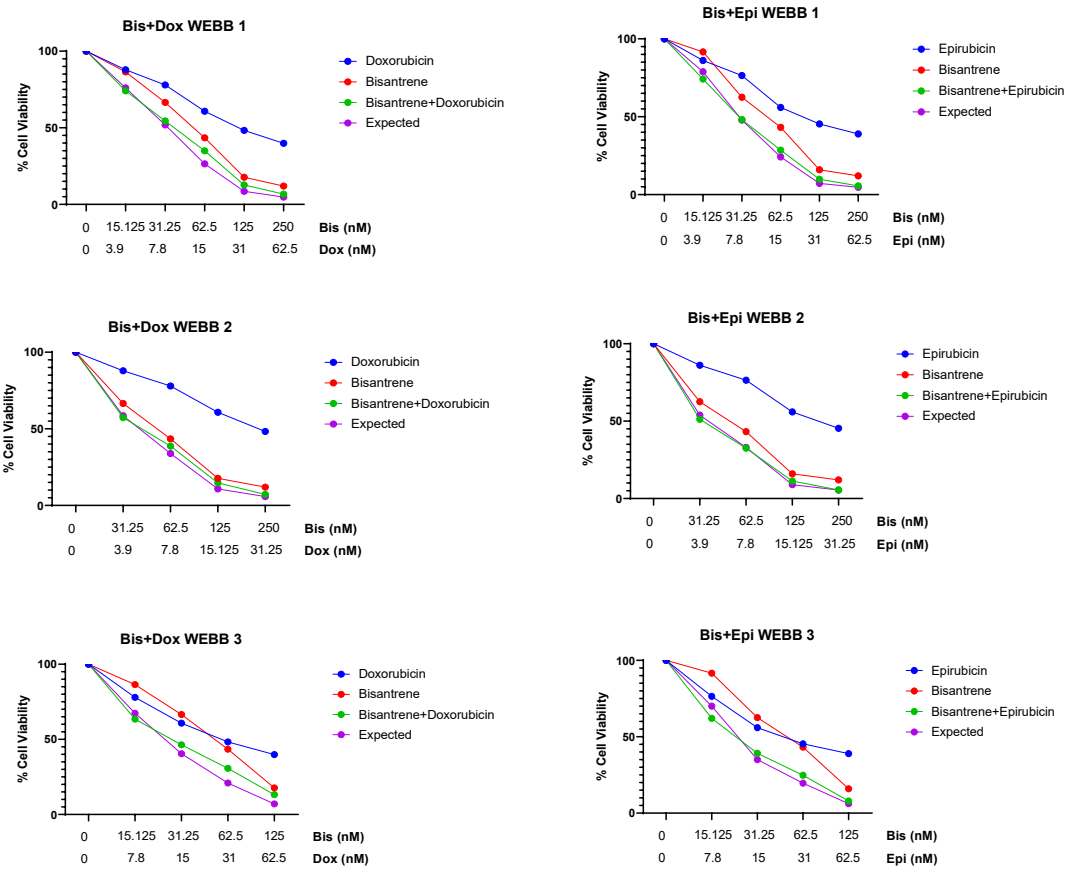


**Figure 6. Webb analysis of MB-231 breast cancer cells treated with Zantrene and doxorubicin (left) or Zantrene and epirubicin (right).** Experimental data is shown for each drug alone and the combinations. The 'Expected value' is calculated using the method of Webb and represents the expected % cell viability if the drug combination is additive.

### *3. Cardio-protective mechanism of Zantrene is independent of FTO inhibition*

The cardio-protective mechanism of Zantrene appears to be independent of its ability to effectively inhibit FTO[14]. Doxorubicin-induced cardiomycocyte cell death was not rescued by either of the other known FTO inhibitors, brequinar[14] or FB23-2[15] (Figs. 6 & 7), suggesting Zantrene is acting via a novel cellular mechanism.

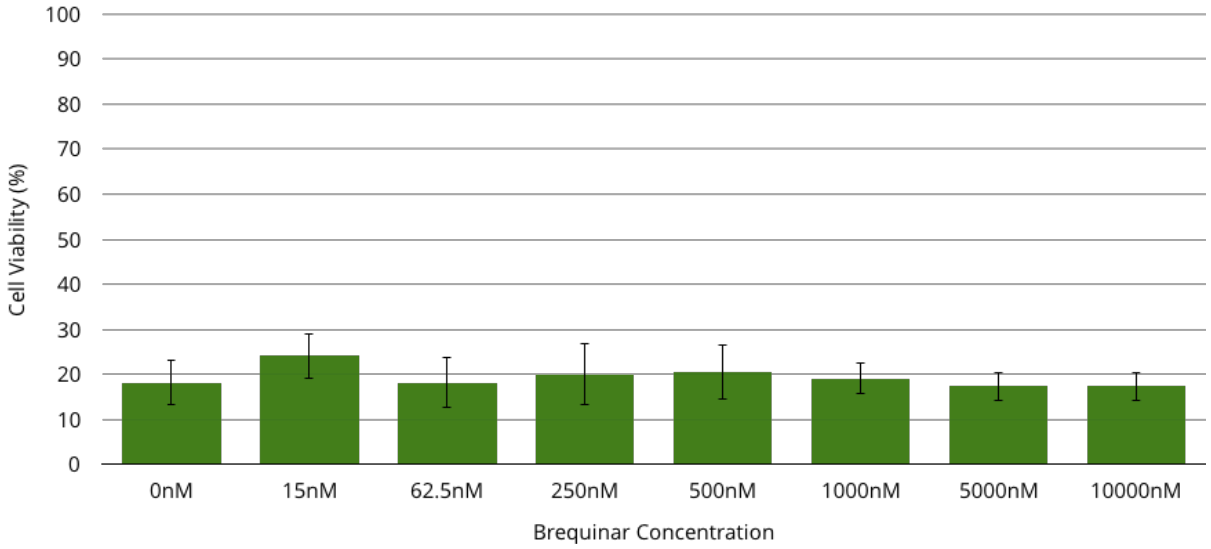


**Figure 6. Primary human cardiomyocyte cell viability when cultured in the presence of 0-10000 nM of the FTO inhibitor brequinar and 1000 nM doxorubicin for 72 hours.** Data represents the average of three full replicates, each performed in triplicate. Bars show the standard error.

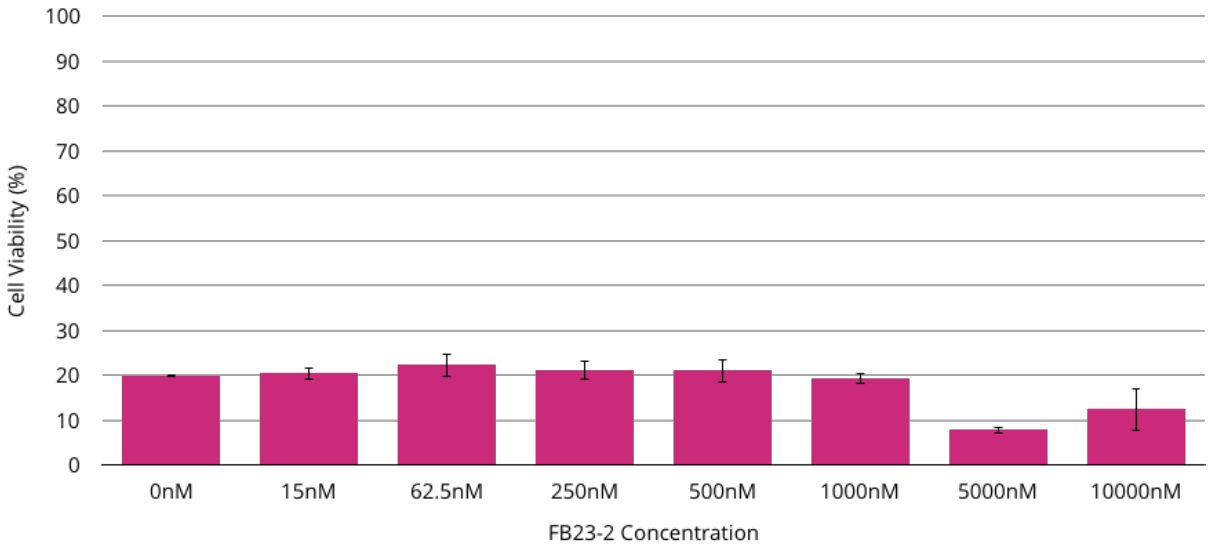


**Figure 7. Primary human cardiomyocyte cell viability when cultured in the presence of 0-10000nM of the FTO inhibitor FB23-2 and 1000 nM doxorubicin for 72 hours.** Data is the average of three full replicates, each performed in triplicate. Bars show the standard error.

## Conclusions

- In this preclinical model, Zantrene protects the heart muscle cells from damage by doxorubicin while synergising with the anthracyclines to better kill breast cancer cells.
- Race has submitted a patent application addressing the combination of Zantrene with an anthracycline for the protection of the heart of patients. This patent (if granted) would provide protection of the drug combination and its clinical use through 2041.
- This new heart protection discovery will be rapidly progressed to the clinic. The extensive clinical history of Zantrene allows this combination to be quickly advanced clinically.
- 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 opens new market opportunities for Zantrene of similar clinical and commercial potential to the earlier discovery that Zantrene is a potent FTO inhibitor.

## Next Steps

- Animal studies to be run in Q4 CY2021/ Q1CY2022.
- Additional preclinical studies to investigate if Zantrene can protect the heart from damage by other chemotherapeutic drugs which are also known to cause cardio-damage.
- Further studies to determine the molecular mechanism of Zantrene's cardio-protective activity. This may allow identification of additional protective functions of Zantrene.
- Development of new and optimised drug combination formulations with improved clinical and commercial value.
- Initiation of a Phase 2b breast cancer clinical trial in 2022.

## Q&A

***What do these cardio-protective results mean for Race?***

While these are preclinical results, if the same results are repeated in patients, they would offer a completely new and unprecedented option for the clinical use of Zantrene. Previous research had identified Zantrene was a heart safer alternative to the effective yet cardiotoxic anthracyclines, and Zantrene was a potent inhibitor of the FTO $m^6A$ RNA demethylase, but its ability to prevent damage to the heart by the anthracyclines was unknown.

Race now has a new and compelling market opportunity that could be used to protect and better treat the millions of cancer patients that receive anthracycline chemotherapy every year.

It should be noted that reduced cardiac damage from Zantrene has been noted in more than 1500 patients in over 60 clinical trials. The heart damage seen in patients after exposure to anthracyclines is well recognized and has been replicated in multiple cell culture studies.

***Have you obtained IP protection for these cardio-protection discoveries?***

**Yes.** A patent application has been filed on this discovery which, if granted, would provide IP protection through 2041.

***What is the market potential of this discovery?***

This is difficult to answer beyond stating that millions of patients are treated every year with multiple cycles of anthracyclines and most are at risk of serious and permanent damage to their hearts. Fears over the damaging side effects of anthracyclines has led many oncologists to limit their use of these extremely effective drugs. This discovery has the potential to revolutionise the use of anthracyclines in the clinic by allowing oncologists to use these powerful drugs to their full anti-cancer potential without fear of permanent damage to the heart.

***When can investors expect the next update?***

We expect to be able to update our investors from late Q4 CY2021 on our further progress.

## Materials and Methods

Primary Human Cardiac Myocytes (HCMs) were purchased from PromoCell® (Heidelberg, Germany) and cultured according to manufacturer's recommendations using PromoCell® Myocyte growth media. Total viable cells were enumerated with Invitrogen™ Countess Cell Counter (Thermo Fisher Scientific, New South Wales, Australia) and 1 X $10^5$ live HCMs were seeded in triplicate into Corning® 96-well white polystyrene microplates (Sigma). Seeded HCMs were incubated at 37°C under 5% $CO_2$ conditions overnight prior to bisantrene treatments (Day -1).

The triple negative breast cancer cell lines MB-231 and MCF7 were used as examples of anthracycline sensitive and resistant breast cancer cell lines, respectively, with known sensitivities to doxorubicin, epirubicin and Zantrene (Fig. 8).

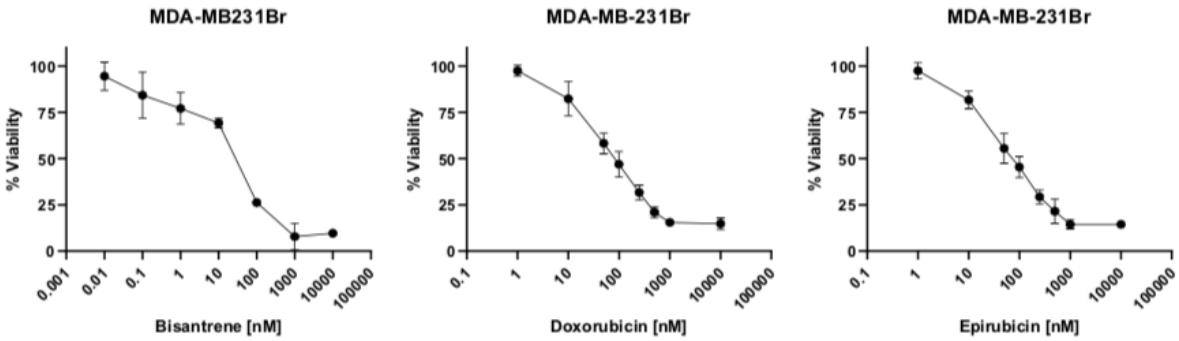


**Figure 8. Single agent cytotoxicity of Bisantrene, doxorubicin and epirubicin in the MB-231 breast cancer cell line.** Cell viability was determined using the resazurin metabolic assay and confirmed by visual inspection under light microscopy.

Human breast adenocarcinoma (MCF7) cells were purchased from In Vitro Technologies (Victoria, Australia) and cultured according to the manufacturer's recommendations using MCF7 growth media, Low glucose Dulbecco's Modified Eagle's medium. Human breast cancer cell line MB-231 was cultured in a humidified chamber at 37°C with 5% $CO_2$ in DMEM supplemented with 10% foetal bovine serum (FBS), 2 mM L-glutamine and 2% HEPES.

Cell viability was then assessed at 24, 48, and 72 hours-post Zantrene treatments using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, New South Wales, Australia) according to the manufacturer's instructions and luminescence recorded with the Cytation™ 3 Cell Imaging Multi-Mode Reader (BioTek Instruments, Vermont, USA).

The breast cancer data was analysed for possible synergistic and additive effects between doxorubicin or epirubicin and Zantrene, respectively, using the Webb[16] and Bliss[17] methods.

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## About Associate Professors Aaron Sverdlov and Doan Ngo

Associate Professors Sverdlov and Ngo lead the dedicated Australian-first, bench-to-bedside "Cancer and the Heart" clinical and basic research program at the University of Newcastle, Hunter Medical Research Institute, Hunter New England Local Health District and Calvary Mater Newcastle Hospitals. This program incorporates basic mechanistic discovery studies looking at mechanisms of cardiotoxicity, drug discovery studies, translational human research, clinical research and clinical inpatient and outpatient service delivery.

In recognition of this important initiative, A/Prof Aaron Sverdlov was awarded the 2018 Ministerial Award for Rising Stars in Cardiovascular Research. A/Prof Doan Ngo, a co-lead of the program, was awarded a NSW Health EMC Fellowship in Cardio-Oncology (2018-2021) and the highly prestigious National Heart Foundation Future Leader Fellowship (2021-2025) for her cardio-oncology program of work.

Both A/Profs Aaron Sverdlov and Doan Ngo have been invited to establish and co-chair the National Cardio-Oncology Working Group under the auspices of the Australian Cardiovascular Alliance (ACvA). The aim of the group is to coordinate clinical and research activities in the field of Cardio-Oncology in Australia and act as a scientific and advocacy body to improve the quality of cardiovascular care for cancer patients.

Associate Professor Sverdlov has over 50 peer-reviewed publications and 4 book chapters (including chapters on Oxidative Stress in Heart Failure in the textbook "Heart Failure: A Companion to Braunwald's Heart Disease") with over 1100 citations and has given more than 80 presentations at international and national meetings. He has received over 30 competitive grants, with >20 in the last 5 years (total >$2.5M AUD).

Associate Professor Ngo is an academic pharmacist and a successful basic and translational scientist with multiple important contributions in the cardiovascular and metabolic field. She has more than 55 publications, of which over 40 were published in the last 5 years.

## About Associate Professor Nikki Verrills

After completing her PhD in 2005 on chemotherapy resistance in childhood leukaemia, Associate Professor Verrills was awarded a Peter Doherty Postdoctoral Fellowship from the National Health and Medical Research Council in 2006. In the same year, she was the inaugural recipient of a Hunter Medical Research Foundation grant for young cancer researchers. Since then, she has established an innovative research lab at the University of Newcastle studying the differences between cancer cells that respond well to drug treatments and those that do not.

Prof Verrills is currently supported by a fellowship from the Australian Research Council and project funding from the National Health and Medical Research Council. She has published over 60 journal articles with an H-index of 24.

**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 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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