ASX Announcement

Impressive Preclinical Bisantrene Breast Cancer Results

- **Bisantrene as a single drug shows similar efficacy to doxorubicin and epirubicin in a range of different breast cancer types.**
- **Bisantrene can kill some breast cancer cells resistant to doxorubicin and epirubicin.**
- **Cyclophosphamide shown to act very similarly when used in combination with either Bisantrene, doxorubicin or epirubicin.**
- **Results provide the necessary preclinical evidence to advance Bisantrene into human breast cancer clinical trials as a potentially safer treatment.**

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24 November 2020 – Race Oncology Limited (ASX: RAC) is pleased to share results of the collaborative preclinical research program between Race and The University of Newcastle. Eminent cancer researcher, Associate Professor Nikki Verrills of the Hunter Medical Research Institute is leading the project.

The aim of this research program is to identify combinations of current breast cancer drugs that when paired with Bisantrene show equivalent efficacy to existing treatment options, but with significantly reduced serious side effects. Bisantrene was the subject of a large Phase III single agent clinical trial in the USA in advanced breast cancer patients in the late 1980s and early 1990s. This Phase III trial showed that Bisantrene had the same efficacy as the standard of care treatment, doxorubicin, but caused significantly less damage to the patient's heart; 23% of the patients who received doxorubicin had serious heart failure compared to only 4% who received Bisantrene [1].

The University of Newcastle's results showed Bisantrene to be an effective chemotherapeutic agent across a wide range of different genetically defined breast cancer subtypes. Bisantrene was able to kill some cancer subtypes that were resistant to the currently used anthracyclines doxorubicin and epirubicin. Importantly, Bisantrene showed near identical additive benefit when used in combination with cyclophosphamide as that seen with doxorubicin and epirubicin. Finally, initial data obtained by the University of Newcastle team on Fat mass and obesity-associated protein (FTO) suggests an association between FTO expression level and cancer sensitivity to Bisantrene.

Associate Professor Nikki Verrills of the University of Newcastle and Hunter Medical Research Institute said *"Our data shows that the combination of Bisantrene and cyclophosphamide is more effective than either drug alone, in killing a wide range of different human breast cancer subtypes. The findings are very encouraging as they show the clinical potential for combining Bisantrene with standard of care for breast cancer patients. While the early clinical data tells us that Bisantrene is effective in breast cancer patients as a single agent, decades of experience tells us that combination therapy is far more effective in eliminating cancers and blocking the development of treatment resistance. Our data provides the necessary preclinical evidence to now test this combination in clinical trials."*

Race's CSO Dr Daniel Tillett commented *"These high quality results from Nikki's team fully support our clinical plans for the use of Bisantrene as a safer alternative to the commonly used anthracyclines which can be very dangerous to the hearts of patients. These result show that Bisantrene is compatible with existing treatment regimes and can be combined with standard chemotherapy drugs. I am very excited that we are now in a position to move Bisantrene into its next clinical trial and offer a potentially less harmful, but equally effective, treatment alternative for breast cancer patients."*

Race's CEO Mr Phillip Lynch added, "*This new research not only underscores our confidence in moving Bisantrene into a Phase II breast cancer trial, but continues to build on the body of evidence we have supporting Bisantrene's potential for broader use and then commercial opportunity. Breast cancer affects about 2.1m women annually and the drug market is valued at US$20 billion globally [2]. We hope to be able to bring a valuable new treatment option to patients with Bisantrene.*"

Study Background

The most common use of anthracyclines (doxorubicin and epirubicin) in standard breast cancer therapy is in combination with cyclophosphamide, often followed by taxane therapy. Given the anthracycline is administered concurrently with cyclophosphamide, this is the key indication where Bisantrene needs to be directly compared for efficacy and toxicity. Therefore, the study tested the *in vitro* efficacy of Bisantrene alone, and in combination with cyclophosphamide, in a range of human breast cancer cell lines that cover the major molecular and clinical subtypes, and span drug sensitive and resistant lines.

Materials and Methods

Ten breast cancer cell lines were screened for their sensitivity to Bisantrene and other common chemotherapeutic drugs used in breast cancer patients. The cell lines were selected from a range of different breast cancers to cover all the common breast cancer sub-types (Table 1).

Table 1. IC50 of anthracyclines and cyclophosphamide in human breast cancer cells

Cell Line	Breast cancer subtype	IC50			
		Bisantrene (nM)	Doxorubicin (nM)	Epirubicin (nM)	4-OH-Cyclophosphamide (μM)
T47D	ER^+, PR^+	726.8	269.6	218.0	7.37
ZR-75-1	ER^+, PR^+, $HER2^+$	323.6	350.7	203.0	8.44
ZR-75-30	ER^+, PR^+, $HER2^+$	1001.5	1573.1	720.8	11.56
DU4475	TNBC	133.9	35.2	28.2	8.40
MB-231	TNBC (claudin low)	90.3	96.6	115.4	19.11
231-Br	TNBC (Brain met)	85.6	90.2	82.7	12.75
MB-468	TNBC	157.9	62.8	61.5	7.51
SKBR3	$HER2^+$	175.5	160.5	140.9	8.01
HMT-3522-T4-2	TNBC	408.3	414.0	612.9	17.10
HMT-3522-S2	TNBC	202.7	373.4	378.0	6.91

Bisantrene was compared to doxorubicin and epirubicin as both a single agent and in combination with cyclophosphamide.

The data was analysed for possible synergetic and additive effects between cyclophosphamide and doxorubicin, epirubicin and Bisantrene, respectively using the Webb [3], Bliss [4], Chou-Talalay [5] and MuSyc [6] methods.

In addition, the protein and gene expression levels of the Fat and Obesity associated protein (FTO) were also measured for 8 of the cancer cell lines and compared to their sensitivity to Bisantrene as a single agent at a concentration of 125nM. FTO has recently been identified as important protein in breast cancer development and progression. Importantly, Bisantrene has been identified by researchers from the City of Hope Hospital as a potent inhibitor of FTO [7] (ASX Release: June 29, 2020).

Study Highlights

1. Bisantrene as a single agent is as cytotoxic as doxorubicin and epirubicin in a wide range of breast cancers

The study found that Bisantrene showed similar cytotoxic (cell killing) effects to doxorubicin and epirubicin on the 10 breast cancer cell lines. An example from one representative cancer cell line is shown in **Figure 1**, below**.**

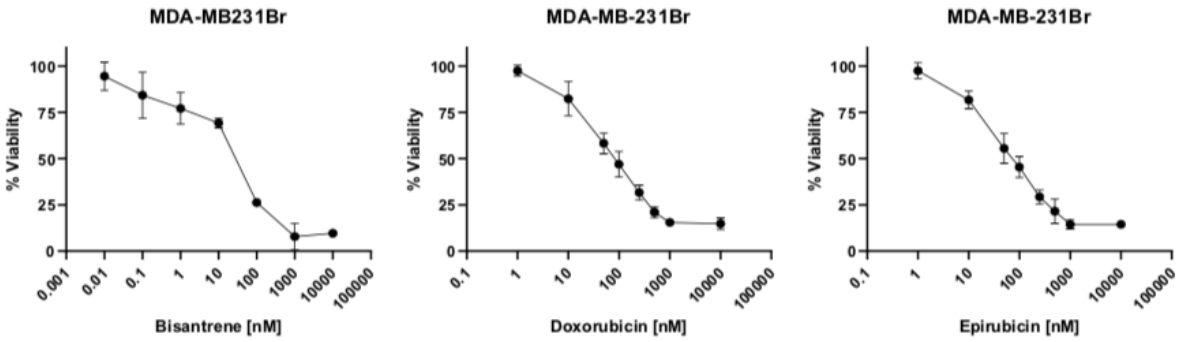


Figure 1. Example of single agent cytotoxicity of Bisantrene, doxorubicin and epirubicin in the MDA-MB231Br breast cancer cell line. Cell viability was determined using the resazurin metabolic assay and confirmed by visual inspection under light microscopy.

2. Bisantrene can kill breast cancer cell lines resistant to doxorubicin and epirubicin

While most cell lines showed similar sensitivity to Bisantrene as they do to doxorubicin or epirubicin, suggesting that Bisantrene can be a viable alternative for these chemotherapeutics in the clinic, a number of the cancer cells (ZR-75-1, ZR75-30, MB-231, 231-Br, SKBR3, HMT-3522-T4-2, and HMT-3522-S2) were more sensitive to Bisantrene than doxorubicin (**Table 1, page 2**). It should be noted that in breast cancer treatment doxorubicin is typically used at a dose of 60-75mg/m^2 once every 21 days while Bisantrene can be used at doses of up to 250mg/m^2.

Two of the cell lines (T47D and DU4475) were less sensitive to Bisantrene than doxorubicin, however, the concentration of Bisantrene required to kill these cancer cells was well below the maximum concentration of Bisantrene that can be achieved in cancer patients [8].

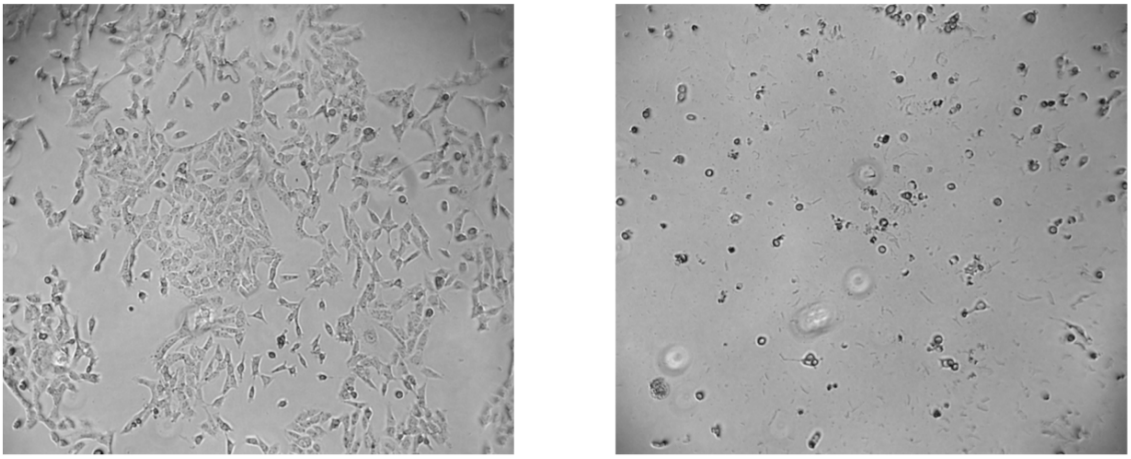
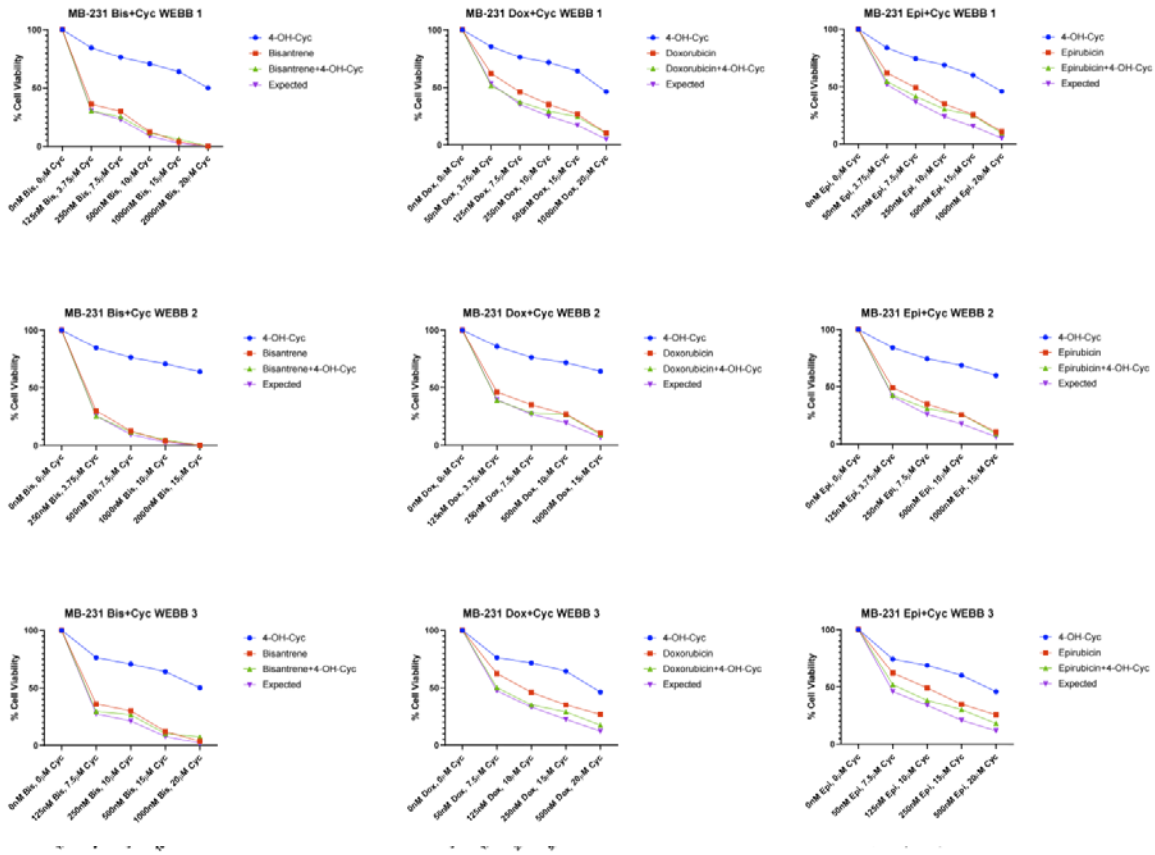


Figure 2. Light microscope example of MB-231Br cells before (left) and after (right) 72h of exposure to 125nM Bisantrene.

3. Bisantrene acts similarly to doxorubicin and epirubicin when in combination with cyclophosphamide on breast cancer cells

All breast cancer cell lines tested displayed a very similar additive cell death response when cyclophosphamide was combined with Bisantrene, doxorubicin or epirubicin. An example of this similarity can be seen from the Webb analysis shown in **Figure 3**, below.

A



B

				MB-231				
Bis	Cyc	Webb	Dox	Cyc	Webb	Epi	Cyc	Webb
125	3.75µM Cyc	-0.00151	50	3.75µM Cyc	-0.01677	50	3.75µM Cyc	0.024985
125	7.5µM Cyc	0.020173	50	7.5µM Cyc	0.025212	50	7.5µM Cyc	0.05813
125	10µM Cyc	0.040306	50	10µM Cyc	0.02963	50	10µM Cyc	0.067278
125	15µM Cyc	0.051148	50	15µM Cyc	0.055123	50	15µM Cyc	0.071095
125	20µM Cyc	0.059637	50	20µM Cyc	0.062996	50	20µM Cyc	0.075406
250	3.75µM Cyc	-0.00107	125	3.75µM Cyc	-0.00693	125	3.75µM Cyc	0.01104
250	7.5µM Cyc	0.023945	125	7.5µM Cyc	0.020644	125	7.5µM Cyc	0.046625
250	10µM Cyc	0.052822	125	10µM Cyc	0.019417	125	10µM Cyc	0.044327
250	15µM Cyc	0.06297	125	15µM Cyc	0.041952	125	15µM Cyc	0.05469
250	20µM Cyc	0.057689	125	20µM Cyc	0.082065	125	20µM Cyc	0.069034
500	3.75µM Cyc	0.015098	250	3.75µM Cyc	-0.00655	250	3.75µM Cyc	0.017207
500	7.5µM Cyc	0.020715	250	7.5µM Cyc	0.013821	250	7.5µM Cyc	0.051736
500	10µM Cyc	0.026211	250	10µM Cyc	0.041987	250	10µM Cyc	0.064022
500	15µM Cyc	0.025093	250	15µM Cyc	0.062911	250	15µM Cyc	0.091675
500	20µM Cyc	0.002467	250	20µM Cyc	0.078473	250	20µM Cyc	0.087265
1000	3.75µM Cyc	0.011388	500	3.75µM Cyc	0.02586	500	3.75µM Cyc	0.033871
1000	7.5µM Cyc	0.016714	500	7.5µM Cyc	0.057456	500	7.5µM Cyc	0.075439
1000	10µM Cyc	0.021809	500	10µM Cyc	0.071731	500	10µM Cyc	0.083562
1000	15µM Cyc	0.035631	500	15µM Cyc	0.074284	500	15µM Cyc	0.097773
1000	20µM Cyc	0.05648	500	20µM Cyc	0.050844	500	20µM Cyc	0.064929
2000	3.75µM Cyc	0	1000	3.75µM Cyc	0.00545	1000	3.75µM Cyc	0.018021
2000	7.5µM Cyc	0	1000	7.5µM Cyc	0.015363	1000	7.5µM Cyc	0.030169
2000	10µM Cyc	0	1000	10µM Cyc	0.020611	1000	10µM Cyc	0.029454
2000	15µM Cyc	0	1000	15µM Cyc	0.02397	1000	15µM Cyc	0.026339
2000	20µM Cyc	0	1000	20µM Cyc	0.051008	1000	20µM Cyc	0.041825

Figure 3. Webb analysis of MDA-MB-231 cells. (A) Cell viability in response to three different dose ranges of cyclophosphamide and anthracycline (Bisantrene, doxorubicin or epirubicin, 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. **(B)** Webb analysis for all drug combination doses tested where a result of <-0.1 indicates synergy (yellow); between -0.1 to 0.1 is additive (green); and >0.1 is antagonistic (red).

At some drug concentrations and ratios there was some limited evidence of synergy between the cyclophosphamide and either Bisantrene, doxorubicin or epirubicin. Importantly, all three drug combinations showed a highly similar synergy response as measured by cell death (Figures 4 & 5).

ZR-75-1
SynergyFinder Analysis

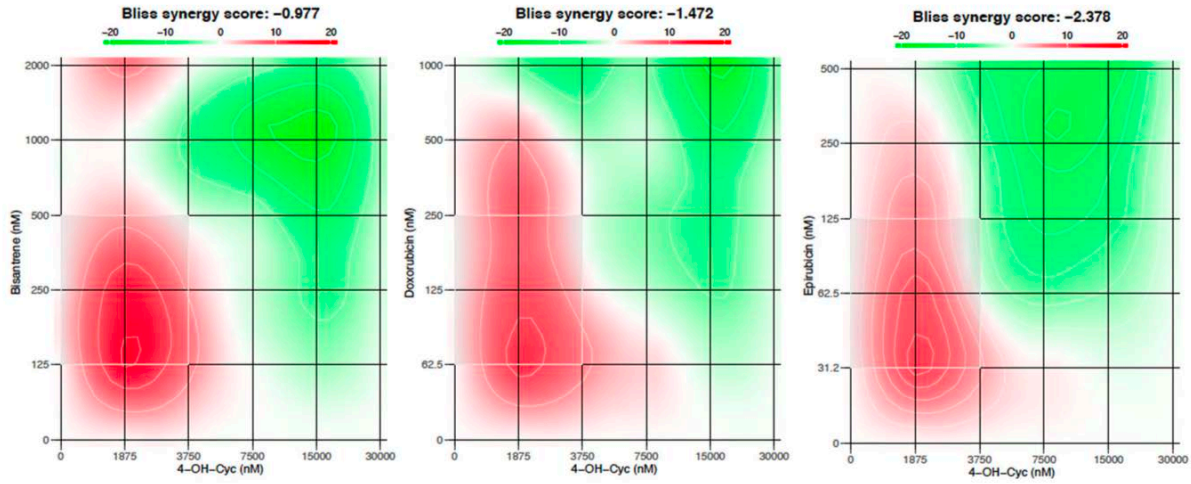


Figure 4. Bliss Synergy Analysis for ZR-75-1 cancer cells. 2D visualisation of the predicted Bliss scores at each dose point, with red to green scale indicating areas of synergy to antagonism, and the average synergy score. The most synergistic 2x2 area is indicated with a white box. Values >10 are considered synergistic (red); values below -10 are considered antagonistic. Values between-10 to 10 are additive.

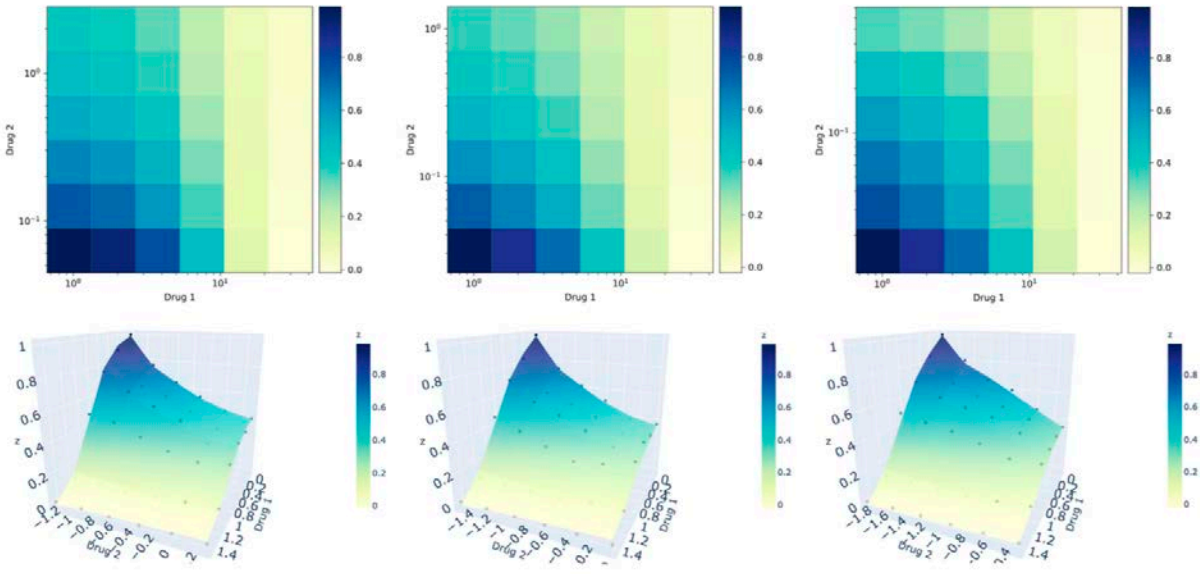


Figure 5. Multidimensional synergy of combinations (MuSyc) Analysis for ZR-75-1 cancer cells.

4. FTO expression level correlates with breast cancer sensitivity to Bisantrene

While this study was too small to give statistically significant results in regards FTO protein expression level and sensitivity to Bisantrene, there was general trend of higher sensitivity to Bisantrene at lower FTO expression levels at 125nM (Figure 6, below).

It should be noted that the FTO data is very preliminary and none of the 8 cell lines examined were high FTO overexpressing cancers. Further studies are planned in breast cell lines with high FTO expression levels to examine the FTO effect of Bisantrene.

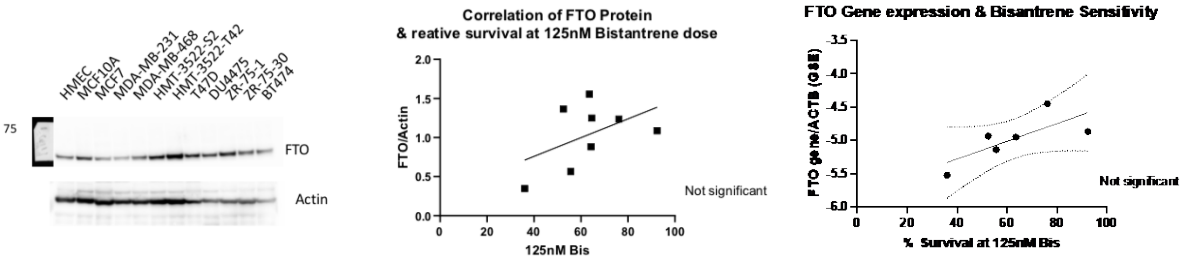


Figure 6. FTO Protein Expression (left) and Bisantrene sensitivity (centre & right). FTO concentrations were normalised to Actin expression levels.

Conclusions

- Bisantrene was found to kill a wide range of different breast cancer cell sub-types similarly to doxorubicin and epirubicin.
- Bisantrene shows additive cell death effects when combined with cyclophosphamide that are very similar to that seen when cyclophosphamide is used in combination with doxorubicin or epirubicin.
- Bisantrene was able to kill some breast cancers cells resistant to the doxorubicin and epirubicin, confirming the historical clinical data from past Bisantrene Phase II/III clinical trials.
- No antagonism was observed between Bisantrene and cyclophosphamide supporting the use of this combination in the clinic.
- These data support the use of Bisantrene as an alternative to doxorubicin or epirubicin in breast cancer patients in human clinical trials.

Next Steps

- These results are highly supportive of Race Oncology's plans for progressing Bisantrene as a safer alternative to the current anthracyclines used in breast cancer treatment.
- Further studies are currently being conducted to elucidate the clinical significance of FTO overexpression in breast cancer, as well as expand the range of clinically usable Bisantrene drug combinations.
- Publication of these results in a relevant scientific journal.
- Update on our clinical trial plans to be released in the near future.

References

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About Race Oncology (RAC: ASX)

Race Oncology (RAC) is a drug development biotech with a Phase II/III cancer drug called bisantrene. RAC has compelling clinical data for Bisantrene in acute myeloid leukaemia (AML) as well as breast and ovarian cancer. RAC is pursuing an exciting '5-Path' clinical development strategy that involves parallel US and Australian clinical trials in AML, breast and ovarian cancer.

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