RACE ONCOLOGY

ASX Announcement

Compelling Preclinical Breast Cancer Results

- **Bisantrene killed breast cancer cells resistant to the current standard of care breast cancer drugs etoposide, palbocicclib, fulvestrant, tamoxifen, doxorubicin, epirubicin and cyclophosphamide**
- **Bisantrene was found to kill breast cancer cells from all common breast cancer subtypes including triple negative, ER+, and Her2+**
- **These results clearly support advancing Bisantrene into human breast cancer clinical trials**

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9 March 2021 – Race Oncology Limited (ASX: RAC) is pleased to share the final results of our collaborative preclinical research program with the eminent cancer researcher, Associate Professor Nikki Verrills of The University of Newcastle and Hunter Medical Research Institute.

The aim of this research program was to identify combinations of existing breast cancer drugs which when paired with Bisantrene show equivalent or better efficacy to existing treatment options, but with reduced side effects. Activity of Bisantrene alone against a range of breast cancer genetic subtypes, including those resistant to standard of care drug treatments, was also explored.

The interim results demonstrated that Bisantrene was an effective chemotherapeutic agent across a wide range of genetically distinct breast cancer subtypes (ASX announcement: 24 Nov 2020). 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 to that seen with either doxorubicin or epirubicin.

Final results showed Bisantrene to be an effective chemotherapeutic agent across a diverse panel of genetically defined breast cancer subtypes and to also kill breast cancer cells resistant to a wide range of breast cancer treatment drugs.

Race's CSO Dr Daniel Tillett commented *"The final results from Nikki's team highlights Bisantrene's potential use in breast cancers resistant to current treatments. Not only does Bisantrene offer a potentially safer alternative to existing chemotherapeutic drugs, it may also help patients who have exhausted other treatment options."*

Race's CEO Mr Phillip Lynch added, *"This new research underscores our confidence in moving Bisantrene into Phase II breast cancer trials and continues to build on the body of evidence we have supporting Bisantrene's broader applications. Our aim here is to bring a valuable new treatment forward for the management of breast cancer."*

Study Background

Anthracyclines agents such as doxorubicin and epirubicin are routinely combined with cyclophosphamide for the management of breast cancer. This is often followed by taxane based therapy.

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 efficacy comparable to standard of care treatment, doxorubicin, but caused significantly less damage to the patient's heart. Some 23% of the patients who received doxorubicin had serious heart failure compared to just 4% with Bisantrene [2].

Past studies have observed that Bisantrene offers a resistance profile different to other anti-cancer drugs [2], suggesting it may prove useful as a salvage agent in heavily pretreated patients as seen in late-stage metastatic breast cancer settings.

This study tested the *in vitro* efficacy of Bisantrene alone and Bisantrene in combination with cyclophosphamide across a range of human breast cancer cell lines covering the major molecular and clinical subtypes and spanning drug sensitive and resistant cell lines.

Materials and Methods

Sixteen breast cancer cell lines were screened for their sensitivity to Bisantrene and other common anti-cancer drugs used in breast cancer patients. These cell lines were selected from a range of different breast cancers to cover all the common breast cancer sub-types and to span both drug sensitive and drug resistant cancers (Table 1).

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

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

Table 1. IC_{50} cell death for Bisantrene, doxorubicin, epirubicin & cyclophosphamide in 16 breast cancer cell lines

Cell Line	Breast cancer subtype	IC50			
		Bisantrene (nM)	Doxorubicin (nM)	Epirubicin (nM)	4-OH-Cyclophos-phamide (μM)
MCF10A	Non-tumourigenic	56.7	16.7	18.2	6.9
MCF7	ER^+, PR^+	362.6	488.6	399.7	6.5
MCF7 VP16	ER^+, PR^+ (etoposide resistant)	195.5	749.0	781.0	5.9
MCF7 PalbR	ER^+, PR^+ (palbocicclib resistant)	267.4	660.1	537.4	5.9
MCF7 FasR	ER^+, PR^+ (fulvestrant resistant)	221.7	1061.0	964.4	12.4
MCF7 TamR	ER^+, PR^+ (tamoxifen resistant)	130.7	560.6	637.8	7.8
*T47D	ER^+, PR^+	713.9	267.9	215.6	7.4
*ZR-75-1	ER^+, PR^+, $HER2^+$	324.9	334.4	191.9	8.4
*ZR-75-30	ER^+, PR^+, $HER2^+$	1001.5	1573.1	720.8	11.6
*DU4475	TNBC	133.9	35.2	21.8	8.4
*MB-231	TNBC (claudin low)	90.3	105.0	119.6	19.1
*231-Br	TNBC (Brain met)	85.6	98.2	92.4	12.8
*MB-468	TNBC	157.9	67.2	63.1	7.5
*SKBR3	$HER2^+$	175.5	163.2	140.3	8.0
*HMT-3522-T4-2	TNBC	408.3	411.1	607.3	17.1
*HMT-3522-S2	TNBC	202.7	368.2	387.4	6.9

**Results previously reported (ASX Announcement 24 Nov 2020).*

Study Highlights

1. Bisantrene kills breast cancer cells resistant to a wide range of different breast cancer drugs

Building on the data presented in the interim report which showed that Bisantrene can kill breast cancer cells resistant to doxorubicin the study was extended to a range of drug resistant MCF7 cell lines developed by repeated treatment with the DNA damaging topoisomerase II inhibitor, etoposide (VP16); the cyclin-dependent kinase 4/6 inhibitor palbocicclib (PalbR); the selective estrogen receptor degrader (SERD), fulvestrant (FasR); or the estrogen receptor modulator (SERM), tamoxifen (TamR). These are informative cellular models to investigate if Bisantrene is likely to be clinically useful in patients who have developed resistance to standard therapies.

Table 2. Relative sensitivity of breast cancer cell lines to Bisantrene compared to doxorubicin and epirubicin[1].

Cell line	Bisantrene	Doxorubicin	Epirubicin
MCF10A	1	0.3	0.3
MCF7	1	1.3	1.1
MCF7 VP16	1	3.8	4.0
MCF7 PalbR	1	2.5	2.0
MCF7 FasR	1	4.8	4.4
MCF7 TamR	1	4.3	4.9
T47D	1	0.4	0.3
ZR-75-1	1	1.0	0.6
ZR-75-30	1	1.6	0.7
DU4475	1	0.3	0.2
MB-231	1	1.2	1.3
231-Br	1	1.1	1.1
MB-468	1	0.4	0.4
SKBR3	1	0.9	0.8
HMT-3522-T4-2	1	1.0	1.5
HMT-3522-S2	1	1.8	1.9

[1]Values < 1 indicate that cell line is more sensitive to doxorubicin or epirubicin than to Bisantrene. Values >1 indicate that cell line is more sensitive to Bisantrene than to doxorubicin or epirubicin.

As expected, the MCF7/VP16 cells were more resistant to doxorubicin and epirubicin than the parental MCF7 cells, due at least in part to their expression of the multidrug resistant protein 1 (MRP1; *ABCC1*) and MRP6 (*ABCC6*) [6, 7]. Intriguingly, the MCF7/VP16 cells were more sensitive to Bisantrene (1.9x) than the parental MCF7 cells (i.e. relative resistance value of 0.5; Table 2), and were more sensitive to Bisantrene than to doxorubicin (3.8x) or epirubicin (4.0x) (Table 3). This result suggests that Bisantrene is not a substrate for the multi-drug resistance proteins, MRP1 and MRP6, which are commonly overexpressed in cancers that develop resistance to treatment. The observation that Bisantrene is even more effective in these resistant cells than the parental line is supportive for the use of Bisantrene as a salvage treatment.

In addition to reduced drug accumulation from MRP overexpression, the topoisomerase II drug target in these resistant cells was less sensitive to drug-induced, cleavable complex formation than the parental MCF7 cells. It is possible that the altered topoisomerase II may mediates hyper-sensitivity to Bisantrene.

Like the MCF7/VP16 cells, MCF7 cells selected for resistance to palbocicclib, fulvestrant and tamoxifen, were 1.4-fold, 1.6-fold and 2.8-fold more sensitive to Bisantrene than the parental MCF7 cells, respectively. All of the resistant lines were also more sensitive to Bisantrene than to doxorubicin or epirubicin. All resistant lines displayed cross-resistance to epirubicin, and all except for the TamR cell line were cross-resistant to doxorubicin. In contrast, no cross-resistance to Bisantrene was observed in any of these resistant cell lines. The mechanism(s) mediating resistance in these lines are not yet established, however this data is important as it suggests that Bisantrene may be clinically useful in estrogen receptor positive (ER+) patients who have developed resistance to a range both traditional chemotherapies and the more recently introduced targeted drugs.

Finally, the effects of each drug were tested on the transformed, but non-cancerous breast epithelial cell line, MCF10A. It is interesting to note while these cells were sensitive to all drugs, they were 3.4-fold and 3.1-fold more sensitive to doxorubicin and epirubicin than to Bisantrene (Table 3), indicating a broader therapeutic window with Bisantrene than other anthracyclines and a possibly more targeted mechanism of action.

Table 3. Relative sensitivity/resistance of drug-selected cell lines to Bisantrene, doxorubicin and epirubicin[2]

	Bisantrene	Doxorubicin	Epirubicin
MCF7	1	1	1
MCF7 VP16	0.5	1.5	2.0
MCF7 PalbR	0.7	1.4	1.3
MCF7 FasR	0.6	2.2	2.4
MCF7 TamR	0.4	0.7	1.6

[2]Values less than 1 indicate that cell line is more sensitive than the MCF7 parental cells to that drug; values >1 indicate that cell line is more resistant to that drug than the parental MCF7 cells.

2. Bisantrene acts very similarly to doxorubicin and epirubicin when used 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.

To determine the effects of the combined drug treatments, three different approaches were used.

Webb analysis revealed that the vast majority of doses of cyclophosphamide and Bisantrene were additive across all the cell lines. There were a few doses of cyclophosphamide and Bisantrene in the ZR-75-1, MDA-MB-468, SKBR3, HMT-3522-S2 and MCF7 (parental and resistant) cell lines that were at the cut-off for synergy (0.1).

Chou-Talalay analysis indicated additivity between Bisantrene and cyclophosphamide, similar to that seen for doxorubicin or epirucibin and cyclophosphamide at the inhibitory concentration 50% (IC50) and 25% (IC25) doses.

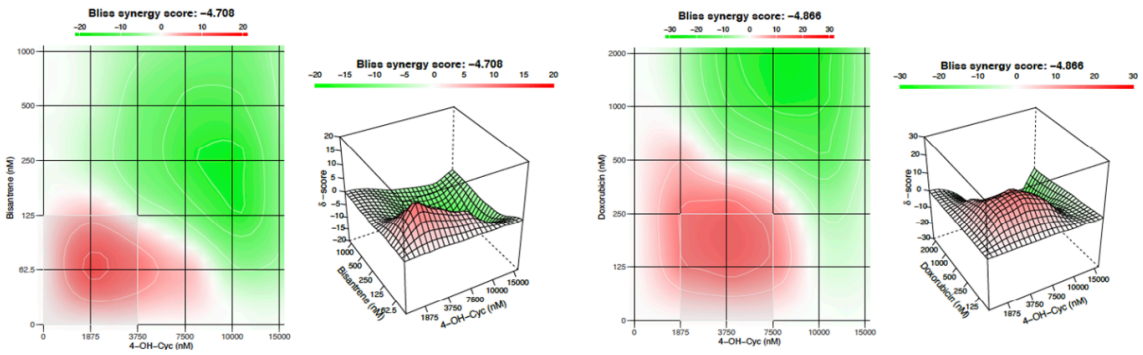
Bliss analysis showed an additive effect for all cell lines and areas of mild synergy in the ZR-75-30 and DU4475 cell lines (Table 4). Individual Bliss scores for each dose combination further revealed a number of doses that were synergistic across most cell lines. An example of the individual analysis is shown in Fig. 1.

Table 4. Bliss Synergy Analysis

	Overall Synergy Score			Most Synergistic Area Score		
Cell Line	Bisantrene + 4-OH-Cyc	Doxorubicin + 4-OH-Cyc	Epirubicin + 4-OH-Cyc	Bisantrene + 4-OH-Cyc	Doxorubicin + 4-OH-Cyc	Epirubicin + 4-OH-Cyc
MCF10A	-0.22	-2.17	-1.29	4.18	2.82	4.75
MCF7	3.19	0.12	-0.45	5.15	4.91	1.90
MCF7 VP16	-4.71	-4.87	-7.38	2.24	6.96	4.60
MCF7 PalbR	-0.43	-0.84	-0.88	1.94	2.01	3.17
MCF7 FasR	0.61	1.85	5.61	3.53	7.19	8.46
MCF7 TamR	1.02	2.09	-1.25	6.76	6.05	5.71
T47D	2.22	2.36	0.29	6.45	9.14	5.53
ZR-75-1	-0.98	-1.47	-2.38	4.49	4.08	2.80
ZR-75-30	5.69	5.73	3.28	12.15*	9.98	6.54
DU4475	6.58	5.35	5.26	13.39*	13.39*	13.91*
MB-231	3.14	4.31	1.84	8.23	11.07*	7.33
231-Br	-0.02	2.02	0.76	4.11	5.99	5.12
MB-468	-3.67	-4.46	-6.00	3.95	4.86	4.57
SKBR3	-1.77	0.43	-1.74	3.74	6.67	3.63
HMT-3522-T42	2.37	4.00	1.51	7.11	7.35	4.43
HMT-3522-S2	1.73	4.80	2.71	5.86	8.151	7.41

**Values above 10 are considered synergistic*

Figure 1. Bliss Synergy Analysis of MCF7 VP16 cancer cells for Bisantrene (left) and doxorubicin (right). 2D & 3D visualisations 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.



Conclusions

- Bisantrene was found to kill breast cancer cells across a wide range of different subtypes, including triple negative, HER2 positive, and estrogen receptor positive.
- Bisantrene showed 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 breast cancers cells resistant to etoposide, palbocicclib, fulvestrant, tamoxifen, doxorubicin, epirubicin & cyclophosphamide
- Bisantrene was less toxic to non-tumorous breast cancer cells than either doxorubicin or epirubicin, suggesting a more precisely targeted mechanism of action
- These data support the use of Bisantrene not only as a heart friendlier alternative to doxorubicin or epirubicin, but also as a possible salvage treatment in heavily pretreated metastatic breast cancer patients.

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 and as a salvage treatment for metastatic breast cancer
- Further studies are currently planned to elucidate the clinical significance of FTO overexpression in breast cancer, and also expand the range of clinically usable Bisantrene drug combinations
- Publication of these results in a high impact scientific journal
- Update on Race's clinical trial plans under the Three Pillar strategy to be released before the end of Q1 2021.

References

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About Race Oncology (ASX: RAC)

Race Oncology is an ASX listed precision oncology company with a Phase II/III cancer drug called Bisantrene.

Bisantrene is a potent inhibitor of the Fat mass and obesity associated (FTO) protein. Over-expression of FTO has been shown to be the genetic driver of a diverse range of cancers. Race is exploring the use of Bisantrene 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 Bisantrene.

See more at www.raceoncology.com.

Release authorised by:

Phil Lynch, CEO/MD on behalf

of the Race Board of Directors

phillip.lynch@raceoncology.com

Media contact:

Jane Lowe

+61 411 117 774

jane.lowe@irdepartment.com.au