RACE ONCOLOGY

ASX Announcement

Cardioprotection & Anticancer Data Presented at the European Society for Medical Oncology Congress 2025

- (E,E)-bisantrene mechanism of action data presented at the prestigious European Society for Medical Oncology (ESMO) Congress held in Berlin, 17-21 October 2025
- Data highlights how combining (E,E)-bisantrene with doxorubicin protects against doxorubicin-induced cardiotoxicity while enhancing anticancer activity
- This preclincial data supports Race's current Phase 1 trial of RC220 in combination with doxorubicin in advanced solid tumour patients.

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20 October 2025 – Race Oncology Limited ("Race") is pleased to announce that CEO and Managing Director, Dr. Daniel Tillett presented data on (E-E)-bisantrene (RCDS1) mechanism of action at the major international oncology meeting, ESMO Congress 2025. The poster presentation summarises the results of preclinical studies and clinical observations that explore the dual anticancer and cardioprotective benefits of (E,E)-bisantrene when combined with anthracyclines like doxorubicin.

The poster presentation entitled "*Discovery of (E,E)-bisantrene as a dual-cardioprotective and anticancer agent in combination with doxorubicin*" is attached to this announcement.

The presented data highlights how (E,E)-bisantrene functions as an anthracycline cardioprotective agent via the clinically proven molecular pathway of decreasing double strand DNA breaks in heart muscle cells (cardiomyocytes) by reducing the activity of Topoisomerase 2β. This molecular mechanism of action is the same as the FDA-approved cardioprotective drug, dexrazoxane. Unlike dexrazoxane, (E,E)-bisantrene additively increases the anticancer activity of anthracyclines by stabilising G4-DNA & RNA structures which leads to the silencing of the key cell growth regulator, MYC (ASX announcement: 2 October 2025).

Race Oncology CEO and Managing Director, Dr Daniel Tillett commented, *"It is an honour for me to be able to share Race's MOA data at such a major international oncology congress. I wish to thank the entire Race preclinical team and all our collaborators for the amazing work they have done and which has enabled us to start treating cancer patients with the potentially practice-changing combination of doxorubicin and RC220."*

-ENDS-

About Race Oncology (ASX: RAC)

Race Oncology (ASX: RAC) is an ASX-listed clinical stage biopharmaceutical company with a dedicated mission to be at the heart of cancer care.

Race's lead asset, RCDS1 (E,E-bisantrene), is a small molecule anticancer agent that primarily functions via G4-DNA & RNA binding, leading to potent inhibition of the important cancer growth regulator MYC. RCDS1 has demonstrated therapeutic activity in cancer patients with a well characterised safety profile.

Race is advancing a proprietary formulation of RCDS1 (RC220) to address the high unmet needs of patients across multiple oncology indications, with a clinical focus on combinations with an anthracycline (doxorubicin), where we aim to deliver both cardioprotection and enhanced anticancer activity in solid tumour indications. Race is also exploring the use of RC220 as a low intensity treatment for acute myeloid leukaemia and other cancers.

Race Oncology has collaborated with Astex, MD Anderson, Sheba City of Health, UNC School of Medicine, University of Wollongong and University of Newcastle, and is actively exploring partnerships, licence agreements or a commercial merger and acquisition to accelerate access to RC220 for patients with cancer across the world.

Learn more at www.raceoncology.com.

If you have any questions on this announcement or any past Race Oncology announcements, please go to the Interactive Announcements page in our Investor Hub announcements.raceoncology.com

Race encourages all investors to go paperless by registering their details with the Company's share registry, Automic Registry Services, at www.automicgroup.com.au.

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Discovery of (E,E)-bisantrene as a dual-cardioprotective and anticancer agent in combination with doxorubicin

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AT THE HEART OF CANCER CARE

BACKGROUND

Doxorubicin (Dox) shows potent anticancer activity in many cancers, but its clinical utility is hampered by dose-limiting cardiotoxicity. Bisantrene was evaluated clinically in the 1980s as a broad anticancer agent with low cardiotoxicity, however, its development was ultimately abandoned due to issues with blood solubility and injection site intolerance. Bisantrene and Dox are both topoisomerase 2 (Top2) inhibitors, although multiple other mechanisms contribute to their anticancer activities. Based on observations from exploratory preclinical studies, we hypothesised that co-administration of Bisantrene with Dox might protect against Dox-induced cardiotoxicity, while simultaneously providing improved anticancer benefits. This poster describes preclinical studies aimed at testing the dual-activity hypothesis.

METHODS

In reviving the clinical development of bisantrene, a new proprietary formulation (RC220) of the active isomer (E,E)-bisantrene[1] was developed with improved blood solubility that enables safe peripheral vein IV administration in humans. RC220 was used for all *in vivo* studies. GMP manufactured (E,E)-bisantrene dihydrochloride (Bis; RCDS1) was used for all *in vitro* studies.

The *in vitro* anticancer activity of Bis and Dox alone and in combination (1:1 molar ratio) was assessed in a panel of 111 cancer cell lines derived from 22 tissue types. The *in vivo* anticancer activity was evaluated in the 4T1 syngeneic mouse breast cancer model.

Cardioprotection was assessed *in vitro* using primary human cardiomyocytes (PHCs) and neonatal rat ventricular myocytes (NRVMs) and *in vivo* using established mouse and dog models of Dox-induced cardiotoxicity. To evaluate effects of the drugs on myocyte function, human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were assessed using the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA). Cardioprotection mechanisms were explored *in vitro* using flow cytometry by quantitating dsDNA breaks (DSBs) in all-trans retinoic acid-differentiated rat H9c2 (RA H9c2) cells by γH2AX staining. Results were corroborated *in vivo* by performing western blots on hearts obtained from drug-treated mice and staining for γH2AX.

RESULTS

Anticancer Activity

Bis and Dox both showed nanomolar cytotoxicity against most human cancer cell lines tested, with Dox typically showing higher potency. For all tissue types within the cell line panel, IC_{50} values for the combination were lower than Dox alone (**Fig 1A**). Across the panel, the combination showed significantly higher potency ($p < 0.0001$) than Dox alone (**Fig 1B**). Synergy analysis suggests in most cases combining Bis with Dox provides additive anticancer activity.

In vivo studies confirmed enhanced survival for the Bis-Dox combination (4:1 molar ratio) over either drug used alone (**Fig 2**).

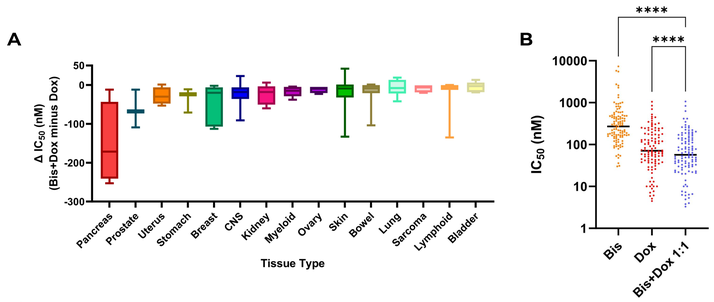

Figure 1. Adding Bis to Dox enhances *in vitro* anticancer activity. **A**, Change in IC_{50} for cancer cell lines (111 in total) treated with Bis + Dox vs Dox alone. **B**, Analysis across 111 cancer cell lines showing that adding Bis to Dox (1:1) lowers the mean IC_{50} below the Dox IC_{50} (****$p < 0.0001$).

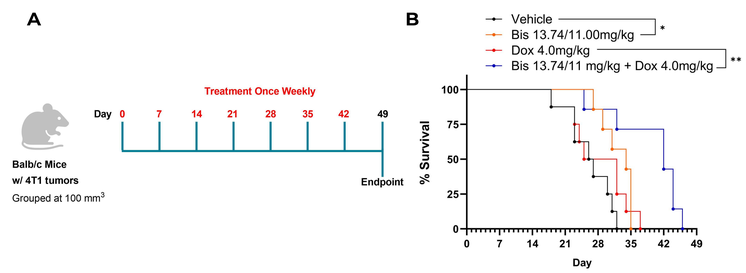

Figure 2. Combining Bis with Dox (1:1 mol) enhances *in vivo* anticancer efficacy. BALB/c mice with 4T1 tumours were treated weekly with vehicle, Bis, Dox, or Bis + Dox **(A)** and survival **(B)** assessed. *$p < 0.05$, **$p < 0.01$.

Cardioprotection

Bis protected PHCs and NRVMs from Dox-induced cytotoxicity in a dose-dependent manner (**Fig 3A,B**) and mitigated Dox-induced increases in beat rate in iPSC-CMs (**Fig 3C**). *In vivo*, the combination showed significant improvements in cardiac ejection fraction compared to Dox alone in mice and dogs (**Fig 4**).

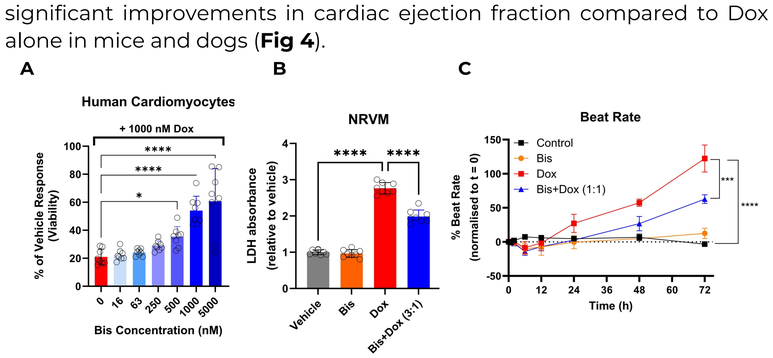

Figure 3. Bis protects against Dox-induced cardiotoxicity *in vitro*. **(A)** Bis protects human cardiomyocytes from Dox-induced cytotoxicity in a concentration dependent manner. **(B)** Bis reduced Dox-induced LDH release (indicator of cytotoxicity) in NRVMs. **(C)** Bis protects against Dox-induced increases in beat rate in iPSC-CMs. * $p < 0.05$, ***$p < 0.001$, **** $p < 0.0001$.

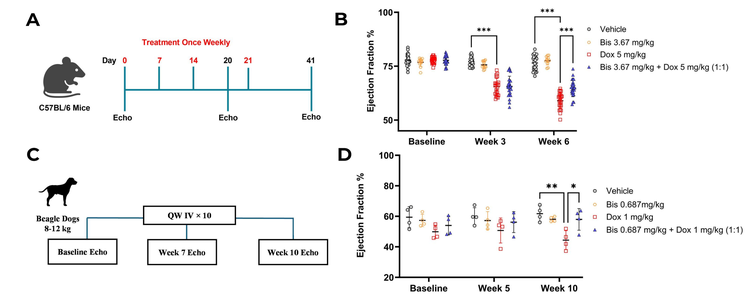

Figure 4. Bis protects against Dox-induced cardiotoxicity *in vivo*. Mouse **(A)** and dog **(C)** models were treated with weekly doses of Bis, Dox, or Bis + Dox and left ventricular ejection fraction **(B, D)** was assessed using echocardiography. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Mechanism of Action

The anticancer activity of Bis has recently been found to arise from G-quadruplex (G4) stabilisation that drives a reduction in *MYC* expression.[2]

Dexrazoxane (Dex), used clinically to protect against Dox-induced cardiotoxicity, reduces Dox-mediated DSBs in cardiomyocytes. Similar reductions in Dox-induced DSBs are observed with Bis-Dox, compared to Dox alone, and at lower concentrations than Dex (γH2AX signal, **Fig 5**). Reduced DSBs were corroborated *in vivo* by western blots on hearts obtained from drug-treated mice and staining for γH2AX (not shown). Thus, like Dex, Bis affords protection from Dox-induced cardiotoxicity by reducing the number of Top2β-mediated DSBs in cardiomyocytes. Studies are underway to elucidate the molecular mechanisms underpinning the reductions in Dox-induced DSBs observed in the presence of Bis, with a focus on MYC.

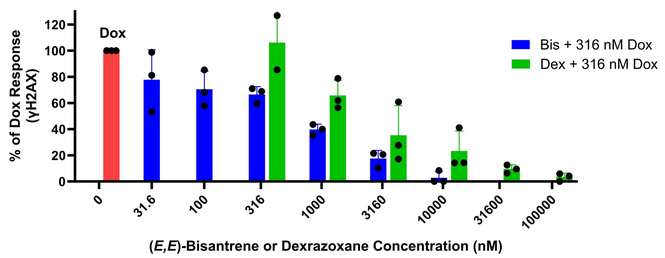

Figure 5. RA-H9c2 cells were pre-treated with Dex or Bis for 2 h prior to treatment with Dox for 4 h. γH2AX (DSB marker) was quantified by flow cytometry and calculated as a percent of the signal produced by Dox alone. *$p < 0.05$, ***$p < 0.001$, ****$p<0.0001$, p-values are compared to Dox alone.

CONCLUSIONS

Co-administration of Bis with Dox protects against Dox-induced cardiotoxicity while enhancing overall anticancer activity through additive, non-overlapping mechanisms of action. RC220 is currently being evaluated in combination with standard doses of Dox (60 mg/m^2) in a Phase I dose escalation trial of patients with diverse solid tumours (NCT06815575).

This study was funded by Race Oncology. Daniel Tillett daniel.tillett@raceoncology.com is employed by and owns stock in Race Oncology.

REFERENCES

1. ASX:RAC - Race Breakthrough Composition of Matter IP Discovery. https://announcements.raceoncology.com/announcements/7157250
2. ASX:RAC - Discovery of the Anticancer Mechanism of (EE)-bisantrene https://announcements.raceoncology.com/announcements/7181421