RACE ONCOLOGY

ASX Announcement

Bisantrene Highly Active in a Mouse Model of Multiple Myeloma in Combination with Carfilzomib

- Bisantrene kills human multiple myeloma cells at clinically relevant drug concentrations in both cell culture and mice
- Bisantrene treatment slows disease progression in a mouse model of multiple myeloma and shows enhanced activity in combination with carfilzomib
- Supports further evaluation of the bisantrene + carfilzomib in combination as a potentially more effective and less cardiotoxic treatment for multiple myeloma.

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13 June 2024 – Race Oncology Limited ("Race") is pleased to share results of preclinical work performed under contract by Labcorp (USA). In these studies, bisantrene, as a single agent treatment, was found to be effective against human multiple myeloma in a mouse model and more effective in combination with the standard of care multiple myeloma proteasome inhibitor, carfilzomib (trademark Kyprolis®, Amgen).

While effective in treating multiple myeloma, carfilzomib has known and serious heart toxicity side-effects that preclude its use in patients with elevated cardiac risk factors[1]. Importantly, Race has previously discovered that bisantrene is able to protect human heart muscle cells from the toxicity caused by carfilzomib (ASX Announcement: 8 December 2021).

In this mouse model, treatment with bisantrene was found to significantly slow multiple myeloma disease progression, whereas carfilzomib at the maximum tolerated dosage showed no single-agent activity. Despite a lack of activity when used alone, carfilzomib, when combined with bisantrene, was able to slow disease progression more than bisantrene treatment alone. These promising results suggest that the combination of bisantrene and carfilzomib is highly active and warrants further investigation as a potentially heart-safer treatment option for multiple myeloma patients.

Race Chief Executive Officer, Dr Daniel Tillett comments: *"It is always exciting to see the potential clinical utility of bisantrene grow. Carfilzomib is a highly active treatment for multiple myeloma, but it comes with very serious cardiotoxicity risks. The potential for using bisantrene to not only better treat multiple myeloma, but also protect patients from the heart damage caused by carfilzomib, is worthy of further investigation."*

Study Highlights

Background

Multiple Myeloma

Multiple myeloma is a dangerous blood cancer caused by the uncontrolled proliferation of white blood cells known as plasma cells. It usually occurs in people aged over 60 and is more common in men. Multiple myeloma accounts for 10% of blood cancers and is responsible for 1.8% of all new cancer cases in the United States, with a lifetime risk of 0.8% and a 5-year survival of 55.6% (2021 data)[2]. A recent (June 2024) estimate predicted global drug sales in multiple myeloma will reach US$33B by 2030[3].

Carfilzomib mechanism of action and use

Carfilzomib is an intravenous drug for treating multiple myeloma in patients with relapsed or refractory disease after at least one previous therapy. It is given in combination with dexamethasone or with lenalidomide and dexamethasone[4].

Originally developed by Proteolix before it was acquired by Onyx Pharmaceuticals in 2009, carfilzomib is currently owned by Amgen, who acquired Onyx in 2013[5]. Carfilzomib's patent protection is expected to expire in 2027[6].

Carfilzomib is a 20S proteasome inhibitor that works by interfering with the system for breaking down unwanted proteins within cells. One of the hallmarks of cancer is loss of control of normal protein synthesis, leading to an increase in misfolded and damaged proteins, which need to be removed from cells. Inhibition of the 20S proteasome impedes disposal of these damaged proteins, ultimately resulting in death of the cancer cells[7].

Carfilzomib is administered to multiple myeloma patients in 28-day cycles. An intravenous infusion is given on two consecutive days each week for three weeks, followed by a 12-day rest period. The annual cost of carfilzomib for a multiple myeloma patient is more than US$300,000, with many patients treated for two or more years[8].

Carfilzomib-induced cardiotoxicity

While effective in treating multiple myeloma, carfilzomib comes with a significant risk of permanent heart damage. An analysis of carfilzomib studies found that of 526 patients treated, 22.1 (n=116) developed cardiac side-effects, 13.3% (n=70) showed arrhythmia, mainly atrial fibrillation, 7.2% (n=38) exhibited heart failure, 2% (n=9) developed treatment-associated cardiomyopathy, and 3% (n=18) suffered from ischemic heart disease[1]. Most cardiovascular events occurred early when the first few doses were administered[9].

A prior history of atrial fibrillation/flutter or heart failure was more prevalent in patients experiencing serious cardiovascular events, emphasising the importance of carefully selecting patients for carfilzomib treatment[9]. Ionising radiation of the chest and/or anthracycline treatment also increases the risk of carfilzomib-induced cardiotoxicity. Biomarkers and echocardiography are not able to identify the patients most at risk of cardiovascular events[10].

In late 2021, in collaboration with researchers from the University of Newcastle, Race reported preclinical results showing that bisantrene can protect human heart muscle cells from carfilzomib-induced cell death (ASX Announcement: 8 December 2021). These data suggested that the use of bisantrene in combination with carfilzomib may provide cardioprotective benefits in addition to possible additional anticancer activity.

Bisantrene is active in multiple myeloma cells *in vitro* at clinically relevant drug concentrations

The human multiple myeloma (MM) cell line, MM.1S-Luc-Neo, is sensitive to both bisantrene and carfilzomib (Figure 1). *In vitro*, carfilzomib was extremely potent, showing an IC_{50} value of only 0.5 nM. The MM.1S-Luc-Neo cell line was also sensitive to bisantrene, showing an IC_{50} of 67.7 nM.

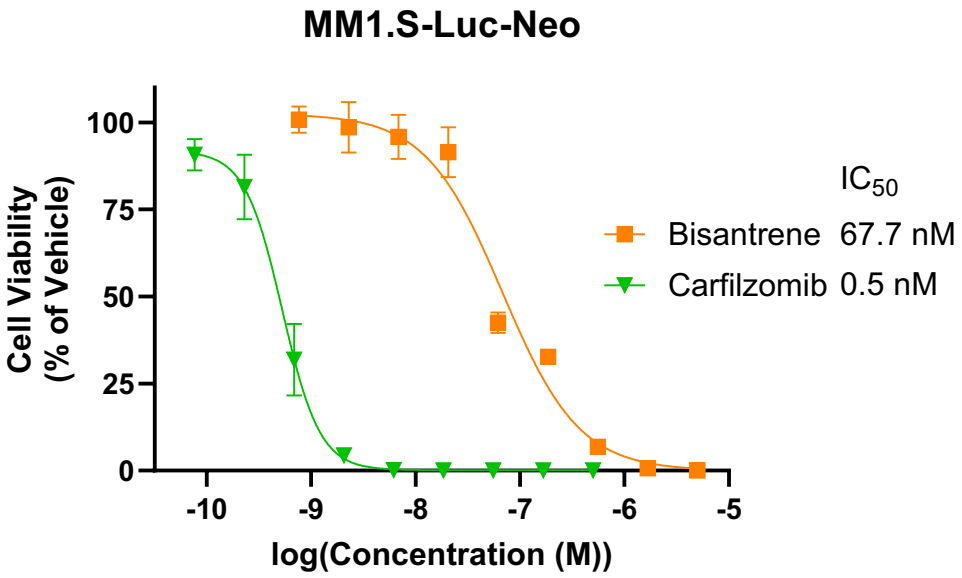

Figure 1. Viability of luciferase (Luc)-tagged human multiple myeloma cell line MM1.S-Luc-Neo following treatment with bisantrene or carfilzomib. Viability was measured by CellTiter® Glo analysis after incubation with drug for 72 hours. Values shown represent the average and standard deviation of n = 3 per condition.

Bisantrene shows significant activity alone and improved activity in combination with carfilzomib in a MM.1S-Luc-Neo mouse model of multiple myeloma

After confirming the sensitivity of human MM.1S-Luc-Neo cells to bisantrene and carfilzomib, immunocompromised NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice (8 mice per treatment group) were injected with 5 x 10^6 cells into the lateral tail vein to generate multiple myeloma disease. Tumour burden was quantitated for animals in each treatment group using periodic whole animal bioluminescence imaging. This technique allows quantitative monitoring of disease progression as the Luc-tagged multiple myeloma cells produce a detectable bioluminescence signal that is proportional to total tumour cell mass. Bisantrene, carfilzomib and the bisantrene plus carfilzomib combination were dosed on a weekly schedule of 2 daily doses followed by 5 days off, repeated five times, to mimic carfilzomib's clinical dosing regimen. Bisantrene at 5 mg/kg and carfilzomib at 1.25 mg/kg were well-tolerated both alone and in combination across all mice, with no weight loss observed for any animal (data not shown). Doses of carfilzomib above 1.25 mg/kg were poorly tolerated in the mice, causing significant weight loss and deaths.

Figure 2 shows that 1.25 mg/kg carfilzomib alone (green) produced no reductions in multiple myeloma tumour burden relative to vehicle-treated controls (blue) for any mouse at any stage during treatment. This indicates that the very high *in vitro* sensitivity of MM.1S-Luc-Neo cells to carfilzomib did not translate to the whole animal setting. In contrast, all mice receiving 5 mg/kg bisantrene showed significantly delayed progression of multiple myeloma disease compared to mice treated with carfilzomib alone or vehicle ($p < 0.0001$). Treating mice with bisantrene and carfilzomib slowed disease progression more than bisantrene treatment alone ($p < 0.05$).

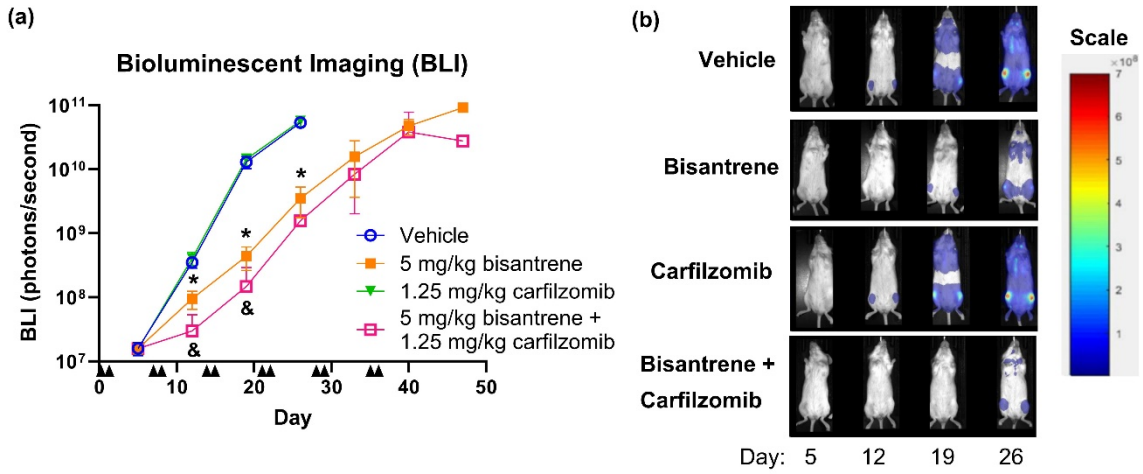

Figure 2. Bisantrene treatment alone or in combination with carfilzomib significantly slows MM disease progression compared to vehicle or carfilzomib alone. **(a)** Multiple myeloma disease burden in NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice was measured weekly using whole body bioluminescence imaging (BLI) for 7 weeks, or until mice succumbed to disease. **(b)** Representative BLI images from each treatment group. Scale bar depicts the photons/second reading for each colour. *represents $p < 0.0001$ at each time point for bisantrene alone vs vehicle or bisantrene + carfilzomib vs carfilzomib alone. &Represents $p < 0.05$ at each time point for bisantrene + carfilzomib vs bisantrene alone. Arrowheads indicate dosing days.

References

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Next Steps

- Additional preclinical studies to investigate the cellular mechanism(s) responsible for delayed multiple myeloma progression and increased survival of mice treated with bisantrene and carfilzomib.
- Complete mouse models of carfilzomib-induced cardiotoxicity to confirm the cardioprotective properties of bisantrene *in vivo*.
- Publication in a high-quality, international, peer-reviewed journal.
- Explore options for clinical studies evaluating bisantrene in combination with carfilzomib as a more effective and cardio-safer treatment for patients with MM.

Q&A

What do these results mean for future bisantrene clinical trials?

Race does not currently have plans to undertake new clinical trials of bisantrene in multiple myeloma patients but is open to supporting investigator-sponsored trials, subject to resourcing. No definitive discussions have taken place with any investigator, but as we advance RC220 bisantrene in the clinic for solid tumours and Acute Myeloid Leukaemia, opportunities for advancing bisantrene in new indications will continue to arise.

What is the commercial significance of this discovery?

Carfilzomib is owned by Amgen and its patent protection from generic competition expires in 2027. The combination of carfilzomib with bisantrene may offer a way of extending the commercial value of carfilzomib beyond 2027. Race has applied for patent protection covering the use of carfilzomib and bisantrene in multiple myeloma.

Why didn't carfilzomib show any effect on its own in the mice, yet its presence improved the efficacy of bisantrene treatment?

Carfilzomib is toxic to mice and displays much lower efficacy in whole animals compared to *in vitro* conditions due to its relatively short half-life in blood. It is an excellent example of how sometimes results from cell culture experiments do not translate to animal models, or the clinic.

Where can I learn more?

CEO Dr Daniel Tillett has released a short video discussing this announcement. Watch the video and ask further question here: https://announcements.raceoncology.com/link/qy11Dy

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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, bisantrene, is a small molecule chemotherapeutic. Bisantrene has a rich and unique clinical history with demonstrated therapeutic benefits in both adult and paediatric patients, a well-characterised safety profile, and compelling clinical data demonstrating an anticancer effect and less cardiotoxicity over certain anthracyclines, such as doxorubicin.

Race is advancing a reformulated bisantrene (RC220) to address the high unmet needs of patients across multiple oncology indications, with a clinical focus on anthracycline combinations, where we hope to deliver cardioprotection and enhanced anticancer activity in solid tumours. Race is also exploring RC220 as a low-intensity treatment for acute myeloid leukaemia.

Race is investigating the effect of bisantrene on the m^6A RNA pathway, following independent research published by the City of Hope identifying bisantrene as a potent inhibitor of FTO (Fat mass and obesity-associated protein). Dysregulation of the m^6A RNA pathway has been described in numerous peer reviewed studies as a driver of a diverse range of cancers.

Race Oncology has collaborated with Astex, City of Hope, 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 bisantrene 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 https://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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