RACE ONCOLOGY

ASX Announcement

Zantrene Highly Effective in a Mouse Model of Extramedullary AML

- **Zantrene in combination with decitabine was found to target extramedullary tumours as well as in the bone marrow and spleen using an AML mouse model.**
- **Zantrene alone found to kill a genetically diverse range of AML cells at low drug concentrations and slow the growth of AML tumours in mice.**
- **Zantrene in combination with decitabine showed significantly greater cell killing across a diverse panel of AML cell lines than either drug on its own (true synergy).**
- **These results provide further robust in vitro and in vivo preclinical support for Race's announced extramedullary AML Phase 1/2 clinical trial.**

17 March 2022 – Race Oncology Limited ("Race") is pleased to share interim results from the extramedullary acute myeloid leukaemia (EMD AML) preclinical program led by eminent cancer researcher, Associate Professor Nikki Verrills of The University of Newcastle and Hunter Medical Research Institute (ASX announcement: 30 March 2021).

This research found that low dose Zantrene in combination with decitabine can kill AML tumours in a mouse model of extramedullary AML and builds on the results of earlier AML clinical trials. Race is rapidly advancing Zantrene into the clinic as a possible new treatment option for patients with EMD AML.

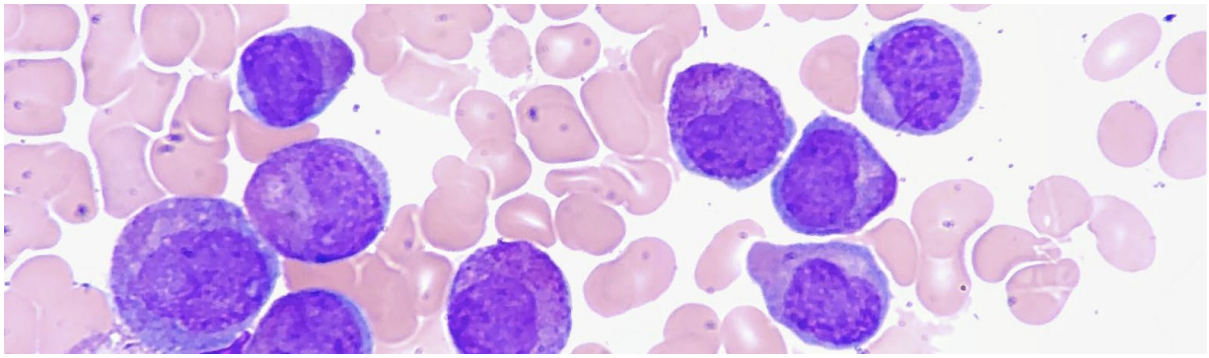

Figure 1. Human AML cells (purple) from a bone marrow aspirate.

Chief Scientific Officer, Dr Daniel Tillett said, *"The results from Prof Verrills laboratory are highly supportive of our upcoming EMD AML Phase 1/2 trial for Zantrene. This work further builds on the 2020 Phase 2 trial of Prof Arnon Nagler, who identified Zantrene as showing encouraging efficacy in EMD AML. The optimised drug combination and schedule identified in this preclinical mouse study will be rapidly translated to the clinic via our EMD AML trial."*

Chief Executive Officer, Mr Phillip Lynch said, *"These results provide support for our well advanced EMD AML clinical trial and provides important guidance for the study's design and treatment protocol."*

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Study Background

Extramedullary AML

Extramedullary AML occurs when the leukaemia spreads from the bone marrow and forms solid tumours in tissues such as the skin, breast, kidney, brain, or other organs[1]. A 2020 prospective positron imaging trial (^{18}FDG PET) identified that up to 22% of AML patients have the extramedullary form[2]. Extramedullary AML patients have no clinically approved treatments and only limited experimental treatment options[3]. Many clinical trials exclude patients with this difficult to treat form of AML.

Zantrene showed positive efficacy in extramedullary AML

In a 2020 Phase 2 clinical trial conducted at Chaim Sheba Medical Centre, Tel Aviv in relapsed and refractory (R/R) AML patients, Zantrene was observed to have clear efficacy in patients with extramedullary AML[4]. In this trial of 10 heavily pre-treated AML patients, all four patients having the EMD AML subtype showed a clinical response to a single cycle of Zantrene as a single agent[4]. An example ^{18}FDG PET image of an EMD AML patient from this trial before and after Zantrene is shown in Figure 2.

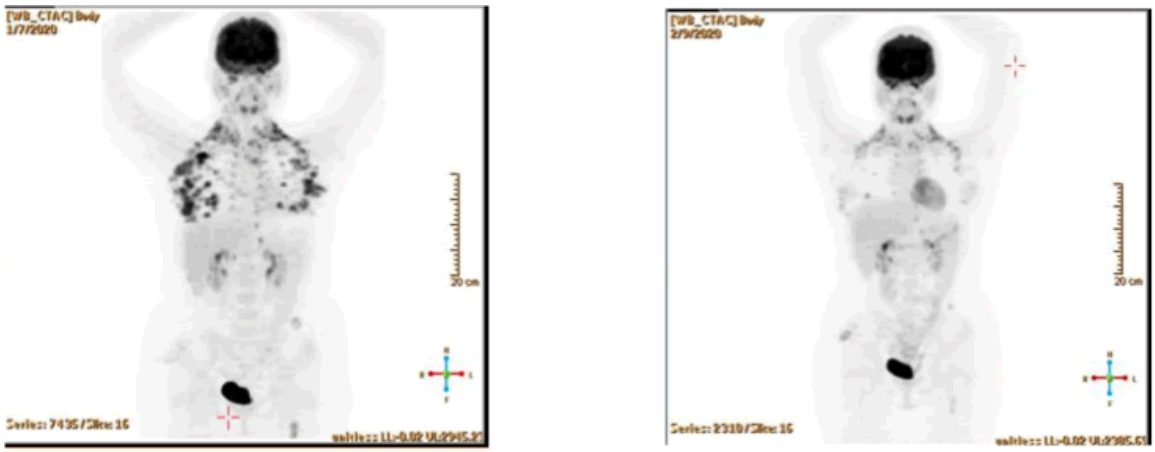

Figure 2. Extramedullary AML ^{18}FDG PET imaging before (*left*) with AML cancer cells appearing as dark /bright spots in breast tissue and after (*right*) a single cycle of treatment with Zantrene alone.

Synergy between FTO inhibition and decitabine

Su *et al.* (2018) observed synergy between FTO inhibition and decitabine against AML cells[5]. Decitabine is often used for the treatment of AML or myelodysplastic syndrome (MDS) in patients unfit for high intensity chemotherapy and stem cell transplantation[6]. On the basis that Zantrene is a potent FTO inhibitor (IC_{50} 142nM)[7], it was hypothesised that Zantrene and decitabine would synergise to better kill AML cells. This hypothesis was tested both *in vitro* in AML cell cultures and *in vivo* in a mouse model of extramedullary AML.

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Study Highlights

1. Zantrene kills AML cells more effectively than decitabine as a single agent

Cells were treated for 72 hours and cell viability determined using a resazurin metabolic assay and visual inspection (Figure 3A). The concentration of drug required to inhibit cell viability by 50% (IC_{50}) or 25% (IC_{25}) was determined (Table 1).

Table 1. Single agent cytotoxicity of Zantrene and decitabine in a diverse range of human AML cell lines.

Cell Line	Driver Mutations	Isolation	Zantrene		Decitabine	
			IC_{25} (nM)	***IC_{50} (nM)***	***IC_{25} (nM)***	***IC_{50} (nM)***
MV4-11	FLT3-ITD	Diagnosis	4	16	81	> 500
Kasumi-1	KIT N822K TP53 ASXL1 c.1934dupG	2nd Relapse	87	563	112	> 500
THP-1	NRAS G12D TP53 del	Relapse	95	152	156	> 500
HL-60	NRAS Q61L MYC amp TP53 del CDKN2A pR8 NOX5	Diagnosis	16	65	191	> 500
MOLM-13	FLT3-ITD (Het)	Relapse	26	42	23	53
OCI-AML3	NPM1, DNMT3A R882C(het) NRAS Q61L	Diagnosis	71	124	>500	>500

All AML cell lines were sensitive to Zantrene in the nanomolar range at drug concentrations of clinical relevance (Table 1). The *MV4-11* cells were the most sensitive to single agent Zantrene, with an IC_{50} of just 16nM, followed by the *MOLM-13* and *HL60* cells at 42nM and 65nM, respectively. The *OCI-AML3* and *THP-1* lines showed similar sensitivity, with IC_{50s} of 124nM and 152nM, respectively. The *Kasumi-1* cells were more resistant to Zantrene with an IC_{50} of 563nM.

In contrast, decitabine treatment alone, up to 500nM for 72 hours, did not reach the IC_{50} level even at the 500nM concentration in 5 out of the 6 cell lines tested (Figure 3B & Table 1). The *MOLM-13* cells were the most sensitive to decitabine with an IC_{50} of 53nM, however, 100% cell death was not achieved for decitabine at any tested dose.

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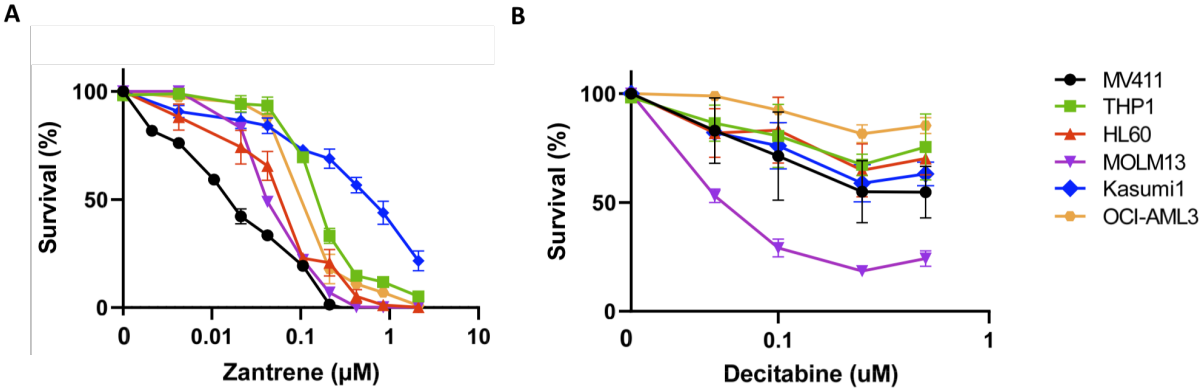


Figure 3. Single agent Zantrene and decitabine cytotoxicity assays in human AML cells. Cells were treated for 72h with different dose combinations of (A) Zantrene or (B) Decitabine. Dose-response curves show cell viability in response to indicated dose ranges. n ≥2.

The *MV4-11* and *MOLM-13* cell lines both contain the *fms-like tyrosine kinase 3 internal tandem duplication* (FLT3-ITD) mutation, suggesting a possible connection between FLT3-ITD mutations and Zantrene sensitivity. These two cell lines, as well as the *THP-1* cells also have MLL rearrangements, suggesting a further possible association of Zantrene sensitivity and MLL rearrangement. The *HL60, THP-1* and *Kasumi-1* lines all have *tumour protein p53* (TP53) mutations, suggesting an association with TP53 mutational status and Zantrene sensitivity. Importantly, these data suggest that TP53 mutation alone does not induce resistance to Zantrene. The three cell lines with *neuroblastoma RAS oncogene* (NRAS) mutations were all sensitive to Zantrene (*THP-1, HL60* and *OCI-AML3*).

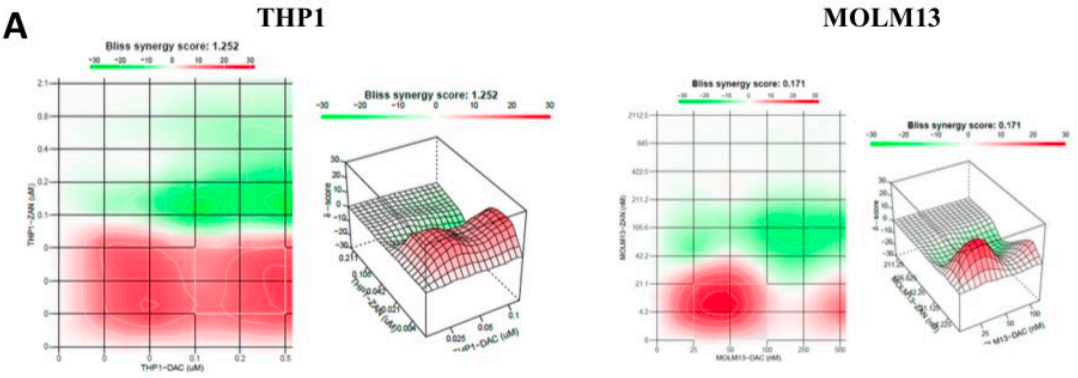
In addition to having a TP53 deletion, the *Kasumi-1* cell line (least sensitive to Zantrene) expresses the *tyrosine-protein kinase KIT* (KIT) carrying a mutation within the tyrosine kinase domain causing constitutive activation of KIT, an oncogenic driver of AML.

The *in vitro* cell line data shows that Zantrene is able to kill AML cells at low concentrations as a single agent irrespective of the cancer driver mutations that are common causes of AML in patients with the possible exception of KIT.

2. Zantrene and decitabine work together to better kill human AML cell lines

The viability of AML cells to combined treatment with Zantrene and decitabine for 72 hours was analysed for synergy using the methods of Bliss which indicated several synergistic dose combinations in all AML cell lines tested (Table 2). An example of the Bliss analysis is shown in Figure 4 for the *THP-1* and *MOLM-13* cell lines.

A



B

Drug1	Drug2	Conc1 nM	Conc2 nM	Relative inhibition	Synergy
ZAN	DAC	0.00	0	1.737	0.000
ZAN	DAC	4.23	0	1.057	0.000
ZAN	DAC	21.13	0	5.750	0.000
ZAN	DAC	42.25	0	6.523	0.000
ZAN	DAC	105.63	0	30.400	0.000
ZAN	DAC	211.25	0	66.763	0.000
ZAN	DAC	422.50	0	85.277	0.000
ZAN	DAC	845.00	0	88.153	0.000
ZAN	DAC	2112.50	0	94.817	0.000
ZAN	DAC	0.00	25	17.510	0.000
ZAN	DAC	4.23	25	20.590	10.513
ZAN	DAC	21.13	25	24.293	9.946
ZAN	DAC	42.25	25	26.243	11.314
ZAN	DAC	105.63	25	32.480	-5.326
ZAN	DAC	211.25	25	69.127	-1.049
ZAN	DAC	422.50	25	85.770	-1.069
ZAN	DAC	845.00	25	89.253	-0.093
ZAN	DAC	2112.50	25	94.713	-0.678
ZAN	DAC	0.00	50	13.633	0.000
ZAN	DAC	4.23	50	22.277	16.919
ZAN	DAC	21.13	50	23.680	13.625
ZAN	DAC	42.25	50	21.613	10.576
ZAN	DAC	105.63	50	27.390	-7.668
ZAN	DAC	211.25	50	68.097	-0.639
ZAN	DAC	422.50	50	84.430	-1.851
ZAN	DAC	845.00	50	88.273	-0.615
ZAN	DAC	2112.50	50	94.610	-0.552
ZAN	DAC	0.00	100	19.483	0.000
ZAN	DAC	4.23	100	22.573	10.353
ZAN	DAC	21.13	100	23.637	7.015
ZAN	DAC	42.25	100	23.840	6.496
ZAN	DAC	105.63	100	19.130	-21.526
ZAN	DAC	211.25	100	67.000	-4.150
ZAN	DAC	422.50	100	83.210	-4.207
ZAN	DAC	845.00	100	87.110	-2.709
ZAN	DAC	2112.50	100	94.540	-0.989
ZAN	DAC	0.00	250	32.727	0.000
ZAN	DAC	4.23	250	39.387	13.300
ZAN	DAC	21.13	250	41.057	11.164
ZAN	DAC	42.25	250	38.923	8.217
ZAN	DAC	105.63	250	37.730	-12.205
ZAN	DAC	211.25	250	68.793	-7.432
ZAN	DAC	422.50	250	83.613	-6.088
ZAN	DAC	845.00	250	88.850	-2.678
ZAN	DAC	2112.50	250	94.580	-1.763
ZAN	DAC	0.00	500	24.483	0.000
ZAN	DAC	4.23	500	33.340	16.211
ZAN	DAC	21.13	500	36.853	15.826
ZAN	DAC	42.25	500	33.347	11.305
ZAN	DAC	105.63	500	22.940	-21.511
ZAN	DAC	211.25	500	63.723	-9.703
ZAN	DAC	422.50	500	82.490	-5.869
ZAN	DAC	845.00	500	87.127	-3.396
ZAN	DAC	2112.50	500	94.757	-1.061

Drug1	Drug2	Conc1 nM	Conc2 nM	Relative inhibition	Synergy
ZAN	DAC	0.00	0	0.520	0.000
ZAN	DAC	4.23	0	0.000	0.000
ZAN	DAC	21.13	0	16.820	0.000
ZAN	DAC	42.25	0	51.050	0.000
ZAN	DAC	105.63	0	77.955	0.000
ZAN	DAC	211.25	0	93.045	0.000
ZAN	DAC	422.50	0	99.765	0.000
ZAN	DAC	845.00	0	99.850	0.000
ZAN	DAC	2112.50	0	99.840	0.000
ZAN	DAC	0.00	25	51.040	0.000
ZAN	DAC	4.23	25	55.115	19.615
ZAN	DAC	21.13	25	59.045	11.828
ZAN	DAC	42.25	25	63.910	-7.904
ZAN	DAC	105.63	25	82.825	-4.702
ZAN	DAC	211.25	25	94.480	-1.609
ZAN	DAC	422.50	25	99.660	-0.245
ZAN	DAC	845.00	25	99.660	-0.309
ZAN	DAC	2112.50	25	99.830	-0.090
ZAN	DAC	0.00	50	46.970	0.000
ZAN	DAC	4.23	50	53.045	23.305
ZAN	DAC	21.13	50	58.905	16.858
ZAN	DAC	42.25	50	68.200	0.478
ZAN	DAC	105.63	50	81.350	-5.152
ZAN	DAC	211.25	50	96.120	0.859
ZAN	DAC	422.50	50	99.595	-0.311
ZAN	DAC	845.00	50	99.735	-0.206
ZAN	DAC	2112.50	50	99.990	0.118
ZAN	DAC	0.00	100	70.895	0.000
ZAN	DAC	4.23	100	66.920	3.728
ZAN	DAC	21.13	100	70.095	0.139
ZAN	DAC	42.25	100	76.100	-7.731
ZAN	DAC	105.63	100	82.790	-11.475
ZAN	DAC	211.25	100	95.445	-2.536
ZAN	DAC	422.50	100	99.520	-0.490
ZAN	DAC	845.00	100	99.525	-0.522
ZAN	DAC	2112.50	100	99.885	-0.071
ZAN	DAC	0.00	250	81.515	0.000
ZAN	DAC	4.23	250	79.290	2.739
ZAN	DAC	21.13	250	79.205	-2.146
ZAN	DAC	42.25	250	81.160	-9.449
ZAN	DAC	105.63	250	87.195	-9.612
ZAN	DAC	211.25	250	95.895	-3.113
ZAN	DAC	422.50	250	99.395	-0.683
ZAN	DAC	845.00	250	99.600	-0.453
ZAN	DAC	2112.50	250	99.725	-0.296
ZAN	DAC	0.00	500	75.710	0.000
ZAN	DAC	4.23	500	78.710	10.944
ZAN	DAC	21.13	500	77.735	3.454
ZAN	DAC	42.25	500	81.125	-5.124
ZAN	DAC	105.63	500	84.495	-10.993
ZAN	DAC	211.25	500	96.805	-1.364
ZAN	DAC	422.50	500	99.535	-0.489
ZAN	DAC	845.00	500	99.695	-0.322
ZAN	DAC	2112.50	500	99.710	-0.300

Figure 4. Bliss Synergy Analysis for human THP-1 and MOLM-13 AML cells. (A) 2D and 3D visualisation of 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. **(B)** Table of Bliss scores for each individual dose combination. Values >10 are considered synergistic (red); values below -10 are considered antagonistic. Values between -10 and 10 are additive.

Table 2. Bliss synergy analysis of cell killing after 72h of combined Zantrene and decitabine treatment.

Cell Line	Best Score
MV4-11	14.16
Kasumi-1	20.42
THP-1	11.08
HL-60	16.91
MOLM-13	23.35
OCI-AML3	20.67

Values >10 are considered synergistic (red).

3. Pre-treatment with decitabine improves AML cell killing by Zantrene.

Previous work suggested that pre-treatment of AML cells with decitabine before exposure to Zantrene would improve cell killing through upregulation of FTO[5,7]. In the current study, improved synergy was observed when AML cells were pre-treated with decitabine for 24 hours prior to 72 hours of Zantrene and decitabine treatment (Table 3). Bliss analysis showed synergy at multiple doses for the *MOLM-13, MV4-11, THP-1, Kasumi-1* and *OCI-AML3* cells, with *MOLM-13* cells displaying the highest synergy score (42.95).

Table 3. Bliss synergy analysis of cell killing after 24h pre-treatment with decitabine followed by 72h of Zantrene and decitabine.

Cell Line	Best Score
MV4-11	14.45
Kasumi-1	16.35
THP-1	12.84
HL-60	9.4
MOLM-13	42.95
OCI-AML3	21.00

Values >10 are considered synergistic (red).

These data suggest that pre-exposing AML cells to decitabine for 24 hours before exposure to the Zantrene/decitabine combination provides greater drug synergy and cell killing.

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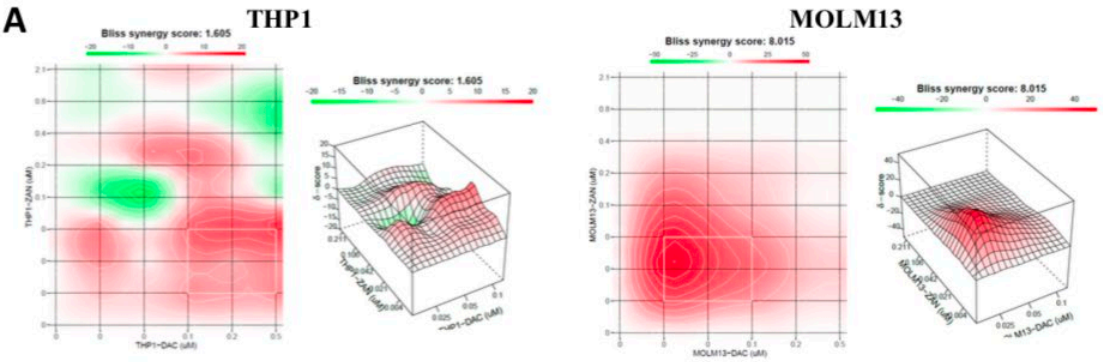

B

Drug1	Drug2	Conc1 nM	Conc2 nM	Relative inhibition	Synergy
ZAN	DAC	0.00	0	-1.642	0.000
ZAN	DAC	4.23	0	7.191	0.000
ZAN	DAC	21.13	0	10.290	0.000
ZAN	DAC	42.25	0	10.347	0.000
ZAN	DAC	105.63	0	61.977	0.000
ZAN	DAC	211.25	0	69.625	0.000
ZAN	DAC	422.50	0	83.528	0.000
ZAN	DAC	845.00	0	84.866	0.000
ZAN	DAC	2112.50	0	89.643	0.000
ZAN	DAC	0.00	25	16.056	0.000
ZAN	DAC	4.23	25	18.846	3.134
ZAN	DAC	21.13	25	23.777	5.425
ZAN	DAC	42.25	25	24.677	6.342
ZAN	DAC	105.63	25	59.255	-6.804
ZAN	DAC	211.25	25	73.097	0.657
ZAN	DAC	422.50	25	84.494	-0.634
ZAN	DAC	845.00	25	85.456	-0.903
ZAN	DAC	2112.50	25	88.108	-2.710
ZAN	DAC	0.00	50	3.978	0.000
ZAN	DAC	4.23	50	6.017	2.342
ZAN	DAC	21.13	50	6.539	-0.560
ZAN	DAC	42.25	50	7.492	0.405
ZAN	DAC	105.63	50	46.979	-14.704
ZAN	DAC	211.25	50	74.667	6.622
ZAN	DAC	422.50	50	84.122	1.282
ZAN	DAC	845.00	50	84.218	-0.111
ZAN	DAC	2112.50	50	91.668	2.587
ZAN	DAC	0.00	100	8.250	0.000
ZAN	DAC	4.23	100	13.168	5.442
ZAN	DAC	21.13	100	15.940	5.122
ZAN	DAC	42.25	100	19.057	8.425
ZAN	DAC	105.63	100	65.521	3.412
ZAN	DAC	211.25	100	76.303	6.876
ZAN	DAC	422.50	100	84.459	0.826
ZAN	DAC	845.00	100	85.081	0.067
ZAN	DAC	2112.50	100	92.660	3.142
ZAN	DAC	0.00	250	28.292	0.000
ZAN	DAC	4.23	250	32.350	4.484
ZAN	DAC	21.13	250	33.483	3.119
ZAN	DAC	42.25	250	39.541	9.608
ZAN	DAC	105.63	250	72.002	1.533
ZAN	DAC	211.25	250	80.227	4.023
ZAN	DAC	422.50	250	85.680	-1.700
ZAN	DAC	845.00	250	86.636	-1.785
ZAN	DAC	2112.50	250	93.332	1.451
ZAN	DAC	0.00	500	27.188	0.000
ZAN	DAC	4.23	500	32.799	6.161
ZAN	DAC	21.13	500	34.209	5.055
ZAN	DAC	42.25	500	41.469	12.840
ZAN	DAC	105.63	500	65.771	-4.702
ZAN	DAC	211.25	500	75.948	-0.204
ZAN	DAC	422.50	500	80.022	-7.593
ZAN	DAC	845.00	500	83.109	-5.397
ZAN	DAC	2112.50	500	91.012	-0.919

Drug1	Drug2	Conc1 nM	Conc2 nM	Relative inhibition	Synergy
ZAN	DAC	0.00	0	0.279	0.000
ZAN	DAC	4.23	0	4.431	0.000
ZAN	DAC	21.13	0	11.918	0.000
ZAN	DAC	42.25	0	35.095	0.000
ZAN	DAC	105.63	0	67.974	0.000
ZAN	DAC	211.25	0	87.813	0.000
ZAN	DAC	422.50	0	100.015	0.000
ZAN	DAC	845.00	0	100.056	0.000
ZAN	DAC	2112.50	0	99.919	0.000
ZAN	DAC	0.00	25	29.403	0.000
ZAN	DAC	4.23	25	43.080	25.512
ZAN	DAC	21.13	25	64.184	42.946
ZAN	DAC	42.25	25	75.734	34.128
ZAN	DAC	105.63	25	90.846	20.132
ZAN	DAC	211.25	25	98.166	9.586
ZAN	DAC	422.50	25	100.070	0.067
ZAN	DAC	845.00	25	100.113	0.078
ZAN	DAC	2112.50	25	100.078	0.169
ZAN	DAC	0.00	50	48.428	0.000
ZAN	DAC	4.23	50	55.782	15.559
ZAN	DAC	21.13	50	70.334	27.285
ZAN	DAC	42.25	50	79.972	22.244
ZAN	DAC	105.63	50	90.863	11.846
ZAN	DAC	211.25	50	99.102	7.519
ZAN	DAC	422.50	50	99.995	-0.017
ZAN	DAC	845.00	50	100.112	0.091
ZAN	DAC	2112.50	50	100.134	0.214
ZAN	DAC	0.00	100	58.084	0.000
ZAN	DAC	4.23	100	63.734	12.264
ZAN	DAC	21.13	100	73.077	18.894
ZAN	DAC	42.25	100	79.920	13.641
ZAN	DAC	105.63	100	90.719	7.463
ZAN	DAC	211.25	100	98.960	5.749
ZAN	DAC	422.50	100	99.978	-0.035
ZAN	DAC	845.00	100	100.063	0.041
ZAN	DAC	2112.50	100	100.068	0.126
ZAN	DAC	0.00	250	80.278	0.000
ZAN	DAC	4.23	250	81.985	4.660
ZAN	DAC	21.13	250	86.221	7.593
ZAN	DAC	42.25	250	89.878	5.632
ZAN	DAC	105.63	250	93.862	1.443
ZAN	DAC	211.25	250	99.102	2.228
ZAN	DAC	422.50	250	99.828	-0.204
ZAN	DAC	845.00	250	100.010	-0.003
ZAN	DAC	2112.50	250	100.043	0.072
ZAN	DAC	0.00	500	85.417	0.000
ZAN	DAC	4.23	500	87.025	3.850
ZAN	DAC	21.13	500	90.316	6.203
ZAN	DAC	42.25	500	92.073	3.648
ZAN	DAC	105.63	500	94.956	0.477
ZAN	DAC	211.25	500	99.538	1.883
ZAN	DAC	422.50	500	99.824	-0.208
ZAN	DAC	845.00	500	99.857	-0.178
ZAN	DAC	2112.50	500	99.998	0.013

Figure 5. Bliss Synergy Analysis for human *THP-1* and MOLM-13 AML cells. Cells were pre-treated with decitabine (DAC) for 24h, followed by 72h of combined Zantrene (ZAN)/DAC treatment at indicated doses. **(A)** 2D and 3D visualisation of predicted Bliss scores at each dose point, with red to green scale indicating areas of synergy to antagonism, and the average synergy score. **(B)** Table of Bliss scores for each individual dose combination. Values >10 are considered synergistic (red); values below -10 are considered antagonistic. Values between -10 and 10 are additive.

4. Zantrene and decitabine work together to better kill AML tumours in mice.

The combination of Zantrene and decitabine was trialed in mice engrafted with *MOLM-13-luc* AML cells (*MOLM-13* cells engineered to express the bioluminescence-producing enzyme luciferase). The mice were dosed for 5 days every week for three weeks with decitabine at 0.5mg/kg intraperitoneally (IP). Zantrene was dosed weekly using a dose escalation strategy on Days 3 and 5 at 5mg/kg IP (Week 1) or 10mg/kg intravenously (IV) (Weeks 2 & 3; formulated using a solubilisation vehicle). Additional groups of mice were dosed with either decitabine alone, Zantrene alone, vehicle, or no treatment (control). The dosing and luciferase-based bioluminescence imaging (BLI) schedule is shown in Figure 6.

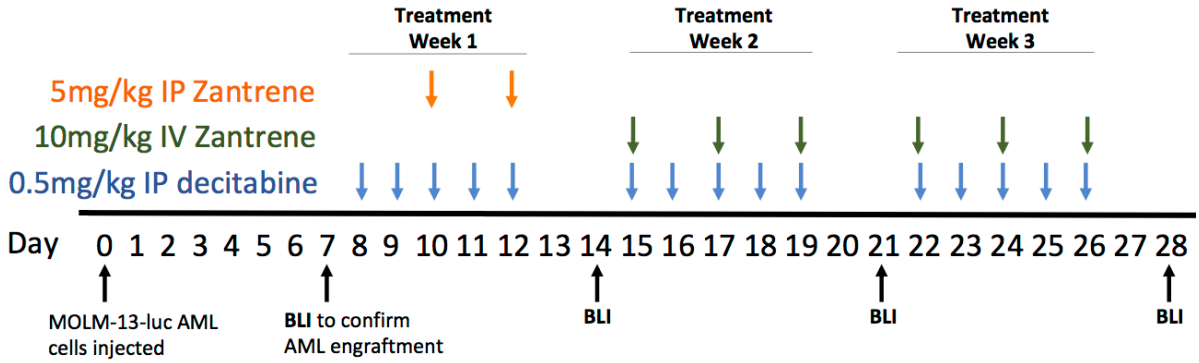

Figure 6. Dosing schedule for MOLM-13-luc mouse xenografts.

Drug treatments were started 7 days post-engraftment when a positive luciferase signal was able to be detected, showing successful engraftment (Figure 7; Day 7). BLI was conducted weekly to quantify tumour burden, tissue location, and response to treatment of the AML cells (Figure 7; Days 14, 21, 28).

Leukemic burden increased rapidly in vehicle control mice, with a median survival of 17 days and no mice alive at Day 21 (Figure 7; Control). Animal survival was increased with single agent decitabine and single agent Zantrene, with 4/5 and 1/5 animals surviving to Day 28, respectively (Figure 7; DAC; Zan).

Quantification of the tumour burden revealed no slowing of AML cell growth for Zantrene treatment alone, or in combination with decitabine, when Zantrene was dosed at 5mg/kg IP, with tumour burden similar to the control animals (Figure 7; Day 14).

Increasing the Zantrene dose to 10mg/kg from Day 15 resulted in survival past Day 21 for the Zantrene alone cohort (5/5). More interestingly, significant shrinkage of tumour burden in the decitabine + Zantrene cohort was observed at Day 21 compared to Day 14 (p-value < 0.05; data not shown), which was not observed in either the decitabine or Zantrene alone cohorts (Figure 7; Day 21).

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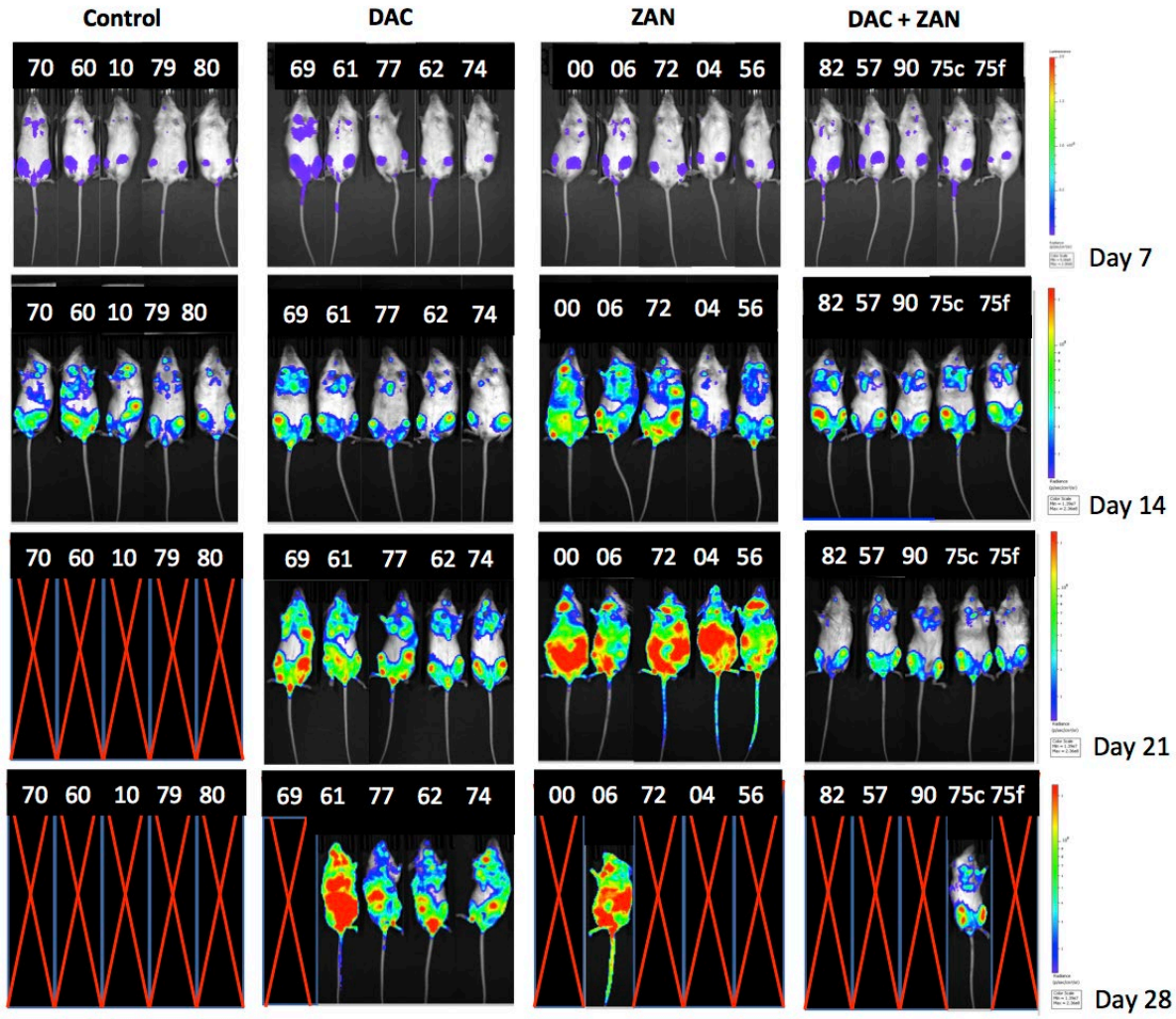

Figure 7. Bioluminescence images of NSG mice engrafted with MOLM-13-luc AML cells. Mice were injected with 3mg of D-luciferin (IP) and the whole-body tumour burden quantified after 10min using a Xenogen IVIS Spectrum Imager. DAC (decitabine) Zan (Zantrene). Red X indicates the animal was sacrificed or died before imaging could be performed.

Importantly, the Zantrene + decitabine combination resulted in substantial regression of EMD AML lesions in most mice at Day 21 that were present at Day 14. For example, *Mouse 82* displayed extensive extramedullary AML tumours in the neck and chest region at Day 14, which almost completely regressed by Day 21 (Figure 8). This regression in tumour burden was also observed in the hind leg long bones (i.e. bone marrow). Animal ethics requirements with respect to weight loss mandated that all but one of the Zantrene + decitabine mice be euthanized before Day 28. Despite this, the last mouse alive in this cohort (75c) had the lowest tumor burden of any mouse in the study at Day 28.

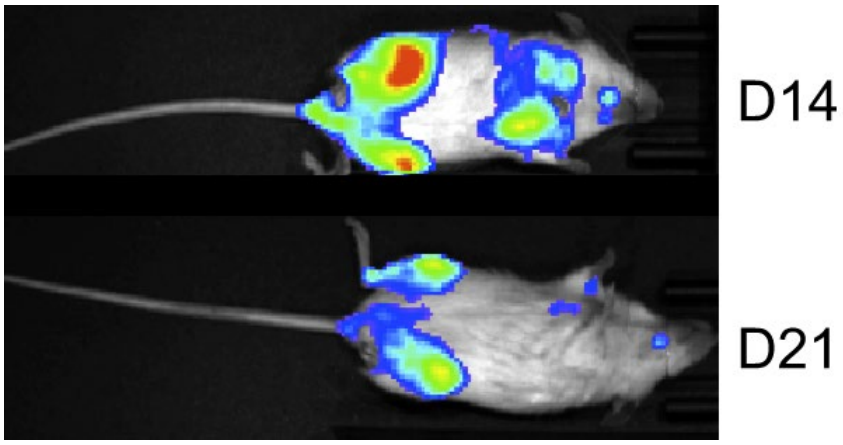

Figure 8. Comparison of Mouse 82 treated with Zantrene + decitabine at Day 14 and Day 21. Mice were injected with 3mg of D-luciferin (IP) and the whole-body tumour burden quantified after 10min using a Xenogen IVIS Spectrum Imager.

Flow cytometric analysis for the presence of human CD45+ AML cells at treatment endpoint revealed a significant reduction (p-value < 0.01) in tumour cell number in both the bone marrow and spleen for the Zantrene + decitabine drug combination (Figure 9).

Zantrene + decitabine was shown to cause significant regression of AML tumours in mice when dosed in combination.

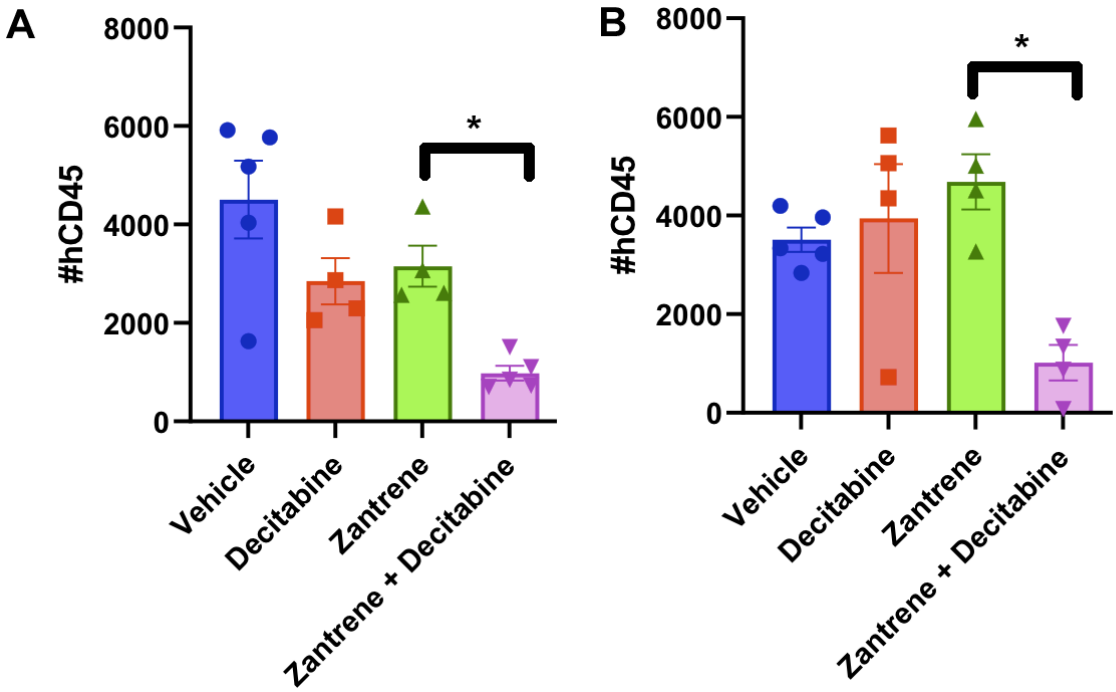

Figure 9. Flow cytometric analysis of human AML (CD45+) cells at treatment endpoint. (A) Bone marrow. **(B)** Spleen. n= 5. *p-value < 0.01.

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Conclusions

- Zantrene is highly effective at killing AML cells as a single agent across a diverse range of genetic subtypes both *in vitro* in cell culture and *in vivo* in mice.
- The combination of Zantrene + decitabine shows strong synergy at a range of drug levels and AML genetic subtypes.
- Dosing cells with decitabine as a single agent before dosing with Zantrene + decitabine provides improved synergy and cell killing *in vitro*.
- Zantrene + decitabine are better able to kill human AML cells found in the bone marrow and spleen when using a mouse model of AML than using either drug alone.
- The combination of Zantrene + decitabine causes regression of EMD AML tumours in a mouse model of extramedullary AML, further supporting the study of the combination Zantrene + decitabine in the clinic.
- Results of this animal study will be rapidly translated to the clinic via a Race sponsored Phase 1b/2 trial of Zantrene + decitabine in patients with extramedullary AML or MDS, as well as part of a high intensity chemotherapy protocol in patient able and willing to tolerate such therapy.

Next Steps

- Further mouse studies to explore optimal dosing schedules to report in Q2 CY2022.
- Publication of results in a high impact, peer reviewed journal.
- Initiation of clinical studies utilising low dose Zantrene and decitabine in combination to treat patients with extramedullary AML or MDS who cannot tolerate, or who are unwilling to tolerate, high intensity chemotherapy. This trial is expected to begin patient recruitment in Q2 CY2022.
- Discussion with relevant pharmaceutical partners to support Race sponsored clinical trials using this drug combination.

Q&A

What do these EMD AML results mean for Race?

The results are highly supportive Stratum 2 of our announced Phase 1b/2 EMD AML clinical trial using the combination of low dose Zantrene and decitabine in patients with extramedullary AML or MDS who are unwilling or unable to tolerate high intensity chemotherapy. Approximately 70% of patients have pre-existing conditions or are unwilling to tolerate the serious side effects of high intensity chemotherapy. Treatment options are limited for these patients, especially when they have the extramedullary form of AML or MDS which often does not respond well to existing AML drugs.

The Zantrene single agent results are also important for Stratum 1 of the EMD AML trial where patients will receive high intensity chemotherapy with Zantrene on its own for a single cycle, before subsequent consolidation cycles in combination with decitabine.

What is decitabine and how is it used to treat AML and MDS?

Decitabine is a modified nucleotide (one of the components of DNA & RNA) that can be incorporated into DNA by the cell as it grows. Decitabine prevents DNA from being methylated and leads to changes in the genes that are expressed. One of the proteins that is increased by decitabine is FTO[5].

Decitabine is commonly used as a first line treatment for AML or MDS in patients who are unfit for high intensity chemotherapy. While it is able to slow the progression of the disease, decitabine is not able to cure patients of AML or MDS as a single agent. New drugs that are able to synergise with decitabine are of high clinical interest.

What is the market potential of this discovery?

Extramedullary AML and MDS are neglected forms of AML and MDS with significant unmet clinical need. AML and MDS offers a potentially accelerated route to regulatory approval under the FDA 505(b)(2) pathway. In addition, Race has been granted Orphan Drug Designation for AML by the FDA, offering the potential for additional IP protection. Orphan Drug Designation provides market exclusivity for up to 7 years in the USA and 10 years in the EU post marketing approval irrespective of patent protection status.

What has been the response of clinicians to these results?

Race has received very positive feedback on the clinical relevance of these results from key opinion leaders in Australia, Europe and the USA. The unmet clinical need for improved treatment options and a growing recognition that extramedullary AML is more common than previous believed, has led to considerable interest from clinicians participating in clinical trial(s) exploring this drug combination.

When can Race investors expect the next update?

We expect to be able to update the market in the near future on the progress of our Phase 1b/2 extramedullary AML and MDS clinical trial. Updates on further mouse EMD AML studies are expected in Q2 CY2022.

Materials and Methods

Drugs

Zantrene (20mM) and decitabine (100mM) were reconstituted in DMSO. Drugs were stored at -20°C in aliquots to reduce the number of freeze-thaw cycles.

Cell Culture

Human AML cell lines were cultured in a humified chamber at 37°C with 5% CO_2 in RPMI (*THP-1, Kasumi-1, MOLM-13, OCI-AML3*) or DMEM (*MV4-11, HL60*) supplemented with 10% foetal bovine serum (FBS) and 2mM l-glutamine. *THP-1, Kasumi-1, MOLM-13, MV4-11* and *HL60* cells were supplemented with 20mM HEPES and THP-1 cells with additional 0.05m beta-mercapto-ethanol. Mouse myeloid early progenitor cell lines transduced with either empty vectors (EV) or oncogenic mutant AML-associated kinase mutations (KIT-D816V, FLT3-ITD KRAS-G12V) were cultured in a humidified chamber at 37°C with 5% CO_2 in DMEM supplemented with 10% foetal bovine serum (FBS), 2mM L-glutamine and 20mM HEPES.

Cytotoxicity Assays

Cell viability was determined using a resazurin metabolic activity assay and visual inspection. Drugs and cells were plated out in duplicate wells of 96-well microtitre plates at $2x10^4$ (*MOLM-13*), $4x10^4$ (*MV4-11, THP-1*), $5x10^4$ (*OCI-AML3*), $8x10^4$ (HL60) and $1.6x10^5$ (*Kasumi-1*) cells/well. Decitabine drug dilutions were either plated out at the same time as Zantrene and cells treated for 72h (standard assay), or cells were pretreated with decitabine for 24h followed by a further 72h in the presence of both Zantrene and decitabine (1-day pretreatment assay). Viability after 72hr for all assays was then determined using the fluorogenic viability dye Resazurin (Ex 544nm, Em 590nm; 0.6mM Resazurin, 78µM Methylene Blue, 1mM Potassium Hexacyanoferrate (III), 1mM Potassium Hexacyanoferrate (II) Trihydrate (Sigma, St Louis, Missouri, USA), dissolved in sterile PBS[8]). Resazurin is metabolised into the red-fluorescent resorufin by metabolically active cells. Fluorescence was measured 5 hours post the addition of resazurin solution (1:10, v/v) at 544nm excitation/ 590nm emission on a FLUOstar OPTIMA plate reader (BMG Labtechnologies, Offenburg, Germany). Graphpad Prism 8 software (La Jolla, CA, USA) was used to generate graphs. At least 2 independent replicates were performed for each cell line and each drug combination unless stated below, and data represent the mean +/-standard error of the mean (SEM).

Synergy Analysis

For combination drug treatments, three different synergy analyses have been conducted, including the fraction product method of Webb[9] and the Bliss synergy method[10] using *SynergyFinder 2.0* software[11].

AML xenograft mouse model

30 female NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ mice (also referred to as NOD scid gamma or NSG), 6.5 ± 0.3 weeks old, were procured from Australian BioResources (ABR) and acclimatised for one week. Animals had unrestricted access to standard diet (provided as pellets) and water, and were kept under 12h light/12h dark cycles in individually ventilated cages. Twenty five animals were xenografted with MOLM-13-luc ($5x10^5$ cells/animal) via tail vein injection. At engraftment, the animals were 10.6 ± 0.1 weeks old. Drug treatment was started immediately after the presence of luc+ cells in the body was confirmed with Bioluminescence Imaging (BLI), which was carried out between Days 5 and 7 post-engraftment, following an IP injection of 3mg luciferin. The 20 animals

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with the highest BLI readings were selected and randomised into four groups of 5 animals. The remaining five animals (i.e. with the lowest BLI readings) were kept for observation but did not receive any drug treatment (untreated control).

Group 1 (Vehicle control). IP injection of 50µL of solubilizing vehicle solution was administered separately. Starting from the beginning of the 2nd week (i.e. Day 8 of treatment), 100 µL of solubilizing vehicle solution was administered every other day, three times a week via tail-vein injection (IV).

Group 2 (Decitabine alone). IP injection of 0.5 mg/kg decitabine in PBS containing 1% DMSO, 5 days a week (100 µL).

Group 3 (Zantrene alone). IP injection of 5 mg/kg Zantrene in solubilizing vehicle solution (50µL) on Day 3 and Day 5. Starting from the beginning of the 2nd week, 10 mg/kg Zantrene in solubilizing vehicle solution was administered every other day, three times a week, via tail-vein injection (100µL).

Group 4 (Zantrene + decitabine combination). IP injection of 0.5 mg/kg decitabine in PBS containing 1% DMSO, 5 days a week (100µL). IP injection of 5 mg/kg Zantrene in solubilizing vehicle solution (50µL) on Day 3 and Day 5. Starting from the beginning of the 2nd week, 10 mg/kg Zantrene in solubilizing vehicle solution was administered every other day, three times a week, via tail-vein injection (100µL).

Group 5 (untreated control). Observed only.

Monitoring and imaging

Body weight was determined, and mortality counts and observations for signs of toxicity were made. Gross autopsy was performed on animals that either died or were sacrificed at humane endpoints.

Bioluminescence imaging was conducted weekly. Mice were injected with 3mg D-luciferin (Promega #P1043, solubilised in PBS) by intraperitoneal injection with a 30-gauge needle and 1mL syringe. Mice were anaesthetised using 2-5% isoflurane (500-1000 CC/min) and imaged approximately 10 minutes after D-luciferin injection using a Xenogen IVIS Spectrum imager (PerkinElmer, Waltham, MA, USA). Images were analysed using Living Image software. A rectangular region of interest (ROI) was placed over each individual mouse, and total flux (p/s) within each ROI was calculated.

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-ENDS-

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.

Professor 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 and proteasome inhibitors 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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