Complementary activity of the vascular disruption agent BNC105 and the hypoxia-activated prodrug Evofosfamide (TH-302) in suppressing the growth of preclinical renal and breast solid tumors

Tina C. Lavranos[1], Daniel Inglis[1], Donna Beaumont[1], Annabell F. Leske[1], Chloe Hawkins[1], Michaela Scherer[1], Charles Hart[2], Jessica Sun[2], Qian Liu[2], Brian Elenbaas[3], Gabriel Kremmidiotis[1]

[1]Bionomics Ltd, Australia, [2]Threshold Pharmaceuticals, USA, [3]EMD Serono Inc, USA

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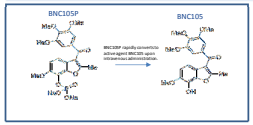
Abstract #B174

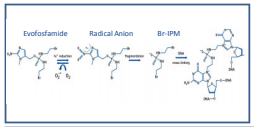
A complementary therapeutic approach

BNC105 is a vascular disruption agent that selectively disrupts tumor blood vessels resulting in hypoxia and necrosis (Kremmidiotis *et al* 2010). Evofosfamide (previously known as TH-302) is an alkylating agent with DNA cross-linking ability (Sun *et al* 2012). Evofosfamide is reduced by intracellular reductases, and under hypoxic conditions selectively releases Br-IPM. Evofosfamide has little activity under normoxic conditions, but is highly cytotoxic under hypoxic conditions. Both BNC105 and Evofosfamide are currently being independently evaluated in Phase I-III clinical trials.

Our studies sought to establish the potential therapeutic combination of BNC105 and Evofosfamide. It was reasoned that tumor hypoxia caused by BNC105 will result in increased intra-tumoral activation of Evofosfamide. A greater localized conversion of Evofosfamide to Br-IPM would increase the tumor kill achieved.

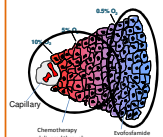
- ***BNC105 causes a dramatic increase in hypoxia within the tumor via selective destruction of tumor neovasculature***
- ***This may potentiate greater hypoxic activation of Evofosfomide to Br-IPM within a larger area of the tumor including diffusion to surrounding non-hypoxic tumor regions***
- ***BNC105 treatment may also cause a "lock-in" effect of Br-IPM, extending retention, exposure and cellular necrosis to a greater tumor area***
- ***Complementary Clinical regimen - Day 1, 8 of a 21 day cycle - as Evofosfamide is dosed the Phase III Soft Tissue Sarcoma Trial (NCT01440088)***





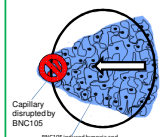
Evofosfamide + chemotherapeutic

The second agent targets a different compartment in the tumor microenvironment



Evofosfamide + BNC105

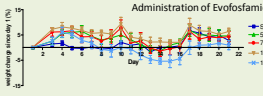
BNC105 increases the magnitude and extent of tumor hypoxia for Evofosfomide activation by selective disruption of tumor vasculature



A well tolerated combination

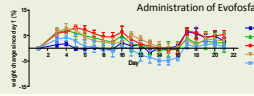
BNC105 causes an acute disruption of tumor vasculature leading to a rapid increase in tumor hypoxia. Studies were designed to balance the interplay between BNC105 induced peak hypoxia and the delivery of Evofosfamide to tumor for maximum activation while maintaining safety. Safety was first monitored using Balb/c mice (non tumor bearing) dosed with BNC105P dosed at 50-150mg/kg - Day 1 and 8 of a 21 day cycle and animals weighed daily. Groups (n=3/group)

Administration of Evofosfamide at increasing dose levels 1 hr **pre** BNC105P administration



Scenario → Delivery of Evofosfamide for activation by acute hypoxia caused by BNC105 without risk of exclusion from the tumor caused by vessel occlusion

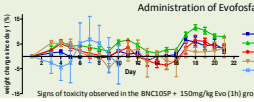
Administration of Evofosfamide at increasing dose levels **concurrently*** with BNC105P



Scenario → Delivery of Evofosfamide for simultaneous activation by acute hypoxia caused by BNC105

*Evofosfamide is dosed i. p. immediately following BNC105P i.v.

Administration of Evofosfamide at increasing dose levels 1 hr **post** administration of BNC105P



Scenario → tumor has begun its transition to a more hypoxic environment and is then treated with Evofosfamide for faster activation

Signs of toxicity observed in the BNC105P + 150mg/kg Evo (1h) group (ruffled fur, lethargy, signs of diarrhoea, hunched posture)

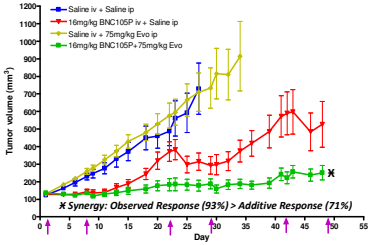
Concurrent administration of Evofosfamide @ 75mg/kg and BNC105 @ 10 mg/kg is well tolerated and has been used in the efficacy studies

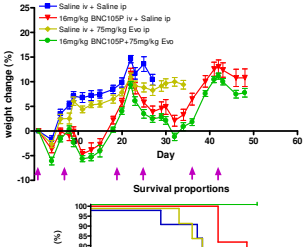
Based on these data, concurrent administration is the best schedule for clinical translation

Efficacy in triple negative breast cancer model

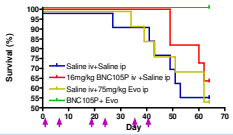
Balb/c nude mice bearing subcutaneous MDA-MB-231 tumors were dosed with 16mg/kg BNC105P and 75mg/kg Evofosfamide. Tumors were measured for volume (width x length x height = mm³) and animals weighed three times weekly. Animals were dosed concurrently on Day 1 and 8 of three 21 day cycles. Groups (n=15/group).

MDA-MB-231 Tumor volume (mm³) +/- SEM





Survival proportions



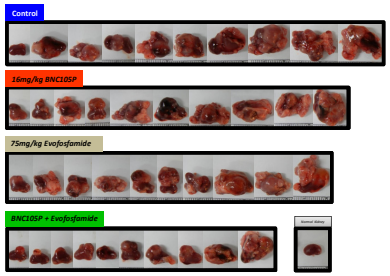
% TGI compared to control at Day 27	
75mg/kg Evofosfamide	9%
16mg/kg BNC105P	68%
16mg/kg BNC105P + 75mg/kg Evofosfamide	**93% Synergistic response** (Additive Response 71%)

The combination synergistically inhibits growth of MDA-MB-231 breast cancer xenografts with a tumor growth inhibition of 93% compared to vehicle control

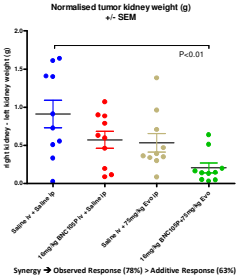
Repeat dosing in tumor bearing animals is well tolerated

Efficacy in orthotopic renal cancer model

Balb/c mice bearing orthotopic renal tumors were dosed with 16mg/kg BNC105P and 75mg/kg Evofosfamide. Animals were dosed concurrently on Day 1 and 8 (n=10/group). Tumor growth inhibition (tumor weight) was measured on Day 10 of treatment.



Normalised tumor kidney weight (g) +/- SEM



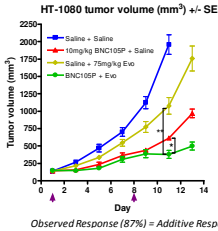
Synergy → Observed Response (78%) > Additive Response (63%)

Combination of BNC105 with Evofosfamide led to synergistic inhibition in an orthotopic model of renal cancer and was well tolerated

Efficacy in soft tissue sarcoma

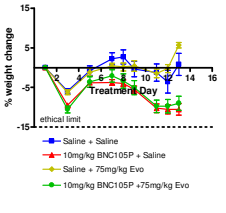
Balb/c nude mice bearing subcutaneous HT-1080 tumors were dosed with 10mg/kg BNC105P and 75mg/kg Evofosfamide. Tumors were measured for volume (width x length x height = mm³) and animals weighed three times weekly. Animals were dosed concurrently on Day 1 and 8 (n=10/group).

HT-1080 tumor volume (mm³) +/- SEM



% TGI compared to control at Day 11	
75mg/kg Evofosfamide	49%
10mg/kg BNC105P	74%
10mg/kg BNC105P+ 75mg/kg Evofosfamide	87%

*p<0.05
**p<0.001

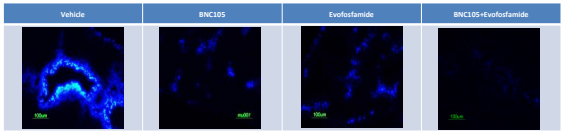


Observed Response (87%) = Additive Response (87%)

The combinations significantly inhibits the growth of HT-1080 soft tissue sarcoma xenografts with a tumor growth inhibition of 87% compared to vehicle control without adding toxicity

Driver of efficacy

Balb/c nude mice bearing subcutaneous HT29 tumors (400-600mm³) were dosed with a single dose of 16mg/kg BNC105P and 75mg/kg Evofosfamide (n=5/group). Animals were dosed concurrently then 24 hours later injected with Hoechst 33342, a marker of tumor perfusion, tumors were collected and sectioned. Images are representative tumor sections.



Following combination treatment of BNC105 with Evofosfamide a substantial reduction in tumor perfusion was observed 24 hours after treatment which would hamper tumor recovery.

Summary

BNC105 and Evofosfamide is a well tolerated combination with dual complementary action designed to leverage the hypoxic activation of Evofosfamide

BNC105 and Evofosfamide can be administered concurrently Day 1 and 8 of a 21 day cycle – compatible from a clinical logistic perspective

The combination delivered synergistic tumor growth inhibition activity in a MDA-MB-231 triple negative breast tumor model and the RENCA renal orthotopic tumor model

Significant tumor growth inhibition was observed in the soft tissue sarcoma model when treated with BNC105 and Evofosfamide compared to treatment with the monotherapies

Evofosfamide and BNC105 are in clinical development and have not been approved for treatment

References:

- Kremmidiotis G1, Leske AF, Lavranos TC, Beaumont D, Gasic J, Hall A, O'Callaghan M, Matthews CA, Flynn. BNC105: a novel tubulin polymerization inhibitor that selectively disrupts tumor vasculature and displays single-agent antitumor efficacy. BMol Cancer Ther. 2010 Jun;9(6):1562-73
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A: 31 Dalgleish Street, Thebarton, South Australia, 5031, Australia **E:** tlavranos@bionomics.com.au **W:** www.bionomics.com.au