Bionomics Ltd

Evaluation of the anti-cancer effects of the tumor selective Vascular Disruption Agent BNC105 in preclinical renal cancer models

Tina C. Lavranos[1,2], Annabell F. Leske[1], Donna M. Beaumont[1], Jelena Gasic[1], Gabriel Kremmidiotis[1]

[1]Bionomics Ltd, 31 Dalgleish Street Thebarton, SA 5031 Australia; [2]Neurofit SAS Bd Sébastien Brant, Bioparc—Parc Innovation 67400 Illkirch France

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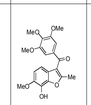
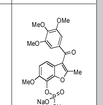
Introduction

The disruption of blood vessels that feed tumors represents one of the most promising therapeutic strategies for treating cancer. Bionomics cancer drug BNC105 is a tubulin targeting dual acting vascular disruption agent with cytotoxic ability in solid tumors. To facilitate intravenous administration of BNC105 in animals the phosphate ester of this compound was synthesized (BNC105P). The structure of BNC105 and BNC105P are shown here.

Compound	Structure	Compound	Structure
BNC105		BNC105P	

Renal cell carcinoma (RCC) is the most common malignant lesion of the kidney. Current treatment options are limited and the 5 year survival rate for metastatic RCC is estimated to be less than 10%.

Targeted therapies have improved clinical outcomes over the past few years with new therapeutics constantly in development. Therapeutics such as Sutent (multi-targeted tyrosine kinase inhibitor) provide patients with 11 months progression free survival (PFS), Axitinib (multi-targeted tyrosine kinase inhibitor) or Tivozanib (VEGF receptor tyrosine kinase inhibitor) provide patients with 7 months PFS and Afinitor (mTOR inhibitor) give patients 4.9 months PFS.

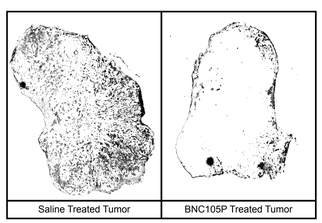
BNC105 is currently in Phase II Clinical trials in mesothelioma and renal cancer. We have conducted a number of preclinical evaluations that provide a strong rationale of potential therapeutic utility of BNC105 in combination with agents that target mTOR signalling or as a follow-on therapeutic option to current therapeutics in use in the clinic

Results: Xenograft model

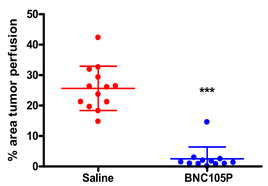
Figure 1: BNC105 acts as a vascular disrupting agent in Caki-1 renal tumor xenografts.

Balb/c *nu/nu* 6-8 week old females were subcutaneously inoculated with Caki-1 cells (human kidney clear cell carcinoma ATCC cat# HTB-46). Tumor xenografts were allowed to grow to a mean volume greater than 340mm^3 prior to treatment. Animals were then treated with a single i.v. administration of BNC105P or saline (vehicle control). At 4 hours post administration of the compound, animals were injected with H33342 which acts as a marker of blood perfusion in tumors and normal tissues.

1A



1B



Vascular shutdown was assessed by quantitation of H33342 staining in tumor sections using ImageJ software and expressed as percentage of total tumor area. Statistical analysis was performed using a Two-tailed T test (***=P<0.001). Representative Caki-1 tumor images from BNC105P and Saline treated animals (Figure 1A). A statistically significant reduction in vascular perfusion following treatment with BNC105P is seen with a mean decrease of 90% (Figure 1B).

References

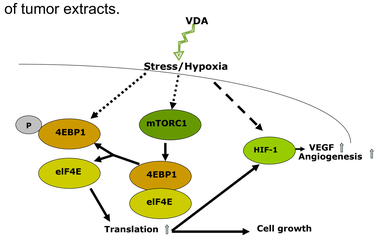
(1) Kremmidiotis *et al.* 2010
Molecular Cancer Therapeutics 9 (6):1562 June *2010*.

Figure 2: Evidence of involvement of mTOR/4EBP1 pathway following vascular disruption of Caki-1 renal tumors with administration of BNC105P.

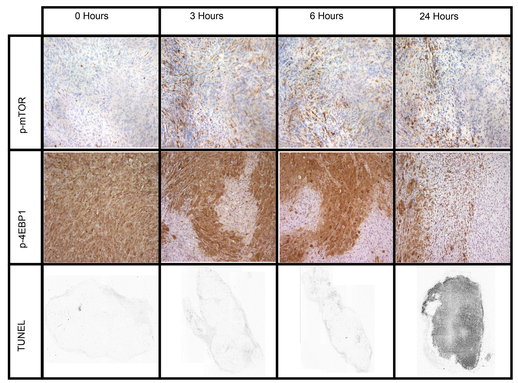
Involvement of the mTOR/4EBP1 pathway was assessed in tumors treated with BNC105P (Figure 2A).
Histological changes were evident in tumor sections examined following i.v. administration of BNC105P to Balb/c *nu/nu* female mice with subcutaneous Caki-1 renal tumor xenografts. A number of time points over a 24 hr period were examined. At 3hrs post BNC105P administration phosphorylated-mTOR (ser2448) (Cell Signaling Technologies, Cat# 29765) staining of sections was up-regulated, with specific staining evident at 6 and 24 hrs around areas of necrosis. Phosphorylated-4E-BP1(Thr37/46) (Cell Signaling Technologies, Cat# 2855) showed down-regulation at 3 and 6 hrs post BNC105P administration, with small areas of expression at 24 hrs. Extensive areas of apoptosis were evident at 24 hrs as detected by TUNEL (Roche, Cat# 11 684 795 910) (Figure 2B).

In addition, a transient increase in expression of HIF-1α (Cell Signaling Technologies Cat# 3716) was observed in tumors at 3 and 6 hrs post BNC105P administration (Figure 2C), following western analysis of tumor extracts.

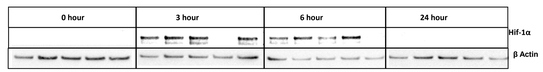
2A



2B



2C



Conclusions

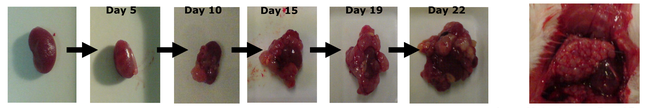
- BNC105 acts as a vascular disrupting agent in Caki-1 subcutaneous renal tumor xenografts.
- BNC105 induced vascular shutdown in Caki-1 renal tumors involves the mTOR/4EBP1 pathway.
- BNC105 treatment induces vascular shutdown in both primary renal tumor lesions and lung metastases in the RENCA orthotopic renal cancer model with normal kidney areas remaining unaffected.
- BNC105 causes tumor growth inhibition in the RENCA orthotopic renal cancer model comparable to inhibition seen with standard of care drug Sutent.
- Vasculature in normal tissues is not affected by BNC105 even at doses 8 times higher than the minimum efficacious dose.

Results: Orthotopic syngeneic renal model

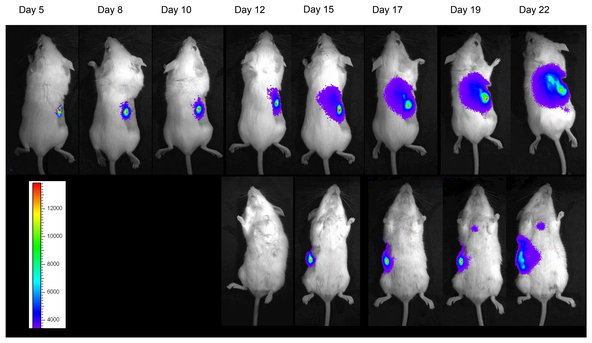
Balb/c 6-8 week old female mice were inoculated with RENCA cells (mouse renal adenocarcinoma; ATCC cat# CRL-2947) under the kidney capsule. Tumor growth was examined in the kidney over 22 days and metastasis observed in the lungs as shown for day 15 (Figure 3A). This mouse tumor model accurately mimics the progression of human adult renal cell carcinoma specifically with reference to spontaneous metastasis to lungs. The orthotopic renal model was also established using the RENCA cell line with a cherry/luciferase tag* and imaging conducted using the Xenogen IVIS 100 observing tumor development for 23 days with lung metastasis also visible using this model (Figure 3B shows a representative mouse). Tumor growth inhibition was assessed following treatment with 32mg/kg BNC105P administered i.v. on Day 1 & 8 of treatment and 40mg/kg Sutent administered p.o. daily. Figure 3C shows examples of tumors at Day 10 of treatment with the graph showing kidney weights of animals treated (n=10). Both BNC105 and Sutent have similar therapeutic benefit in reducing tumor growth, with BNC105P giving tumor/kidney weight of 0.33± 0.15 compared to Sutent 0.30± 0.24. Weights in corresponding controls were 0.67± 0.14 (saline control) and 0.75± 0.17 (Sutent vehicle control).
A vascular disruption assay (1) was conducted to evaluate the vascular disruption activity of BNC105 in the RENCA orthotopic syngeneic renal tumor model. Figure 3D shows disruption of tumor vasculature as early as 3hrs following i.v. administration of BNC105P compared to saline treated animals. The vasculature in the normal tissue of the inoculated kidney was not affected by BNC105. Lung metastatic lesions were seen in a number of animals. Vasculature in these metastatic lesions was disrupted by BNC105P treatment (Figure 3E). BNC105P does not affect the perfusion in normal tissues at doses eight times higher than the minimal efficacious dose when assessed in the heart, un-inoculated kidney, liver and spleen (Figure 3F). BNC105P has a NOAEL of 80mg/kg.

Figure 3: BNC105 Evaluation in the RENCA orthotopic renal tumor model in mice.

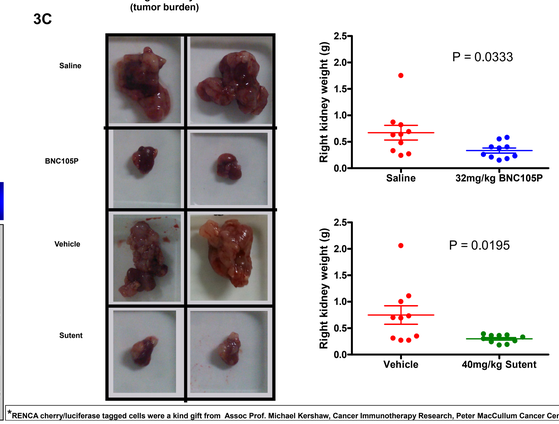
3A



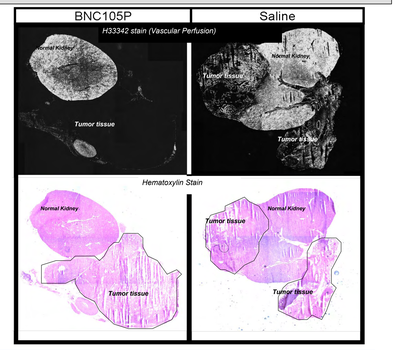
3B



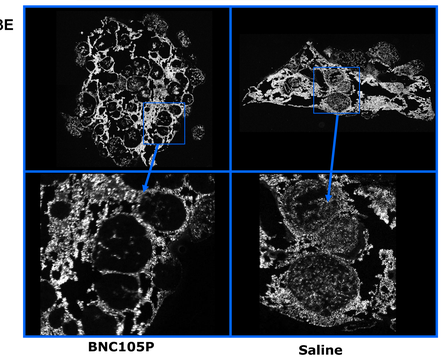
3C



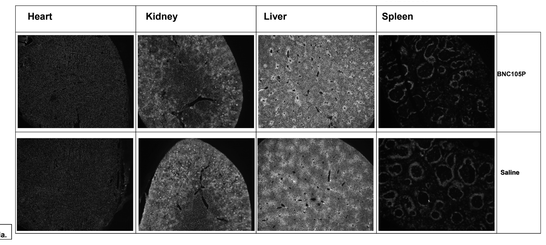
3D



3E



3F



*RENCA cherry/luciferase tagged cells were a kind gift from Assoc Prof. Michael Kershaw, Cancer Immunotherapy Research, Peter MacCullum Cancer Centre, Victoria, Australia.