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

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Abstract

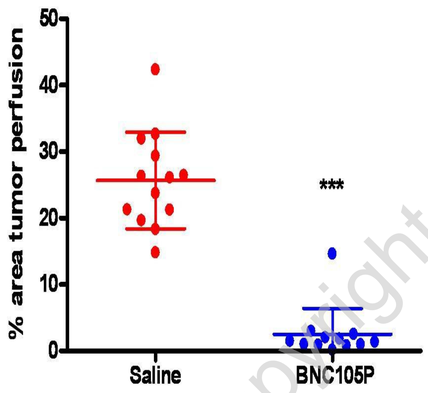
The disruption of blood vessels that feed tumours represents one of the most promising therapeutic strategies for treating cancer. Bionomics cancer drug BNC105 is a tubulin targeting dual acting vascular disruption agent with cytotoxic ability in solid tumours. It is currently in Phase II Clinical trials in mesothelioma and renal cancer. We have conducted a number of preclinical evaluations that provide a strong rationale for combining BNC105 with agents that target mTOR signalling. BNC105 activity operates through the selective disruption of tumor blood vessels. Over 95% of blood flow is disrupted in tumors grown in xenograft or orthotopic syngeneic models. In the renal cancer setting we evaluated the anti-cancer effects of BNC105 in a Caki-1 xenograft model and a syngeneic RENCA orthotopic model. Animals carrying Caki-1 solid tumors were treated with a single dose of BNC105P (prodrug formulation). Disruption of blood flow within the tumors was observed as early as 3 hr post-treatment. Similarly, blood flow disruption was seen in mice carrying solid tumors orthotopically inoculated in the kidney capsule using the mouse renal cancer cell line RENCA. Interestingly, BNC105 also caused blood flow disruption in lung metastatic lesions seen in a number of the animals inoculated with RENCA. Vasculature in all normal tissues examined remained intact. Tumor re-vascularisation following BNC105P administration was observed 2 days following treatment. Immunohistochemical analysis in BNC105 treated tumors revealed that a number of proteins involved in the mTOR signalling pathway exhibit expression changes consistent with activation of this pathway. Up-regulation in phosphorylated mTOR, Hif1α, VEGF and down-regulation of phosphorylated 4EBP1 were observed. The consequences of targeting mTOR or VEGF in combination with BNC105 are currently under investigation in these preclinical models.

Results:Xenograft model

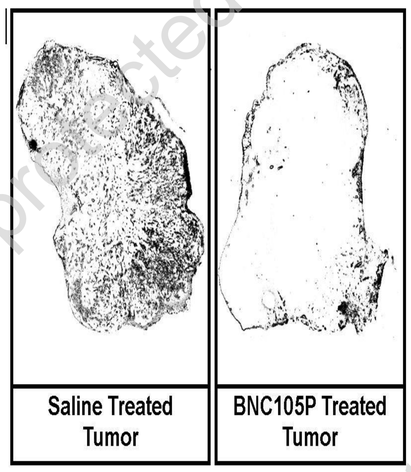
Figure 1: BNC105 acts as a vascular disrupting agent in Caki-1 renal tumor xenografts.

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 tumours 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). A statistically significant reduction in vascular perfusion following treatment with BNC105P is seen with a mean decrease of 90% (Figure 1A). Representative Caki-1 tumor images from BNC105P and Saline treated animals (Figure 1B).

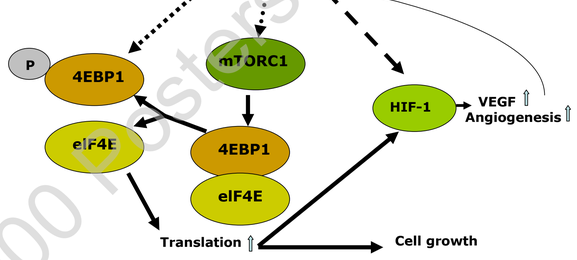
Figure 2: Evidence of involvement of mTOR/4EBP1 pathway following vascular disruption of Caki-1 renal tumors with administration of BNC105P.

Involvement of the mTOR/4EBP1 pathway was assessed in tumors treated with BNC105P (Figure 2A).

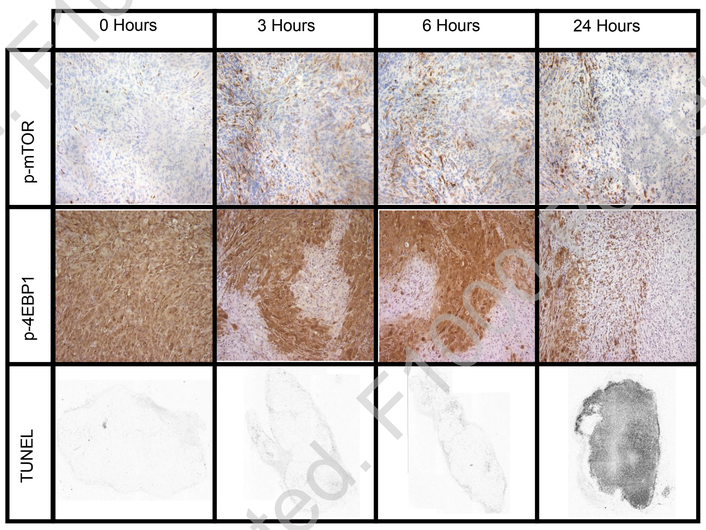
Histological changes were evident in tumor sections examined following i.v. administration of BNC105P to Balb/c *nu/nu* female mice with subcutaneous Caki-1 renal tumor xenografts. A number of time points over a 24 hr period were examined. At 3hrs post BNC105P administration p-mTOR (ser2448) (Cell Signaling Technologies, Cat# 29765) staining of sections was up-regulated, with specific staining evident at 6 and 24 hrs around areas of necrosis. Phosphorylated-4EBP1 (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.

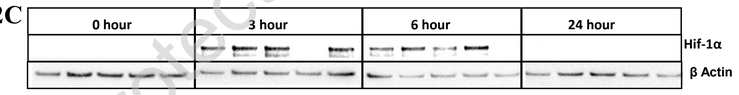
2A



2B



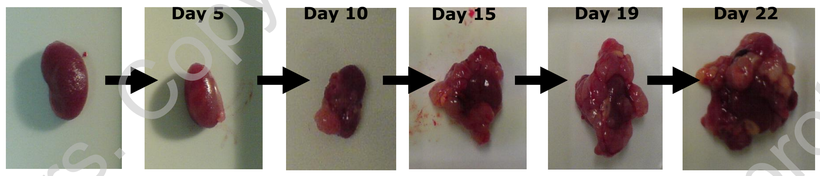
2C

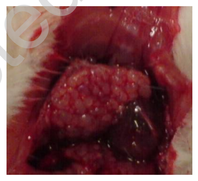


Results: Orthotopic syngeneic model

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

3A





Balb/c 6-8 week old female mice were inoculated with RENCA cells (mouse renal adenocarcinoma; ATCC cat# CRL-2947) under the kidney capsule. Tumour growth was examined in the kidney over 22 days and metastasis observed in the lungs (Figure 3A) as shown at day 15.

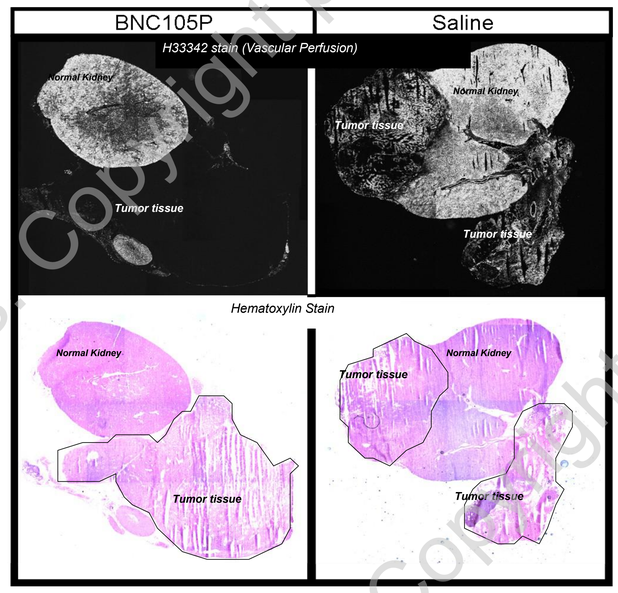
This mouse tumor model accurately mimics the progression of human adult renal cell carcinoma specifically with reference to spontaneous metastasis to lungs.

A vascular disruption assay (Kremmidiotis et al; 2010) was conducted to evaluate BNC105 in the RENCA orthotopic syngeneic renal tumor model.

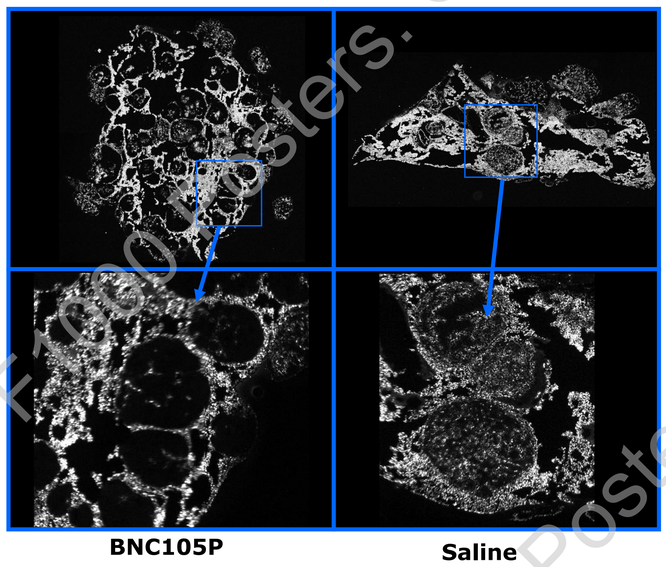
Figure 3B shows disruption of tumour vasculature as early as 3hrs following i.v. administration of BNC105P compared to saline treated animals. The vasculature in the normal part of the inoculated kidney was not affected by BNC105.

Dual site, tumor vascular disruption was seen in animals with metastasis in the lung following BNC105P treatment (Figure 3C).

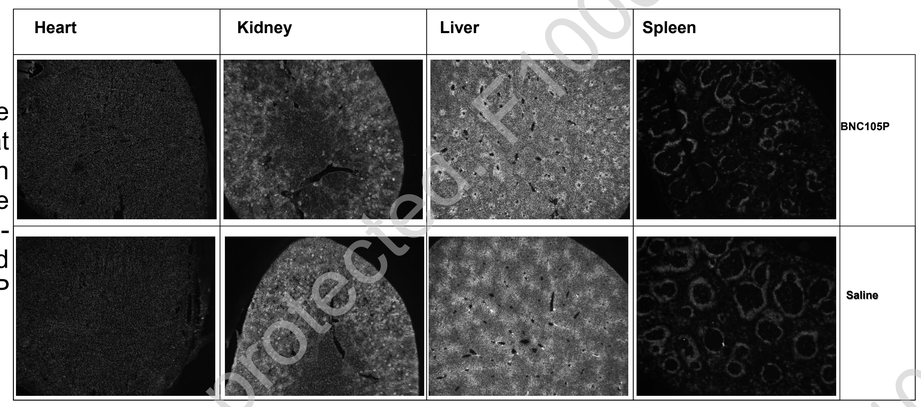
3B



3C



3D



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 3D). BNC105P has a NOAEL of 80mg/kg.

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 tumor lesions in the RENCA orthotopic renal cancer model with normal kidney areas remaining unaffected.
- BNC105 causes vascular shutdown in lesions of lung metastasis in the RENCA orthotopic renal cancer model.
- Vasculature in normal tissues is not affected by BNC105 even at doses 8 times higher than the minimum efficacious dose.

References

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