BNC105 is a tubulin polymerisation inhibitor that selectively disrupts tumor vasculature. BNC105P is the phosphorothioate analog of BNC105. A single iv administration of BNC105P is able to disrupt >90% of blood perfusion in tumors in a number of animal models, including breast, colon, prostate and brain cancer. Disruption of tumor vasculature occurs 1 to 3 days after BNC105P treatment and persists for 48 hrs. In in vitro evaluations, BNC105P was shown to have selective activity for human umbilical vein endothelial cells (HUVEC) cultured under angiogetic growth factor stimulated conditions compared to cells cultured to induce a quiescent growth state (Kremesti et al 2010). In comparison to vascular disrupting agents (VDAs) developed, AVTs and other tubulin targeting agents, BNC105P selectively reduces endothelial cell ability to switch quiescent states (Table 1). BNC105 is currently under clinical evaluation in melanoma patients as a single agent and in combinations with other immunotherapies in patients with metastatic melanoma.

To support a clinical rationale for the evaluation of BNC105 in lung and breast cancer, we have conducted preclinical evaluation in animal models of melanoma and lung cancer. The parameters assessed included the ability of BNC105 to cause vascular disruption in both primary tumors and metastatic lung lesions, tumor growth inhibition and animal survival. Evaluations were also undertaken to examine the comparative efficacy of BNC105 against Pemetrexed and Cisplatin, the standard of care for patients with melanoma.

BNC105 was assessed as a combination partner with the NLCLC standard of care Gemcitabine and Cisplatin.

Table 1. BNC105 (P)<0.0001 compared to BNC105P or Cisplatin monotherapies.

Vascular Disruption

Complete vascular shutdown in lung xenografts is observed 24 hours after BNC105P treatment.

A single iv administration of BNC105P is able to disrupt >90% of blood perfusion in tumors in a number of animal models, including breast, colon, prostate and brain cancer. Disruption of tumor vasculature occurs 1 to 3 days after BNC105P treatment and persists for 48 hrs. In in vitro evaluations, BNC105P was shown to have selective activity for human umbilical vein endothelial cells (HUVEC) cultured under angiogenic growth factor stimulated conditions compared to cells cultured to induce a quiescent growth state (Kremesti et al 2010). In comparison to vascular disrupting agents (VDAs) developed, AVTs and other tubulin targeting agents, BNC105P selectively reduces endothelial cell ability to switch quiescent states (Table 1). BNC105 is currently under clinical evaluation in melanoma patients as a single agent and in combinations with other immunotherapies in patients with metastatic melanoma.

Tumor models

- MCT-211H (human melanoma), ATCC CRL-2118 cells at 1 x 10^6 cells were inoculated subcutaneously in Balb/c nu/nu mice. For perfusion experiments, animals were allowed to develop a small tumor volume of 150mm^3. For survival studies, tumors were allowed to reach a mean tumor volume of 180mm^3 prior to treatment commencement.
- C57 BL/6J mouse lung xenograft model: ATCC CRL-2268 cells at 1 x 10^6 cells were inoculated subcutaneously in Balb/c nu/nu mice. Tumors were allowed to reach a mean tumor volume of 200mm^3 prior to treatment commencement.
- RENCA cells (mouse renal adenocarcinoma; ATCC CRL 6815) were subcutaneously transplanted in the right flank of Balb/c nu/nu mice. Tumors were allowed to develop a small tumor volume of 100mm^3 prior to treatment commencement.

Immunohistochemistry

- H33342 fluorescence micrographs were obtained using Olympus BX51 microscope with Magnafire image acquisition software. Composite tumor images were prepared using Image J Image Processing and Analysis software.
- Apoptosis was evaluated using TUNEL staining (Click-IT Assay) in vehicle-treated and BNC105P treated tumors. TUNEL positive nuclei were identified and counted using aqimage software.
- BNC105P treated tumors show high levels of CD31 and CD34 staining indicative of vessel recruitment in tumor sections was performed using a biotinylated CD31 (BD Biosciences) antibody, ExtrAvidin Peroxidase and counter stained with Mayers hematoxylin. Images were captured on an Olympus BX51 microscope with Magnafire image acquisition software.

Conclusions

- Significant vascular disruption was demonstrated in primary mesothelioma lesions.
- Vascular disruption in mesothelioma tumors lead to widespread increases in apoptosis and loss of CD31 and CD34 positive staining vessels with repeat dosing leading to improved survival comparable to perfusion treatment.
- BNC105P demonstrated significant vascular disruption in animal models of primary lung tumors and metastatic lung lesions.
- BNC105P in combination with standard of care agents (Gemcitabine and Cisplatin) for NSCLC resulted in prolongation of tumor regression, enhanced delays in tumor growth and increased survival of animals bearing Calu-6 NSCLC xenografts.

Figure 1. Evaluation of survival with therapy in MCT-211H xenograft model. Balb/c nude mice bearing MCT-211H tumors treated with BNC105P (30mg/kg) on days 2 and 9 of 20 day cycle. Pemetrexed 3 x weekly (A), 3 x days of 5 x 10^6 for 2 weeks in Calu-6 xenografts (B) and 1x weekly (C) in 20 day cycle. A. Survival curve for BNC105P and Control animals. B. Survival Curve for Pemetrexed and Control animals. C. Survival Curve for combination therapy.

Figure 4. Evaluation of survival with therapy in MCT-211H xenograft model. Balb/c nude mice bearing MCT-211H tumors treated with BNC105P (30mg/kg) on days 2 and 9 of 20 day cycle. Pemetrexed 3 x weekly (A), 3 x days of 5 x 10^6 for 2 weeks in Calu-6 xenografts (B) and 1x weekly (C) in 20 day cycle. A. Survival curve for BNC105P and Control animals. B. Survival Curve for Pemetrexed and Control animals. C. Survival Curve for combination therapy.

Figure 5. Combination therapy with BNC105P and Gemcitabine. Balb/c nude mice bearing Calu-6 tumors treated with BNC105P (30mg/kg) on days 2 and 9 of 20 day cycle. Gemcitabine (100mg/kg) on days 1, 8, 15 and 22 of cycle. BNC105P + Gemcitabine as described for monotherapies. A. Mean Tumor volume (mm^3) ± SE. B. Survival curves (Median Survival: months). Vehicle (V) vs. BNC105P + Gemcitabine (B+G).

Figure 6. Combination therapy with BNC105P and Cisplatin. Balb/c nude mice bearing Calu-6 tumors treated with BNC105P (30mg/kg) on days 2 and 9 of 20 day cycle. Cisplatin (150mg/kg) on days 1, 8, 15 and 22 of cycle. BNC105P + Cisplatin as described for monotherapies. A. Mean Tumor volume (mm^3) ± SE. B. Survival curves (Median Survival: months). Vehicle (V) vs. BNC105P + Cisplatin (B+C).