The tubulin-targeting agent BNC105 potentiates the efficacy of immune checkpoint inhibitors in preclinical models of colorectal cancer

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Abstract #B92

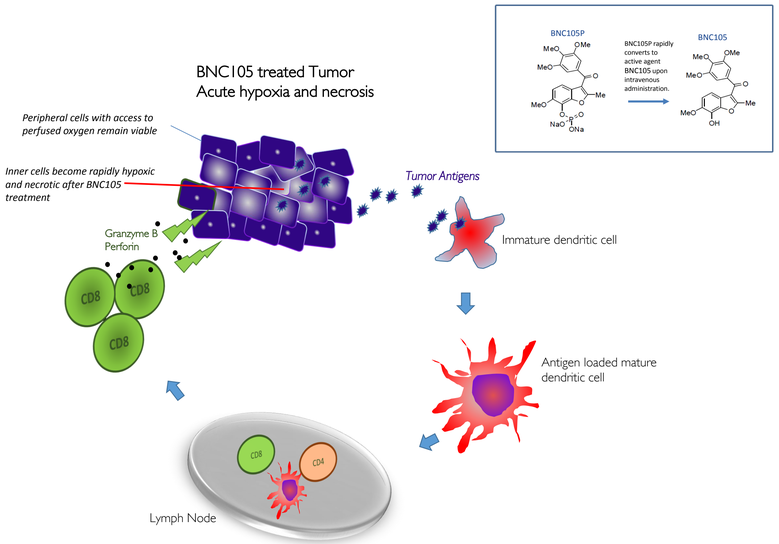
A complementary therapeutic approach

BNC105 is a a tubulin depolymerisation agent that exerts anti-cancer efficacy through the selective destruction of solid tumor microvasculature and direct suppression of tumor cell proliferation (Kremmiditios *et al* 2010). A single IV dose of BNC105 causes a very high degree of tumor hypoxia leading to >95% necrosis in rodent models.

Despite the tumor necrosis, tumor recovery becomes evident by day 2 following BNC105 treatment. Recent evidence demonstrates the utility of microtubule-depolymerizing agents in enhancing the conversion of dendritic cells into antigen presenting cells for greater anti-tumor immunity. These observations led us to investigate the potential therapeutic benefit of combining BNC105 with the checkpoint inhibitors that target PD-1 or CTLA-4.

- ***The tumor growth inhibition efficacy of BNC105 is driven by the selective destruction of tumor vasculature which has been shown to result in acute hypoxia and tumor necrosis.***
- ***The rapid induction of tumor stress and necrosis can lead to an increased release of tumor antigens which are taken up by immature dendritic cells activating them to generate tumor specific cytotoxic T cells***
- ***The up-regulation of hypoxic factors such as HIF-1a which co-localise with PD-L1 act as a protectant of the tumor against the resulting cytotoxic T lymphocytes***
- ***A level of immune-tolerance may also be established by the large antigen release after BNC105 treatment without further intervention***
- ***Co-treatment of BNC105 with cytotoxic T cell protectants such as anti-PD-1 or anti-CTLA-4 therapy could leverage the effectiveness of both treatments making full use of their complementary action.***

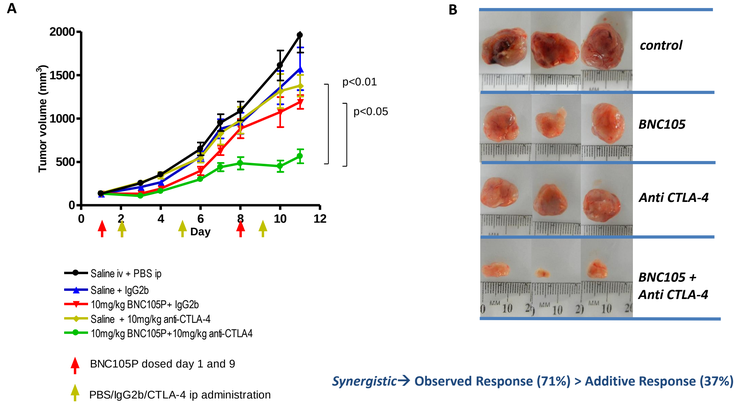
Studies were conducted to assess the potential *in vivo* therapeutic utility of these combinations with BNC105. The *in vivo* models utilised were xenografts of the murine colorectal cancer cell lines MC38 and CT26.



Complementary treatment of BNC105 with anti PD-1 or CTLA-4 potentiates the viability and effectiveness of tumor specific cytotoxic T cells

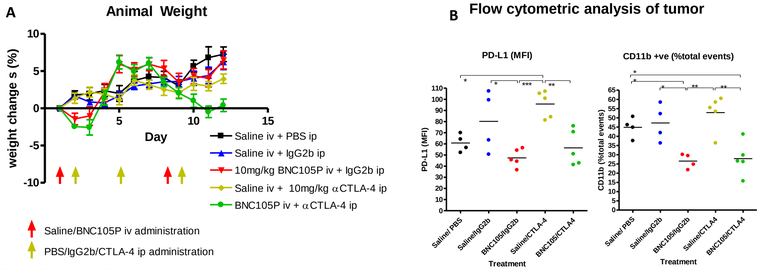
Efficacy in a murine colon cancer model with anti CTLA-4

Balb/c mice were inoculated subcutaneously with CT26 cells. When tumors reached an average volume of approximately 100 - 150mm^3 animals were randomised into 5 groups of 10 mice per group. BNC105P (10mg/kg) i.v. was administered on Days 1 and 8 and the anti-CTLA-4 antibody (Clone 9D9) was administered at 10mg/kg i.p. on Days 2, 5, and 9.



***Synergistic*→ Observed Response (71%) > Additive Response (37%)**

Animals treated with the BNC105 + anti-CTLA-4 combination in the CT26 colorectal cancer model experienced greater inhibition of tumor growth compared to animals treated with either BNC105 or anti-CTLA-4 alone. **A)** Significant tumor growth inhibition over the treatment period in the combination group compared to the control groups was demonstrated. On Day 11 a 27% tumor growth inhibition was observed in BNC105 treated animals, 14% tumor growth inhibition in anti-CTLA-4 treated animals and 71% tumor growth inhibition in animals treated with the BNC105 + anti-CTLA-4 combination. **B)** representative tumors from each group at termination.



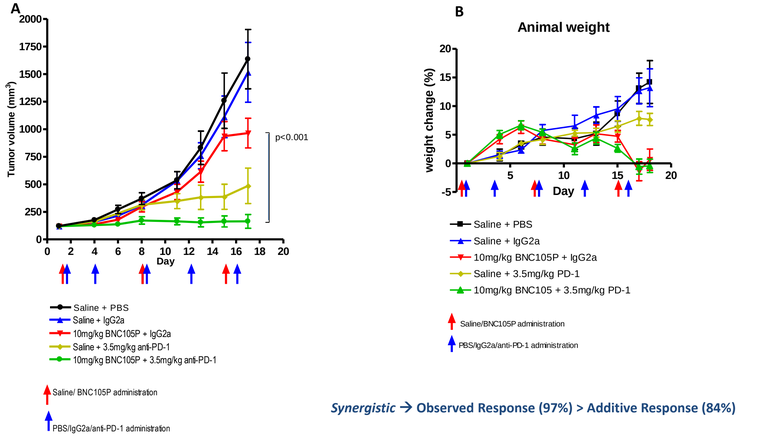
BNC105 + anti CTLA-4 is a well tolerated combination

Animal weights and health monitored throughout the treatment period demonstrate that BNC105 + anti CTLA-4 is a well tolerated combination. Previous literature shows that CTLA-4 blockade results in PD-L1 up-regulation, which drives the escape of tumors from anti CTLA-4 treatment, we demonstrated this change in the regulation of tumor cell PD-L1 but also showed that BNC105 treatment maintained PD-L1 at control levels potentially reducing the ability of tumors to escape via this mechanism when used in combination. There was a significant reduction in the number of tumor infiltrating macrophages (CD11b+) after treatment with BNC105, in both the monotherapy and combination group. In the context of the reported tumor-promoting properties of tumor infiltrating macrophages, this observation suggests that the reduction of CD11b+ cells in tumors by BNC105 may be one of the mechanisms of its underlying anti-cancer action. (*p<0.05, **p<0.01, ***p<0.0001)

Combination of BNC105 with anti-CTLA-4 led to synergistic tumor growth inhibition in a murine model of colon cancer

Efficacy of combination treatment: BNC105 + anti- PD-1 in a colorectal tumor model

C57/BL6 mice were inoculated subcutaneously with MC38 cells and treatment commenced when tumors reached a volume of approximately 100-150mm^3. BNC105P was administered at 10 mg/kg i.v. on Days 1, 8 and 15 and anti-PD-1 antibody (Clone RMP1-14) was administered at 3.5mg/kg i.p. on Days 1, 4, 8, 12 and 16.



***Synergistic* → Observed Response (97%) > Additive Response (84%)**

Evaluation of combining BNC105 with anti-PD-1 in the MC38 colorectal cancer model. **A)** Tumor growth inhibition was evident as early as Day 8 of the treatment period especially in the combination group compared to control group (p<0.05). On Day 17 of the treatment period, animals treated with BNC105 as a monotherapy experienced 40% inhibition of tumor growth, anti-PD-1 treated animals experienced 74% inhibition in tumor growth. Animals treated with the combination of BNC105+anti-PD-1 therapy experienced 97% inhibition in tumor growth. **B)** Animal weight and health monitored throughout the treatment period demonstrate that BNC105 + anti PD-1 is a well tolerated combination

Study outsourced to CrownBio

Combination of BNC105 with anti PD-1 led to synergistic inhibition in an murine model of colon cancer

Summary

BNC105 is a potent and highly selective disruptor of tumor microvasculature causing rapid onset of tumor hypoxia and tumor cell necrosis.

This rapid generation of tumor antigens potentially arms immature dendritic cells to generate a strong tumor specific cytotoxic T cell response

The combination of BNC105 and the checkpoint inhibitors anti PD-1 and CTLA-4 have a complementary activity to leverage the immuno-stimulatory action of BNC105 and maintain the cytotoxic T cell attack of the tumor while avoiding immunotolerance.

The combinations delivered synergistic tumor growth inhibition activity in two syngeneic models of murine colorectal cancer CT26 and MC38

These findings support the investigation of combining BNC105 with immune checkpoint inhibitors in further preclinical models with the view of progressing such combinations to clinical evaluation

Synergy defined to occur when the Observed Response (R_{obs}) > Additive Response (R_{add}). R_{add} = Fraction $(F)^{Treatment\ 1} + F^{Treatment\ 2} - (F^{Treatment\ 1} \times F^{Treatment\ 2}) \times 100$*

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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