# BNC420 is a novel VEGFR3 selective inhibitor, which unlike the pan-VEGFR inhibitor Sunitinib, suppresses lymphatic metastasis in a model of metastatic melanoma

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## Background

Cancer metastasis is the primary cause of cancer-related deaths with dissemination of cancer cells from the primary tumor occurring via the blood stream and lymphatic vessels to the draining lymph nodes. Metastatic melanoma spreads through local lymphatic invasion and dissemination to regional lymph nodes. Intra-tumoral and peri-tumoral lymphatics represent a prognostic indicator for metastasis and overall patient survival (Karaman *et al* 2014). Induction of tumor associated lymphatic vessel growth through expression of VEGFC and signaling through the vascular endothelial growth factor receptor VEGFR3 are key molecular elements underpinning the formation of tumor lymphatics. Targeting VEGFC/VEGFR3 inhibits lymphatic mediated metastasis. Several tyrosine kinase inhibitors of the VEGFR family receptors have been approved for use in a number of cancer indications (e.g. Sunitinib). Despite the demonstrated therapeutic benefit gained by such agents, there have been reports of undesirable increased metastasis occurring as a consequence of inhibiting the function of VEGFR2. It has been previously reported that inhibition of VEGFR2 in preclinical models augments metastasis through increased hypoxia within the tumor microenvironment (Pàez-Ribes *et al* 2009). Furthermore, clinical evidence has demonstrated that VEGFR2 inhibition can induce a more invasive metastatic disease (Ebos *et al* 2009). We have discovered BNC420 (*Abstract #4029, AACR 2014*), a tyrosine kinase inhibitor that potently inhibits VEGFR3 while displaying selectivity over VEGFR2 and VEGFR1. BNC420 is very effective in suppressing lymphatic metastasis in a preclinical model of melanoma.

## BNC420 is a potent inhibitor of VEGFR3 and displays selectivity over VEGFR2

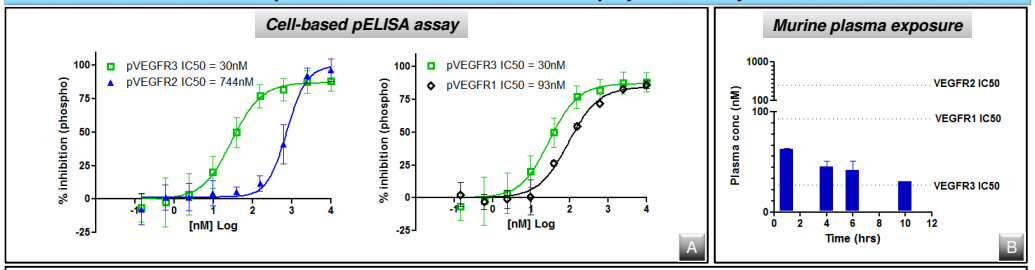


**Figure 1. (A)** BNC420 activity in inhibiting phosphorylation of VEGFR3,VEGFR2 and VEGFR1 in cells was evaluated using an ELISA-based phosphorylation detection assay utilising lymphatic or vascular endothelial cells exposed to VEGFC or VEGFA. BNC420 displays 25-fold selectivity over VEGFR2 and 3-fold selectivity over VEGFR1. **(B)** Drug exposure in mice was determined following a single oral dose of BNC420 at a dose of 75mg/kg. Plasma protein binding and unbound drug were determined by ultracentrifugation/UPLC-MS.

## Melanoma in Transit

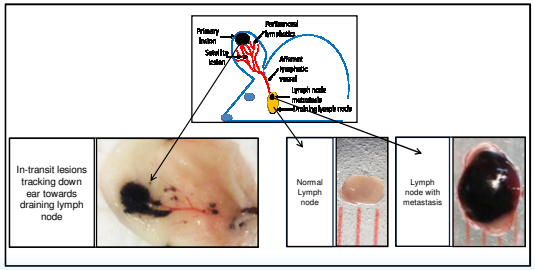


**Figure 2.** Balb/c nude mice were injected intradermally with $4x10^5$ B16F10 murine melanoma cells (ATCC CRL-6475) in the left ear. Treatment (p.o.) with BNC420 (75mg/kg, b.i.d), Sunitinib (40mg/kg, q.d.) or vehicle commenced 24 hours post inoculation for 14 days. Ears and draining lymph nodes were collected, measured and imaged. Adapted from Bobek *et al* 2010.

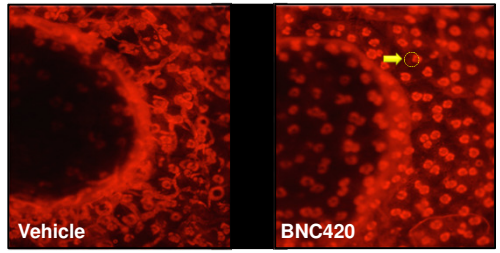


**Figure 3.** Ears from animals treated with BNC420 or vehicle were paraformaldehyde fixed and stained using an anti-mouse-Lyve-1 antibody (red stain) for identification of lymphatic vessels surrounding the primary melanoma lesion. Hair follicle autofluorescence is observed in both images (example shown - yellow arrow). Ears from mice treated with BNC420 were devoid of peri-tumoral lymphatic vessels in comparison to the extensive vessel development seen in vehicle treated animals.

## BNC420 suppressed the development of lymph node metastasis and in-transit lesions

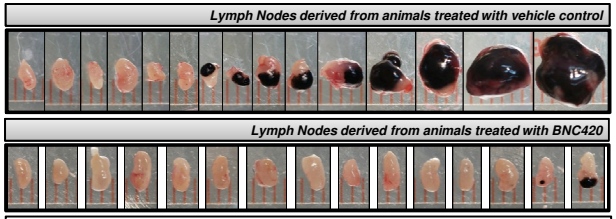


**Figure 4.** Draining lymph nodes from BNC420 or vehicle treated mice were collected and examined for the presence of characteristic black B16F10 metastatic lesions. Mice treated with BNC420 had significantly fewer visible metastatic lesions observed in the draining lymph nodes compared to the vehicle treated animals.

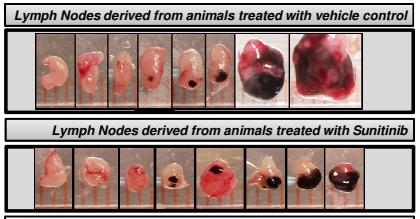


**Figure 6.** Draining lymph nodes from Sunitinib or vehicle treated mice were examined for the presence of metastatic lesions. Sunitinib did not alter the development of lymph node lesions

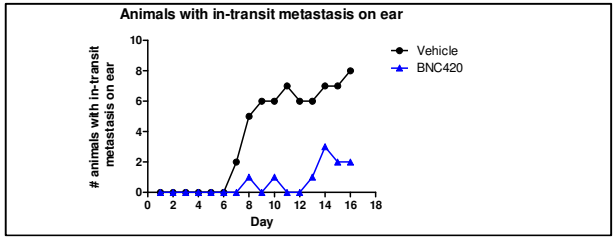


**Figure 5.** In-transit metastasis on ears of mice treated with BNC420 or vehicle were counted daily. Graph depicts the total number of animals where in-transits lesions were observed each day. Development of in-transit metastatic lesions on ears was inhibited in BNC420 treated animals. BNC420 treated animals displayed fewer in-transit lesions on the ear compared to vehicle treated animals.

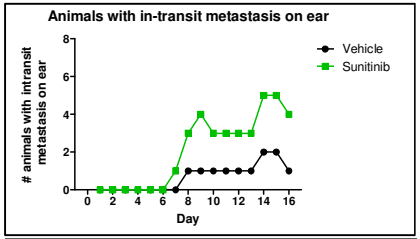


**Figure 7.** The number of animals where in-transit metastasis were observed on the ears of vehicle or Sunitinib treated mice. Sunitinib treated animals displayed enhanced in-transit lesions on the ear compared to vehicle treated animals.

## Conclusions

- **BNC420 selectively inhibits VEGFR3 phosphorylation and displays significant selectivity over VEGFR2 and VEGFR1**
- **In a murine model of melanoma, BNC420 suppressed the development of peri-tumoral lymphatics, the growth of in-transit metastatic lesions and the spread of metastasis to the draining lymph nodes**
- **The pan-VEGFR inhibitor Sunitinib failed to suppress lymph node metastasis and appeared to enhance formation of in-transit metastatic lesions. These data are consistent with previous report showing that pan-VEGFR inhibitors augment metastasis through increased hypoxia within the tumor microenvironment (Pàez-Ribes *et al* 2009)**
- **Unlike pan-VEGFR inhibitors, BNC420 suppresses metastasis - this is potentially due to its selectivity for VEGFR3 over VEGFR2**

## References

1. Karaman *et al*. Mechanisms of lymphatic metastasis. 2014. Journal of Clinical Investigation 124 (3): 922-928
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3. Pàez-Ribes *et al*. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. 2009. Cancer Cell: 15 (3): 220-231
4. Ebos *et al*. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. 2009. Cancer Cell: 15 (3): 232-239