

Functional and molecular biomarker analysis demonstrates pharmacological activity for the novel vascular disrupting agent BNC105 in a first in human clinical study.

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Abstract

BNC105 is a novel agent which inhibits tubulin polymerisation and causes tumour vascular disruption. Preclinical evaluations have demonstrated selective activity for tumour vasculature in a number of xenograft and syngeneic models, where complete vascular shutdown was seen in both primary and metastatic lesions as early as 3 h following BNC105 administration. A multicentre Phase I clinical trial was conducted under an IND utilising the network of Cancer Trials Australia. BNC105P (prodrug formulation) was administered IV over 10 min on Day 1 & 8 every 21 days to patients with advanced solid tumours. Dose levels administered were 2.1, 4.2, 8.4, 12.6, 16 and 18.9 mg/m². Decreases in DCE-MRI parameters, used to measure changes in tumour blood flow consistent with VDA activity, were observed. In a newly developed biomarker assay Peripheral Blood Mononuclear cells (PBMCs) were utilized as a surrogate cell population for measuring the activity of BNC105 on its molecular target. A dose related decrease in the levels of polymerised tubulin was detected, reaching a peak at the 3 h time point (>80%) and returning to normal levels by 24 h. This finding represents the first reported "on target" activity of a VDA in cancer patients. The clinical data are consistent with preclinical observations and suggest that the drug exposure observed in cancer patients achieves modulation of polymerised tubulin, is sufficient to cause tumour damage, does not cause myelosuppression and is well tolerated by patients. BNC105 is currently under evaluation in two phase II clinical trials for the treatment of mesothelioma and renal cell carcinoma.

Study Design

Drug Administration BNC105P was administered IV over 10 min on Day 1 & Day 8 every 21 days. Six dose levels were investigated: 2.1, 4.2, 8.4, 12.6, 16 and 18.9 mg/m². 2 x 21 day cycles were administered unless patients were withdrawn due to disease progression or toxicity. Treatment was continued for additional cycles if clinical benefit outweighed risk.

Pharmacokinetics & Pharmacodynamics Assessments

LC-MS/MS - The levels of BNC105P and BNC105 in plasma samples were measured by LC-MS/MS. Samples were collected during Cycle 1 following Day 1 & Day 8 administration.

CT scans - Response by RECIST was determined following completion of the first 2 cycles of treatment. For additional cycles tumor assessments were carried before every 3rd cycle.

DCE-MRI - Measurement of blood flow and vascular permeability in preselected tumor lesions was carried out twice prior to treatment (pre-dose), at the 3-6 h and 24 h time intervals following Cycle 1 Day 1 treatment.

Immunoblot densitometry assay - Measurement of the amount of polymerised tubulin in peripheral blood mononuclear cells (PBMCs) was undertaken as a biomarker of BNC105 on-target action. (Matthews *et al*, 2010)

Plasma/serum biomarkers - Vascular Endothelial Growth Factor (VEGF), Plasminogen Activator Inhibitor 1 (PAI-1) and Cytokeratin 18 (CK18) plasma/serum levels were measured as indicators of pharmacological response.

Patient Details & Pharmacokinetics

Twenty one patients (13M; 8F; median 60 yr) were enrolled to the study (Table 1). Cycles administered ranged from 0.5-7.5. BNC105P was well tolerated at doses ≤16 mg/m².

Dose Level mg/m ²	Patient ID#	Primary Tumor
2.1	1	ascending colon
4.2	2	ovarian
8.4	3	thyroid
	4	renal
12.6	5	mesothelioma
	6	renal
	7	ascending colon
	8	duodenal
	9	rectosigmoid
	10	leiomyosarcoma
16	11	upper G.I. (unknown primary)
	12	colorectal
	13	head & neck
	14	adrenocortical
	15	colon
18.9	16	leiomyosarcoma
	17	mesothelioma
	18	esophageal
	19	colorectal
	20	salivary
	21	melanoma

Table 1: Patient Details (N=21).

BNC105P was rapidly converted to BNC105.

Mean half-life of BNC105P = 0.13 h.

Mean half-life of BNC105 = 0.57 h.

The BNC105 plasma levels achieved correlate with active preclinical plasma exposure (Kremmidiotis *et al*, 2010).

Biomarkers - Tubulin Polymerisation (PBMC)

BNC105 acts as a tubulin depolymerising agent. Treatment with BNC105P should induce changes in the total amount of polymerised tubulin in patient PBMCs. Such changes demonstrate that BNC105 acts on its intended target and reaches pharmacologically active plasma levels. Blood samples for PBMC tubulin analysis were obtained prior to dosing (baseline) and at 1, 2, 3-5, 7 and 24 h post BNC105P treatment (Cycle 1). PBMCs were isolated and polymerised tubulin extracted. Western blot analysis and densitometry was used to quantify polymerised tubulin (Fig 1A). Polymerised tubulin levels decreased to as low as 1% of the pre-dose levels within 1 to 7 h post BNC105P administration and reverted back to pre-dose levels by 24 h (Fig 1B). Polymerised tubulin reached lowest levels at the 3-5 h timepoint, with the effect becoming more pronounced with increasing dose levels (Fig. 1C). Analysis indicated a dose response relationship with no trend between Day 1 and 8 of Cycle 1.

References

Matthews *et al*. Abstract 2768. AACR Annual Meeting, Washington DC, 2010.
Kremmidiotis *et al*. *Molecular Cancer Therapeutics*, 9 (6), June 2010.

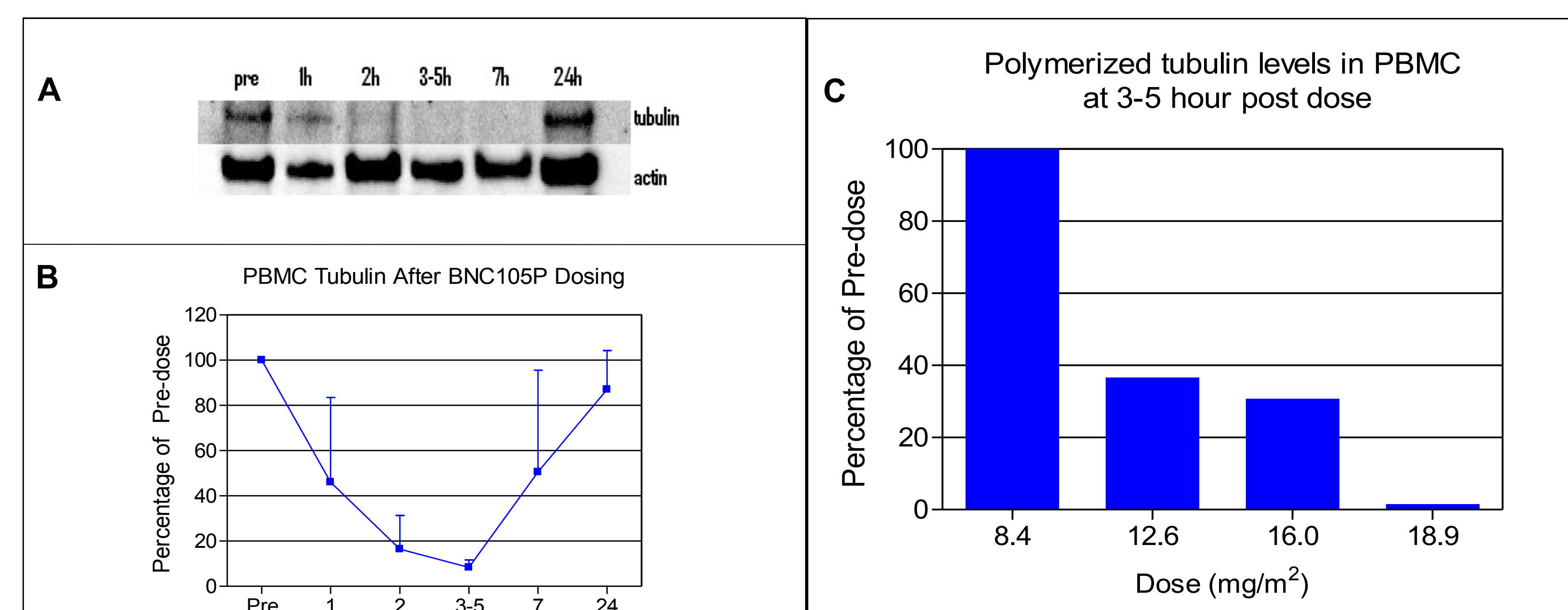


Figure 1: Measurement of polymerised tubulin levels in PBMCs extracted from patients treated with BNC105P. (A) Representative Western blot analysis for patient 18 (Cycle 1 Day 1). (B) Average % changes relative to pre-dose. (C) Average % changes relative to pre-dose at the 3-5 h timepoint. Patients included in this analysis were: Cohort 3 8.4 mg/m² (patients 3, 4), Cohort 4 12.6 mg/m² (patients 6-9), Cohort 5 16 mg/m² (patients 12-15), Cohort 6 18.9 mg/m² (patients 18,19).

Biomarkers - serum/plasma

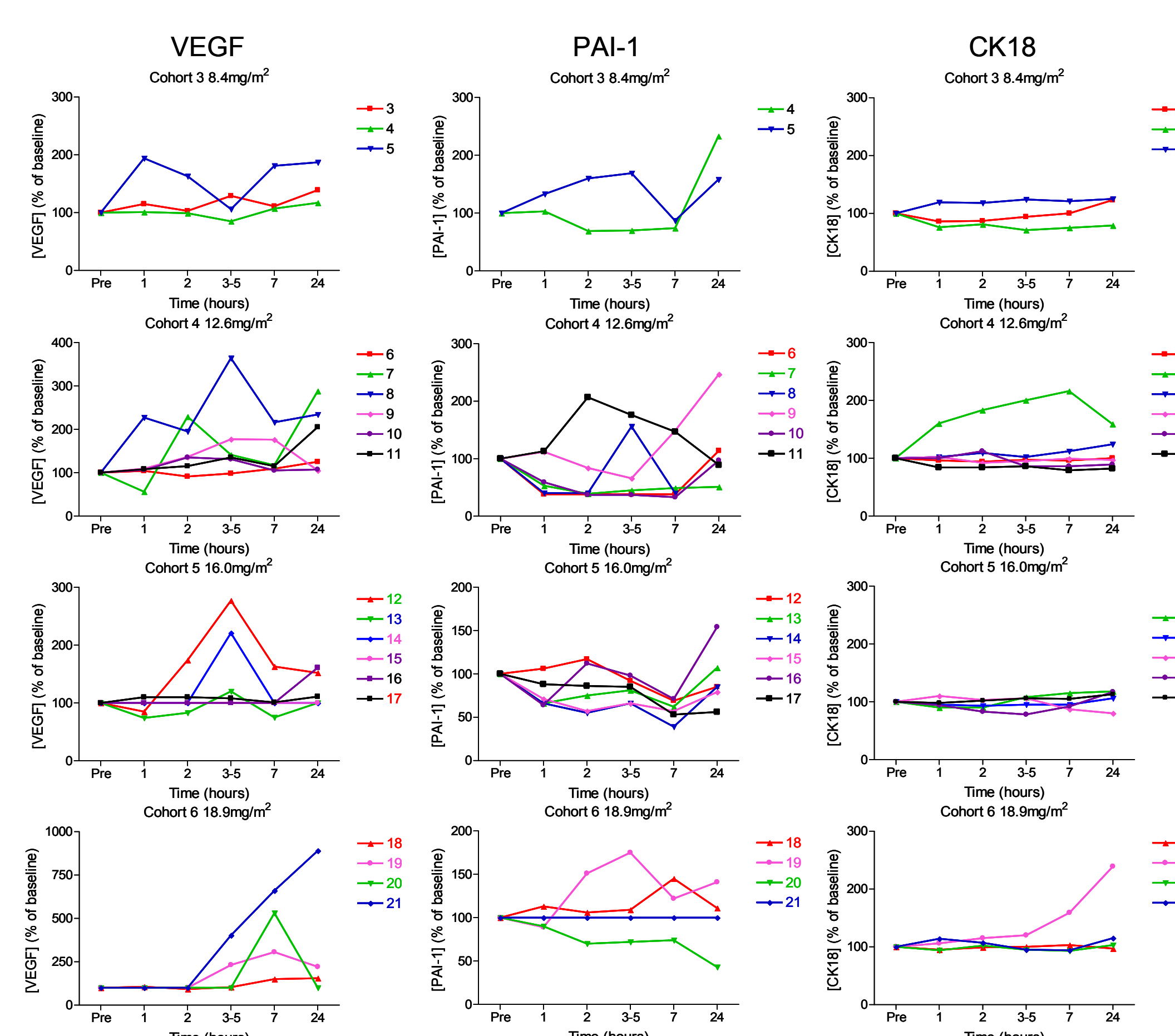


Figure 2: Measurement of VEGF, PAI-1 and CK-18 relative to baseline concentrations in patients following BNC105P treatment on Day 1 Cycle 1.

Plasma/serum samples were obtained at baseline, and 1, 2, 3-5, 7 and 24 h after dosing on Days 1 and 8 of Cycle 1.

Samples were analysed for VEGF, PAI-1 and CK18 levels (Fig. 2).

Increased levels of VEGF were observed in a number of patients dosed at ≥8.4 mg/m². Highest increases in VEGF levels were seen in the 18.9 mg/m² dose cohort with levels up to 8 times the baseline (patient 21).

PAI-1 plasma level changes were seen in several patients. In the majority of cases PAI-1 levels decreased following BNC105P treatment. PAI-1 levels dropped as low as 33% compared to baseline in some cases.

In general there were no major changes in the serum CK18 levels.

DCE-MRI

DCE-MRI qualitative analysis suggested changes in a number of patients. Decreases in tumor pixel initial area under the gadolinium curve from 0 to 90 seconds (IAUGC90) were seen. A correlation between DCE-MRI changes and clinical benefit was observed in patient 6. In the IAUGC90 pixel color maps shown in Fig. 3 for patient 6, the underlying gray scale MRI image demonstrates a large right renal mass, and multiple confluent liver metastases. The area shown demonstrates decreasing tumour vascularity in the superior aspect relative to the inferior portion of the lesion. This is reflected by declines on IAUGC90 of 9% and 21% at 3-6 h and 24 h post treatment, respectively. K^{trans} declines were 49% at 3-6 h and 23% at 24 h in this instance. Statistically significant changes in K^{trans} were seen in a total of four patients (Table 2).

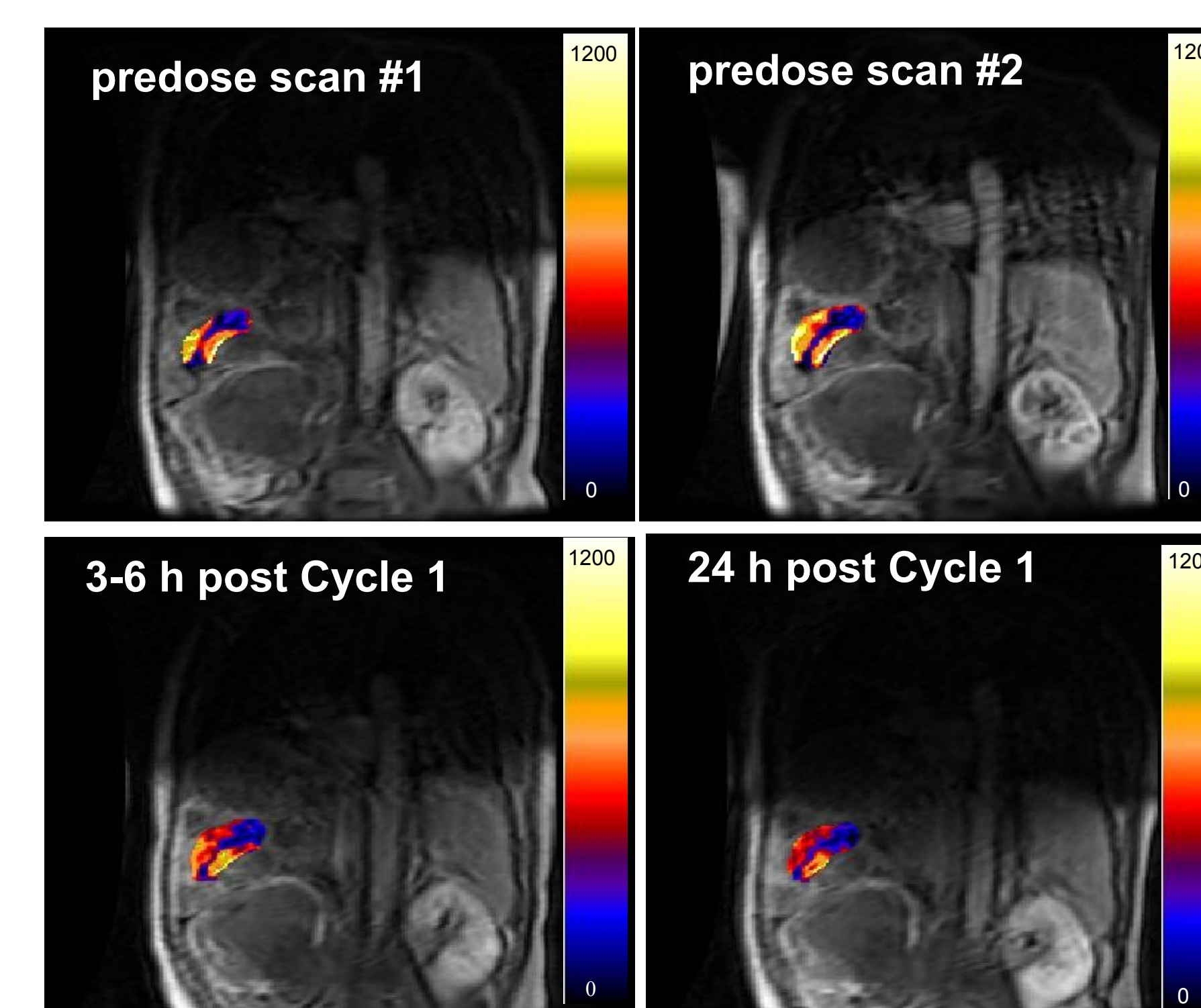


Figure 3: Patient 6 IAUGC90 Color Map (renal cancer, liver metastasis shown; RECIST classification: SD).

Dose level mg/m ²	Patient ID	% Decrease in K ^{trans} 3-6 h Cycle 1 Day 1	% Decrease in K ^{trans} 24 h Cycle 1 Day 1
12.6	6	49 #	29
16	12	-	42 #
18.9	18	25	61 #
	21	40	43 #

Table 2: Patients with decline in K^{trans} (compared to baseline) at 3-6 h and/or 24 h post Cycle 1 Day 1 dose. [# = statistically significant]

Conclusions

- BNC105P was well tolerated with a recommended phase II dose of 16 mg/m².
- Stable disease was observed in 4 of 16 patients who completed ≥2 cycles at doses of 8.4 mg/m² or higher.
- Preliminary evidence suggest that BNC105 attains pharmacodynamically active plasma levels:
 - DCE-MRI changes in tumour lesions
 - Reduced levels of polymerised tubulin in patient PBMCs
 - Changes in patient plasma/serum levels of VEGF, PAI-1 and CK18