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Clinical, Pharmacodynamic, and Pharmacokinetic Evaluation of BNC105P: A Phase I Trial of a Novel Vascular Disrupting Agent and Inhibitor of Cancer Cell Proliferation

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Abstract

Purpose: To determine the recommended phase II dose and evaluate the safety and toxicity profile and pharmacokinetic (PK) and pharmacodynamic (PD) effects of BNC105P, an inhibitor of tubulin polymerization that has vascular disrupting and antiproliferative effects.

Experimental Design: BNC105P was administered as a 10-minute infusion on days 1 and 8 of a 21-day cycle in a first-in-human phase I study. A dynamic accelerated dose titration method was used for dose escalation. Plasma concentrations of BNC105P (phosphate prodrug) and BNC105 (active agent) were determined. PD assessments were carried out using dynamic contrast enhanced (DCE)-MRI and analysis of a blood-borne biomarker.

Results: Twenty-one subjects with advanced solid tumors were enrolled on 6 dose levels (range: 2.1–18.9 mg/m^2). The recommended dose level was 16 mg/m^2 and was well tolerated. BNC105P (prodrug) rapidly converted to BNC105 with a half-life of 0.13 hours. Plasma concentrations of BNC105 generally increased in proportion to dose with a half-life of 0.57 hours. Pharmacodymanically active plasma levels were obtained with a dose dependant reduction in the levels of polymerized tubulin (on-target action) being observed in PBMCs. DCE-MRI also indicated blood flow changes in the tumor lesions of a number of subjects.

Conclusions: BNC105P has a favorable toxicity profile at the recommended dose of 16 mg/m^2 and is associated with PD changes consistent with its known mechanism of action. Phase II studies in renal cancer and mesothelioma have commenced. *Clin Cancer Res; 17(15); 5152–60. ©2011 AACR.*

Introduction

Vascular disruption agents (VDA) cause a rapid occlusion of tumor vasculature, leading to hypoxic stress within the tumor and induction of cell death. Their selective action on tumor vasculature endothelium distinguishes them from other vaso-active agents such as the antiangiogenics. Taking advantage of the differences between newly formed blood vessels in tumors and mature vessels in normal tissues is a concept that has been outlined for some time (1). However, VDAs have gained increased prominence in recent years as compounds have progressed in their development. At present, there are at least 12 compounds that have been evaluated in the clinic. These agents can be grouped into 2 broad classes: the flavonoids (such as ASA404) and the tubulin-binding agents (2, 3).

BNC105P is the disodium phosphate ester prodrug of BNC105, a tubulin polymerization inhibitor (TPI) that exhibits a high degree of selectivity for tumor endothelial cells (4, 5). BNC105P disrupts the vasculature within solid tumors in mice and acts as a direct antiproliferative, suppressing tumor cell growth in culture. BNC105P shows evidence of good efficacy in mice-bearing xenografts of several human-tumor types, causing the regression of tumors, and in some cases tumor clearance. Evaluations in preclinical models of cancer have shown that BNC105P is more potent and displays a significantly improved therapeutic index compared with other VDAs in development (4). BNC105P achieves >95% vascular disruption as early as 3 hours post administration in tumor bearing mice when dosed at 10 mg/kg. This dose is well tolerated as a Day 1, Day 8 dose in a 21-day treatment cycle.

In addition, BNC105P displays 80-fold higher potency against endothelial cells that are actively proliferating or are engaged in the formation of *in vitro* capillaries compared with nonproliferating endothelial cells or endothelium found in stable capillaries. This selectivity was not observed

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Translational Relevance

BNC105P is an inhibitor of tubulin polymerization that has vascular disrupting and antiproliferative effects. The study reported herein is the first-in-human trial of this agent. The experimental design incorporated a number of PK and PD assessments to elucidate the actions of BNC105P and investigate potential predictive markers for this class of agent. Dynamic contrast enhanced–MRI was used to assess tumor blood flow following exposure to this vascular disruption agent (VDA). A novel immunoblot densitometry assay was also developed which assessed the level of polymerized tubulin levels in PBMCs isolated from trial subjects. This assay shows the "on-target" action of drug. This trial showed a favorable toxicity profile of this agent at the recommended dose level and has led to further evaluation in phase II trials.

with combretastatin, a VDA currently under evaluation in phase III clinical trials. Further, while combretastatin produces 90% vascular disruption at its no-observed adverse event level (NOAEL), BNC105P causes 95% vascular disruption at one eighth of its NOAEL (4).

Reported herein is the first-in-human study of BNC105P. This was an open-label, two-stage, phase I dose escalation study of BNC105P monotherapy for advanced solid tumors. A determination of the safety and tolerability of this novel agent was the primary focus of this study. Other key objectives were the evaluation of the pharmacokinetic (PK) and pharmacodynamic (PD) actions of the molecule. PD effects were investigated in a number of ways. (i) Response was evaluated using RECIST-defined criteria and standard imaging techniques. (ii) The level of polymerized tubulin in peripheral blood mononuclear cells (PBMCs) isolated from blood was determined. (iii) Dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI), an emerging technology used to investigate microvascular structure and function, was employed in all patients to inform on changes in the tumor microvasculature within a defined target lesion.

Patients and Methods

Eligibility criteria

Patients with advanced solid tumors for whom no standard therapy was available were considered eligible for the study.

Inclusion criteria included the following: (a) disease that was amenable to evaluation by DCE-MRI (lesion $\geq$3 cm if located in anatomically mobile regions, $\geq$2 cm if located in nonmobile region), and was not cystic nor largely necrotic in nature; (b) had an Eastern Co-Operative Oncology Group (ECOG) performance status $\leq$2 and a life expectancy of at least 12 weeks; (c) had adequate organ function, (d) corrected QT interval (QTc) $\leq$450 msec; and left-ventricular ejection fraction (LVEF) within the institutional reference range.

Exclusion criteria included: (a) treatment with any investigational agent, radiation therapy to a visceral organ, immunotherapy, biological therapy, or chemotherapy or major surgery within 4 weeks prior to study entry; (b) prior radiotherapy to more than 25% of bone marrow or high-dose chemotherapy requiring hematopoietic stem cell support; (c) having contraindications to the DCE-MRI (such as pacemaker, metallic fragments, non-MRI-compatible implants); (d) having not recovered from the toxic effects of previous therapy [Common Terminology Criteria for Adverse Events (CTCAE) grade $\leq$1]; (e) known brain or leptomeningeal disease; (f) myocardial infarction, angina, cerebro-vascular accident or transient ischemic attack within the last twelve months, significant cardiac comorbidity or poorly controlled hypertension; (f) deep-vein thrombosis, pulmonary embolism, or arterial thrombosis within 5 years; (g) glomerulonephritis; (h) squamous non-small cell lung cancer with a central lesion; (i) receiving full dose, therapeutic anticoagulation with warfarin or related anticoagulants; (j) $\geq$ grade 2 peripheral neuropathy.

Drug administration and dose escalation

This study was designed as a 2-stage, open label, dose-escalation study. Patients received BNC105P monotherapy on Days 1 and 8 of a 21-day cycle as a 10 minute IV injection. Dosing began at a level of 2.1 mg/m^2 (one tenth the severely toxic dose in 10% of rats). Dosing was escalated in 100% increments during stage 1 (single patient cohorts) and in 33% to 100% increments in stage 2 (depending on observed toxicity). Stage 1 continued until at least one CTCAE grade $\geq$2 adverse event attributable to the study drug was observed, at which point stage 2 (expanded cohorts) began. Dose escalation in stage 2 continued with three patients per dose level until a dose-limiting toxicity (DLT) was observed whereupon an additional three patients were added. If a second DLT was observed in the 6-patient cohort no further escalation was undertaken and the cohort of one dose level below was expanded to confirm that level as the MTD. Continuation of therapy beyond 2 cycles was permitted in the absence of disease progression. The follow-up period was 28 days post administration of last dose.

Assessments

Patients were assessed for safety by means of monitoring of adverse events and graded according to the National Cancer Institute Common Toxicity Criteria version 3.0. Laboratory tests, electrocardiogram (ECG), multigated acquisition scan (MUGA) for determination of LVEF (echocardiogram was substituted if clinically necessary), vital signs (heart rate, systolic, and diastolic blood pressure, respiration rate, and temperature), and assessment of ECOG performance status were done at baseline and repeated on treatment. Neurological examinations were carried out at baseline, before treatment on Day 8 of Cycles 1 and 2, every other cycle thereafter and at termination. Baseline neurological examination included mental status, cranial nerves, motor, and sensory examinations.

Tumor measurements were assessed using response criteria in solid tumors (RECIST) criteria version 1.0. These were conducted prior to treatment and in the 7 days prior to every third cycle.

The "As Treated" population was comprised of all patients who received at least one dose of BNC105P, and were used for the generation of all efficacy and safety data.

Treatment emergent adverse events (TEAE) are defined as new or worsening AEs (both drug and nondrug related), which commenced or worsened, on or after the time of first administration of the study drug.

Pharmacokinetic analysis of BNC105 and BNC105P

PK samples were obtained prior to dosing (baseline), at the end of the injection, at 10, 20, 40, 60, and 90 minutes, and at 2, 3–5 (subject to MRI requirements), 7, and 24 hours after completion of the injection on Days 1 and 8 of the Cycle 1.

BNC105 (active agent) analysis utilized the extracts of BNC105 and the internal standard (IS) from human plasma using liquid–liquid extraction. Analytes were separated by high performance liquid chromatography (HPLC; Shimadzu Scientific Instruments) on a Waters XBridge C18 reverse phase column and the eluates were monitored by an Applied Biosystems API3000 mass spectrometer using tandem mass spectrometry (MS/MS) detection. The detection range was 0.5 to 200 ng/mL.

BNC105P (prodrug) analysis used a protein precipitation extraction. The analytes were also separated by HPLC (Waters XBridge C18 reverse phase column) and the eluates monitored by an Applied Biosystems API3000 mass spectrometer. The detection range was 5 to 2000 ng/mL.

Data were acquired and processed by the data acquisition system analyst 1.4 (Shimadzu) linked directly to the API3000 MS/MS detector.

Both methods adhered to the Good Laboratory Practice (GLP) principles and the Food and Drug Administration (FDA) guidance on bioanalytical method validation.

Tubulin depolymerization in peripheral blood mononuclear cells

Blood samples for blood-borne biomarker analysis were obtained prior to dosing (baseline), at 1, 2, 3–5 (subject to MRI requirements), 7, and 24 hours after completion of the IV infusion on Days 1 and 8 in Cycle 1. PBMCs were isolated from blood and suspended in lysis buffer (glycerol, Triton-X100, DMSO, protease inhibitor cocktail, guanosine triphosphate). Following cell lysis polymerized tubulin was extracted by centrifugation at 180,000 $\times$ g for 1 hour at 37°C. Standard western blotting procedures were then followed. Fractionated tubulin samples were resolved in SDS-PAGE gels, transferred to nitrocellulose membranes and detected using primary anti-β1 tubulin antibodies (Sigma) and primary anti-β actin antibodies (Sigma) as the loading control. Protein bands were photographed and analyzed using ImageJ software (6; National Institutes of Health) to generate numerical representations of normalized protein band density.

Tumor-based pharmacodynamic biomarker–dynamic contrast enhanced–MRI

DCE-MRI was carried out twice in the 7 days prior to receiving the first dose of BNC105P, allowing statistical comparison with post-dose assessments. During Cycle 1, DCE-MRI was carried out 3–6 and 24 hours after Day 1 administration (i.e., on Days 1 and 2). DCE-MRI images were reviewed on the MiSTAR version 3.2 software package (Apollo Medical Imaging Technology Pty Ltd.). The reader remained blinded throughout the analytical period with time points being presented in random order, and date/dosing information removed prior to image viewing.

Results

Patient demographics

A total of 21 subjects were enrolled in the study. Patient characteristics are shown in Table 1. Patients with a range of advanced and/or metastatic solid tumors were enrolled.

Safety and tolerability

Patients received a median of 2 cycles of BNC105P (range 0.5–7.5). Of the 21 patients enrolled in the study, 16 received at least two cycles of BNC105P. As per stage 1 of the protocol, single patient cohorts were enrolled until dose level 3 (8.4 mg/m^2), where Grade 2 toxicity was observed. Subsequent enrolment involved a minimum of 3 patients per cohort. No DLTs were seen until the 18.9 mg/m^2 dose level, where 2 patients developed DLTs. An 83-year old female with metastatic colorectal cancer experienced a Grade 4 acute myocardial infarction (2 hours post-dose) and also developed a Grade 4 peripheral sensory neuropathy. She recovered from the myocardial infarction without any complications and the neuropathy had improved to Grade 2 by Day 30. This patient had a calculated creatinine clearance of 54 mL/minute estimated by the Cockcroft–Gault formula which was below the protocol stipulated inclusion criterion of

Table 1. Patient characteristics ($N = 21$)

Males, *n* (%)	13 (62)
Median age, years (range)	60 (42–83)
Race, n (%)	Caucasian: 20 (95)
	Arabic: 1 (5)
ECOG performance status, *n* (%)	0–1: 19 (90)
	2: 2 (10)
Median number of prior chemotherapy regimens, *n* (range)	3 (0–7)
Diagnosis, *n*	
Colorectal	6
Other gastro-intestinal	2
Renal	2
Mesothelioma	2
Leiomyosarcoma	2
Other	7

≥55mL/minute (and a glomerular filtration rate measured by Cr-51 EDTA technique of 50 mL/minute), but had been given a waiver by the medical monitor. She had no history of ischemic heart disease, but had hypertension well controlled on candesartan. She had experienced mild oxaliplatin-related neuropathy in the past but there was no evidence of neuropathy at the time of study entry. The second DLT occurred in a 63-year old male with metastatic salivary gland tumor who developed an asymptomatic Grade 3 elevation in alanine aminotransferase (ALT), and Grade 2 elevations in aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT). Drug was withheld for 7 days, ALT/AST resolved to Grade ≤2.

In view of 2 out of 4 patients experiencing a DLT, dose escalation was ceased and the dose level below (12.6 mg/m^2) expanded to 6 patients to confirm safety at this level. We then elected to explore a dose level intermediate between the 2 previous dose levels and enrolled patients at 16 mg/m^2. 6 patients were treated at 16 mg/m^2 and no DLTs were observed. Hence this dose level was deemed to be the recommended dose level. At this dose level and below the drug was well tolerated with no drug-related Grade 3 or 4 events, an acceptable cardiovascular profile (consisting of one Grade 1 CPK-MB increase, and one Grade 2 LVEF decrease) and only a single hematological event (anemia, Grade 1). Those TEAEs considered to have a potential causal relationship to the study drug (possibly, probably or definitely related to study drug) are listed in Table 2.

Pharmacokinetics of BNC105P (prodrug) and BNC105 (active agent)

PK results (Table 3 and Supplementary Table S1) showed that BNC105P was rapidly converted to BNC105, with a mean half-life of 0.13 hours for both Day 1 and Day 8, averaged across all patients.

The concentration profiles for BNC105P and BNC105 were similar for Day 1 and Day 8. There was no trend observed in the plasma levels of either analyte between the Day 1 and Day 8 profiles, indicating no change in exposure between the two administrations of drug in Cycle 1.

The maximal plasma concentration of BNC105 occurred at the end of the infusion in all patients. The mean half-life of BNC105, averaged across all patients was 0.99 hours for Day 1, and 0.63 hours for Day 8. A contributing factor to the apparent difference in half-life of BNC105 between Day 1 and Day 8 was the significantly prolonged half-life in one patient (18.9 mg/m^2; half-life: 9.47 hours) who experienced a DLT (Grade 4 N-STEMI) within a few hours of dosing on Cycle 1 of Day 1 and was subsequently withdrawn from the study. Exclusion of this outlier results in an overall half-life of 0.57 hours (being 0.52 hours for Day 1, and 0.63 hours for Day 8).

The elimination rate of BNC105 immediately postdose appears to be first order, with the rate becoming slower by 30 minutes postdose, for those patients with quantifiable concentrations at those sampling times.

Antitumor effects

There were no objective responses, but stable disease was observed in 4 patients. Stable disease was achieved in one patient at 8.4 mg/m^2 dose (primary tumor type: mesothelioma, evidence of progression at study entry), one patient in the 12.6 mg/m^2 dose group (renal) and two patients in the 16mg/m^2 dose group (adrenocorticol carcinoma, leiomyosarcoma). Stable disease was maintained in these patients for 3 to 7.5 cycles.

Blood and tumor-based pharmacodynamic biomarkers

PBMCs were isolated and polymerized tubulin extracted using high-speed centrifugation. Western blotting analysis and densitometry was used to quantify polymerized tubulin (Fig. 1). This analysis was carried out in samples obtained following the Cycle 1 Day 1 and/or Day 8 dosing. Polymerized tubulin levels decreased to as low as 1% of the predose levels within 1 to 7 hours post BNC105P administration and reverted back to predose levels by 24 hours (Fig. 1B). Polymerized tubulin reached lowest levels at the 3 to 5 hours timepoint, with the effect becoming more pronounced with increasing dose levels (Fig. 1C). Analysis of a number of patients from each cohort indicated a dose response relationship with no trend between Day 1 and 8 of Cycle 1.

DCE-MRI quantitation (Tables 4 and 5; Supplementary Fig. S1) yielded declines in whole tumor K^{trans} values at a number of dose levels. Significant changes in K^{trans} were seen in a total of 4 patients administered between 12.6 and 18.9 mg/m^2 of drug, with declines ranging from 42% to 61% compared with baseline. Qualitative changes in tumor pixel initial area under the gadolinium curve from 0 to 90 seconds ($IAUGC^{90}$) maps were also seen in several patients.

Discussion

In this first-in-human phase I study of BNC105P, the MTD of BNC105P administered as a 10-minute infusion on Days 1 and 8 of a 21-day cycle was deemed to be 16 mg/m^2. At this dose level and below, BNC105P was well tolerated with a safety profile that compared favorably with other VDAs. Increases in blood pressure and changes in QTc remain an expected risk of certain VDAs and these class effects were closely monitored. Such effects were not prominent within this study. No hematogical toxicities were observed, with the exception of 3 events of anemia (Grade 1–2). There were no instances of infusion related reactions.

Drug-related DLTs were observed in two patients administered 18.9 mg/m^2. The first occurred in an 83-year old female with metastatic colorectal cancer who experienced a Grade 4 N-STEMI, and a Grade 4 peripheral sensory neuropathy. It is important to note that this patient had the highest $AUC_{0\text{-last}}$ of BNC105 of any subject at 252 ng.h/mL (the next highest being 97 ng.h/mL). The patient appeared to clear BNC105 at a significantly slower rate compared with all other subjects with a $t_{1/2}$ of 9.47 hours

Table 2. Drug-related treatment emergent adverse events by CTCAE grade

Dose level	No. of patients	Toxicity	Grade			
			1	2	3	4
2.1 mg/m^2	1	Nausea	1			
		Vomiting	1			
		Fatigue	2			
		Headache	1			
4.2 mg/m^2	1	Palmar erythema	1			
8.4 mg/m^2	3	Anemia	1			
		Fatigue	1	1		
		Mucosal inflammation	1	1		
		Rash	1			
		Vibration test abnormal	1			
12.6 mg/m^2	6	CPK-MB increase	1			
		LVEF decrease		1		
		Diarrhea	1			
		Fatigue	1			
		Chills	1			
		Feeling hot	1			
		Pyrexia	2			
		Pleuritic pain	1			
		Urinary bladder hemorrhage		1		
16 mg/m^2	6	Constipation	2			
		Nausea	1	1		
		Vomiting	1			
		Fatigue	5			
		Chills	1			
		Pyrexia	2			
		Infection (cold sore, oral thrush)	1	1		
		Elevated liver function test (AST)		1		
		Chest pain (musculoskeletal)	1			
		Hyperesthesia	1			
		Rash	1	1		
18.9 mg/m^2	4	Abdominal pain	1			
		Anemia	1	1		
		Diarrhea		1	1	
		Nausea			1	
		Vomiting		1		
		Fatigue	2	2	1	
		Feeling cold	1			
		Edema	1			
		LVEF decrease	1			
		Acute myocardial infarction[a]				1
		Hypertension		1		
		Peripheral sensory neuropathy[a]				1
		Vision blurred		1		
		Paraesthesia	1			
		Elevated liver function test[b]		2	1	
		Myalgia	2			
		Dysgeusia	1			

NOTE: Possibly, probably, or definitely-related to drug.
[a]DLT in Patient 19.
[b]DLT in Patient 20.

Table 3. The pharmacokinetic parameters of BNC105 (active agent)

Dose level, mg/m^2		C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (ng.h/mL)	k_{el} (h^{-1})	$t_{1/2}$ (h)	CL_{tot} (L/h)	V (L)
DAY 1 CYCLE 1							
2.1	$N = 1$	58	10.7	11.45	0.06	321.4	28.1
4.2	$N = 1$	31	7.8	5.92	0.12	684.6	115.5
8.4	*N*	3	3	3	3	3	3
	Average	66	15.8	6.88	0.11	927.7	144.4
	SD	31	7.9	2.30	0.04	324.1	65.6
12.6	*N*	6	6	6	6	6	6
	Average	175	39.6	2.13	0.45	517.7	339.4
	SD	55	8.5	1.14	0.30	107.7	237.7
16.0	*N*	6	6	5	5	5	5
	Average	164	42.5	4.14	0.24	703.3	189.8
	SD	70	14.5	3.68	0.10	451.2	36.8
18.9	*N*	4	4	3	3	3	3
	Average	267	107.3	1.17	4.79	571.7	1700.6
	SD	116	99.7	1.84	4.63	510.2	1544.0
DAY 8 CYCLE 1							
2.1	$N = 1$	75	16.3	NA	NA	NA	NA
4.2	$N = 1$	41	8.0	10.52	0.07	659.5	62.7
8.4	*N*	3	3	3	3	3	3
	Average	93	21.0	6.44	0.15	693.0	138.5
	SD	37	10.3	5.18	0.09	218.7	67.7
12.6	*N*	6	6	5	5	5	5
	Average	189	43.1	2.79	0.92	466.8	571.7
	SD	72	11.1	2.10	1.39	82.5	837.7
16.0	*N*	6	6	6	6	6	6
	Average	179	42.6	1.56	0.78	692.7	691.5
	SD	76	18.7	1.38	0.50	384.5	558.3
18.9	*N*	3	3	1	1	1	1
	Average	249	67.4	2.10	0.33	1822.7	866.7
	SD	169	45.8	NA	NA	NA	NA

NOTE: The time of C_{max} (T_{max}) corresponded with the time of first sampling in all cases.

Clearance for BNC105 was calculated by converting the actual dose of BNC105P to an apparent dose of BNC105 determined by adjusting the dose by the ratio of the molar weights.

Abbreviations: The maximum observed concentration, (C_{max}); The area under the concentration–time curve from time 0 to last detection of analyte, $AUC_{0\text{-last}}$; the elimination rate constant, k_{el}; the elimination half-life, $t_{1/2}$; systemic clearance, CL_{tot}; and volume of distribution (V). NA = Not Applicable (Elimination rate not estimable).

(across all other subjects the $t_{1/2}$ was 0.57 hours). The cardiotoxicity and neurotoxicity observed in this subject should be viewed in the context of exceptionally high and prolonged levels of analyte detected in her plasma. Although renal elimination is not known to play a role in BNC105 clearance, we can not exclude the possibility that this patient's mild renal impairment may have contributed to the slow clearance and toxicity. In addition, her prior oxaliplatin neuropathy may have predisposed her to neurotoxicity. The second DLT occurred in a 63-year old male with metastatic salivary gland tumor with asymptomatic Grade 3 elevation in alanine aminotransferase (ALT). No other cases of drug-related liver dysfunction were observed in the study. Other tubulin modulating agents have also shown clinical elevations in transaminases, e.g., paclitaxel (7) and the tubulin-targeting VDA, ABT-751 (8, 9).

Four patients achieved stable disease, but there were no objective responses. This is not unexpected based on previous clinical trials of this class of drug when administered as a single agent, e.g., in three phase I trials of single agent combretastatin, only 2/96 patients achieved an objective response (10–12). In preclinical models these agents leave a peripheral rim of viable tissue from which rapid regrowth can occur. Hence it is not expected that a high rate of objective responses could be achieved, but preclinical data suggest that these agents may be more promising when combined with other agents.

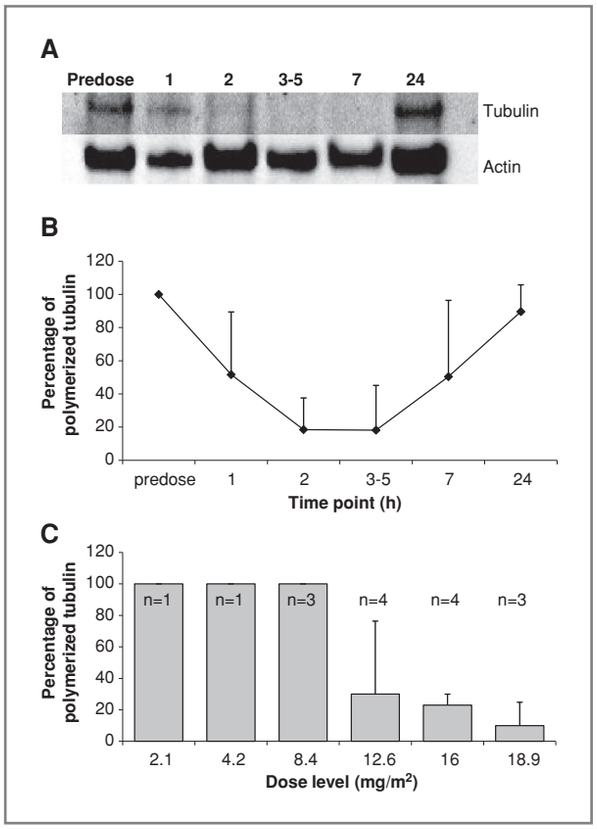

Figure 1. A, representative western blotting of PBMC pellet tubulin following BNC105P administration. Wells are labeled to represent predose, or the time (h) post drug administration (Cycle 1, Day 1; Patient # 18, 18.9 mg/m^2 BNC105P). The top band was detected using an anti–βI-tubulin antibody, while the lower band was detected with an anti–β-actin antibody and used as a loading control. B, combined results of Western blot densitometry analyses from patients dosed at 12.6 mg/m^2 ($n = 4$), 16 mg/m^2 ($n = 4$), and 18.9 mg/m^2 ($n = 3$). Normalized western blotting densitometry value converted to a percentage of the predose value. Each data point contains 11 measurements across cohorts plus standard deviation. C, tubulin concentration in cell pellets from each dose cohort at the 3 to 5 hours postdose time point. Each bar on the graph represents western blotting densitometry values converted to a percentage of the predose value plus standard deviation. No tubulin reduction was observed at or below the 8.4 mg/m^2 dose level. A reduction in PBMC pellet tubulin was observed at doses $\geq$12.6 mg/m^2.

PK sampling occurred over the 24-hour period following each administration in Cycle 1, though in the majority of patients both analytes (BNC105P and BNC105) were below detectable levels within 3 hours of dosing. The removal of the phosphate moiety of the BNC105P prodrug generates active BNC105, likely a result of exposure to nonspecific phosphatases within the blood. The analysis of plasma confirmed the rapid generation of BNC105 with the analyte being detected at the first time point (immediately upon completion of the prodrug infusion). At <1 hour, the elimination $t_{1/2}$ of BNC105 is shorter than that reported for combretastatin ($t_{1/2}$ = 4.3 hours; 10). Relatively high volumes of distribution and clearance were also observed. A "lock-in" effect remains a possibility, where a reduction in perfusion within target lesions occurs upon exposure to the VDA, trapping drug within the tumor matrix (4). The full metabolic profile of BNC105P remains unconfirmed, but preclinical studies suggest that cytochrome P450 (CYP)-regulated glucoronidation of BNC105 is likely a major metabolic pathway. This glucoronide has been shown to be inactive (4, 13).

Tumoral vascular disruption was elucidated by DCE-MRI. The technique has been employed in the assessment of several VDAs including ASA404, combrestatin, and ZD6126 (14). However, the technique remains exploratory in nature and requires relatively large changes in perfusion to occur before meaningful conclusions can be made. As the post-dose assessments were both conducted within 24 hours of administration of the Cycle 1 Day 1 dose, only immediate changes to a single administration could be observed. While no instances of marked tumor devascularization in whole or part were encountered, DCE-MRI quantitation yielded statistically significant declines in whole tumor K^{trans} values in some patients, largely at the 24 hours post-treatment DCE-MRI time point (Cycle 1, Day 2), and at the higher dose levels. Qualitative changes in tumor pixel $IAUGC^{90}$ maps were also seen in selected patients.

A western blotting densitometry analysis of PBMC tubulin was used to quantify changes in polymerized tubulin in PBMCs in response to BNC105. The method exploits the difference in mass between tubulin polymers and tubulin monomers, and was used to show that cell exposure to

Table 4. DCE-MRI. Changes in $IAUGC^{90}$ by dose cohort

Dose level mg/m^2	Mean $IAUGC^{90}$ baseline mM.sec	Mean $IAUGC^{90}$ 3–6 h (C1D1) mM.sec	% Change from baseline	Mean $IAUCG^{90}$ 24 h (C1D2) mM.sec	% Change from baseline
2.1–4.2	773.5	583.6[a]	−24.6[a]	397.8	−36.4[b]
8.4	502.6	411.1	−18.2	383.8	−23.6
12.6	546.2	588.4	7.7	562.0	2.9
16	571.3	599.2	4.9	486.5	−8.3[b]
18.9	218.3	271.8	24.5	247.0	13.1

[a]Indicates a statistically significant change.
[b]Percent change calculated from a different pre-treatment mean based on number of analyzable patients at 24 h post Cycle 1 Day 1.

Table 5. DCE-MRI. Changes in K^{trans} by dose cohort

Dose level mg/m^2	Mean K^{trans} baseline min^{-1}	Mean K^{trans} 3–6 h (C1D1) min^{-1}	% Change from baseline	Mean K^{trans} 24 h (C1D2) min^{-1}	% Change from baseline
2.1–4.2	0.368	0.235[a]	−36.1[a]	0.253	−2.7[b]
8.4	0.530	0.398	−25.0	0.457	−13.9
12.6	0.361	0.357	−1.1	0.318	−12.0
16	0.387	0.434	12.2	0.526	24.0[b]
18.9	0.173	0.137	−20.9	0.106[a]	−38.7[a]

[a]Indicates a statistically significant change.
[b]Percent change calculated from a different pre-treatment mean based on number of analyzable patients at 24 h post Cycle 1 Day 1.

BNC105 results in a reduction in tubulin polymer concentration. Reduced tubulin polymer levels are additional evidence of target engagement, albeit not in the tumor. A trend toward dose-response was also evident for this surrogate marker, with large reductions in polymerized tubulin being detected at the three highest dose levels (Fig. 1C). To our knowledge this is the first report of a consistent decrease in polymerized tubulin following exposure to a tubulin-targeting VDA. Previously, the effect of ABT-751 on tubulin polymerization was evaluated in neuroblastoma patients using an antidetyrosinated α-tubulin antibody. However, a consistent pattern was not observed across all patient samples (15). The search continues for validated biomarkers within this compound class. Cell adhesion molecules, von Willebrand factor, and circulating endothelial cells have all been evaluated (10, 16, 17). Quantitation of polymerized tubulin from PBMCs represents another option for the assessment of PD effect.

In summary, this first-in-human study has established the recommended dose level of 16 mg/m^2 of BNC105P administered as a 10-minute IV infusion on Days 1 and 8 of a repeating 21-day cycle. This dose level was well tolerated. "On target" activity was confirmed by a decrease in the levels of polymerized tubulin in PBMCs, a surrogate marker. In addition, at the higher dose levels significant changes on DCE-MRI were observed in some patients. Phase II and I/II studies in mesothelioma and renal cell carcinoma are now underway to investigate this agent as monotherapy and in combination with the mTOR inhibitor everolimus, respectively.

Disclosure of Potential Conflicts of Interest

D.C. Bibby, G. Kremmidiotis, A.F. Leske, and C.A. Matthews are employed by and have ownership interest in Bionomics Limited. The other authors disclosed no potential conflicts of interest.

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