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Therapeutic Discovery

Molecular Cancer Therapeutics

Safety, Pharmacokinetics, and Activity of GRN1005, a Novel Conjugate of Angiopep-2, a Peptide Facilitating Brain Penetration, and Paclitaxel, in Patients with Advanced Solid Tumors

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Abstract

GRN1005 is a novel peptide–drug conjugate composed of paclitaxel covalently linked to a peptide, angiopep-2, that targets the low-density lipoprotein receptor-related protein 1. This first-in-human study evaluated the safety, tolerability, pharmacokinetics, and efficacy of GRN1005 in patients with advanced solid tumors. Patients in sequential cohorts (one patient per cohort until grade 2 toxicity, then 3 + 3 design) received intravenous GRN1005 at escalating doses between 30 and 700 mg/m^2 once in every 21 days. In the maximum tolerated dose (MTD) expansion group, patients were required to have brain metastases. Fifty-six patients received GRN1005, including 41 with brain metastases (median number of prior therapies = 4). MTD was 650 mg/m^2; the main dose-limiting toxicity was myelosuppression. Sixteen of 20 patients dosed at the MTD had brain metastases. Pharmacokinetics was dose linear and the mean terminal-phase elimination half-life was 3.6 hours. No evidence of accumulation was observed after repeat dosing. No anti-GRN1005 antibodies were detected. Five of the 20 patients (25%) dosed at 650 mg/m^2 (MTD), three of whom had previous taxane therapy, achieved an overall partial response (breast, $n = 2$; non–small cell lung cancer, $n = 2$; and ovarian cancer, $n = 1$); responses in all five patients were also accompanied by shrinkage of brain lesions (−17% to −50%). In addition, six patients (11%; doses 30–700 mg/m^2) experienced stable disease that lasted 4 months or more. GRN1005 was well tolerated and showed activity in heavily pretreated patients with advanced solid tumors, including those who had brain metastases and/or failed prior taxane therapy. *Mol Cancer Ther; 11(2); 308–16. ©2011 AACR.*

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Introduction

As many as 170,000 patients with solid tumors develop brain metastases in the United States each year (1). In the absence of effective treatment options, the prognosis for patients with brain metastases is poor, with an estimated median survival less than 4 months (1). Many studies exclude patients with brain metastases, despite the urgent need to develop treatments for this critical problem.

Treatment of brain metastases sometimes includes steroids to control edema, anticonvulsants to control seizures, resection when appropriate, whole brain irradiation, radiosurgery, and/or chemotherapy with efficacy limited by the difficulty posed by the blood–brain barrier (2–5).

GRN1005 (formerly known as ANG1005) is a conjugate of angiopep-2 (peptide backbone) and 3 molecules of paclitaxel that contribute approximately 50% of its molecular weight (MW; ref. 6). While standard paclitaxel is marketed in a formulation that contains Cremophor EL, GRN1005 is Cremophor free (7). This is advantageous, as many toxicities, such as hypersensitivity reactions, have been attributed to the Cremophor EL associated with paclitaxel (8).

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GRN1005 can actively penetrate into the brain compartment by targeting low-density lipoprotein receptor-related protein-1 (LRP-1), which is highly expressed on the surface of the blood–brain barrier. *In vivo* and *in vitro* data show that the brain's uptake rate of GRN1005 is 86-fold greater than paclitaxel and approximately 10-fold greater than temozolomide (6, 9). Using the same receptor-mediated transporter, GRN1005 enters tumor cells, many of which also express high levels of LRP-1. The bonds between angiopep-2 and paclitaxel are cleaved by esterases found in large concentrations in lysosomal compartments, releasing free paclitaxel to exert its antimitotic functions at the site of disease. Paclitaxel does not reach high concentrations in brain tumor tissue following normal intravenous injection, potentially due to transport by phospho-glycoprotein (10, 11). The potential for GRN1005 to cross the blood–brain barrier and specifically target tumor cells in patients with brain metastases would offer significant therapeutic advantages over currently available treatments.

Herein, we report the results of a first-in-human, open-label, phase I clinical study conducted to assess the safety, tolerability, pharmacokinetics, and preliminary evidence of efficacy of GRN1005 in adult patients with advanced solid tumors.

Patients and Methods

Eligibility criteria

To be eligible, patients had to be 18 years or more of age and have progressing and measurable metastatic or advanced solid tumor not amenable to established forms of therapy, an Eastern Cooperative Oncology Group performance status 0 to 2, and adequate hematologic, hepatic, and renal function; patients enrolled into the expanded maximum tolerated dose (MTD) cohort were required to show evidence of progressing brain metastases by computed tomography (CT) or MRI scan before study entry. Patients with symptomatic brain metastases were not excluded from the study. Patients with unstable or uncompensated organ system dysfunction, known severe hypersensitivity to paclitaxel, severe toxicity with previous taxane treatment, and/or persistent $\geq$ grade 2 neurotoxicity were excluded. Treatment with P450, CYP3A4, and 2C8 enzyme-inducing anticonvulsant drugs during the study and within 14 days of day 1, and chemotherapy, immunotherapy, radiotherapy, and investigational agents during the study and within 4 weeks of day 1 were prohibited. Institutional Review Board approval and written informed consents were obtained before study-related procedures were started.

Study design

This first-in-human, phase I, open-label study used a rapid dose-escalation design (12). The starting dose, 30 mg/m^2, was calculated on the basis of toxicology studies in rats and dogs. Escalation by dose doubling was carried out for 2 dose levels (60 and 120 mg/m^2), after which a modified Fibonacci escalation scheme (i.e., increases of 67%, 50%, 40%, and 33%) was used to guide dose increases. GRN1005 was administered by intravenous infusion at a concentration of 1.5 mg/mL and a rate of 8.0 to 8.5 mL/min (~1 hour) once every 21 days. Premedication was not allowed during cycle 1 and was allowed only as medically indicated thereafter.

In the dose-escalation phase, patients were enrolled sequentially into cohorts of 1 to 3 patients each, until a $\geq$ grade 2 drug-related toxicity was observed, at which point that and subsequent cohorts were expanded to a minimum of 3 patients. If one patient experienced a dose-limiting toxicity (DLT), a minimum of 6 patients were enrolled at that dose. Dose escalation continued until more than 1 of 6 patients in a cohort experienced a DLT during cycle 1, after which a lower dose was explored. Once identified, the MTD cohort was expanded to obtain additional safety and pharmacokinetics data and to explore potential antitumor activity in patients with brain metastases. Patients remained on study until disease progression, death, unacceptable toxicity, or consent withdrawal.

DLT and MTD

DLT was defined as any of the following occurring during cycle 1 that were treatment emergent and possibly related to GRN1005: any grade 3 or 4 nonhematologic toxicity; febrile neutropenia; grade 4 neutropenia lasting 7 days or more; grade 4 thrombocytopenia; grade 2 peripheral neuropathy lasting 7 days or more or $\geq$ grade 3 peripheral neuropathy of any duration. The MTD was defined as the dose level at which 1 or less of 6 patients in a cohort developed a DLT during cycle 1. Dose reductions (one dose level) were permitted for patients who experienced a DLT, and patients were allowed 2 dose reductions.

Evaluation of safety

Adverse events were recorded for patients who received at least one dose of GRN1005. Severity was assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 3.0.

Vital signs were measured at various time points up to 4 hours after infusion and regularly between infusions. Electrocardiograms were obtained before infusion and 30 minutes and 3 hours after infusion during cycle 1. Hematology, blood chemistry, and urine values were monitored regularly, and physical and neurologic examinations were also carried out.

Neurocognitive testing

Neurocognitive testing was done at baseline and every 6 weeks during treatment to assess potential neurotoxic effects. The battery included the following tests: Hopkins Verbal Learning Tests–-Revised (HVLT-R); Trail Making; Controlled Oral Word Association (COWA); and Grooved Pegboard (13, 14). Test results

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were sent for central evaluation and reading by an independent reviewer.

Immunogenicity

Serum for assessment of anti-GRN1005 antibodies was collected predose at each treatment cycle and at the final visit. Anti-GRN1005 antibodies were assayed by a validated ELISA with a sensitivity of 0.56 μg/mL or less (15, 16).

Pharmacokinetics

Plasma samples for pharmacokinetics characterization were collected before infusion, at end of infusion, and 30 minutes, 1, 2, 3, 4, and 24 hours after infusion during cycles 1 and 3. GRN1005 and paclitaxel concentrations were determined with validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) methods (17, 18).

Concentrations of ANG1005 in human EDTA K_2 plasma were determined with LC/MS-MS with a limit of quantitation of 0.50 to 2.00 μg/mL (17, 18). GRN1005 (MW of 5,109.14 Da) was extracted by protein precipitation and quantified by peak area ratio. A weighed $1/X^2$ linear regression was carried out to determine the concentration of GRN1005.

Concentration of free paclitaxel was determined with LC/MS-MS with a limit of quantitation of 100 ng/mL. Paclitaxel (MW of 853.9 Da) was extracted from an aliquot of human EDTA plasma containing GRN1005 using an automated liquid–liquid extraction, then injected into a liquid chromatograph equipped with a tandem mass spectrometry detector and quantified by peak area ratio. A weighed $1/X^2$ linear regression was carried out to determine the concentration of paclitaxel.

Evaluation of efficacy

Treatment efficacy was evaluated by CT or MRI per Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 (19) in all organs in which disease was present, including brain, before treatment, and every 6 weeks thereafter. Briefly, complete response was disappearance of all lesions; partial response was 30% or more reduction in the sum of the longest diameters of the lesions; stable disease was sum of longest diameters not decreased more than 30% and not increased more than 20%; and progressive disease was 20% or more increase in the sum of the longest diameters of the lesions. Overall response was determined by the primary physician; scans from patients dosed more than 300 mg/m^2 and who experienced tumor shrinkage per RECIST were later sent to an independent radiologist for examination of lesion response per organ system.

Statistical analysis

Descriptive statistics are provided for demographic, safety, pharmacokinetics, and efficacy data. Categorical data are summarized by frequency and percentages; continuous data are summarized by mean and SD, or median and range, as appropriate. Changes from baseline in neurocognitive test results were assessed using paired *t* tests.

Results

Patient characteristics

Fifty-six patients were enrolled across 3 study centers in the United States (University of Texas MD Anderson Cancer Center, Houston, TX; Institute for Drug Development, Cancer Therapy and Research Center at University of Texas Health Science Center San Antonio, San Antonio, TX; and Gabrail Cancer Center, Canton, OH). A total of 160 doses of GRN1005 were administered, for a median of 2 doses per patient (range: 1–11). All 56 patients were included in safety and efficacy evaluations.

Demographics and clinical characteristics at study entry are summarized in Table 1. Forty-one patients (73%) had brain metastases at the time of enrollment, including both patients who had and had not received prior brain radiation and patients treated with steroids,

Table 1. Patient demographics

Characteristics ($n = 56$)	
Age, y	
Median	54
Range	23–81
Gender, *n* (%)	
Men	24 (43)
Women	32 (57)
Primary tumor site, *n* (%)	
Breast	14 (25)
Melanoma	13 (23
Lung (NSCLC)	8 (14.5)
Lung (SCLC)	8 (14.5)
Head and neck	7 (12.5)
Other[a]	6 (10.5)
Brain metastases, *n* (%)	
Yes	41 (73)
No	15 (27)
No. of prior therapies, *n* (%)	
≤2	15 (27)
3—5	23 (41)
≥6	18 (32)
Prior radiotherapy, *n* (%)	
Yes	44 (79)
No	12 (21)
ECOG performance status score, *n* (%)	
0	12 (21.5)
1	35 (62.5)
2	9 (16)

Abbreviation: SCLC, small-cell lung cancer; ECOG, Eastern Cooperative Oncology Group.

[a]Other includes hepatocellular carcinoma ($n = 2$), colorectal ($n = 2$), cervical ($n = 1$), and ovarian ($n = 1$).

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Table 2. Adverse events ≥ possibly related to GRN1005

Dose, mg/m^2	30 ($n = 1$)		60 ($n = 3$)		120 ($n = 3$)		200 ($n = 3$)		300 ($n = 7$)[a]		420 ($n = 6$)[a]		500 ($n = 4$)[a]		550 ($n = 3$)		650[b] ($n = 20$)		700 ($n = 6$)	
NCI-CTCAE grade	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**	**1–2**	**3–4**
Hematologic																				
Neutropenia	0	0	1	0	0	0	2	0	1	1	2	2	1	0	1	1	1	18	0	4
Febrile neutropenia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (DLT)	1 (DLT)	0	0
Anemia	1	0	3	0	1	0	3	0	6	1	6	0	4	0	3	0	16	3	4	1
Thrombocytopenia	0	0	1	0	0	0	0	0	1	1	1	1	1	0	1	0	8	3	1	2 (1 DLT)
Nonhematologic																				
Fatigue	1	0	0	0	0	0	1	0	1	0	3	0	1	0	1	0	5	2	1	0
Alopecia	0	0	0	0	0	0	0	0	1	0	2	0	1	0	1	0	7	0	0	0
Neuropathy	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	7 (1 DLT)	1	1	1
Dehydration	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	2 (1 DLT)[d]	0	0
Hypotension	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1 (DLT)
Infusion reaction[c]	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0
Mucositis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	6	0	1	1
Rash	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
Pneumonia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (DLT)	0	0
Gastrointestinal																				
Nausea/vomiting	0	0	1	0	1	0	1	0	2	0	1	0	1	0	2	0	6	0	0	1
Diarrhea	0	0	0	0	0	0	0	0	1	0	2	0	0	0	2	0	6	0	1	0
Anorexia	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	2	1	1	0

[a]Because of discovery of cloudy infusion solutions, dosing was repeated in additional patients at the 300 to 500 mg/m^2 dose levels and escalated again from that point.

[b]A total of 650 mg/m^2 administered once every 3 weeks was determined to be the MTD.

[c]Infusion reactions were sometimes characterized by facial flushing, bradycardia, hypotension, dizziness, shortness of breath, chest tightening, and rash.

[d]This patient experienced grade III dehydration and grade III stomatitis during cycle 1 of treatment.

including while receiving GRN1005. The median number of prior therapies was 4 (range: 0–22).

Reasons for study withdrawal were: disease progression ($n = 33$), adverse events (all causalities including not related to drug; $n = 12$), investigator decision ($n = 4$), consent withdrawal ($n = 6$), and death ($n = 1$).

Dose escalation

Patients were enrolled sequentially into the following dose cohorts: 30, 60, 120, 200, 300, 420, 500, 550, 650, and 700 mg/m^2. At the 60 mg/m^2 dose, grade 2 neutropenia and anemia occurred, and the dose level and subsequent dose-escalation cohorts were expanded to include 3 to 6 patients. At the 500 mg/m^2 dose, the study sites noticed that the dosing solution was turning cloudy during the infusion period. On the basis of reported adverse events, the cloudy solutions did not jeopardize patient safety; enrollment was nevertheless temporarily interrupted to investigate the formulation. Analyses determined that at higher concentrations, GRN1005 was precipitating out of solution; this did not affect the purity of GRN1005 but did influence its potency. These patients were still included in safety and efficacy evaluations. A modified dilution process was subsequently implemented and dosing was repeated as of the 300 mg/m^2 dose level in the interest of rigor. These findings were reported to the Institutional Review Boards and the U.S. Food and Drug Administration.

DTLs and MTD

No DLTs were reported at doses 550 mg/m^2 or less. The first DLT (febrile neutropenia) was reported in one of 3 patients initially enrolled at 650 mg/m^2. This patient was treated with filgrastim and intravenous antibiotics, and febrile neutropenia resolved in 2 days. Dose escalation continued after no DLTs were observed in the additional 3 patients enrolled at this dose level. A DLT occurred in 2 of 6 patients enrolled at the next dose level (700 mg/m^2). One patient experienced grade 4 thrombocytopenia that was treated with a platelet transfusion to resolution the same day. Another patient with metastatic breast cancer to the bone, brain, liver, and lung experienced grade 3 hypotension accompanied by multiorgan failure within 6 days of the first infusion. Relationship to GRN1005 could not be ruled out because of the temporal relationship between events, although disease state could have also been a cause. Because criteria for MTD were exceeded at 700 mg/m^2, the 650 mg/m^2 dose was determined to be the

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MTD for GRN1005 and the dose cohort was expanded to 20 patients, including 16 with brain metastases. Among the 20 patients treated at 650 mg/m^2, 5 patients experienced DLTs in the first cycle: febrile neutropenia ($n = 2$), dehydration with stomatitis ($n = 1$), neuropathy ($n = 1$), and pneumonia ($n = 1$).

Safety

All 56 patients received at least one dose of GRN1005 and were evaluated for safety and tolerability. Dose reductions were required in 11 patients (20%). Myelosuppression was the major toxicity (Table 2) although most incidences were manageable and reversible with standard treatments. Dose-limiting neutropenia and febrile neutropenia was managed by the addition of granulocyte colony-stimulating factor (G-CSF), dose reduction, or both. Peripheral neuropathy was reported only at doses 420 mg/m^2 or more; it occurred in 21.5% of patients ($\geq$ grade 2 in 12.5% of patients). Infusion reactions were observed in 9% of patients ($\geq$ grade 3 in 3.5% of patients); they occurred at doses ranging from 200 to 650 mg/m^2. Symptoms included facial flushing, bradycardia, hypotension, and dyspnea; they occurred sporadically and despite premedication with acetylsalicylic acid, hydrocortisone, ranitidine, and diphenhydramine in one patient. Rash ($\leq$ grade 2 in all cases) was reported only at 650 mg/m^2; it occurred in 9% of patients. Rash, typically erythema, was not associated with infusion reaction except in one patient.

Immunogenicity

One hundred and fifty-five samples from 45 patients were analyzed for presence of anti-GRN1005 antibodies. No antibodies were detected, even in patients who received up to 11 treatment cycles and/or patients who reported infusion reactions and/or rashes.

Neurocognitive testing

Twenty patients carried out at least one posttreatment neurocognitive test battery and were included in the analysis. Results revealed no evidence that GRN1005 causes central nervous system (CNS) toxicity. One patient who experienced stable disease for more than 7.5 months showed significant improvement in memory, processing speed, and executive function after 6, 12, 18, and 24 weeks of therapy. Radiographic images of the patient's brain lesion showed changes in tumor size from baseline of −9%, +3%, −6%, and −3% at 6, 12, 18, and 24 weeks, respectively.

Pharmacokinetics

Fifty-five patients had at least one posttreatment pharmacokinetics sample drawn and were included in the analysis. The pharmacokinetics of GRN1005 seemed to be dose proportional. After single dose intravenous infusion, GRN1005 showed an overall mean time to maximum observed plasma concentration (T_{max}) ranging from 0 to 0.5 hours after infusion (Table 3). The mean terminal-phase elimination half-life ($t_{1/2}$) was approximately 3.6 hours. Plasma concentrations of GRN1005 during and after cycle 1 infusion are shown in Fig. 1A. No evidence of accumulation was observed after repeat dosing, as indicated by comparable mean maximum concentration (C_{max}) and area under the concentration–time curve to infinity (AUC_{inf}) values at cycles 1 and 3 (Fig. 1B). Plasma concentrations of free paclitaxel measured at the MTD (650 mg/m^2) revealed that most paclitaxel in plasma remained associated with angiopep-2 over the time course

Table 3. Pharmacokinetic data for GRN1005

Parameter	Mean[a]								
Dose, mg/m^2	**60**	**120**	**200**	**300**	**420**	**500**	**550**	**650**	**700**
n	3	3	3	7	5 or 6[b]	3	3	20	6
C_{max}, μg/mL	30.0	55.2	119	160	232	210	238	306	376
C_{max}, μmol/L	5.87	10.8	23.3	31.3	45.4	41.1	46.6	59.9	73.6
T_{max}, h	0.17	0.17	0.17	0.14	0.40	0.17	0.00	0.33	0.08
AUC_{inf}, μg h/mL	140	261	664	818	1,369	1,532	1,810	2,571	3,322
AUC_{inf}, μmol/L h	27.4	51.1	130	160	268	300	354	503	650
$t_{1/2}$, h	2.57	2.94	3.52	3.19	3.73	3.72	3.57	4.00	3.96
CL, mL/m^2 h	428	470	302	381	324	343	326	285	228
V_d, mL/m^2	1,590	1,947	1,537	1,769	1,565	1,799	1,675	1,666	1,312

Abbreviations: AUC_{inf} = area under the drug concentration–time curve from time zero to infinity; CL = clearance; C_{max} = maximum observed drug concentration; $t_{1/2}$ = half-life; T_{max} = time to maximum observed drug concentration; and V_d = apparent volume of distribution.

[a]Data from one patient treated at 30 mg/m^2 and one patient treated at 500 mg/m^2 were not included in the pharmacokinetic data as these patients did not receive a complete infusion.

[b]$n = 5$ for C_{max}, T_{max}, AUC_{inf}, V_d and $n = 6$ for half-life and CL.

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analyzed (Fig. 1C). Pharmacokinetic measures of paclitaxel exposure (Cmax and AUClast) showed that free paclitaxel exposures were approximately 14- to 15-fold lower than those of ANG1005-associated paclitaxel in plasma during both cycles (AUC = 1,447 μmol/L for GRN1005 versus 101 μmol/L for paclitaxel and C_{max} = 1,187 μmol/L for GRN1005 versus 13 μmol/L for paclitaxel). There was no evidence of accumulation of unconjugated paclitaxel based on pharmacokinetics analyses for cycle 1 versus cycle 3.

Efficacy

All 56 patients were included in the efficacy evaluation. Response data were available for 43 patients: 39 patients had at least one posttreatment evaluation and 4 patients came off study early for clinical progression. The remaining 13 patients were not reevaluated posttreatment for the following reasons: consent withdrawal ($n = 5$), adverse events ($n = 4$), investigator decision ($n = 3$), and death ($n = 1$); these patients were considered treatment failures.

Best overall responses ($n = 43$) are shown in Fig. 2A and Table 4. Five patients (9%) achieved a partial response (breast cancer, $n = 2$; non–small cell lung cancer (NSCLC), $n = 2$, 1 unconfirmed; and ovarian cancer, $n = 1$). All partial responses were observed at the 650 mg/m^2 dose (MTD; Table 4). Among the 5 patients achieving partial response, all also experienced shrinkages in their brain lesions (Fig. 2B and C) as assessed by an independent radiologist; brain responses ranged from −17% to −50%. Furthermore, 3 of these patients had failed prior taxane therapy. No patients in Fig. 2B who had increase in brain lesions achieved partial response or stable disease for 4 months or more.

The median duration of partial response was 1.5 months (range: 1–5 months). The greatest overall tumor reduction was observed in a 45-year-old woman with breast cancer metastasized to the brain, liver, lung, and lymph nodes who was treated at 650 mg/m^2 for 5 cycles then 420 mg/m^2 for 2 cycles. The patient achieved a partial response (−60%) after 1.5 months of GRN1005. Measurement of her brain lesions by an independent radiologist showed shrinkage of −48% after 4.5 months of treatment [Fig. 2C (i)]. The patient withdrew from the study after approximately 6.5 months due to neuropathy.

In addition to the 5 patients with partial response, 6 patients (11%) had stable disease for 4 months or more, with the longest duration being 8 months (a patient with NSCLC and brain metastasis dosed at 420 mg/m^2).

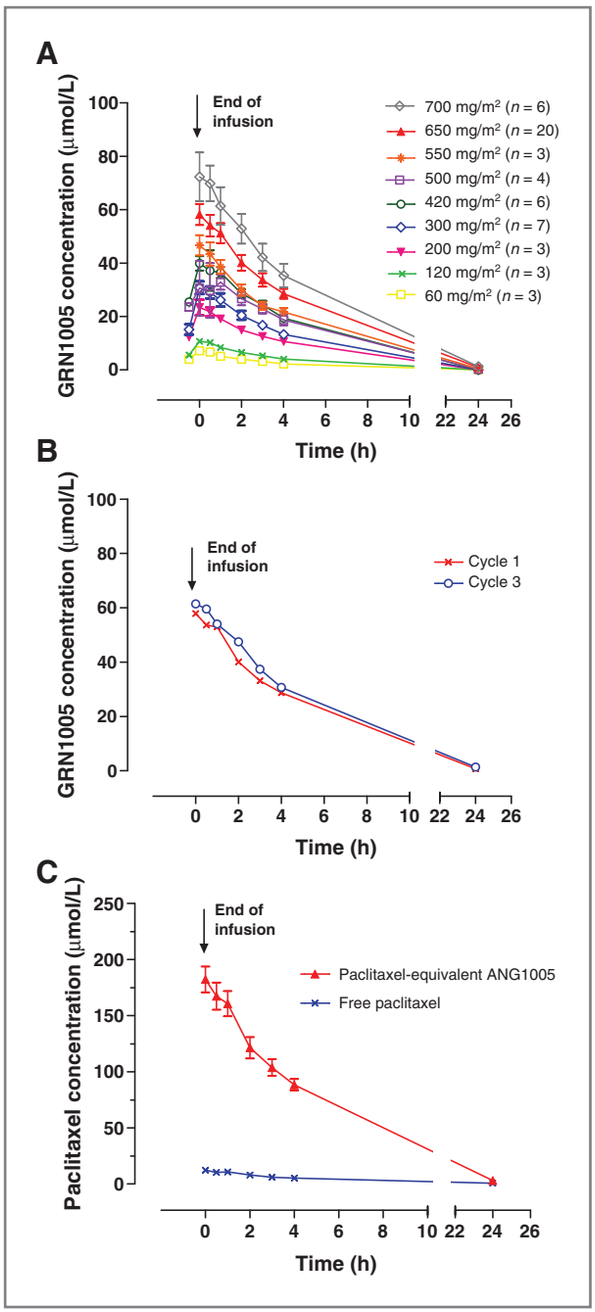

Figure 1. A, mean (±SEM) plasma concentrations of GRN1005 during and after infusion at cycle 1 ($n = 3$–20). B, mean plasma concentrations of GRN1005 in the MTD group ($n = 8$) at cycles 1 and 3. C, mean (±SEM) paclitaxel concentrations (free and associated with angiopep-2 expressed as paclitaxel-equivalent GRN1005) during cycle 1 in the MTD Group ($n = 16$).

Discussion

GRN1005 administered as monotherapy by intravenous infusion once every 3 weeks was well tolerated up to 650 mg/m^2. The most common DLT was febrile neutropenia.

The most frequently observed toxicity was myelosuppression, which was generally manageable and reversible with standard treatments. Adverse events such as peripheral neuropathy, infusion reactions, and rashes were seen in a minority of patients, and no CNS toxicity was revealed by neurocognitive testing and neurologic examination. Reversal of neurologic deficits after treatment with GRN1005 was observed in one patient who showed

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Table 4. Overall best response by dose cohort

GRN1005 dose	30 mg/m^2	60 mg/m^2	120 mg/m^2	200 mg/m^2	300 mg/m^2	420 mg/m^2	550 mg/m^2	650 mg/m^2	700 mg/m^2
Overall best response ($n = 43$)	($n = 1$)	($n = 3$)	($n = 3$)	($n = 3$)	($n = 6$)	($n = 5$)	($n = 1$)	($n = 16$)	($n = 5$)
CR									
PR								5	
SD	1	1	1	2		2	1	4	4
PD		2	2	1	6	3		7	1

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; and PD, progressive disease.

marked improvements in memory, processing speed, and executive function after only 6 weeks of therapy; these improvements were accompanied by a 9% shrinkage of brain metastases.

The pharmacokinetics of GRN1005 showed dose proportional increases in C_{max} and AUC_{inf} at doses ranging from 30 to 700 mg/m^2. The mean $t_{1/2}$ was 3.6 hours; in contrast, the $t_{1/2}$ for nab-paclitaxel is 21.6 hours and 20.5 hours for paclitaxel (20). Repeat dosing revealed no evidence of accumulation, lending evidence to support the safety of long-term GRN1005 use. Analyses at the MTD showed that most paclitaxel in plasma remains associated with the angiopep-2 peptide over a period of at least 24 hours postinfusion. The C_{max} for GRN1005 at the MTD was 3,06,000 ng/mL versus 22,968 and 3,543 μg/mL for nab-paclitaxel and paclitaxel, respectively, at doses of 260 and 175 mg/m^2 (which are their MTDs; ref. 20). This supports preclinical testing results and correlates well with the favorable safety profile observed in patients to date.

Although GRN1005 has a peptide backbone, no anti-GRN1005 antibodies were detected even after repeat

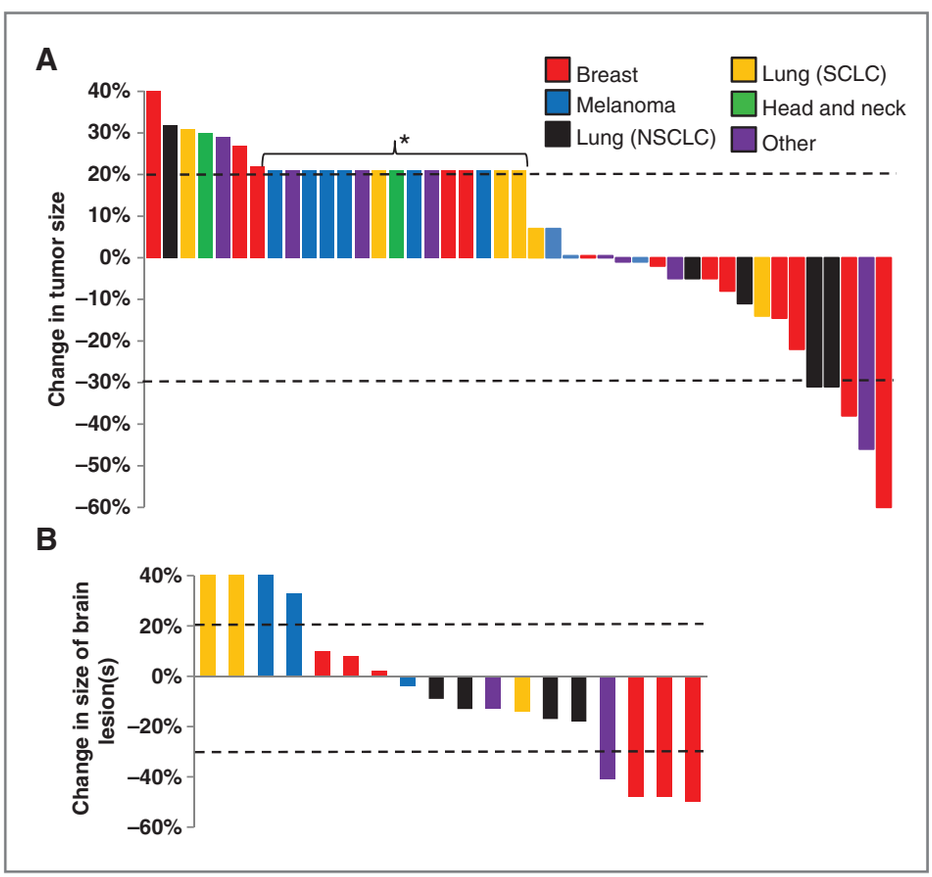

Figure 2. A, best response in 43 of 56 patients treated. Patients with early clinical progression or new lesions are indicated on the graph as +21% and marked with an asterisk. Thirteen patients were not reevaluated posttreatment because of consent withdrawal ($n = 5$), adverse events ($n = 4$), investigator decision ($n = 3$), and death ($n = 1$). All these patients are considered treatment failures. B, brain responses in patients were assessed for nonprogressing patients who received doses from 420 mg/m^2 and higher, including all patients who were treated at the MTD.

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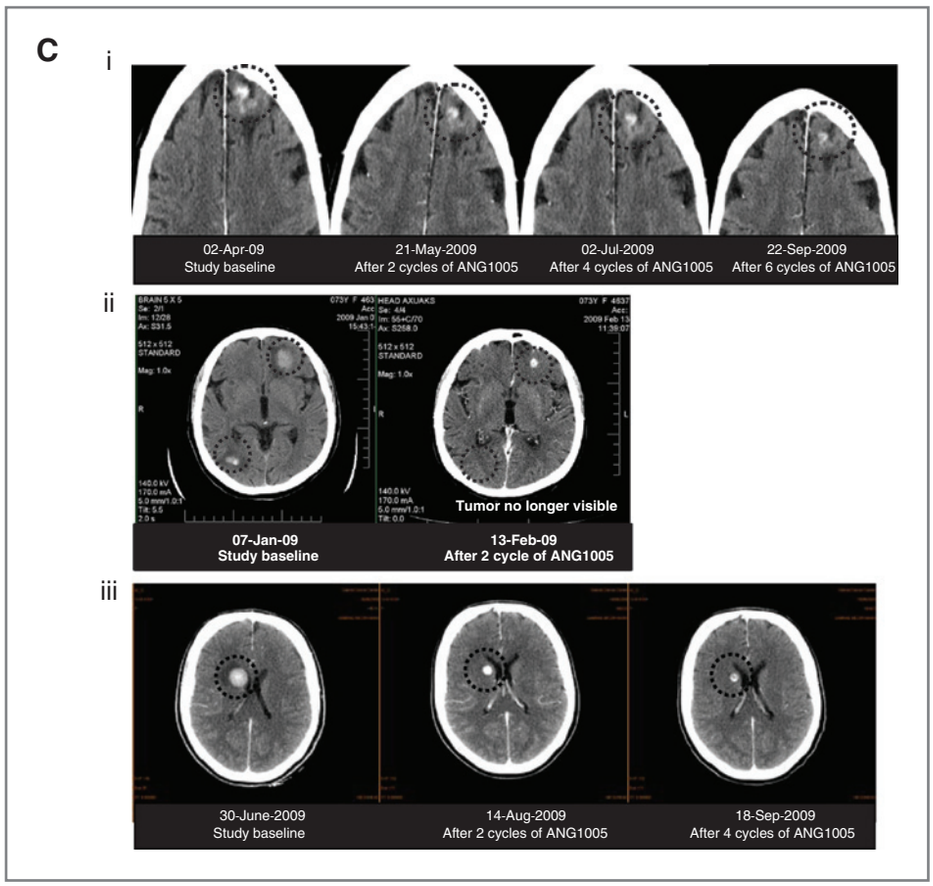

Figure 2. (*Continued*) C, radiographic images of the brain of 3 patients treated at the MTD (650 mg/m^2) who achieved an overall best response of partial response: i, 45-year-old woman with breast cancer; ii, 73-year-old woman with ovarian cancer; and iii, 38-year-old woman with breast cancer.

dosing and in cases where infusion reactions and rashes were observed. These results suggest that GRN1005 does not elicit an antibody response.

In this heavily pretreated patient population, treatment with GRN1005 showed evidence of efficacy with tumor stabilization and several cases of significant reductions in tumor size. While paclitaxel is efficacious against various cancers, the clinical use of paclitaxel to treat brain cancer has been hampered by the inability of molecule to cross the blood–brain barrier and reach the tumor. In this study, 5 of 20 patients (25%) dosed at the MTD (650 mg/m^2) achieved an overall partial response; in each of these patients, there was also shrinkage in brain metastases by RECIST criteria (−17% to −50%). Three of these patients had failed prior taxane therapy. Future phase II studies could also evaluate brain response by Macdonald and RANO criteria (21, 22).

The receptor that facilitates GRN1005's penetration of the brain, LRP-1, is not only highly expressed on the surface of the blood–brain barrier, it is also upregulated in various cancer cell types (23). Lesion response by organ system was therefore assessed by an independent radiologist in patients dosed more than 300 mg/m^2 and who experienced tumor shrinkage per RECIST (data not shown). Interesting antitumor effects were observed in metastatic locations including the liver, lungs, lymph nodes, and bones. Notably, 7 of 8 patients with liver metastases showed shrinkage in their liver lesions (maximum = −100%) and all 9 patients with lung lesions had tumor shrinkage within the lung (maximum = −100%). Lung metastases and liver lesion shrinkage occurred even in patients who did not have an overall response of partial response, including one patient with a complete response in liver lesions and one patient who achieved a partial response in the lung. In addition, one patient who did not achieve an overall partial response had a partial response in her brain lesions. These data points to a possible distinct ability of GRN1005 to effectively treat patients with brain metastases as well as active systemic disease.

In summary, GRN1005 is a new chemical entity with a unique targeting mechanism. It was well tolerated and showed evidence of activity in patients with advanced solid tumors and brain metastases. These results suggest that GRN1005 is able to penetrate the blood–brain barrier and has activity in both the brain and other metastatic sites, despite failure of multiple previous lines of therapy including taxanes. Further study of this molecule at the recommended phase II starting dose of 650 mg/m^2 given intravenously once every 3 weeks is warranted and studies in patients with breast and NSCLC with brain metastases are planned.

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Disclosure of Potential Conflicts of Interest

K. Elian was employed by Angiochem Inc. at the time the research was conducted. R. Kurzrock received commercial research support from Angiochem Inc. No potential conflicts of interest were disclosed by the other authors.

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