

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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Abbreviations: AE (adverse event), AUC (area under the curve), C_{max} (maximum concentration), COWA (controlled oral word association), CR (complete response), CT (computed tomography), CTCAE (Common Terminology Criteria for Adverse Events), DLT (dose-limiting toxicity), ECOG (Eastern Cooperative Oncology Group), EDTA (ethylenediaminetetraacetic acid), ELISA (enzyme-linked immunosorbent assay), FDA (Food and Drug Administration), HVLt-R (Hopkins Verbal Learning Tests-Revised), IRB (Institutional Review Board), IV (intravenous), LC/MS/MS (liquid chromatography/mass spectrometry/mass spectrometry), LRP-1 (lipoprotein receptor-related protein-1), MRI

(magnetic resonance imaging), MTD (maximum tolerated dose), NCI (National Cancer Institute), NSCLC (non-small cell lung cancer), PK (pharmacokinetics), PR (partial response), RECIST (Response Evaluation Criteria in Solid Tumors), SD (stable disease), SD (standard deviation), $t_{1/2}$ (half-life), T_{max} (time to maximum observed concentration)

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ABSTRACT

GRN1005 is a novel peptide-drug conjugate (PDC) comprised of paclitaxel covalently linked to a peptide, Angiopep-2, that targets the low-density lipoprotein receptor-related protein 1 (LRP-1). This first-in-human study evaluated the safety, tolerability, pharmacokinetics (PK) 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 mg/m² and 700 mg/m² once 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/m²; the main dose-limiting toxicity was myelosuppression. Sixteen of 20 patients dosed at the MTD had brain metastases. PK 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/m² (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/m²) experienced stable disease that lasted \geq 4 months.

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.

INTRODUCTION

As many as 170,000 patients with solid tumors develop brain metastases in the US 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 three molecules of paclitaxel which contribute approximately 50% of its molecular weight (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).

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 IV injection, potentially due to transport by p-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 (PK) 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 of age and have progressing and measurable metastatic or advanced solid tumor not amenable to established forms of therapy, an Eastern Cooperative Oncology Group (ECOG) performance status 0-2 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 magnetic resonance imaging (MRI) scan prior to 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 CYP 3A4 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 (IRB) 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/m², was calculated based on toxicology studies in rats and dogs. Escalation by dose-doubling was performed for two dose levels (60 and 120 mg/m²), 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 (IV) infusion at a concentration of 1.5 mg/mL and a rate of 8.0-8.5 mL/minute (~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 1-3 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 three patients. If one patient experienced a dose-limiting toxicity (DLT), a minimum of six patients were enrolled at that dose. Dose escalation continued until > 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 PK 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.

Dose-Limiting Toxicity and Maximum Tolerated Dose

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; Grade 4 thrombocytopenia; Grade 2 peripheral neuropathy lasting ≥ 7 days or \geq Grade 3 peripheral neuropathy of any duration. The MTD was defined as the dose level at which ≤ 1 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 two dose reductions.

Evaluation of Safety

Adverse events (AEs) 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 timepoints up to 4 hours after infusion and regularly between infusions. Electrocardiograms were obtained prior to 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 performed.

Neurocognitive testing

Neurocognitive testing was done at baseline and every six 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 were sent for central evaluation and reading by an independent reviewer.

Immunogenicity

Serum for assessment of anti-GRN1005 antibodies was collected pre-dose at each treatment cycle and at the final visit. Anti-GRN1005 antibodies were assayed using a validated enzyme-linked immunosorbent assay (ELISA) with a sensitivity ≤ 0.56 $\mu\text{g/mL}$ (15, 16).

Pharmacokinetics (PK)

Plasma samples for PK characterization were collected prior to 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 using validated liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methods (17, 18).

Concentrations of ANG1005 in human ethylenediaminetetraacetic acid (EDTA) K₂ plasma were determined using Liquid Chromatography/Mass Spectrometry/ Mass Spectrometry (LC/MS/MS) with a limit of quantitation of 0.50 – 2.00 µg/mL (17, 18). GRN1005 (MW of 5109.14 daltons) was extracted by protein precipitation and quantified by peak area ratio. A weighed 1/X² linear regression was performed to determine the concentration of GRN1005.

Concentration of free paclitaxel was determined using LC/MS/MS with a limit of quantitation of 100 ng/mL. Paclitaxel (MW of 853.9 daltons) 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/X² linear regression was performed 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 six weeks thereafter. Briefly, complete response (CR) was

disappearance of all lesions; partial response (PR) was $\geq 30\%$ reduction in the sum of the longest diameters of the lesions; stable disease (SD) was sum of longest diameters not decreased more than 30% and not increased more than 20%; and progressive disease (PD) was $\geq 20\%$ increase in the sum of the longest diameters of the lesions. Overall response was determined by the primary physician; scans from patients dosed $> 300 \text{ mg/m}^2$ 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, PK and efficacy data. Categorical data are summarized by frequency and percentages; continuous data are summarized by mean and standard deviation (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 three study centers in the US (University of Texas M. D. 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 two 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, including while receiving GRN1005. The median number of prior therapies was 4 (range: 0-22).

Reasons for study withdrawal were: disease progression (n = 33), AEs (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/m². At the 60 mg/m² dose, Grade 2 neutropenia and anemia occurred, and the dose level and subsequent dose-escalation cohorts were expanded to include 3-6 patients. At the 500 mg/m² dose, the study sites noticed that the dosing solution was turning cloudy during the infusion period. Based on reported AEs, 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/m² dose level in the interest of rigor. These findings were reported to the Institutional Review Boards and FDA.

Dose-Limiting Toxicities and Maximum Tolerated Dose

No DLTs were reported at doses \leq 550 mg/m². The first DLT (febrile neutropenia) was reported in one of three patients initially enrolled at 650 mg/m². This patient was treated with filgrastim and IV antibiotics, and febrile neutropenia resolved in two days. Dose escalation continued after no DLTs were observed in the additional three patients enrolled at this dose level. A DLT occurred in two of six patients enrolled at the next dose level (700 mg/m²). One patient experienced Grade 4 thrombocytopenia which 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 multi-organ failure within 6 days of the first infusion. Relationship to GRN1005 could not be ruled out due to the temporal relationship between events, although disease state could have also been a cause. Because criteria for MTD were exceeded at 700 mg/m², the 650 mg/m² dose was determined to be the 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/m², five 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 G-CSF, dose reduction, or both. Peripheral neuropathy was reported only at doses \geq 420 mg/m²; 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 200-650 mg/m². 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/m²; 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 performed at least one post-treatment neurocognitive test battery and were included in the analysis. Results revealed no evidence that GRN1005 causes CNS toxicity. One patient who experienced SD for > 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 post-treatment PK sample drawn and were included in the analysis. The PK of GRN1005 appeared to be dose proportional. After single dose IV 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 **Figure 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 (**Figure 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 analyzed (**Figure 1C**). Pharmacokinetic measures of paclitaxel exposure (C_{max} and AUC_{last}) demonstrated that free paclitaxel exposures were approximately 14 to 15-fold lower than those of ANG1005-associated paclitaxel in plasma during both cycles ($AUC = 1447 \text{ } \mu\text{M}$ for GRN1005 versus 101

μM for paclitaxel and $C_{\text{max}} = 1187 \mu\text{M}$ for GRN1005 versus $13 \mu\text{M}$ for paclitaxel). There was no evidence of accumulation of unconjugated paclitaxel based on PK analyses for Cycle 1 versus Cycle 3.

Efficacy

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

Best overall responses ($n = 43$) are shown in **Figure 2A and Table 4**. Five patients (9%) achieved a PR (breast cancer, $n = 2$; non-small cell lung cancer (NSCLC), $n = 2$, 1 unconfirmed; ovarian cancer, $n = 1$). All PR's were observed at the 650 mg/m^2 dose (MTD; **Table 4**). Among the five patients achieving PR, all also experienced shrinkages in their brain lesions (**Figure 2B, 2C**) as assessed by an independent radiologist; brain responses ranged from -17% to -50%. Furthermore, three of these patients had failed prior taxane therapy. No patients in Figure 2B who had increases in brain lesions achieved PR or SD ≥ 4 months.

The median duration of PR 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 PR (-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 (**Figure 2Ci**). The patient withdrew from the study after ~6.5 months due to neuropathy.

In addition to the five patients with PR, six patients (11%) had SD \geq 4 months, with the longest duration being eight months (a patient with NSCLC and brain metastasis dosed at 420 mg/m²).

DISCUSSION

GRN1005 administered as monotherapy by IV infusion once every three weeks was well-tolerated up to 650 mg/m². 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 neurological deficits after treatment with GRN1005 was observed in one patient who showed 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 PK of GRN1005 showed dose proportional increases in C_{max} and AUC_{inf} at doses ranging from 30 to 700 mg/m². 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 demonstrated that most paclitaxel in plasma remains associated with the Angiopep-2 peptide over a period of at least 24 hours post-infusion. The C_{max} for GRN1005 at the MTD was 306,000 ng/ml, versus 22,968 and 3543 µg/ml for nab-paclitaxel and paclitaxel, respectively, at doses of 260 and 175 mg/m² (which are their MTDs) (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 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 molecule's inability to cross the blood-brain barrier and reach the tumor. In this study, five of 20 patients (25%) dosed at the MTD (650 mg/m²) achieved an overall PR; 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 > 300 mg/m² 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, seven of eight patients with liver metastases showed shrinkage in their liver lesions (maximum = -100%) and all nine 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 PR, including one patient with a CR in liver lesions and one patient who achieved a PR in the lung. Additionally, one patient who did not

achieve an overall PR had a PR in her brain lesions. This 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/m² given IV once every three weeks is warranted and studies in patients with breast and non-small cell lung cancer with brain metastases are planned.

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Table 1. Patient Demographics

| Characteristics (n=56) | |
|--------------------------------------|------------|
| Age (years) | |
| 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* | 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%) |

Abbreviations: NSCLC, Non Small Cell Lung Cancer; SCLC, Small Cell Lung Cancer; ECOG, Eastern Cooperative Oncology Group
*Other includes hepatocellular carcinoma (n=2), colorectal (n=2), cervical (n=1), ovarian (n=1)

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)* | | 420 (n=6)* | | 500 (n=4)* | | 550 (n=3) | | 650† (n=20) | | 700 (n=6) | |
|-------------------------|-------------|-----|-------------|-----|--------------|-----|--------------|-----|---------------|-----|---------------|-----|---------------|-----|--------------|-----|----------------|------------|--------------|-----------|
| | 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) |
| Non-hematologic | | | | | | | | | | | | | | | | | | | | |
| 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‡) | 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¥ | 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 |

* Due to discovery of cloudy infusion solutions, dosing was repeated in additional patients at the 300-500 mg/m² dose levels and escalated again from that point.

† 650 mg/m² administered once every 3 weeks was determined to be the MTD

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

‡ This patient experienced Grade 3 dehydration and Grade 3 stomatitis during Cycle 1 of treatment.

Table 3. Pharmacokinetic Data for GRN1005

| Parameter | Mean ¹ | | | | | | | | |
|------------------------------|-------------------|------|------|------|---------------------|------|------|------|------|
| | 60 | 120 | 200 | 300 | 420 | 500 | 550 | 650 | 700 |
| n | 3 | 3 | 3 | 7 | 5 or 6 ² | 3 | 3 | 20 | 6 |
| C _{max} (ug/mL) | 30.0 | 55.2 | 119 | 160 | 232 | 210 | 238 | 306 | 376 |
| C _{max} (uM) | 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} (ug·h/mL) | 140 | 261 | 664 | 818 | 1369 | 1532 | 1810 | 2571 | 3322 |
| AUC _{inf} (uM·h) | 27.4 | 51.1 | 130 | 160 | 268 | 300 | 354 | 503 | 650 |
| T _½ (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 |
| Vd (mL/m ²) | 1590 | 1947 | 1537 | 1769 | 1565 | 1799 | 1675 | 1666 | 1312 |

¹ Data from 1 patient treated at 30 mg/m² and one patient treated at 500 mg/m² were not included in the pharmacokinetic data, as these patients did not receive a complete infusion.

² n=5 for C_{max}, T_{max}, AUC_{inf}, Vd and n=6 for half-life and CL

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; Vd = apparent volume of distribution.

Table 4. Overall Best Response by Dose Cohort

| GRN1005 Dose | 30 mg/m² | 60 mg/m² | 120 mg/m² | 200 mg/m² | 300 mg/m² | 420 mg/m² | 550 mg/m² | 650 mg/m² | 700 mg/m² |
|-------------------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 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 |

CR = Complete Response; PR = Partial Response; SD = Stable Disease; PD = Progressive Disease

FIGURE LEGENDS

Figure 1. Mean (\pm SEM) plasma concentrations of GRN1005 during and after infusion at Cycle 1 (n=3-20) **(A)**. Mean plasma concentrations of GRN1005 in the MTD group (n=8) at Cycles 1 and 3 **(B)**. Mean (\pm SEM) paclitaxel concentrations (free and associated with Angiopep-2 expressed as paclitaxel-equivalent GRN1005) during Cycle 1 in the MTD Group (n=16) **(C)**.

Figure 2. 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 re-evaluated post-treatment 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 **(A)**. Brain responses in patients were assessed for non-progressing patients that received doses from 420 mg/m² and higher, including all patients that were treated at the MTD. **(B)**. Radiographic images of the brain of three patients treated at the MTD (650 mg/m²) who achieved an overall best response of PR **(C)**: **(i)** 45-year old woman with breast cancer; **(ii)** 73-year old woman with ovarian cancer; **(iii)** 38-year old woman with breast cancer.

FIGURE 1

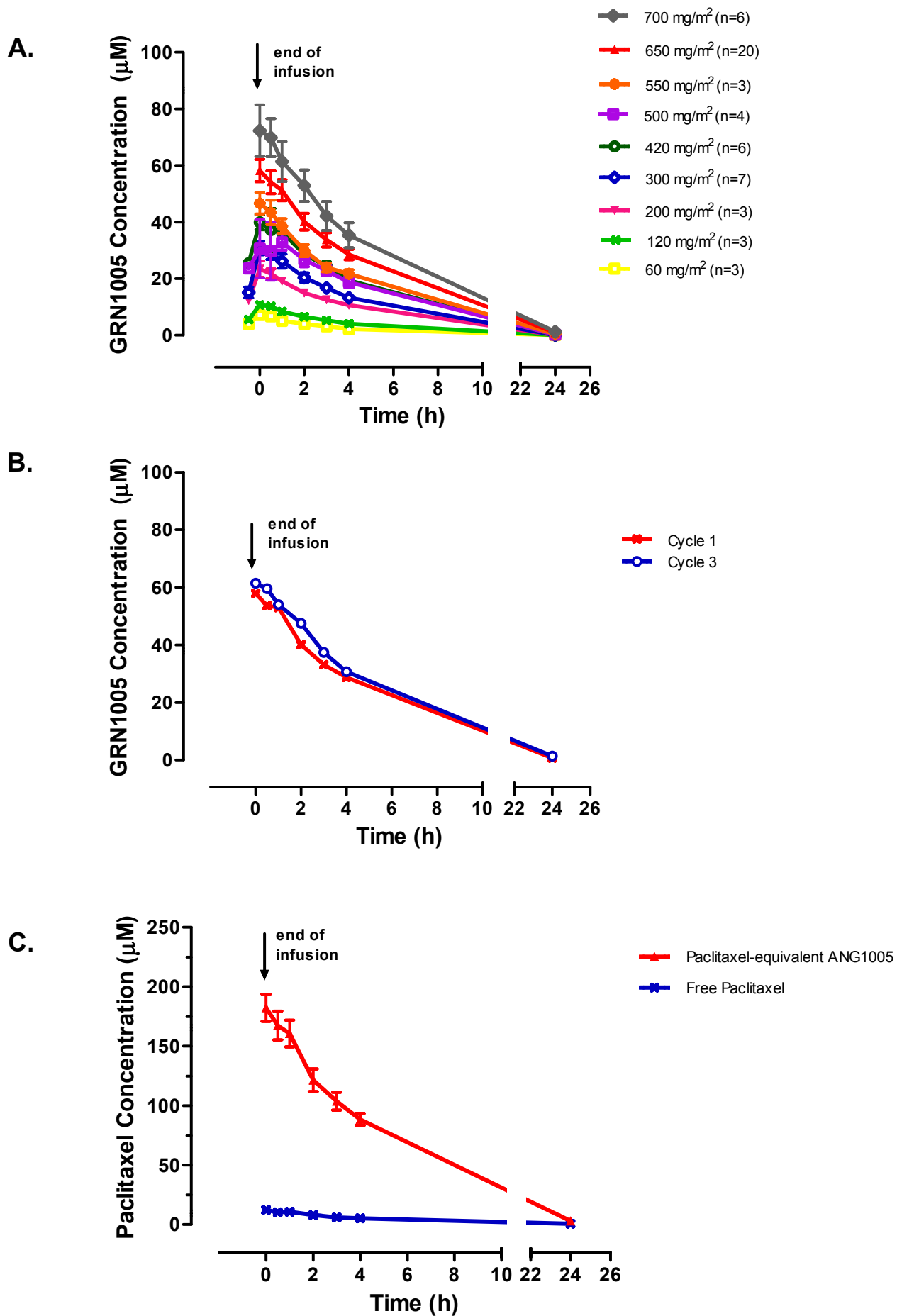
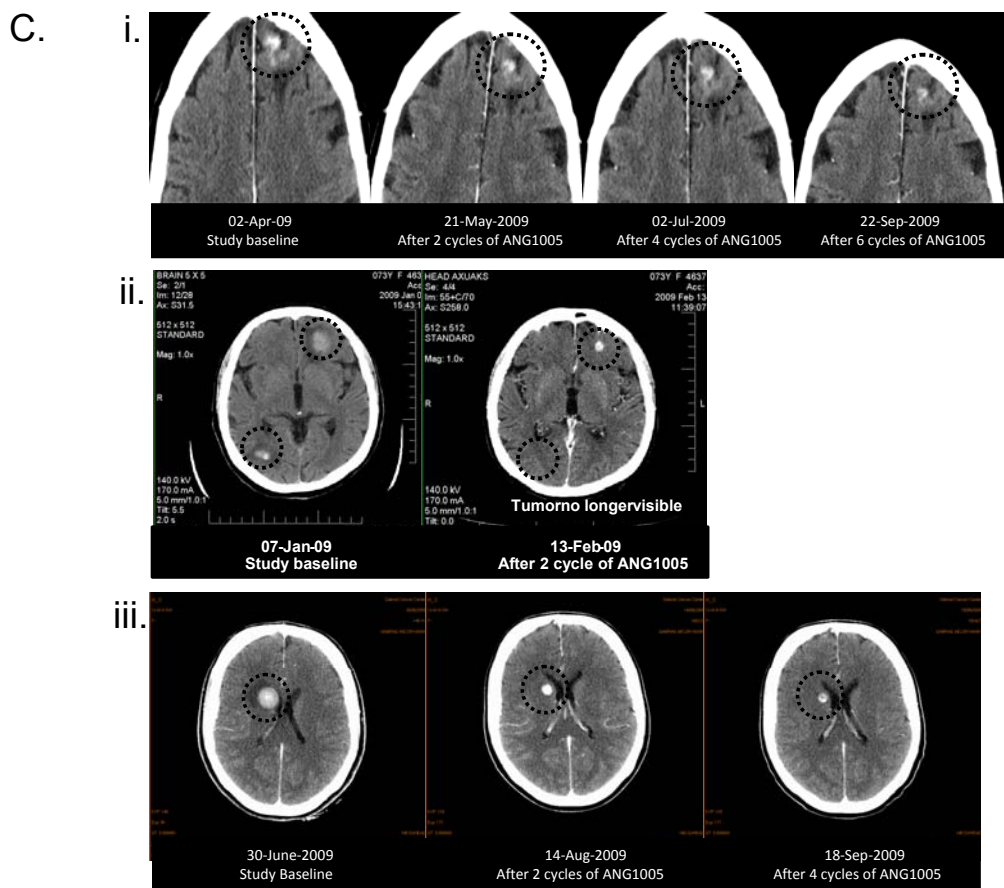
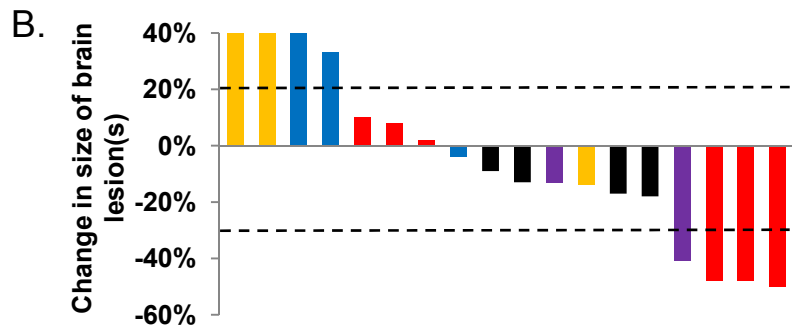
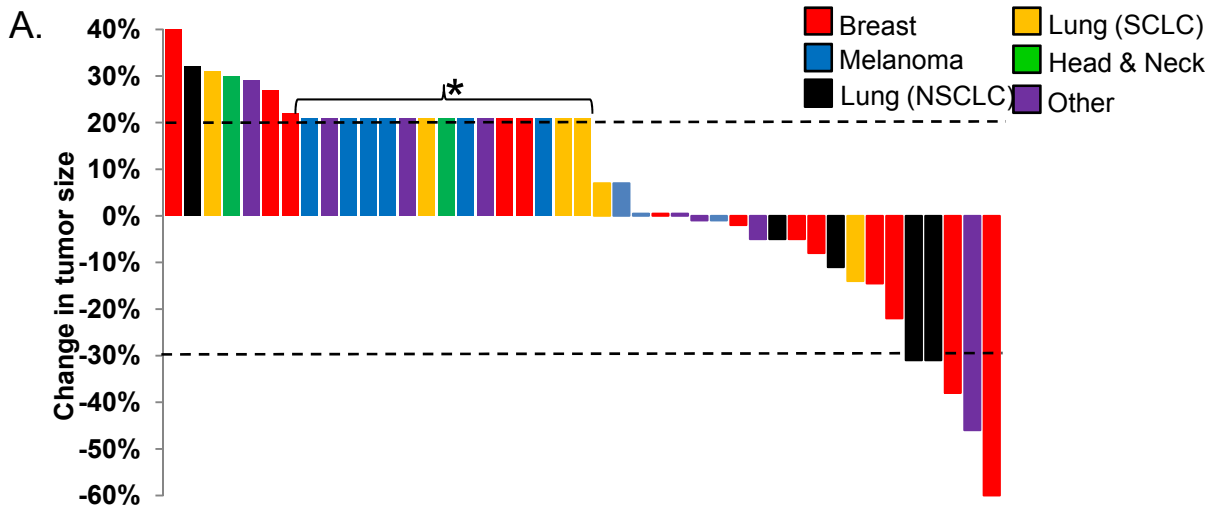


FIGURE 2



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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