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Phase I Study of GRN1005 in Recurrent Malignant Glioma

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Abstract

Purpose: GRN1005 is a peptide–drug conjugate with the ability to penetrate the blood–brain barrier (BBB) and tumor cells by targeting the low-density lipoprotein receptor–related protein-1. We conducted a first-in-human phase I trial of GRN1005 in patients with recurrent glioma.

Methods: Patients received GRN1005 by intravenous infusion every 3 weeks. Doses were escalated using a modified Fibonacci scheme. Study objectives included safety, tolerability, identification of the maximum tolerated dose (MTD), pharmacokinetics, and preliminary evidence of efficacy. Tumor extracted from patients undergoing surgery following administration of GRN1005 was analyzed to determine whether therapeutic concentrations of GRN1005 were achieved.

Results: Sixty-three patients received GRN1005 at doses of 30 to 700 mg/m^2 every 3 weeks. Therapy was well tolerated with neutropenia, leucopenia, and fatigue as the most frequent drug-associated grade 3/4 or higher toxicities. The MTD was 650 mg/m^2 every 3 weeks. Dose-limiting toxicities were grade 3 mucositis and grade 4 neutropenia. There was no evidence of central nervous system toxicity or antibody production. Pharmacokinetic analysis showed that exposure to GRN1005 was dose proportional. We observed one complete and two partial responses. Eight of 27 patients dosed $\geq$420 mg/m^2 had stable disease, which lasted a median of 51 days. Therapeutic concentrations of GRN1005 and free paclitaxel were shown in tumor tissue of surgical patients dosed with $\geq$200 mg/m^2.

Conclusion: GRN1005 delivers paclitaxel across the BBB and achieves therapeutic concentrations in tumor tissue. It has similar toxicity to paclitaxel and appears to have activity in recurrent glioma. The recommended phase II dose is 650 mg/m^2 every 3 weeks. *Clin Cancer Res; 19(6); 1567–76. ©2013 AACR.*

Introduction

High-grade gliomas (HGG) are resistant to treatment and carry a poor prognosis (1). Standard treatment with radiotherapy and temozolomide provides only limited benefit and patients inevitably develop recurrent disease (2). At relapse, conventional chemotherapies have not improved survival (3). Although bevacizumab produces high response rates and improvement in progression-free survival (PFS) in recurrent glioblastoma (4, 5), responses are rarely durable (4–6) and the effect on overall survival remains controversial (7).

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Translational Relevance

Malignant glioma is the most common primary brain tumor, with poor survival despite standard therapies. Novel therapeutic regimens are needed. This study is the first report showing safety and tolerability, pharmacokinetics, and preliminary efficacy of GRN1005, a paclitaxel–Angiopep-2 peptide–drug conjugate that binds to the LRP-1 receptor, for the treatment of recurrent glioma. We provide evidence that GRN1005 is able to cross the blood–brain barrier and achieve therapeutic concentrations of paclitaxel within brain tumor tissue. Although efficacy in our heavily pretreated study population was modest, our research highlights that LRP-1 receptor–mediated transcytosis may serve as a paradigm to improve drug delivery across the blood brain barrier. On the basis of these data, further studies of GRN1005 for primary and metastatic brain tumors are warranted.

A major reason for the poor results of current therapy is the blood–brain barrier (BBB), which restricts the entry of many drugs into the brain, especially agents that are substrates of drug efflux transporters such as P-glycoprotein and the ABC efflux transporters (8–10). Various mechanisms have been studied to circumvent the BBB for the delivery of therapeutic agents with thus far disappointing results (11).

Angiopep-2

The low-density lipoprotein receptor–related protein-1 (LRP-1) mediates BBB transcytosis (12, 13) for normal ligands such as thyroglobulin, lactoferrin, tissue-type plasminogen activator, and $\alpha2$ macroglobulin (14–20). It is expressed on the surface of the BBB (20–22) and on a variety of neoplastic cells including HGG (23, 24). It has been shown that β-amyloid precursor protein lacking the Kunitz protease inhibitor domain was a poor substrate for LRP-1, suggesting an important function for this domain in recognition, internalization, and clearance via LRP-1 (19). Subsequent protein homology searches identified a 19–amino-acid sequence called Angiopep-2 that exhibits high transcytosis capacity (18), crossed the BBB through an LRP-1–mediated mechanism (25), and distributed broadly throughout brain parenchyma (18, 26). In addition to its well-determined role in ligand binding and endocytosis, LRP-1 has emerged as a central molecular regulator of cytoskeleton organization and adhesive complex turnover in malignant cells (27).

GRN1005

Paclitaxel, a broad-spectrum antitumor microtubule-stabilizing agent, has activity against several malignancies (28). However, despite potent activity against HGG cell lines in vitro (29), clinical trials of paclitaxel in recurrent HGG have shown no benefit (30, 31). While inherent resistance to apoptosis due to taxanes has been observed in gliomas (32), the difference between in vivo and in vitro activity is likely due to low penetration of the drug across the BBB (33). In fact, while tumor cells are sensitive to paclitaxel with a 90% lethal dose (LD90) in vitro in the range of 0.3 μmol/L (29), brain parenchymal concentrations barely reach the LD90 once prior reports of intratumoral concentrations are corrected for vascular content (34–36).

Development of a drug with the functional properties of paclitaxel that has the ability to cross the BBB and is not a P-glycoprotein substrate would be expected to increase efficacy in vivo. GRN1005 is a novel agent designed to cross the BBB by exploiting a novel Engineered Peptide Compound (EPiC) platform, combining Angiopep-2 and paclitaxel (37). Following intravenous administration, GRN1005 presumably binds to LRP-1 and is transported across the BBB via the Angiopep-2-LRP-1 complex (ref. 26; Fig. 1A). Because LRP-1 is expressed on the surface of brain tumors (23, 38), GRN1005 is taken up into tumor cells and is subsequently cleaved by esterases to release paclitaxel from Angiopep-2 (Fig. 1B). As a produg, GRN1005 is not a substrate for P-glycoprotein–mediated drug efflux (37), but after conversion to active drug, it exhibits comparable properties to paclitaxel including inhibition of tumor cell proliferation, blockade of tumor cells in G_2–M phase, and induction of β-tubulin polymerization (26, 37). Furthermore, GRN1005 has shown antitumor activity in orthotopic glioma models (37). Here, we report the results of a first-in-human multicenter phase I trial of GRN1005 in patients with recurrent gliomas.

Materials and Methods

Patient eligibility

Eligibility included histologically confirmed World Health Organization (WHO) grades 2 to 4 glioma with unequivocal radiographic progression, informed consent, age $\geq$ 18 years, measurable disease, Eastern Cooperative Oncology Group (ECOG) performance status score $\leq$ 2; MRI $\leq$ 21 days of registration on a stable steroid dose; life expectancy $\geq$ 3 months; adequate bone marrow function (neutrophils $\geq$ 1,500/mL; platelets $\geq$ 100,000/mL), adequate hepatic and renal function [alanine aminotransferase (ALT)/alkaline phosphatase < 2.5 × normal; bilirubin < 1.5 × normal, creatinine clearance > 60 mL/min]; $\geq$2 weeks from cytochrome P450 enzyme–inducing anti-epileptic drugs (EIAED), $\geq$28 days from cytotoxic or investigational agents; and $\geq$6 weeks from bevacizumab. Patients who were pregnant or nursing, had severe concurrent illness, prior malignancy, or $\geq$grade 2 pre-existing neuropathy were excluded. The study was approved by the Institutional Review Boards of each site.

Treatment plan

GRN1005 was administered intravenously over 1 hour every 3 weeks until tumor progression, intolerable toxicity, or withdrawal of consent. From 30 mg/m^2, doses were escalated according to a modified Fibonacci scheme (39) until the maximum tolerated dose (MTD) was reached. Three patients were treated at each dose level; if 1 patient
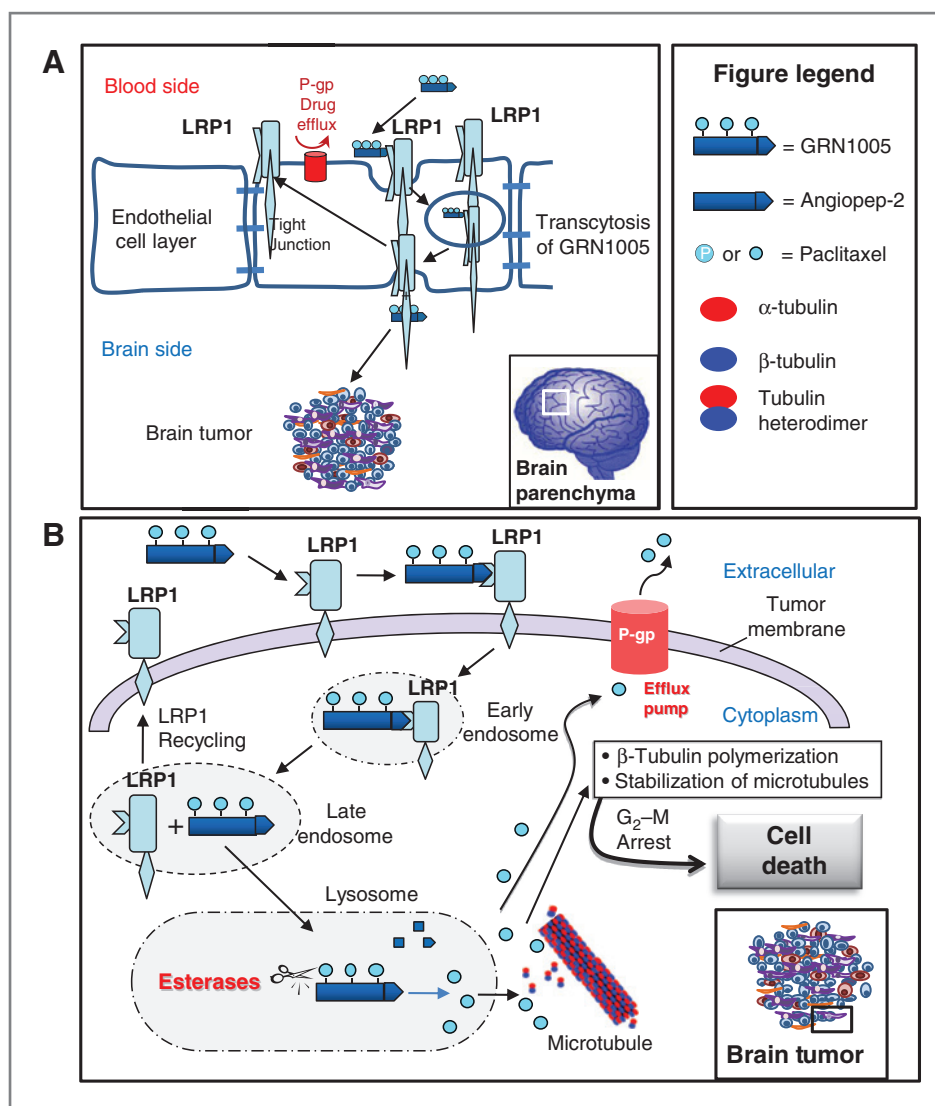
Figure 1. A, transcytosis through the BBB. B, uptake into a tumor cell via endocytosis.

experienced a dose-limiting toxicity (DLT) in the first 21-day cycle, the cohort was expanded to 6. Dose escalation continued until ≥2 of 6 patients experienced a DLT. Patients were allowed up to 2 dose reductions for toxicities.

In a separate surgical cohort, patients received a single infusion of GRN1005 before debulking surgery at one dose level below the enrolling dose of the main study and resumed treatment between 21 and 35 days postoperatively.

Evaluations during study

Complete blood count with differential and serum chemistries was obtained weekly. Physical and neurologic examinations were conducted weekly for the first cycle and then every 3 weeks. A validated neurocognitive testing battery including Hopkins Verbal Learning Test–Revised (HVLT-R), Trail Making Test Parts A and B, Controlled Oral Word Association (COWA) test, and Grooved Pegboard test was used to assess potential effects on cognition and fine motor function (40). MRI evaluations were conducted at baseline

and every 6 weeks. The National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 was used to evaluate adverse events (AE). Response was assessed using modified Macdonald Criteria (41) and confirmed by independent review.

Objectives and endpoint

The primary objective was to identify the MTD of GRN1005. Secondary objectives were to determine the pharmacokinetics (PK) of GRN1005 and paclitaxel, immunogenicity, and preliminary efficacy. A separate cohort of patients underwent resection of their tumor after treatment with GRN1005 to determine whether the drug crosses the BBB to achieve therapeutic tumor concentrations. DLT was defined as any drug-related grade 3/4 nonhematologic toxicity, febrile neutropenia, grade 4 neutropenia ≥ 7 days, grade 4 thrombocytopenia, grade 2 peripheral neuropathy > 7 days, or ≥ grade 3 peripheral neuropathy of any duration. The MTD was defined as that dose level at which ≤1 of 6

patients developed a DLT. Once identified, the MTD cohort was expanded to 18 patients to further assess safety, tolerability, PK profile, and preliminary efficacy of GRN1005.

Pharmacokinetic and immunogenicity analyses

Plasma samples for PK analysis were collected before infusion, and at 0.5, 1, 2, 3, 4, and 24 hours after the end of infusions during cycles 1 and 3. Concentrations of GRN1005 were determined using a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) assay with a calibration range of 0.50 to 40 µg/mL for patients dosed ≤ 75 mg/m^2 and 2.00 to 200 µg/mL for patients dosed ≥ 105 mg/m^2. Plasma samples from patients dosed at the MTD were also analyzed for free paclitaxel using a qualified LC/MS/MS assay with a limit of quantification of 100 ng/mL. Individual patient plasma concentration versus time data for GRN1005 and free paclitaxel were analyzed using noncompartmental methods. Pharmacokinetic calculations were conducted using Microsoft Excel 2007 and RSTRIP (MicroMath), version 5.0. The presence of GRN1005 antibodies was tested using a validated direct ELISA with an estimated sensitivity ≤ 0.56 µg/mL. The assay was developed by EMD Millipore for GRN1005 and validated in compliance with good laboratory practice and Millipore standard operating procedures (unpublished data).

Tumor tissue bioavailability analysis

Tumor tissue samples were obtained within 6 hours after the end of the infusion, rinsed in saline to remove gross blood, snap-frozen in liquid nitrogen, and stored at $-80°$C until analysis. Areas of necrosis were avoided for sampling. Plasma PK samples for GRN1005 analysis were drawn at the time of tumor removal. Tumor tissue was homogenized with buffer solution (0.1 mol/L Tris-HCl pH $= 5.0$, 50 m mol/L sucrose, 0.003% Tween 80, and protease inhibitor cocktail; Calbiochem). GRN1005 and paclitaxel were extracted by addition of acetonitrile with trifluoroacetic acid. Following centrifugation, the organic phase was removed and analyzed by liquid chromatography mass spectroscopy methods that had been qualified for GRN1005 and paclitaxel in tumor extracts. The concentrations were corrected for blood volume (≤ 0.05 mL/g tumor) to more accurately determine only the parenchymal content, which is the true amount of GRN1005 in tumor tissue and the amount of paclitaxel released. The vascular and parenchymal contents in the tumor were determined as follows: vascular content in tumor (µg/g) $=$ blood volume of tissue (Vv, mL/g) $\times$ circulating blood concentration (Cbl, µg/g); parenchymal content (µg/g) $=$ total amount in tumor (µg/g) $-$ (Vv $\times$ Cbl). The following assumptions were used for the correction: blood volume in tissue (Vv) ≤ 0.05 mL/g (42–44), blood/plasma concentration ratio for paclitaxel $\sim$ 1.0 (45, 46), and blood/plasma concentration ratio for GRN1005 ~ 0.55 (Smith, unpublished data; ref. 26).

Determination of LRP-1 expression

The presence of LRP-1 on tumors was determined by Western blot analysis. Specimens were loaded on SDS gels and electrophoretically transferred on polyvinylidene fluoride (PVDF) membranes. The 85-kDa subunit of LRP-1 was detected using the anti-LRP-1 monoclonal antibody from Calbiochem. Proteins were visualized using a secondary anti-mouse IgG linked to peroxidase and enhanced chemiluminescence reagent. The analysis was conducted by Angiochem. We used human U87 cells as an indicator of positive LRP-1 expression. The assay was not quantitative.

Statistical considerations

All efficacy analyses were based on the intent-to-treat principle. Safety analyses included all patients who received at least one dose of GRN1005. Descriptive statistics appropriate for continuous variables [e.g., sample size (n), mean, SD, median, range] and categorical variables [e.g., n(%)] were used for demographic, safety, pharmacokinetics, and efficacy data. Changes from baseline in neurocognitive test results were assessed using paired t tests.

Results

Patient characteristics

Sixty-three patients from 6 institutions were enrolled. Median age was 50 (range, 22–78) years. Median ECOG performance status was 1 (range, 0–2). Histology was confirmed as glioblastoma (WHO grade 4) in 68% of patients and anaplastic gliomas (WHO grade 3) in 29% of patients; 2 patients had WHO grade 2 glioma. Fifty-four percent of patients had 3 or more prior courses of chemotherapy (Table 1).

Summary of GRN1005 treatment

A total of 195 doses were administered to 63 enrolled patients at doses between 30 and 700 mg/m^2. Twenty-nine patients received 2 doses, 15 patients received between 3 and 6 doses, and 6 patients received >6 doses (range, 7–29) of GRN1005. Ten patients had only 1 cycle of GRN1005 treatment. Three other subjects who were enrolled in the substudy were given a single infusion at the time of surgery but did not continue on to the main study. Thirty-eight patients (60%) terminated the study due to disease progression and 18 patients (29%) discontinued due to AEs. There was 1 withdrawal each due to sponsor decision and withdrawal of consent; and 2 patients were removed at investigator discretion.

The following GRN1005 dose levels were explored: 30, 50, 75, 105, 200, 300, 420, 550, 650, and 700 mg/m^2. Dose of 650 mg/m^2 was determined to be the MTD following one event of grade 3 mucositis and one event of grade 4 neutropenia that required medical intervention with granulocyte colony stimulating factor; both occurred during cycle 1 of treatment at 700 mg/m^2. In total, 3 patients were treated at 700 mg/m^2. The MTD cohort was expanded to include a total of 18 patients. None of the 18 patients treated at 650 mg/m^2 experienced DLTs in the first cycle.

Safety

GRN1005 was generally safe and relatively well-tolerated. Table 2 summarizes AEs at the MTD. DLTs

Table 1. Baseline patient characteristics

Age, y	
Median	50
Range	22–78
Sex, *n* (%)	
Male	36 (57%)
Female	27 (43%)
WHO tumor grade,[a] *n* (%)	
Grade IV (GBM)	43 (68%)
Grade III (AA, AOA, and AO)	18 (29%)
Grade II (astrocytoma, ependymoma)	2 (3%)
No. of prior therapies, *n* (%)	
≤ 2	29 (46%)
3–5	24 (38%)
≥ 6	10 (16%)
Prior radiotherapy, *n* (%)	
Yes	62 (98%)
No	1 (2%)
Prior surgery, *n* (%)	
0 = biopsy only	10 (16%)
1	30 (48%)
≥ 2	23 (36%)
ECOG performance status score, *n* (%)	
0	20 (32%)
1	29 (46%)
2	14 (22%)

Abbreviations: AA, anaplastic astrocytoma; AOA, anaplastic oligoastrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme.

Table 2. AEs of interest occurring at $\geq 15\%$ at the MTD dose of 650 mg/m^2

	GRN1005 ($N = 18$)		
System/organ class	Grade III	Grade IV	All grades[a]
Blood and lymphatic system disorders			
Neutropenia[b]	2 (11%)	10 (56%)	14 (78%)
Leukopenia[c]	5 (28%)	8 (44%)	16 (89%)
Thrombocytopenia[d]	6 (33%)	1 (5.6%)	15 (83%)
Anemia[e]	0	0	14 (78%)
Gastrointestinal disorders			
Constipation	0	0	5 (27.8%)
Nausea	0	0	5 (27.8%)
Dyspepsia	0	0	3 (16.7%)
Diarrhea	0	0	2 (11.1%)
Stomatitis	0	0	2 (11.1%)
Vomiting	0	0	1 (5.6%)
General disorders and administration site conditions			
Infusion-related reaction	2 (11.1%)	0	2 (11.1%)
Infections and infestations			
Urinary tract infection	1 (5.6%)	0	3 (16.7%)
Oral candidiasis	0	0	2 (11.1%)
Pneumonia bacterial	0	0	2 (11.1%)
Candidiasis	0	0	1 (5.6%)
Nervous system disorders			
Headache	0	0	4 (22.2%)
Peripheral neuropathy	2 (11.1%)	0	5 (27.8%)
Somnolence	2 (11.1%)	0	4 (22.2%)
Ataxia	1 (5.6%)	0	3 (16.7%)
Convulsion	0	1 (5.6%)	3 (16.7%)
Hemiparesis	2 (11.1%)	0	3 (16.7%)
Dizziness	0	0	2 (11.1%)

[a]Includes AEs of all grades (I–V).
[b]Neutropenia: 1 (5.6%) grade I, 1 (5.6%) grade II, 2 (11%) grade III, 10 (56%) grade IV, and 14 (78%) for grade I–V.
[c]Leukopenia: 2 grade I (11%), 1 (5.6%) grade II, 5 (28%) grade III, 8 (44%) grade IV, and 16 (89%) for grade I–V.
[d]Thrombocytopenia: 6 grade I (33%), 2 (11%) grade II, 6 (33%) grade III, 1 (6%) grade IV, and 15 (83%) for grade I–V.
[e]Anemia: 8 grade I (44%), 6 grade II (33%), 0 grade III, 0 grade IV, and 14 (78%) for grade I–V. There was 1 grade V event of bacterial meningitis at the MTD.

encountered below the MTD included grade 3 seizures in a patient dosed at 50 mg/m^2, grade 3 elevated ALT at 105 mg/m^2, and grade 3 fatigue at 300 mg/m^2. The majority of AEs were mild to moderate (grades 1–2 in 84% of events). The most common toxicities were related to transient bone marrow suppression: anemia developed in 49 patients (78%) and neutropenia and thrombocytopenia in 34 patients (54%) each. The most common nonhematologic toxicities were fatigue (28 patients, 44%), peripheral neuropathy (16 patients, 25%), rash (16 patients, 25%), nausea (13 patients, 21%), and dizziness (12 patients, 19%). Twenty-seven patients underwent neurocognitive testing at baseline and at least one other time point. We observed no significant differences in neurocognitive performance at any time point relative to baseline on any test.

Pharmacokinetics

GRN1005 PK profiles are shown in Fig. 2. Plasma GRN1005 concentrations reached maximum levels (C_{max}) at the end of the infusion and then decreased mono-expo-nentially with a half-life for both cycles of 3.56 ± 0.84 hours ($n = 71$). We observed a linear increase of C_{max} and AUC$_{inf}$ proportionally with the dose. The mean C_{max} and AUC$_{inf}$ at the MTD (650 mg/m^2) for cycle 1 were 378 ± 90 µg/mL ($n = 18$) and $3,198 \pm 859$ µg h/mL ($n = 15$), respectively. In cycles 1 and 3, PK results were similar. There was no evidence of accumulation after repeated cycles.

GRN1005 had a low clearance and a low volume of distribution. The overall clearance (321 ± 159 mL/m^2/h in cycle 1 and 286 ± 193 mL/m^2/h in cycle 3) was less than 8% of the human glomerular filtration rate and
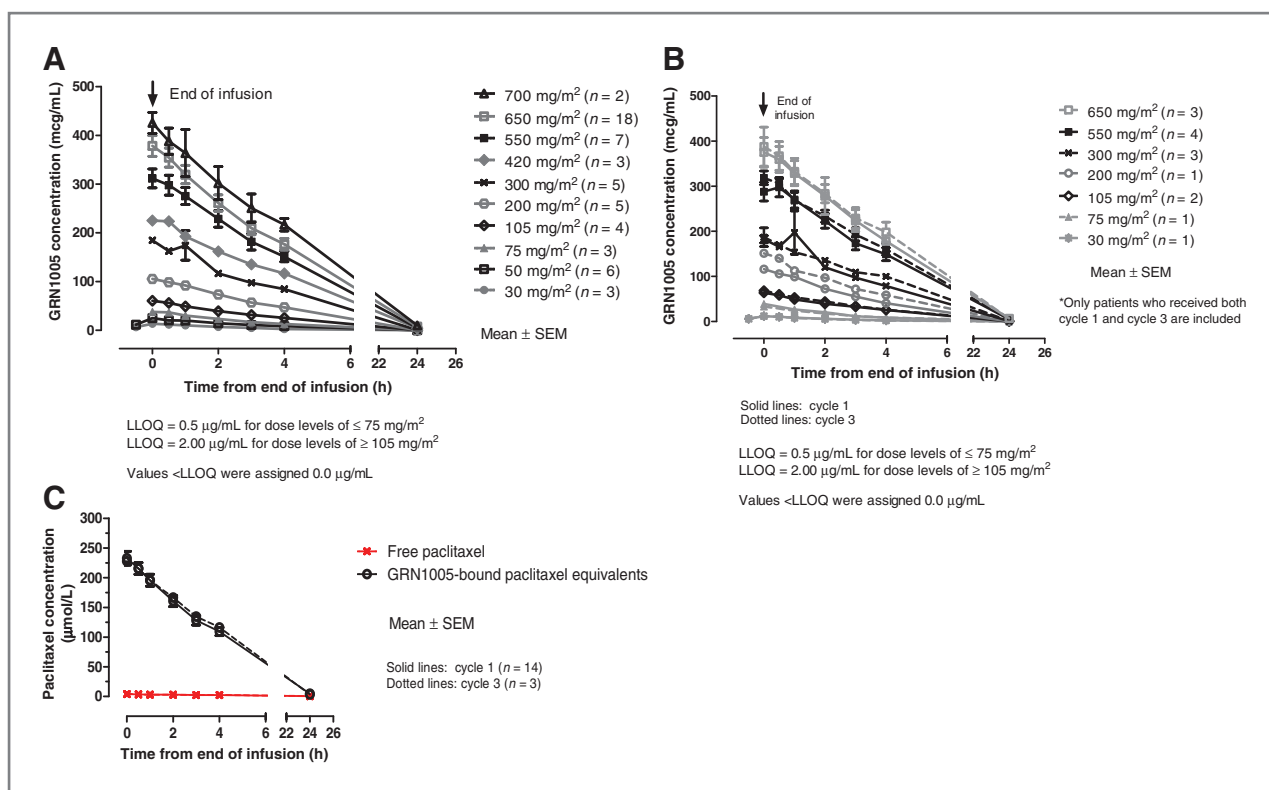
Figure 2. A, mean GRN1005 concentrations at cycle 1. B, mean plasma concentrations at cycle 1 versus 3. C, plasma concentrations of free paclitaxel versus GRN1005-bound paclitaxel equivalents in the MTD group (650 mg/m^2).

less than 2% of human liver plasma flow. The overall volume of distribution (1,456 ± 448 mL/m^2 in cycle 1 and 1,323 ± 358 mL/m^2 in cycle 3) was approximately equal to the plasma volume in humans. These comparisons show that intravenous GRN1005 does not passively distribute outside the plasma compartment and is not rapidly cleared by glomerular filtration or metabolism.

The plasma profile of free paclitaxel, determined in a subset of patients in the MTD group ($n = 14$), was similar in both cycles. Plasma-free paclitaxel reached peak levels (C_{max}) around the end of the infusion and then decreased mono-exponentially with a longer half-life (6.47 ± 1.6 hours in cycle 1 and 6.16 ± 2.0 hours in cycle 3) than that of GRN1005. The mean paclitaxel C_{max} was similar for cycle 1 (4.20 ± 1.0 μmol/L) and cycle 3 (4.30 ± 1.2 μmol/L). Free paclitaxel concentrations were lower than GRN1005-bound paclitaxel equivalents (based on a paclitaxel to peptide backbone molar ratio of 3:1) throughout the 24-hour sampling period, although the proportion increased slowly with time as a result of the longer half-life of free paclitaxel relative to GRN1005 (≤2% for the first 4 hours, 7.7% at 24 hours). Free paclitaxel exposure (C_{max} and AUC_{inf}) was more than 40-fold lower than that of GRN1005-bound paclitaxel equivalents in plasma (Fig. 2C), showing that a large majority of the paclitaxel in plasma remained associated with GRN1005 following intravenous infusion.

Immunogenicity

A total of 53 patients had posttreatment serum samples collected for immunogenicity testing. All samples were negative for anti-GRN1005 antibody including patients who received up to 18 cycles of treatment and samples from patients who experienced infusion reactions or rashes.

Efficacy

Of the 63 patients, 46 received at least 2 doses of GRN1005 and had at least one posttreatment MRI. One patient with glioblastoma experienced a complete response at 700 mg/m^2 and 2 patients with anaplastic anaplastic gliomas had partial responses (PR) at 420 and 650 mg/m^2. Eight of 27 patients dosed ≥420 mg/m^2 had stable disease which lasted a median of 51 days (Fig. 3). One patient with glioblastoma remained on study for 89 weeks (29 cycles).

Tissue concentrations and LRP-1 expression

Nine patients with HGG were enrolled on the surgical cohort (7 glioblastoma, 1 anaplastic astrocytoma, and 1 anaplastic oligodendroglioma) and underwent resection of their tumor within 3.5 to 6 hours of receiving their first GRN1005 infusion. One patient each was treated at dose levels of 200, 300, 420, and 650 mg/m^2, whereas 5 patients were treated at 550 mg/m^2. Intratumoral concentrations of GRN1005 varied from 8% to 379% (median, 27.3%) of plasma concentration (Fig. 4). Intratumoral concentrations

Figure 3. Best response assessment by dose and histology in patients who were treated at 420 mg/m^2 or above and who had at least one MRI response assessment. Each bar represents one patient, depicting the best percentage change from baseline [patients ($n = 9$) from surgical debulking substudy were excluded]. A, astrocytoma; AA, anaplastic astrocytoma; AE, anaplastic ependymoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; GBM, glioblastoma.
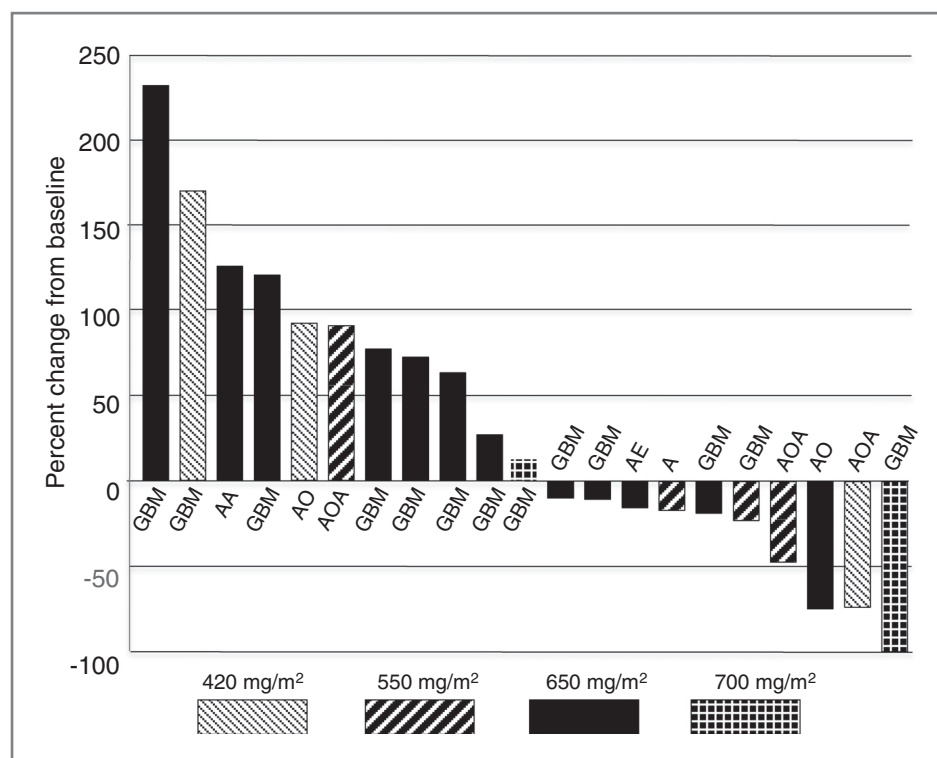
of free paclitaxel ranged from 0.57 to 8.61 µmol/L (median, 0.93 µmol/L), showing that cytotoxic levels (> 0.3 µmol/L) of drug were achieved in all tumors. These results indicate active transport of drug across the BBB into tumor cells and enzymatic release of paclitaxel from GRN1005. Additional qualitative analyses showed the presence of LRP-1 expression in these samples (Fig. 4). LPR-1 levels were variable and did not correlate with drug concentration at resection.

Discussion

In the present study, we describe the safety and tolerability, PK, and preliminary efficacy of GRN1005, a paclitaxel–Angiopep-2 peptide–drug conjugate that binds to the LRP-1 receptor for the treatment of recurrent glioma.

The MTD and recommended phase II dose of GRN1005 is 650 mg/m^2 every 3 weeks in patients with recurrent glioma. This is consistent with a concurrent phase I study of GRN1005 in patients with advanced solid tumors, including brain metastases (47). In that study, 5 of 20 patients (25%) treated with 650 mg/m^2 of GRN1005, including 3 patients who had received prior taxanes, achieved a PR in their systemic disease and reduction in the size of coexisting brain metastases (47). An additional 6 patients achieved SD for 4 months or longer.

GRN1005 was fairly well-tolerated with a toxicity profile qualitatively similar to paclitaxel including hematologic toxicity, mucositis, fatigue, and peripheral neuropathy. These toxicities appear to be cumulative and only partially reversible. Infusion reactions were infrequent and mostly minor. Central nervous system neurotoxicity was not observed on the basis of neurocognitive testing. There was no evidence of immunogenicity.

Efficacy observed in this study was limited. Eight of 27 patients treated at 420 mg/m^2 or above had stable disease which lasted a median of 51 days. Three patients achieved objective responses associated with clinical improvement. However, the patient population was very heavily pretreated with 54% of patients receiving 3 or more prior chemotherapies and 22 patients who had prior therapy with bevacizumab. Whether GRN1005 will have greater activity in a less heavily pretreated population and in patients who have not received prior therapy with bevacizumab will be determined in a planned phase II study of the agent in patients with recurrent glioblastomas.

Intratumoral concentrations of free paclitaxel (0.57–8.61 µmol/L) generally exceeded plasma concentrations of free paclitaxel (0.34–2.3 µmol/L), providing evidence that GRN1005 was able to cross the BBB. In addition, there was a trend for increased relative concentrations with higher doses. Higher absolute tumor concentrations of both GRN1005 and paclitaxel, along with a higher percentage of free paclitaxel in tumors relative to plasma, suggest accumulation in the tumors. In all cases, tumor concentrations of free paclitaxel exceeded the reported *in vitro* cytotoxic concentration for this drug (0.3 µmol/L) and were higher than those reported previously following naked paclitaxel administration (35). These data suggest that the combination of Angiopep-2 with paclitaxel can improve the delivery of this chemotherapeutic agent across the BBB. It raises the possibility that LRP-1 receptor–mediated
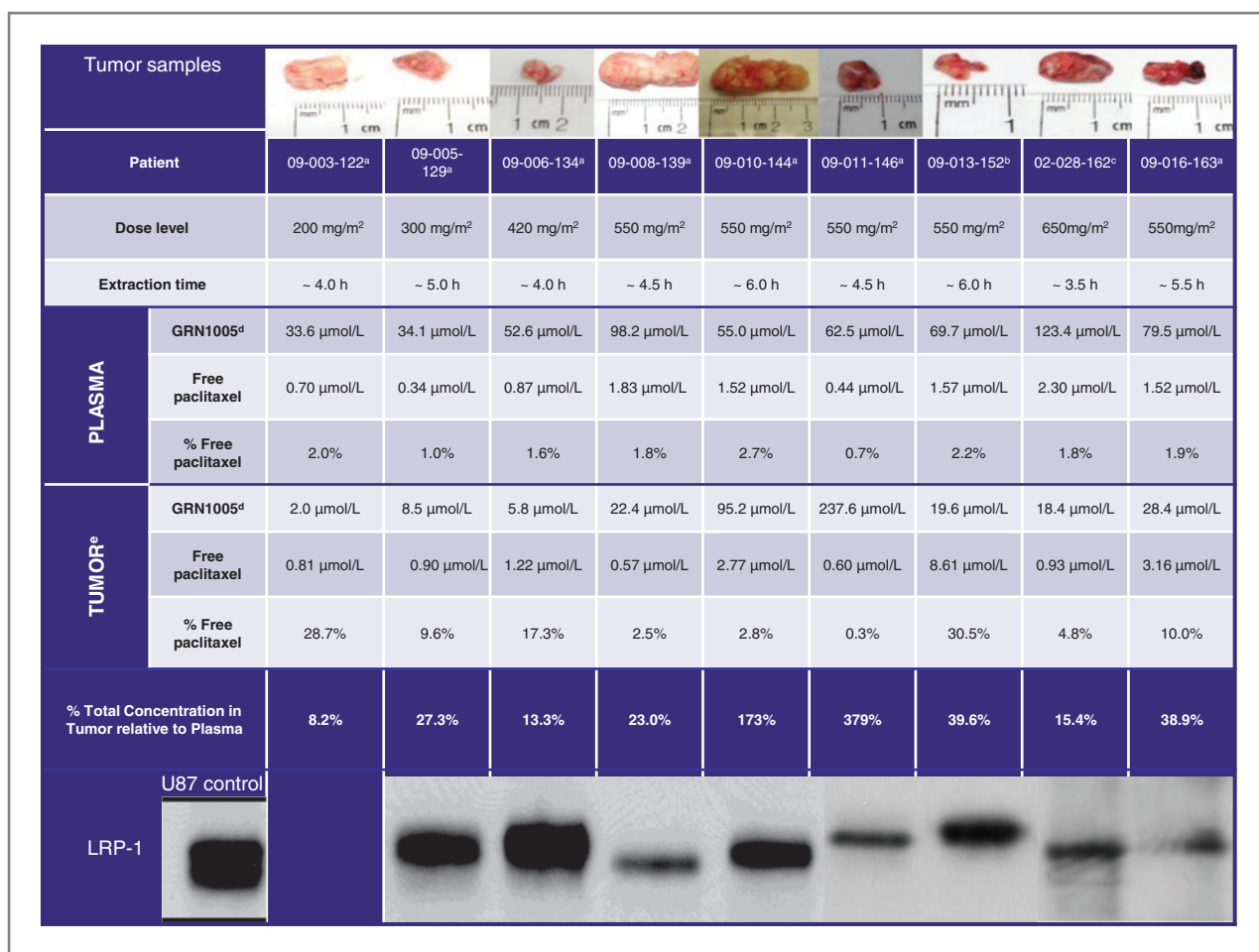
Figure 4. Levels of GRN1005 and free paclitaxel measured in excised tumor samples and concurrent plasma samples. a, GBM; b, poorly differentiated anaplastic oligodendroglioma; c, anaplastic astrocytoma; d, paclitaxel equivalents; e, corrected for vascular content. Immunodetection of LRP-1 in human brain tumors extracted from patients treated with GRN1005 (bottom row). Sample 09-003-122 was not analyzed for LRP-1 expression.

transcytosis may serve as a paradigm for drug delivery improvements for both primary and metastatic brain tumors.

In summary, GRN1005 delivers paclitaxel across the BBB and achieves therapeutic concentrations in tumor tissue. It is reasonably well-tolerated and has activity in recurrent glioma. The MTD and recommended phase II dose of GRN1005 is 650 mg/m^2 every 3 weeks. Phase II trials of GRN1005 in brain metastases from breast and non–small cell lung cancer are underway. Randomized phase II studies of GRN1005 for recurrent glioblastoma are planned.

Disclosure of Potential Conflicts of Interest

J. Drappatz is a consultant/advisory board member of Angiochem. M.D. Groves has received a commercial research grant from Genentech and honoraria from speakers bureau of Merck, and is a consultant/advisory board member of Genentech. R.M. Fielding is a consultant/advisory board member of Angiochem. Immediate family members of K. Elian have ownership interest (including patents) in Angiochem. S. Kelsey has ownership interest (including patents) in Geron Corpora-

tion. P.Y. Wen has other commercial research support from Angiochem and Geron Corporation. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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