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First-in-Man Phase I Study of GC33, a Novel Recombinant Humanized Antibody Against Glypican-3, in Patients with Advanced Hepatocellular Carcinoma

Andrew X. Zhu[1], Philip J. Gold[3], Anthony B. El-Khoueiry[4], Thomas A. Abrams[2], Hideo Morikawa[5], Norihisa Ohishi[5], Toshihiko Ohtomo[5], and Philip A. Philip[6]

Abstract

Purpose: GC33 is a novel recombinant fully humanized monoclonal antibody that binds to human glypican-3 (GPC3). The antitumor activity of GC33 was shown in preclinical models of hepatocellular carcinoma (HCC). This first-in-man clinical trial was conducted to evaluate the safety, pharmacokinetic characteristics, and preliminary efficacy of GC33 in patients with advanced HCC.

Experimental Design: Patients with measurable, histologically proven, advanced HCC were enrolled to a dose-escalation study of GC33 (2.5–20 mg/kg) given intravenously weekly. The primary endpoint was to determine the maximum tolerated dose of GC33 for further development. Pharmacokinetic characteristics were measured in serum samples. Immunohistochemistry was conducted on tumor biopsies to evaluate GPC3 expression. Tumor response was assessed every 8 weeks using Response Evaluation Criteria in Solid Tumors criteria.

Results: Twenty patients were enrolled and treated with GC33. A maximum tolerated dose was not reached as there were no dose-limiting toxicities (DLT) up to the highest planned dose level. Common adverse events with all grades included fatigue (50%), constipation (35%), headache (35%), and hyponatremia (35%). The incidence of adverse events seemed not to be dose dependent. Trough serum concentrations at steady state were in excess of target concentration at doses of 5 mg/kg or greater. Median time to progression (TTP) was 26.0 weeks in the GPC3 high expression group and 7.1 weeks in the low expression group ($P = 0.033$).

Conclusion: This study shows that GC33 was well tolerated in advanced HCC and provides preliminary evidence that GPC3 expression in HCC may be associated with the clinical benefit to GC33 that warrants prospective evaluation. *Clin Cancer Res; 19(4); 920–8. ©2012 AACR.*

Introduction

Hepatocellular carcinoma (HCC) is one of the most common fatal malignancies globally with more than 630,000 new cases diagnosed annually (1). It is currently the sixth most common solid tumor and the third leading cause of cancer-related deaths worldwide (1). The only approved therapy for advanced HCC is sorafenib, as shown by improved overall survival (OS) in 2 phase III randomized placebo-controlled trials (2, 3). Despite the positive results with sorafenib and its wide clinical application, it is clear that the treatment benefit is modest, and there is an ongoing need for developing novel and more effective treatment(s) for advanced HCC, particularly those with unique mechanism of action targeting the relevant molecular signature of HCC.

Glypican-3 (GPC3) is a member of the glypican family, a group of heparan sulfate proteoglycans linked to the cell surface through a glycosyl-phosphatidylinositol anchor (4). GPC3 protein is expressed in a wide variety of tissues during development, but not expressed in most adult tissues due to suppression by DNA methylation within the promoter region (5). Recently, it was shown that GPC3 is highly expressed, both at the mRNA and protein levels, in HCC (6–11). Immunohistochemical studies have shown that GPC3 is expressed in approximately 70% to 100% of surgically removed or biopsied HCC tissues, whereas it is not or less detectable in adjacent nontumoral lesions (6–11). The function of GPC3 in HCC is not very clear, but GPC3 has been reported to promote tumor growth by stimulating the canonical Wnt signaling pathway (12), and

Authors' Affiliations: [1]Massachusetts General Hospital Cancer Center, Harvard Medical School; [2]Dana-Farber Cancer Institute, Boston, Massachusetts; [3]Swedish Cancer Institute, Seattle, Washington; [4]University of Southern California, Norris Comprehensive Cancer Center, Los Angeles, California; [5]Chugai Pharmaceutical Co. Ltd., Tokyo, Japan; and [6]Karmanos Cancer Institute, Wayne State University, Detroit, Michigan

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Corresponding Author: Dr. Andrew X. Zhu, Massachusetts General Hospital Cancer Center, 55 Fruit Street, LH/POB 232, Boston, MA 02114. Phone: 617-643-3415; Fax: 617-724-3166; E-mail: azhu@partners.org

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Translational Relevance

Sorafenib remains the current standard of care in advanced hepatocellular carcinoma (HCC), with modest efficacy. Recently, several antivascular endothelial growth factor receptor tyrosine kinase inhibitors have failed in HCC, emphasizing the urgent need for developing other targeted agents with novel mechanism of action. Glypican-3 (GPC3) is a novel and potentially a critical molecular target in HCC as it is specifically expressed in HCC and associated with poor prognosis. In this first-in-man phase I study, we examined the safety, tolerability, pharmacokinetics, and antitumor activity of GC33, a novel recombinant humanized antibody against GPC3, in patients with HCC. Our study showed the favorable tolerability of GC33 and preliminary evidence that GPC3 expression in HCC may be associated with the clinical benefit of GC33. It has provided the initial clinical experience and rationale for further developing GC33 in advanced HCC with GPC3 expression signature.

it may also interact with the insulin-like growth factor (IGF) receptor pathway (13). Other study has suggested that it may play a role in fibroblast growth factor (FGF)-mediated signaling (14). GPC3 expression correlated with poor prognosis in HCC as membranous patients with GPC3-positive HCC have a significantly lower disease-free survival rate than patients with GPC3-negative HCC after surgical resection (15). Therefore, GPC3 represents a specific tumor marker and a potential therapeutic target in HCC (16).

GC33 is a recombinant, fully humanized monoclonal antibody that binds to human GPC3 with high affinity (17). The preclinical pharmacologic assessments have shown that GC33 elicits antibody-dependent cellular cytotoxicity through human peripheral blood mononuclear cells against GPC3-expressing human HCC cell lines *in vitro* (18). GC33 at 5 mg/kg i.v. weekly showed antitumor activity in several mouse xenograft models inoculated with human HCC cell lines expressing GPC3 (18). Activity is correlated with cell surface expression of GPC3 across 3 xenograft models (19). Because of these preclinical data showing the relevance of GPC3 as a potential therapeutic target in HCC, we conducted the first-in-man phase I clinical trial to assess the safety, tolerability, and pharmacokinetic characteristics of GC33 in patients with advanced HCC. The primary objective of the study was to determine the maximum tolerated dose (MTD) of GC33 given intravenously at weekly intervals. Preliminary antitumor activity and exploratory biomarker analysis of GPC3 expression in biopsied tumor specimens using immunohistochemistry (IHC) as well as its association with clinical outcome were also assessed.

Materials and Methods

This was a multicenter, open-label, dose-escalation phase I trial in patients with advanced HCC (ClinicalTrials.gov: NCT00746317). The trial was conducted at 6 sites across the United States. It was approved by Institutional review boards at participating institutions. All patients gave written informed consent.

Patient population

Patients of at least 18 years of age, with measurable, histologically confirmed, and inoperable HCC were eligible for enrollment into the study. Inclusion criteria included the Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, Child–Pugh class of A or B, life-expectancy of at least 3 months, ability to provide a tumor sample for GPC3-IHC testing, and at least one measurable lesion based on the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.0 (20). In addition, patients were required to have adequate hematologic, hepatic, and renal function tests as evidenced by platelets $\geq$ 50,000/μL, absolute neutrophil count $\geq$ 1,500/μL, transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] $\leq$ 5.0 the upper limit of normal (ULN), total bilirubin $\leq$ 3.0 ULN, prothrombin time and international normalized ratio (PT-INR) $\leq$ 2.0, and serum creatinine $\leq$ 2.0 ULN. Patients who had received prior surgery or systemic treatment (including sorafenib) were allowed in the study.

Exclusion criteria included patients known to be positive for human immunodeficiency virus infection, patients with active infectious diseases requiring treatment except for hepatitis B and C, history of transplantation, patients with brain metastases, other central nervous system (including psychiatric) diseases, or patients with any residual effect of tumor biopsy, which could interfere with study drug administration or evaluation of safety. Patients who had received major surgery, regional therapy for HCC, chemotherapy, radiotherapy, hormone therapy, immunotherapy, or any other investigational drug within 4 weeks before day 1 (6 weeks for nitrosoureas, mitomycin, 2 weeks for sorafenib, and 1 week for tumor biopsy) were also excluded. In addition, patients were excluded if they had been treated within 2 weeks before day 1 with anticoagulant, thrombolytic agents for therapeutic purposes (low-dose administration of these drugs for catheter clearance or for prophylactic purposes were allowed), systemic antiviral therapy for hepatitis C, or blood transfusion.

Treatment and dose escalation schedule

GC33 was provided by Chugai Pharmaceutical Co. Ltd.

Patients were assigned to receive GC33 at 1 of 4 sequentially increasing dose levels, 2.5, 5, 10, and 20 mg/kg given every week by intravenous infusion over a period of 30 to 90 minutes. Each cycle was defined by 4 weeks of treatments. GC33 treatment would continue until disease progression or unacceptable drug-related toxicities occurred.

The starting dose of 2.5 mg/kg was chosen based on an estimate of the dose of GC33 expected to still have potential for some antitumor activity in xenograft mouse models. For the highest tested dose of 20 mg/kg, the safety margin was more than 6-fold of the highest dose used in the multiple dose toxicology studies in cynomolgus monkeys.

Conventional 3 + 3 dose escalation study design was used. At least 3 patients at 2.5 to 10 mg/kg cohort, and at least 6 patients at 20 mg/kg cohort were treated and monitored during the first cycle and at least one week thereafter, before additional patients could be started at the next dose level. If any patient experienced dose-limiting toxicity (DLT), 3 more patients were to be added at the same dose level. DLT was defined as any Common Terminology Criteria for Adverse Events CTCAE (version 3.0) grade 3 or higher toxicity during cycle 1, except for grade 3 cytokine release syndrome/acute infusion reaction, and grade 3 hepatic biochemical dysfunction (ALT, AST, alkaline phosphatase, albumin, total bilirubin, and PT-INR). The independent Data Safety Monitoring Board reviewed safety data of each cohort to determine dose escalation.

Tolerability and safety

The incidence and severity of all adverse events (AE) were graded by NCI-CTCAE version 3.0. DLTs, changes in vital signs, laboratory parameters, physical examination findings, electrocardiography, and medical conditions during and after GC33 infusions were assessed throughout the study. Special attention was paid to the possibility of infusion-associated symptoms following GC33 infusion. Laboratory evaluations included hematology, blood biochemistry, blood coagulation, C-reactive protein, and natural killer (NK) cell count. Anti-GC33 antibodies were measured at screening and during study (at cycle 1, cycle 4, and 4 and 8 weeks after last GC33 infusion).

Tumor assessments

RECIST version 1.0 was used to assess objective response, time to disease progression, and duration of response. Tumor burden and response to treatment were evaluated at baseline and every 8 weeks by physical examination and imaging (CT or MRI). α-fetoprotein (AFP) was also measured at baseline and during the study.

Tumoral GPC3 expression testing

Tumor expression of GPC3 was examined in biopsied specimens by IHC using mouse antihuman GPC3 monoclonal antibody (21; at Charles River Laboratories Inc., Nevada). Table 1 describes the immunohistochemical scoring system for GPC3 expression. Three pathologists who were blinded to clinical information conducted a peer review of immunohistochemical findings. A total score of 7 or more was defined as a high GPC3 expression, and a score of less than 7 was defined as low/no GPC3 expression.

Pharmacokinetic analysis for GC33

Blood samples for serum GC33 measurements were drawn at predose, at the end of infusion, and 1, 8, 24, 48, 72, 120, and 168 hours after the first infusion. In addition, trough concentrations were measured before each subsequent infusion. GC33 in the serum was assayed by validated (ELISA) using human GPC3 core protein (22) as a capture antigen and rabbit anti-GC33 antibody, and goat anti-rabbit IgG (H+L)-HRP as detector antibodies. Pharmacokinetic parameters for GC33 in serum were calculated by noncompartmental methods.

Table 1. Scoring system of GPC3-IHC

		Score
Positive cell rate (PR)	No staining	0
	<20%	1
	20%–49%	2
	>50%	3
Staining intensity (SI)[a]		
Cytoplasm (SI-Cp)	No staining	0
	Minimal staining	1
Cell membrane (SI-Cm)	Weak staining	2
	Moderate staining	3
	Strong staining	4
Staining pattern of cell membrane (SP-Cm)[b]	No cell membrane pattern	0
	Incomplete or partly complete (<20%)	1
	Focally complete (20%–49%)	2
	Rather complete (>50%)	3
Scoring:	IHC total = PR + SI-Cp + SI-Cm + Sp-Cm	

[a] Minimal or weak staining is identified when slight staining can be recognized at low-power objective, ×10 or ×4, respectively. Strong staining is ruled when apparent strong staining was observed with low-power objective (×4), and moderate staining ruled as staining of GPC3 is found easily at ×4, but weaker than that of strong staining.

[b]Complete means circular (ring-like) staining of tumor cells, and incomplete staining shows focal staining of rim of the cells.

Statistical analysis

Statistical analyses were primarily descriptive. In addition, exploratory analyses were conducted. The Kaplan–Meier estimate was calculated for 95% confidence interval (CI) of time to progression (TTP) and OS. And we used the Wald test for calculating *P* value.

Results

Patient characteristics

A total of 20 patients (16 males and 4 females) with a median age of 62 years (50–78) were enrolled between November 2008 and August 2010. Patient characteristics are listed in Table 2. A total of 13 (65%) patients were Child–Pugh A, and 17 (85%) patients had extrahepatic disease. Fifteen (75%) patients had received sorafenib.

GPC3-IHC was conducted using core-needle biopsied specimens from primary lesions in 19 patients (Fig. 1A). Of those, samples from 3 patients were deemed not evaluable due to the insufficient number of tumor cells in the specimens. Nine patients (56%) had total GPC3-staining score of 7 or more (high GPC3).

Dose and duration of therapy

GC33 was given to a minimum of 3 patients at each of the planned dose levels. Four patients (3 with high GPC3-expressing tumors) received 2.5 mg/kg; 3 patients (1 with high GPC3-expressing tumors) received 5 mg/kg; 4 patients (1 with high GPC3-expressing tumors) received 10 mg/kg, and 9 patients (4 with high GPC3-expressing tumors) received 20 mg/kg. Five patients did not complete the first cycle of GC33 and were replaced because of worsening underlying liver disease or rapid progression (4 received only 1 dose and 1 received 3 doses). All 20 patients enrolled in the study were evaluable for safety analyses, and 15 patients were assessable for DLT assessment.

Safety and tolerability

GC33 was generally well tolerated at all dose levels. There were no DLT up to the highest tested dose (20 mg/kg), and an MTD dose was consequently not reached. All patients had at least 1 adverse event, and 85% of the patients had at least 1 drug-related adverse event (Table 3). Fifteen of 20 (75%) patients experienced at least one grade 3 or more adverse event and of these, 6 (30%) patients were thought to have had drug-related adverse events. Grade 1 and 2 infusion reactions occurred in 40% of the patients only after the first infusion, there were no grade 3 or 4 infusion reactions.

Adverse events across the study and by GC33 dose group are listed in Table 3. The most common adverse events were fatigue (50%), constipation (35%), headache (35%), and hyponatremia (35%). The incidence of adverse events seemed not to be dose dependent. There was no evidence of cumulative toxicity and, with the exception of infusion-related adverse events that only occurred during the first infusion, there was no difference in the incidence or severity of adverse events between high and low/no GPC3-expressing groups.

GC33 was associated with a decrease in NK cells (CD16+/CD56+ cells) and a transient decrease of lymphocytes in peripheral blood. Anti-GC33 antibodies were not detected in any of the patients.

Pharmacokinetics

The computed pharmacokinetic parameters of GC33 are shown in Table 4. Mean half-life ($t_{1/2}$) was 2.94, 3.46, 5.16, and 6.47 days, and mean total clearance (CL) was 1.62, 1.14, 0.799, and 0.784 L/days at 2.5, 5, 10, and 20 mg/kg, respectively. Predose GC33 concentrations became stable after 6 infusions for all dose groups. C_{trough} were maintained above 30 μg/mL, the predicted effective concentration in animal studies (18), in all patients who received more than 2 cycles at 5 mg/kg or more.

Antitumor activity

Fifteen patients who completed at least one cycle were evaluable for tumor response assessment (Fig. 1B and C). Across all patients, median TTP was 8.0 weeks (95% CI: 7.1–13.1 wks) and median OS was 16.1 weeks (95% CI: 11.6–54.9 wks). Median TTP was 26.0 weeks (95% CI: 7.1–N/A wks) in the GPC3 high expression group with decreased or stable serum AFP levels and 7.1 weeks (95% CI: 3.3–8.9 wks) in the GPC3 low or none expression group ($P = 0.033$). Stable disease of more than 26 weeks was observed in 4 of 15 (16.7%) patients and all of them were in the GPC3 high expression group. Median OS in GPC3 high was longer than that in GPC3 low or none expression group but this was not statistically significant [49.4 wks; (95% CI: 12.6–81.0 weeks) vs. 13.0 wks (95% CI: 10.6–54.9 wks), $P = 0.142$].

Discussion

Development of effective therapy for advanced HCC remains a challenge. Here, we report the first-in-man, first-in-class, phase I study with GC33, a recombinant fully humanized monoclonal antibody that selectively targets GPC3, in patients with advanced HCC. Our study provided the initial clinical experience of the safety profile and pharmacokinetic features of GC33 and a potential antitumor activity that may be associated with the level of expression of GPC3 in tumors.

GPC3 is a novel and potentially a critical molecular target in HCC. First, overexpression of GPC3 in NIH3T3 cells led to a full cancer cell phenotype, including the ability to grow in serum-free medium and to form colonies in soft agar (13), whereas another study showed GPC3 as a negative regulator of cell proliferation (23). In addition, knockdown of GPC3 by siRNA results in loss of oncogenecity in HCC cells (13). Second, GPC3 may stimulate the growth of HCC cells by upregulating autocrine/paracrine Wnt signaling (24). Two human heparin-degrading endosulfatases, sulfatase 1 (SULF1) and SULF2 have been shown to play a role in liver carcinogenesis (25). In the liver, SULF2 acts as an oncogenic protein and is activated in 60% of the HCC cell lines (25). SULF2 has been shown to upregulate GPC3,

Table 2. Patients characteristics

		Patients ($n = 20$)	
Characteristic		**n (%)**	
Demographic	Age, y		
	Median		62
	Range		50–78
	Sex		
	Male	16 (80%)	
	Female	4 (20%)	
	Ethnicity		
	Caucasian	13 (65%)	
	African-American	3 (15%)	
	Asian	4 (20%)	
	ECOG PS		
	0	10 (50%)	
	1	10 (50%)	
Liver characteristic	Etiology		
	Hepatitis C	10 (50%)	
	Hepatitis B	4 (20%)	
	Alcohol	3 (15%)	
	Other (NASH Cirrhosis)	1 (5%)	
	Cirrhosis	14 (70%)	
	Child–Pugh		
	A	13 (65%)	
	B	7 (35%)	
HCC characteristic	Extrahepatic spread		
	Yes	17 (85%)	
	No	3 (15%)	
	Vascular invasion		
	Yes	9 (45%)	
	No	11 (55%)	
	BCLC staging		
	B	0 (0%)	
	C	20 (100%)	
Laboratory values			Median (range)
	Total bilirubin (mg/dL)		0.8 (0.4–2.8)
	AST (U/L)		82.5 (33–309)
	AFP (ng/mL)		644.85 (6.8–62766)
Previous therapy	Treatment naïve	4 (20%)	
	Surgical resection	8 (40%)	
	Radiotherapy	3 (15%)	
	TACE	3 (15%)	
	Radiofrequency ablation	2 (10%)	
	Intra-arterial chemotherapy	2 (10%)	
	Systemic therapy	15 (75%)	
	Sorafenib	15 (75%)	
	Prior regimens		
	1	3 (15%)	
	2	9 (45%)	
	3	1 (5%)	
	4	2 (10%)	

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; NASH, nonalcoholic steatohepatitis; TACE, transarterial chemoenbolization.

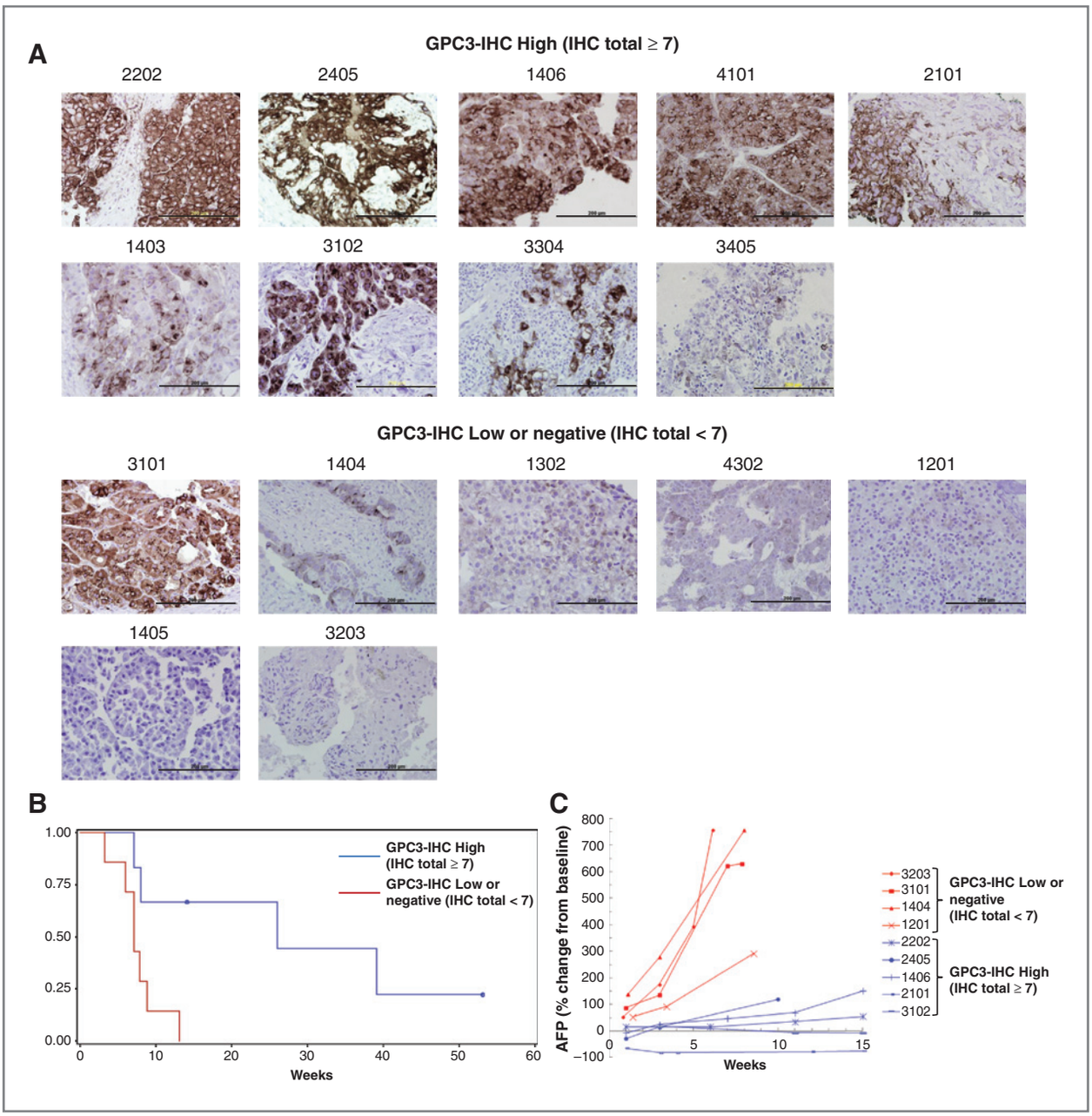

Figure 1. GPC3 expression and antitumor activity of GC33. A, GPC3-IHC staining features observed in each biopsied specimens [photos are taken with microscope (BX41, Olympus, PA) and camera (DP70) under x200 magnification and scale bars in the photos are 200 μm]. B, Kaplan–Meier plots for TTP by retrospectively assessed GPC3 status. Patients with GPC3-IHC high ($n = 7$) had a longer TTP than those with GPC3-IHC low/none ($n = 7$). C, AFP changes in GPC3-IHC high (2202, 2405, 1406, 2101, and 3102) or GPC3-IHC low/none (3203, 3101, 1404, and 1201) were shown as percentage change from their baseline values. Five patients (GPC3-IHC high: 1403 and 3405; GPC3-IHC low/none: 1302, 4302, and 1405) were excluded because of their baseline AFP below 100 ng/mL.

promote FGF signaling, and decrease survival in HCC (14). GPC3 may also confer oncogenecity through activation of the IGF signaling pathways (26). Third, in a study of 194 patients with HCC with resected tumors, GPC3 expression was observed in 77% of the HCC samples in 20% or more of tumor cells using the mouse GC33 monoclonal antibody (15). This study also showed that high membranous GPC3 immunoreactivity was an independent poor prognostic factor for disease-free survival.

In this first-in-man phase I clinical trial, GC33 was generally well tolerated by patients with advanced HCC with Child–Pugh A or B cirrhosis. An MTD was not reached. Grade 1 or 2 infusion reactions occurred only after the first infusion in less than half the patients. The most common

Table 3. The incidence of adverse event

All AEs							Grade 3 AEs				
		GC33 Dose group				Drug-related AEs		GC33 Dose group				Drug-related ≥ grade 3 AEs
Adverse event	Total No. of Patients (*n*=20; %)	2.5 mg/kg (*n*=4)	5 mg/kg (*n*=3)	10 mg/kg (*n*=4)	20 mg/kg (*n*=9)	No. of Patients (*n*=20; %)	Total No. of Patients (*n*=20; %)	2.5 mg/kg (*n*=4)	5 mg/kg (*n*=3)	10 mg/kg (*n*=4)	20 mg/kg (*n*=9)	No. of Patients (*n*=20; %)
Any AEs	20 (100%)	4	3	4	9	17 (85%)	15 (75%)	3	3	2	7	6 (30%)
Infusion-related AEs	8 (40%)	2	1	2	3	7 (35%)	0 (0%)	0	0	0	0	0 (0%)
Fatigue	10 (50%)	2	2	2	4	7 (35%)	1 (5%)	0	1	0	0	1 (5%)
Constipation	7 (35%)	0	2	2	3	2 (10%)	0 (0%)					0 (0%)
Headache	7 (35%)	1	1	0	5	3 (15%)	0 (0%)					0 (0%)
Hyponatremia	7 (35%)	1	0	2	4	2 (10%)	5 (25%)	0	0	1	4	2 (10%)
Diarrhea	6 (30%)	1	2	1	2	3 (15%)	0 (0%)					0 (0%)
Hyperbilirubinemia	6 (30%)	1	0	3	2	1 (5%)	6 (30%)	1	0	3	2	1 (5%)
Disease progression	6 (30%)	2	0	2	2	0 (0%)	4 (20%)	2	0	1	1	0 (0%)
Insomnia	6 (30%)	1	3	0	2	1 (5%)	0 (0%)					0 (0%)
Pyrexia	6 (30%)	1	1	1	3	4 (20%)	0 (0%)					0 (0%)
Decreased appetite	5 (25%)	1	1	0	3	1 (5%)	0 (0%)					0 (0%)
Anemia	4 (20%)	1	0	1	2	2 (10%)	1 (5%)	0	0	0	1	0 (0%)
Chills	4 (20%)	1	1	1	1	4 (20%)	0 (0%)					0 (0%)
Nausea	4 (20%)	0	0	2	2	0 (0%)	0 (0%)					0 (0%)
Edema peripheral	4 (20%)	1	0	0	3	0 (0%)	0 (0%)					0 (0%)
Abdominal pain	3 (15%)	0	1	1	1	0 (0%)	2 (10%)	0	1	0	1	0 (0%)
Ascites	3 (15%)	1	0	0	2	0 (0%)	1 (5%)	0	0	0	1	0 (0%)
AST increased	3 (15%)	0	0	2	1	2 (10%)	2 (10%)	0	0	1	1	1 (5%)
Confusional state	3 (15%)	2	0	0	1	1 (5%)	0 (0%)					0 (0%)
Dehydration	3 (15%)	0	2	1	0	1 (5%)	0 (0%)					0 (0%)
Dyspnea	3 (15%)	0	0	0	3	0 (0%)	0 (0%)					0 (0%)
Hyperglycemia	3 (15%)	0	1	0	2	0 (0%)	1 (5%)	0	1	0	0	0 (0%)
Influenza-like illness	3 (15%)	1	0	1	1	2 (10%)	0 (0%)					0 (0%)
Jaundice	3 (15%)	2	0	0	1	0 (0%)	0 (0%)					0 (0%)
Edema	3 (15%)	1	0	1	1	0 (0%)	0 (0%)					0 (0%)
Pruritus	3 (15%)	2	1	0	0	2 (10%)	0 (0%)					0 (0%)

Abbreviations: AE, adverse event; Pts, patients.

Table 4. Pharmacokinetics parameters of GC33

	C_{max} (mcg/mL)	CL (L/d)	V_{dss} (L)	$t_{1/2}$ (d)	$AUC_{0\text{-inf}}$ (day*mcg/mL)	C_{trough} (Wk 4; mcg/mL)	C_{trough} (Wk 6; mcg/mL)
2.5 mg/kg	46.60 ± 13.09	1.619 ± 1.075	5.356 ± 1.169	2.939 ± 1.766	151.61 ± 62.71	13.7 ± 10.4	13.3 ± 9.41
5 mg/kg	89.87 ± 24.64	1.138 ± 0.402	5.531 ± 1.753	3.464 ± 0.449	372.10 ± 101.00	46.0 ± 14.7	85.7[a]
10 mg/kg	148.00 ± 24.36	0.799 ± 0.319	5.868 ± 2.563	5.155 ± 1.106	736.47 ± 244.56	104 ± 77.1	196 ± 50.2
20 mg/kg	353.89 ± 91.94	0.784 ± 0.207	6.519 ± 2.441	6.471 ±4.144	2425.58 ± 802.32	311 ± 121	369 ± 151

NOTE: Quantitative range of GC33 is 0.05 to 3.2 μg/mL in the serum. Interassay accuracy and precision were within ± 20% and CV <20%. PK parameters for GC33 in serum were calculated by noncompartmental methods. The estimated PK parameters following first GC33 infusion are $t_{1/2}$ (terminal half-life), $AUC_{0\text{-inf}}$ (area under the serum concentration time curve extrapolated to infinity), V_{dss} (voume of distribution at steady state), and C_{max} (maximum serum concentration). In addition, C_{trough} (predose concentrations) at week 4 and 6 are summarized. All values are indicated as mean ± SD.

[a]Mean of C_{trough} at week 6 for 2 patients.

adverse events were fatigue (50%), constipation (35%), headache (35%), and hyponatremia (35%), most of which were either grade 1 or grade 2. NK cell numbers in plasma were reduced following GC33 administration, but no increased incidence of infection was observed.

Pharmacokinetic study showed that total clearance was faster and $t_{1/2}$ was shorter at the 2.5 mg/kg compared with other higher dose groups suggesting nonlinear elimination. At 10 and 20 mg/kg, total clearance and $t_{1/2}$ became comparable indicating that linear clearance is a predominant pathway for GC33 clearance in these dose ranges. Xenograft mouse models (human HCC and hepatoblastoma; ref. 18) suggested that 5 mg/kg/wk of GC33 was effective for tumor growth inhibition, which was associated with trough serum concentrations of 30 μg/mL. In the present study, the trough concentrations of GC33 were above 30 μg/mL in all patients who received more than 2 cycles at 5 mg/kg or greater, suggesting that potentially effective doses were reached.

In this study, no pharmacodynamic (PD) analysis was conducted in tumor as posttreatment biopsy was not obtained. Soluble GPC3 was measured and was below the detection limit in more than half of the serum samples tested. More sensitive assays capable of measuring soluble GPC3 will be needed for further evaluation of its value as a potential PD marker for GC33. On the basis of our current understanding of the mechanism of action of GC33 and its lack of evidence of modulating tumor vasculature and glucose metabolism, functional imaging with MRI or positron emission tomography scan was not conducted in our study and should be further explored in future studies.

Potential antitumor activity that was associated with the target GPC3 expression was observed in this study. Stable disease was seen in 4 patients, all of whom had high GPC3 expression. The median TTP was significantly longer in patients with high GPC3-expressing tumors than in patients with low GPC3-expressing tumors. While the small number of patients and single-arm phase I nature of this study precluded any definitive conclusion, our study suggests that targeting GPC3 antigen in HCC by GC33 warrants further investigation as a therapeutic target in treating advanced HCC. Simultaneous development of a companion diagnostic test based on GPC3 expression would be crucial to enrich the population that benefits from GC33 treatment.

In conclusion, we showed that GC33 could be safely administered in patients with advanced HCC in this study. Meaningful drug concentrations predicted from animal studies could be reached at the doses tested following weekly infusion of 5 to 20 mg/kg of GC33. Dose schedule at 10 mg/kg/wk would consistently achieve a trough level of GC33 above 30 μg/mL and serves as a basis for future dose selection. Preliminary evidence for a potential antitumor activity was observed in patients with high expression of the target protein GPC3 in their tumors. Our study has provided the initial clinical experience and rationale for further developing GC33 in advanced HCC with GPC3 expression signature. Currently, GC33 has moved to a phase II clinical trial in second line patients with HCC who have progressed after one line of systemic therapy and whose tumors have

GPC3 immunohistochemical staining. A fixed dose strategy of 1,600 mg every 2 weeks after 2 weekly loading was adopted in the phase II as impact to AUC by patients' weight is not significant, and the simulated pharmacokinetic model suggests that such dosing strategy could achieve a plasma trough level above the target 30 μg/mL in more than 85% of the patients.

Disclosure of Potential Conflicts of Interest

A. El-khoueiry has a commercial research grant, honoraria from speakers' bureau from, and is a consultant/advisory board member for Genentech Roche. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: A.X. Zhu, H. Morikawa, T. Ohtomo, P.A. Philip
Development of methodology: A.X. Zhu, T.A. Abrams, P.A. Philip
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.X. Zhu, P.J. Gold, A. El-khoueiry, T.A. Abrams, H. Morikawa, T. Ohtomo, P.A. Philip
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.X. Zhu, H. Morikawa, N. Ohishi, T. Ohtomo, P.A. Philip
Writing, review, and/or revision of the manuscript: A.X. Zhu, P.J. Gold, A. El-khoueiry, T.A. Abrams,H. Morikawa, N. Ohishi, T. Ohtomo, P.A. Philip
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.X. Zhu, H. Morikawa
Study supervision: A.X. Zhu, P.A. Philip

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