Clinical Trial Brief Report

Clinical Cancer Research

Phase Ib Study of PEGylated Recombinant Human Hyaluronidase and Gemcitabine in Patients with Advanced Pancreatic Cancer

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Abstract

Purpose: This phase Ib study evaluated the safety and tolerability of PEGylated human recombinant hyaluronidase (PEGPH20) in combination with gemcitabine (Gem), and established a phase II dose for patients with untreated stage IV metastatic pancreatic ductal adenocarcinoma (PDA). Objective response rate and treatment efficacy using biomarker and imaging measurements were also evaluated.

Experimental Design: Patients received escalating intravenous doses of PEGPH20 in combination with Gem using a standard 3+3 dose-escalation design. In cycle 1 (8 weeks), PEGPH20 was administrated twice weekly for 4 weeks, then once weekly for 3 weeks; Gem was administrated once weekly for 7 weeks, followed by 1 week off treatment. In each subsequent 4-week cycle, PEGPH20 and Gem were administered once weekly for 3 weeks, followed by 1 week off. Dexamethasone (8 mg) was given pre- and post-PEGPH20 administration. Several safety parameters were evaluated.

Results: Twenty-eight patients were enrolled and received PEGPH20 at 1.0 ($n = 4$), 1.6 ($n = 4$), or 3.0 μg/kg ($n = 20$), respectively. The most common PEGPH20-related adverse events were musculoskeletal and extremity pain, peripheral edema, and fatigue. The incidence of thromboembolic events was 29%. Median progression-free survival (PFS) and overall survival (OS) rates were 5.0 and 6.6 months, respectively. In 17 patients evaluated for pretreatment tissue hyaluronan (HA) levels, median PFS and OS rates were 7.2 and 13.0 months for "high"-HA patients ($n = 6$), and 3.5 and 5.7 months for "low"-HA patients ($n = 11$), respectively.

Conclusions: PEGPH20 in combination with Gem was well tolerated and may have therapeutic benefit in patients with advanced PDA, especially in those with high HA tumors. *Clin Cancer Res; 22(12); 2848–54. ©2016 AACR.*

Introduction

Pancreatic ductal adenocarcinoma (PDA) or, more commonly, pancreatic cancer, incidence is projected to rise to 62,000 diagnoses and 48,000 deaths in the United States by 2020, which would elevate it to the second leading cause of cancer-related mortality (1). This reality together with an unusual degree of therapeutic resistance lends increased urgency to the search for more effective treatment strategies for patients with advanced PDA. The deoxycytosine analogue gemcitabine (Gem) was approved in 1997 as a first-line treatment for stage IV PDA (2) and has served as the backbone for a number of subsequent regimens that have largely failed to advance the bar (3). The two best current standard-of-care regimens for metastatic PDA, *nab*-paclitaxel+Gem (4) and FOLFIRINOX (5), have reported median overall survival (OS) rates of 8.5 and 11.1 months, respectively.

Pancreas cancers possess a degree of resistance not anticipated by studies *in vitro* or in transplantable tumor systems, suggesting unique features to the autochthonous disease (6,7). PDAs are also characterized by a robust fibroinflammatory infiltrate, or desmoplasia, that evolves with disease progression. This desmoplastic reaction includes stromal fibroblasts, various subpopulations of immunosuppressive cells, and a complex extracellular matrix comprised of glycosaminoglycans, proteoglycans, and collagens. Predominant among the glycosaminoglycans is hyaluronan, or hyaluronic acid (HA). HA is a widely distributed primary constituent of the normal extracellular matrix and accumulates significantly in a variety of solid

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer-related death in the United States, with an overall 5-year survival rate of less than 5%. Late stage at diagnosis, early metastasis, and a lack of effective therapies contribute to the lethality of this disease. Pegylated recombinant human hyaluronidase (PEGPH20) offers an innovative approach to the treatment of PDA by depleting interstitial hyaluronan (HA), a glycosaminoglycan polymer that accumulates in the desmoplastic stroma of PDA. Increased interstitial HA in PDA significantly increases fluid pressures, causing vascular collapse and hindering perfusion. Here, we report the results of a phase 1b study of PEGPH20 in combination with gemcitabine to treat stage IV metastatic PDA. PEGPH20 plus gemcitabine was well-tolerated and showed promising clinical activity, particularly in patients with tumors expressing high HA levels.

malignancies (8). Increasing evidence demonstrates that elevated HA content in the tumor microenvironment plays a primary pathophysiologic role in tumor progression and metastasis, and HA levels in PDA may also be predictive of survival (9). Intratumoral HA also figures prominently in resistance to systemically administered agents by contributing to elevated interstitial fluid pressure (IFP) and subsequent compression of blood vessels (10, 11).

Enzymes that degrade HA (hyaluronidases) exist naturally and can be found, for example, in spermatozoa that use it to access the HA-enshrouded oocyte during fertilization (12). PEGylation of recombinant human hyaluronidase PH20 (PEGPH20; Halozyme Therapeutics) prolongs the circulatory half-life from minutes to over 20 hours, permitting sustained enzymatic breakdown of HA in tissues (10). Single-agent PEGPH20 inhibited tumor growth in xenograft models of PDA (10,13). Preclinical studies have also been conducted in murine models that faithfully recapitulate the progression of human PDA, including high intratumoral HA content. Weekly intravenous (i.v.) treatment with PEGPH20+Gem depleted intratumoral HA, dramatically lowered IFP, and increased drug delivery, which collectively led to significantly prolonged survival compared with Gem monotherapy in the *Kras*$^{LSL\text{-}G12D/+}$;*Trp53*$^{LSL\text{-}R172H/+}$;*Cre* (*KPC*) GEMM of PDA (11). Similar results were independently reported with PEGPH20 plus intraperitoneal administration of Gem (8). Together with earlier clinical studies involving animal-derived hyaluronidase products (14), these findings provided the rationale for the clinical development of PEGPH20.

The safety, pharmacokinetics, and pharmacodynamics of PEGPH20 administration in patients with a variety of advanced solid tumors were characterized in phase I clinical studies (15) and informed the dose range and regimen used in the present study. These previous trials also established a maximum tolerated dose (MTD) of PEGPH20 monotherapy of 3.0 μg/kg once or twice weekly when given with dexamethasone (4 or 8 mg) (16). The current report presents the safety and efficacy results of a phase Ib dose-escalation study of PEGPH20 combined with gemcitabine in patients with previously untreated stage IV PDA (ClinicalTrials.gov Identifier: NCT01453153).

Materials and Methods

Patients

The study was conducted at five centers in the United States and four centers in Russia in accordance with the Declaration of Helsinki and Good Clinical Practice, Guidelines of the International Conference on Harmonization, and was approved by the local institutional review board at each study site. Written informed consent was obtained from all patients. Eligible patients were ≥18 years of age with a Karnofsky score of ≥70% and a life expectancy of at least 3 months with newly diagnosed, previously untreated, histologically confirmed stage IV PDA and documented metastasis to the liver and/or lung. Patients were required to have one or more metastatic tumors measurable on CT scan per Response Evaluation Criteria in Solid Tumor (RECIST) v1.1.

Study design and treatment

Eligible patients were enrolled sequentially into three cohorts in a standard 3+3 dose-escalation design and received PEGPH20 at 1.0, 1.6, and 3.0 μg/kg, with an expansion cohort at the highest dose level. In cycle 1 (8 weeks), PEGPH20 was administered i.v. twice weekly 24 hours prior to Gem during weeks 1 to 4 and once weekly 2 to 24 hours prior to Gem during weeks 5 to 7, followed by 1 week off; Gem (1,000 mg/m^2 i.v.; Sun Pharmaceuticals) was administered once weekly during weeks 1 to 7 followed by 1 week off. Preclinical data demonstrate significant tumor depletion of HA and a 4-fold increase in microvessel luminal area within 24 hours after PEGPH20 treatment, which enhance the delivery of chemotherapy. Preclinical studies have also repeatedly demonstrated an increase in the intratumoral concentration of partner chemotherapies, as well as tumor growth inhibition and improved survival following PEGPH20 administration (8, 11).

For the remaining cycles, PEGPH20+Gem was administered once weekly for the first 3 weeks of each 4-week cycle. Dexamethasone was given orally, by intramuscular injection, or by i.v. injection, as dictated by physician and patient preference, 1 hour pre- and 8 to 12 hours after PEGPH20 administration. Treatment was discontinued for disease progression, intolerable side effects, or withdrawn consent. Patients returned for an end-of-study visit within 1 week of treatment discontinuation.

Assessments

Treatment-related adverse events (AE) were assessed using Common Terminology Criteria for Adverse Events (CTCAE) v4.0. A dose-limiting toxicity (DLT) was defined as any treatment-emergent AE (TEAE) ≥grade 3 occurring within the first 4 weeks of treatment considered to be PEGPH20 related. Any PEGPH20 treatment–related AE that resulted in drug interruption or reduction may have been considered a DLT at the Investigator's or Sponsor's discretion.

Response was assessed by CT scans performed at baseline and every 8 weeks during treatment and evaluated by an independent radiologist in a central imaging laboratory using RECIST v1.1. CA19-9 values were monitored at baseline and every 4 weeks, and the best CA19-9 response was defined as the maximum percent decrease from baseline.

The effects of PEGPH20 on vascular perfusion and metabolic activity were assessed in selected patients using Dynamic Contrast Enhancement-Magnetic Resonance Imaging (DCE-MRI; ref. 17) and [^{18}F]fluorodeoxyglucose positron emission tomography/CT scans (^{18}F-FDG-PET/CT), respectively, and evaluated by an

independent investigator at a central imaging laboratory. DCE-MRI studies were performed at baseline (predose), 8 and 24 hours postdose, and at the end of cycle 1 (EOC1) by measuring the mean and median exchange rate constant (K^{trans}) on target lesions >2 cm in diameter.

Baseline HA content in tumor biopsies was assessed in a CLIA-certified pathology laboratory using a validated histochemical staining method (10,18). HA levels were assigned an H-score calculated as the sum of the products of the percentage of positive staining areas and the staining intensity (0, 1, 2, or 3), with scores ranging from 0 to 300. For example: [90%×1 (weak)]+[10%×2 (moderate)]+[0%×3 (strong)]= H-score of 110. H-scores were generated for areas immediately surrounding the tumor epithelium (pericellular areas), as well as in stroma. Based on exploratory analyses of the distribution of HA surrounding tumor cells in PDA biopsies from this study, an H-score of ≥100 was defined as the provisional cutoff for high HA.

Statistical analysis

Patients exposed to any dose of PEGPH20 were included in the safety population. The DLT-evaluable population included patients enrolled during the dose-escalation portion of the study who received at least 6 of 8 planned doses of PEGPH20 and 3 of 4 doses of Gem in the first 4 weeks, or had a DLT in the first 4 weeks. The intent-to-treat (ITT) population included all enrolled patients and was used for baseline characteristics and safety evaluation. The efficacy-evaluable (EE) population included patients who received 1.6 or 3.0 μg/kg PEGPH20. Objective complete response (CR) or partial response (PR) percentages were assessed using RECIST v1.1 and summarized using descriptive statistics. OS and progression-free survival (PFS) of the EE population were analyzed using the Kaplan–Meier estimation method.

Results

Patient population

Twenty-eight patients were enrolled (United States, $n = 20$; Russia, $n = 8$) and evaluated for safety. Efficacy was evaluated in the 28 ITT patients, as well as the 24 EE patients who received PEGPH20 doses of 1.6 ($n = 4$) or 3.0 μg/kg ($n = 20$). Mean time-on-study for ITT patients was 3.1 months, and the median was 2.5 months (range, 0.03–11.0).

The median patient age was 59 years (range, 27–82) and median weight was 65 kg (range, 42.5–103.5; Table 1). Each dose group enrolled an equal number of males and females. Twelve patients (43%) had a Karnofsky performance status of 90% to 100% and 16 (57%) had a performance status of 70% to 80%. The median time from diagnosis to treatment was 3.4 months (range, 1.0–17.7). Eighty-nine percent (25/28) of patients had liver metastases at diagnosis, and 21% (6/28) had metastases in the lungs or lymph nodes. Seventy-nine percent (22/28) had baseline levels of CA19-9 ≥upper limit of normal (ULN: ≥59 U/mL). Twenty patients had a baseline biopsy assessed for HA content: 40% (8/20) had a high

Table 1. Patient demographic and disease characteristics (ITT population)

	PEGPH20 dose group (μg/kg)			All patients
	1.0 ($n = 4$)	1.6 ($n = 4$)	3.0 ($n = 20$)	$N = 28$
Age, y				
Median	48	55	63	59
Min/max	44.0/62.0	54.0/72.0	27.0/82.0	27.0/82.0
Gender, *n* (%)				
Female	2 (50.0)	2 (50.0)	10 (50.0)	14 (50.0)
Male	2 (50.0)	2 (50.0)	10 (50.0)	14 (50.0)
Weight, kg				
Median	69.6	64.3	65.0	65.0
Min/max	64.0/84.8	49.9/103.0	42.5/103.5	42.5/103.5
Karnofsky performance status (%)				
100	0 (0.0)	0 (0.0)	2 (10.0)	2 (7.1)
90	1 (25.0)	1 (25.0)	8 (40.0)	10 (35.7)
80	2 (50.0)	3 (75.0)	7 (35.0)	12 (42.9)
70	1 (25.0)	0 (0.0)	3 (15.0)	4 (14.3)
Time since stage IV PDA diagnosis (weeks)				
Median	3.8	5.9	2.8	3.4
Min/max	2.6/5.0	4.7/17.7	1.0/13.9	1.0/17.7
Site of metastasis (%)				
Abdomen/peritoneal	2 (50.0)	1 (25.0)	7 (35.0)	10 (35.7)
Liver	4 (100)	4 (100)	17 (85.0)	25 (89.3)
Lung	1 (25.0)	1 (25.0)	4 (20.0)	6 (21.4)
Lymph nodes	2 (50.0)	1 (25.0)	3 (15.0)	6 (21.4)
Other	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CA19-9 (%)				
Median	297.6	31152.9	2560	1139
Min/max	1.4/88300	1.4/64000	1.4/1000000	1.4/1000000
Normal (<59 U/mL)	1 (25.0)	2 (50.0)	3 (15.0)	6 (21.4)
Elevated (≥59 U/mL)	3 (75.0)	2 (50.0)	17 (85.0)	22 (78.6)
HA staining tumor (%)				
n	3	2	15	20
Median	200	180	40	50
Min/max	0.0/240.0	170.0/190.0	0.0/280.0	0.0/280.0
High: ≥ 100	2 (66.7)	2 (100)	4 (26.7)	8 (40.0)
Low: < 100	1 (33.3)	0 (0.0)	11 (73.3)	12 (60.0)

Abbreviations: CA, cancer antigen; HA, hyaluronan; max, maximum; Min, minimum.

Table 2. Adverse events (safety population)

		(*n* = 28)	
	Grade 1/2	**Grade 3/4/5**	**All grades**
Treatment-emergent AEs (≥25% in total incidence)[a]			
Number of patients with AE, *n* (%)	4 (14.3)	23 (82.1)	27 (96.4)
Edema peripheral	16 (57.1)	1 (3.6)	17 (60.7)
Muscle spasms	13 (46.4)	2 (7.1)	15 (53.6)
Fatigue	12 (42.9)	2 (7.1)	14 (50.0)
Thrombocytopenia	12 (42.9)	2 (7.1)	14 (50.0)
Myalgia	12 (42.9)	0 (0.0)	12 (42.9)
Anemia	4 (14.3)	6 (21.4)	10 (35.7)
Nausea	10 (35.7)	0 (0.0)	10 (35.7)
Decreased appetite	9 (32.1)	0 (0.0)	9 (32.1)
Arthralgia	8 (28.6)	0 (0.0)	8 (28.6)
Abdominal pain upper	6 (21.4)	1 (3.6)	7 (25.0)
Asthenia	6 (21.4)	1 (3.6)	7 (25.0)
Insomnia	7 (25.0)	0 (0.0)	7 (25.0)
Diarrhea	7 (25.0)	0 (0.0)	7 (25.0)
Vomiting	7 (25.0)	0 (0.0)	7 (25.0)
Thromboembolic TEAEs (all events)			
Basal ganglia infarction	1 (3.6)	0	1 (3.6)
Cerebrovascular accident	0	2 (7.1)	2 (7.1)
Deep vein thrombosis	2 (7.1)	0	2 (7.1)
Jugular vein thrombosis	1 (3.6)	0	1 (3.6)
Pulmonary embolism	2 (7.1)	3 (10.7)	5 (17.9)
Splenic infarction	0	1 (3.6)	1 (3.6)
Venous thrombosis	1 (3.6)	0	1 (3.6)
PEGPH20 treatment-related TEAEs (≥10% in total incidence)[a]			
Number of patients with AE, *n* (%)	17 (60.7)	7 (25.0)	24 (85.7)
Edema peripheral	7 (25.0)	1 (3.6)	8 (28.6)
Arthralgia	8 (28.6)	0 (0.0)	8 (28.6)
Fatigue	6 (21.4)	1 (3.6)	7 (25.0)
Pain in extremity	5 (17.9)	0 (0.0)	5 (17.9)
Asthenia	3 (10.7)	0 (0.0)	3 (10.7)
Muscle spasms	13 (46.4)	2 (7.1)	15 (53.6)
Myalgia	11 (39.3)	0 (0.0)	11 (39.3)

[a]AEs reported by worst grade.

HA score (provisional H-score cutoff ≥100) and 60% had a low-HA score (H-score <100). Additional demographic data and baseline characteristics are shown in Table 1.

Safety results

During the dose-escalation portion of the study, a total of 14 patients were treated with PEGPH20 at 1.0 ($n = 4$), 1.6 ($n = 4$), or 3.0 μg/kg ($n = 6$); an additional 14 patients were subsequently treated at 3.0 μg/kg during the dose-expansion portion. All patients were included in the safety analyses. The median PEGPH20 treatment duration was 1.2 (range, 0.1–3.1), 2.5 (range, 1.1–8.9), and 3.3 (range, 0.03–11.0) months for the 1.0, 1.6, and 3.0 μg/kg dose groups, respectively. Patients received a median 2.5 cycles of PEGPH20 (range, 1–11) and 3 cycles of gemcitabine (range, 1–11). The median duration of treatment was 2.49 months (range, 0.03–11).

No patient experienced a DLT. The recommended phase II dose for PEGPH20 when administered with Gem was therefore determined to be 3.0 μg/kg. Twenty-seven patients (96%) experienced ≥1 TEAE. The most common TEAEs (all causality) were peripheral edema (61%), muscle spasm (54%), fatigue (50%), and thrombocytopenia (50%). Other TEAEs occurring in more than 25% of patients were myalgia, anemia, nausea, decreased appetite, and arthralgia (Table 2). PEGPH20-related TEAEs were reported in 24 patients (86%). The most common PEGPH20-related TEAEs were musculoskeletal, including muscle spasm (54%), myalgia (39%), arthralgia (29%), and pain in an extremity (18%), peripheral edema (29%), and fatigue (25%). None of these events was serious or led to PEGPH20 discontinuation. A total of 13 thromboembolic events (TE) occurred in 8 patients (29%), not dissimilar to rates seen in other reports on this especially hypercoagulable malignancy (19,20). The TEs included pulmonary emboli ($n = 5$), deep vein thromboses or venous thromboses ($n = 4$), cerebrovascular accidents ($n = 2$), basal ganglia infarction ($n = 1$), and splenic infarction ($n = 1$). The events were generally managed with anticoagulation and/or aspirin. There were two cases of cerebrovascular accident, 1 resulting in death. Musculoskeletal AEs were predominantly grade 1/2 in severity. Grade 3 events (all causality) were reported in 3 patients (11%): 2 patients with muscle spasm (7%) and 1 with pain in an extremity (4%; Table 2).

Efficacy results

Response rate. Objective response was assessed per RECIST v1.1 by an independent radiologist. Partial responses (PRs) occurred in 0 of 4 patients at 1.0 μg/kg, 2 of 4 (50%) at 1.6 μg/kg, and 8 of 20 (40%) at 3.0 μg/kg. PRs were confirmed in 7 of the 10 patients by repeat imaging. No CRs were observed. The best overall confirmed response rates for ITT and EE patients were 25% and 29%, respectively. The overall disease control rate [CR+PR+stable disease (SD)] was 68% (95% CI, 48–84) for the ITT population and 75% (95% CI, 53–90) for the EE population (Table 3). Sixteen of the 22 patients with CA19-9 ≥59 U/mL at baseline had follow-up measurements; 38% (6/16) of these patients had a

Table 3. Overall response rate (ITT population)

Response by RECIST v1.1	PEGPH20 dose group (μg/kg)				All patients
	1.0 ($n = 4$)	1.6 ($n = 4$)	3.0 ($n = 20$)	1.6 + 3.0 ($n = 24$)	($N = 28$)
Complete response (CR), *n* (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Partial response (PR), *n* (%)	0 (0.0)	2 (50.0)	8 (40.0)	10 (41.7)	10 (35.7)
Stable disease (SD), *n* (%)	1 (25.0)	2 (50.0)	6 (30.0)	8 (33.3)	9 (32.1)
Progressive disease (PD), *n* (%)	3 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (10.7)
Unknown, *n* (%)	0 (0.0)	0 (0.0)	6 (30.0)	6 (25.0)	6 (21.4)
Disease control rate (CR + PR + SD)					
n (%)	1 (25.0)	4 (100)	14 (70.0)	18 (75.0)	19 (67.9)
95% CI	1%–81%	40%–100%	46%–88%	53%–90%	48%–84%

maximum >70% decrease from baseline, half of whom (3/16) had a confirmed PR (Supplementary Fig. S1).

Perfusion and metabolic imaging studies. DCE-MRI and ^{18}F-FDG-PET/CT imaging analyses were conducted on subsets of patients pre- and post-treatment. DCE-MRI analyses estimate blood flow in tumors as an indication of tumor perfusion (K^{trans}). Increases in K^{trans} occurred in 5 of 6 patients ranging from 16% to 547% at 24 hours after PEGPH20 administration compared with baseline (Supplementary Table S1 and Supplementary Fig. S2). ^{18}F-FDG-PET/CT imaging was performed to measure metabolic activity pre- and post-treatment in 5 patients, 4 of whom demonstrated decreases in tumor metabolism compared with baseline (Supplementary Table S1).

Progression-free survival and overall survival. Kaplan–Meier estimates of median PFS and OS were 3.5 (95% CI, 1.6–5.3) and 6.0 (95% CI, 4.0–11.5) months, respectively, for ITT patients ($n = 28$), and 5.0 (95% CI, 1.6–5.4) and 6.6 (95% CI, 4.0–12.1) months, respectively, for EE patients ($n = 24$). Exploratory response analyses classified by the tumor HA level were conducted in EE patients who had an archived tumor sample collected at baseline ($n = 17$). Tumor HA was evaluated histochemically and categorized as high or low based on a provisional cutoff H-score of ≥100 (Fig. 1A and B). Median PFS and OS were 7.2 months (95% CI, 5.2–9.0) and 13.0 months (95% CI, 6.9–19.0), respectively, for high HA patients; and 3.5 months (95% CI, 0.5–5.3) and 5.7 months (95% CI, 1.1–9.6), respectively, for low-HA patients (Fig. 1C and D). Confirmed PRs occurred in 4 of 6 (67%) patients with documented high HA tumors and 3 of 11 (27%) patients with documented low HA tumors. Median PFS and OS by dose level and HA level for individual patients are presented in Supplementary Table S2. These results suggest that high HA levels may be predictive of tumor response to PEGPH20 in combination with Gem, a possibility currently being formally addressed in ongoing studies.

Discussion

The present study was designed to evaluate the safety and efficacy of PEGPH20 in combination with gemcitabine chemotherapy in untreated stage IV PDA. We show here that a modified recombinant form of a natural product,

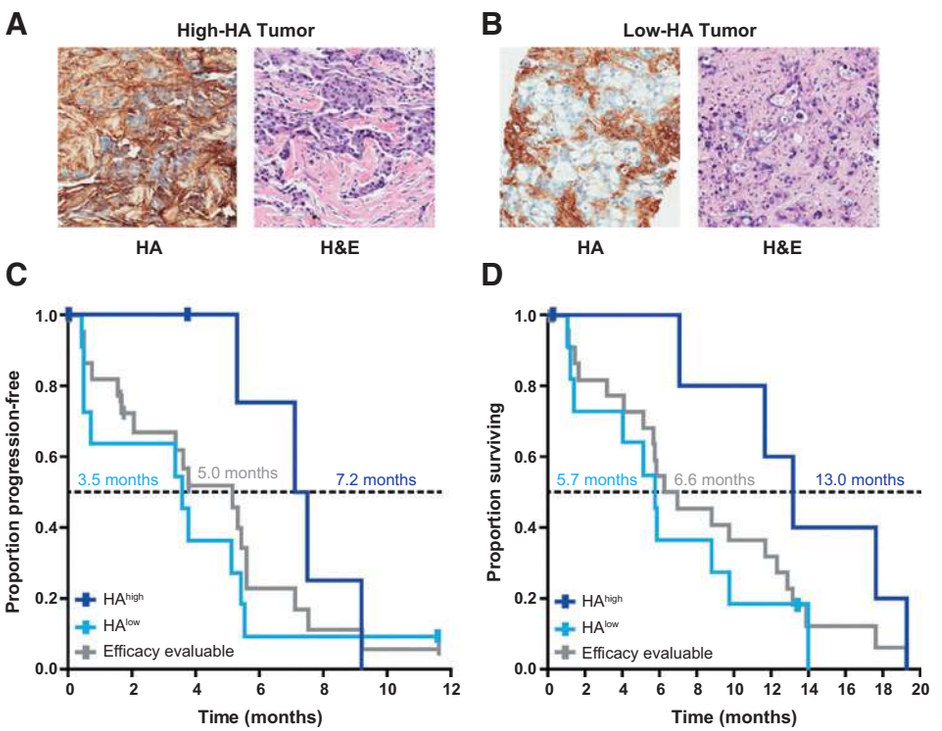

Figure 1.
Representative micrographs of tumor biopsies with high HA (A) and low HA (B) content. HA is detected with HA binding protein, and nuclei are counterstained with hematoxylin. Corresponding hematoxylin and eosin (H&E) stains are also shown. PFS (C) and OS (D) of patients who received either 1.6 or 3.0 μg/kg of PEGPH20+Gem. Gray line, all patients in the efficacy-evaluable population ($n = 24$); dark blue line, high HA ($n = 6$, 1 patient withdrew consent and was censored for PFS and OS, 1 patient withdrew consent and was censored for PFS); light blue line, low HA ($n = 11$, 1 patient who was censored for PFS and OS was withdrawn from study by investigator decision). Magnification, ×200.

hyaluronidase, can be safely administered systemically in combination with a conventional cytotoxic agent to improve efficacy. Of 28 patients enrolled, 10 had a best response of PR (36%); 7 of these PR were confirmed (25%) by RECIST v1.1. Notably, all patients achieving PR were treated with either 1.6 or 3.0 μg/kg PEGPH20. This overall response rate (ORR) compares favorably with that reported historically across a number of studies in patients treated with Gem alone (7%–13%). A recent Bayesian meta-analysis comparing nine treatment regimens suggested that FOLFIRINOX provided the best treatment response for advanced PDA (21). The FOLFIRINOX regimen achieved a 32% ORR in metastatic PDA; however, it was accompanied by a high toxicity rate, effectively limiting its use to patients with excellent performance status (5).

Kaplan–Meier estimates of median PFS and OS for EE patients in the current study were 5.0 and 6.6 months, respectively, and 21% (5/24) had PFS >5.5 months. However, when patients with an available biopsy (n= 17) were stratified for baseline tumor HA content, median PFS, and OS were longer in patients with high HA tumors (7.2 and 13.0 months, respectively) than those with low HA tumors (3.5 and 5.7 months, respectively). The PFS and OS in low HA patients were similar to the single-agent Gem arm in the NCIC CTG PA.3 trial of Gem+erlotinib and the MPACT trial of Gem+*nab*-paclitaxel (4,22). The ORR of 67% in high HA patients versus 29% in low HA patients is consistent with specific target ablation and suggests that high intratumoral HA levels may be predictive of response to PEGPH20+Gem.

Consistent with previous findings (15), exploratory imaging analyses in selected patients demonstrated a rapid increase in tumor perfusion by DCE-MRI 24 hours after systemic single-agent PEGPH20 treatment and a sustained reduction in maximum standardized uptake value (SUV_{max}) by ^{18}F-FDG PET/CT after PEGPH20+Gem treatment. We note that metastases, even when relatively small, appear to be able to recreate the unfavorable biophysics of the primary tumors (Supplementary Fig. S2), suggesting that metastatic disease may also be targeted to advantage with PEGPH20. These preliminary findings are consistent with our preclinical studies and support a mechanism of action in which depletion of intratumoral HA by PEGPH20 leads to increased penetration and antitumor activity of Gem.

The most common PEGPH20-related AEs were musculoskeletal and/or connective tissue symptoms. The majority of patients with PEGPH20-related musculoskeletal events reported more than 1 event. The most frequent musculoskeletal events occurring together were muscle spasms and myalgia in 8 patients and muscle spasms and arthralgia in 7 patients, consistent with the PEGPH20 safety profile established in two prior single-agent phase I clinical studies. None of these events was categorized as serious in the current study. The rationale for dexamethasone use to ameliorate musculoskeletal AEs was based upon preclinical studies in canines, which demonstrate similar sensitivities to PEGPH20. Complementary studies in nude mice have shown that dexamethasone does not interfere with the activity of PEGPH20 *in vivo*. In general, the Gem-related events were mild to moderate in severity (grade 1/2) and were typical of those previously seen with cytotoxic therapies. Thus, the frequency of AEs following treatment with PEGPH20+Gem was not higher than previously reported in studies of single-agent Gem (2,4). The rate was also similar to that previously seen in this disease setting, and there was one grade 5 cardiopulmonary arrest (CVA) attributed to metastatic pancreatic cancer. The second patient with a CVA had a prior history of basal ganglia infarction and came off study due to being medically unstable. Overall, the TE rate of 29% was perhaps higher than expected but within the range reported historically (17%–57%) for this notoriously hypercoagulable malignancy (23). The relatively small number of patients characteristic of a phase Ib trial also results in a wide confidence interval (11.8%, 45.3%). In a real-world analysis of TEs in PDA patients treated with chemotherapy, 11% experienced a TE within 3.5 months of treatment initiation (24). However, this analysis includes patients across all stages of disease and is therefore not directly comparable. A wide range of TE rates has been reported in studies specifically examining the role of thromboprophylaxis in patients receiving chemotherapy for advanced PDA. In the CONKO-004 trial, the cumulative incidence rate for TEs was 10.2%. In contrast, in a phase IIb a study of patients with advanced PDA, the TE rate was 23% in the first 100 days (20). Thus, the apparent increase in the TE rate observed in an ongoing randomized phase II study of PEGPH20+*nab*-paclitaxel+Gem (and now ameliorated with low-molecular-weight heparin) was not readily apparent in this small phase Ib study.

In conclusion, treatment of metastatic PDA with PEGPH20 at a dose of 3.0 μg/kg with gemcitabine is well tolerated with manageable musculoskeletal AEs. The safety profile together with the promising preliminary efficacy results strongly support further exploration of this novel strategy of targeting HA in the tumor microenvironment with PEGPH20 in combination with other chemotherapeutics. These data warrant follow-up randomized studies combining PEGPH20 with other agents in larger cohorts of patients with advanced PDA. Indeed, two national, randomized phase II trials are currently under way in previously untreated stage IV PDA evaluating *nab*-paclitaxel plus gemcitabine (NCT01839487) or FOLFIRINOX (SWOG S1313; NCT01959139) with and without systemic PEGPH20. These trials should further illuminate the efficacy and tolerability of this strategy for overcoming a principal therapeutic barrier in PDA.

Disclosure of Potential Conflicts of Interest

S.R. Hingorani is a consultant/advisory board member for Halozyme Therapeutics. W.P. Harris reports receiving a commercial research grant from Halozyme. S. Tjulandin reports receiving speakers bureau honoraria from AstraZeneca and Pfizer. R. Korn has ownership interest (including patents) in Imaging Endpoints. H.M. Shepard reports receiving a commercial research grant from Halozyme and has ownership interest (including patents) in Halozyme. No potential conflicts of interest were disclosed by the other authors.

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