

A Phase I-II Study Using Rexin-G Tumor-Targeted Retrovector Encoding a Dominant-Negative Cyclin G1 Inhibitor for Advanced Pancreatic Cancer

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Rexin-G is a replication-incompetent retroviral vector displaying a cryptic SIG-binding peptide for targeting abnormal Signature (SIG) proteins in tumors and encoding a dominant-negative human cyclin G1 construct. Herein we report on the safety and antitumor activity of escalating doses of Rexin-G in gemcitabine-refractory pancreatic adenocarcinoma, with one 10-year survivor. For the safety analysis (n = 20), treatment-related grade 1 adverse events included fatigue (n = 6), chills (n = 2), and headache (n = 1), with no organ damage and no DLT. No patient tested positive for vector-neutralizing antibodies, antibodies to gp70, replication-competent retrovirus (RCR), or vector integration into genomic DNA of peripheral blood lymphocytes (PBLs). For the efficacy analysis (n = 15), one patient achieved a complete response (CR), two patients had a partial response (PR), and 12 had stable disease (SD). Median progression-free survival (PFS) was 2.7, 4.0, and 5.6 months at doses 0-I, II, and III, respectively. Median overall survival (OS) and 1-year OS rate at dose 0-I were 4.3 months and 0%, and at dose II-III they were 9.2 months and 33.3%. To date, one patient is still alive with no evidence of cancer 10 years after the start of Rexin-G treatment. Taken together, these data suggest that Rexin-G, the first targeted gene delivery system, is uniquely safe and exhibits significant antitumor activity, for which the FDA granted fast-track designation.

INTRODUCTION

Pancreatic adenocarcinoma (PDAC) is projected to become the second leading cause of cancer death in the United States and is rising worldwide.^{1,2} For patients with advanced disease, first-^{3,4} and second-line⁵ treatment options improve survival modestly, but they are not curative. Unfortunately, there have been few successful targeted therapy options,⁶ in part because the most common mutations (KRAS and TP53) have not been targetable, and others are uncommon (BRCA1, BRCA2, and MSI). As with most cancers, genetic dysfunction of the normal cell division cycle and its checkpoint control elements is critical to progression of PDAC;⁷ therefore, targeting the executive elements of cell cycle checkpoint control represents a promising strategy.⁸

Rexin-G (former names: Mx-dnG1) is the first targeted injectable vector to be approved for clinical trials in the treatment of metastatic cancers.⁹ Rexin-G (Figure 1) is a non-replicative MLV-based amphotropic retrovector displaying a cryptic collagen-binding motif on its gp70 surface membrane for targeting abnormal Signature (SIG) proteins in the tumor microenvironment (TME)¹⁰ and encoding a dominant-negative mutant construct (dnG1) of human cyclin G1 (CCNG1).¹¹ The vector also contains a neomycin resistance (neo^r) gene, which is driven by the SV40 early promoter. When injected intravenously, Rexin-G seeks out and accumulates in cancerous lesions by binding to exposed abnormal collagenous SIG proteins deposited as a result of tumor invasion, tumor-associated angiogenesis, and stroma formation, elevating the vector concentration in the TME in the vicinity of cancer cells. Upon gaining entry into the rapidly proliferating cells, within the TME,¹² Rexin-G produces a cytotoxic dnG1 protein that effectively blocks a pivotal checkpoint of the cell division cycle, resulting in apoptosis and, thus, the elimination of cancer cells, proliferative tumor vasculature, and associated malignant fibroblasts.^{13,14}

Based on encouraging clinical data from the Philippines in patients with metastatic PDAC,^{15,16} clinical trials began in the United States using Rexin-G for standard chemotherapy-resistant PDAC, sarcoma, osteosarcoma, and breast cancer.¹⁷⁻¹⁹ In this report, we provide updated results, along with new mechanistic and pharmacological insights, from an advanced phase I-II study evaluating overall safety and potential antitumor activity of intravenous infusions of Rexin-G in metastatic gemcitabine-resistant PDAC.

RESULTS

Patients and Treatment

This phase I-II trial enrolled 20 patients with metastatic gemcitabine-refractory PDAC. Table 1 shows the patient demographics. The

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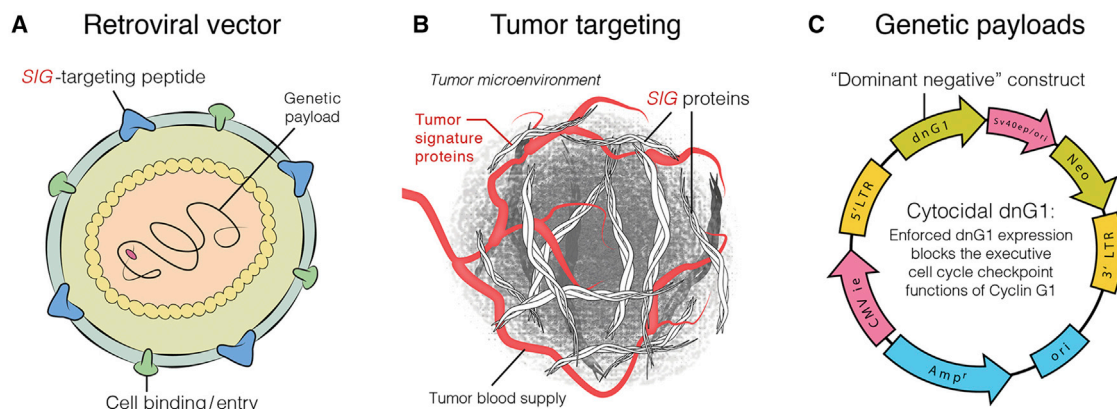


Figure 1. Graphic Illustration of Rixin-G Vector

The Rixin-G vector displaying a S/G-targeting peptide (A), for binding to Signature (S/G) proteins in the tumor microenvironment (TME) (B), and encoding a dominant-negative human cyclin G1 inhibitor gene (C). Injected intravenously, Rixin-G nanoparticles seek out and bind to abnormal S/G proteins in the TME, which augments effective vector concentration in tumors.

patients had failed a median of two regimens, one of which contained gemcitabine. All patients exhibited metastatic disease. Two patients had one target lesion, and 17 patients had 2–7 target lesions in the pancreas, lymph node, omentum, mesentery, adrenal, bone, lung, and the liver in 16 patients (Table 2). Aside from the target lesions in Table 2, all patients had many non-target lesions, malignant ascites, pleural effusion, and peritoneal carcinomatosis. Therefore, target lesions alone do not reflect the patients' total tumor burden.

Dose Escalations

Six patients were treated at dose levels 0–I; 7 were treated at dose level II; and 7 were treated at dose level III. One patient was included in the dose II cohort because he received an (FDA-approved) inpatient dose escalation from dose 0 to dose II. The number of Rixin-G infusions, the number of completed cycles of Rixin-G, and the total exposure (colony-forming units [CFU]) to Rixin-G are summarized by dosage group in Table 3.

The median number of infusions varied from 9 in dose group 0–I to 52 in dose group III. A total of 832 infusions were administered for all patients. The total number of completed infusion cycles varied from 5 in dose group 0–I to 31 in dose group III. The median cumulative dose of Rixin-G increased from 9×10^{11} CFU in dose group 0–I to 60×10^{11} CFU in dose group II to 156×10^{11} CFU in dose group III. Total exposure to Rixin-G for all patients was $1,927 \times 10^{11}$ CFU, with a range from 30 to 453×10^{11} CFU.

Safety Analysis

There were no dose-limiting toxicities observed at any dose level. Unrelated adverse events were reported for all 20 patients. Related but clinically non-significant adverse events occurred in 7 patients, and all were grade 1 (Table 4). These consisted of chills (1 patient), fatigue (2 patients), and headache (1 patient) at dose level II and fatigue (4 patients) at dose level III. There was no treatment-related loss of hair; nausea; vomiting; anemia; thrombocytopenia; neutrope-

nia; or liver, lung, or kidney dysfunction reported. There were no serious drug-related adverse events (AEs).

The most frequent clinically non-significant unrelated grade 3 AEs were hypoalbuminemia (4 patients) and increased alanine aminotransferase (3 patients). Anemia, hyperglycemia, increased aspartate aminotransferase, and hypocalcemia were reported in 2 patients each. Other clinically non-significant unrelated grade 3 AEs were reported in 1 patient each. Several types of unrelated AEs appeared to be more frequent at higher doses: anemia, hyperbilirubinemia, increased aspartate aminotransferase, and decreased appetite (Table 5).

13 patients experienced 25 serious AEs, all of which were deemed not related to the study drug. Details regarding these AEs are provided in Table 6.

No patient tested positive for any of the following: vector-neutralizing antibodies, antibodies to gp70, replication-competent retrovirus in peripheral blood lymphocytes (PBLs), or vector integration into genomic DNA of PBLs.

To date, 19 of the 20 patients enrolled in the study have died. None of the deaths were considered related to Rixin-G. The cause of death was progressive disease in all but one patient where the cause of death was sepsis. Remarkably, the long-term survivor exhibited lymphatic metastasis prior to intravenous Rixin-G infusions as salvage therapy.

Efficacy Analysis

Of the 20 enrolled patients, 15 received at least one complete cycle (4 weeks) of treatment and had a follow-up positron emission tomography-computed tomography (PET-CT) scan, and, therefore, they were considered evaluable for efficacy (modified intent-to-treat [mITT] population) in terms of response, progression-free survival, and overall survival. In the first cohort (dose level I), three patients were withdrawn from the study prior to completion of one treatment

Table 1. Patient Demographics (N = 20)

Patient Characteristics	Number, range, or n (% of N)
Age	
Median	62
Range	50–83
Gender	
Female	12 (60%)
Male	8 (40%)
Race	
White	16 (80%)
Asian	4 (20%)
Disease stage	
Metastatic (1–7 target lesions + non-target lesions, ascites, pleural effusion, peritoneal carcinomatosis)	20 (100%)
Performance score (ECOG)	
1	20 (100%)
Number of previous chemotherapy regimens	
Median	2
Range	1–7

cycle either due to disease-related complications (n = 1; worsening malignant pleural effusion) or due to a personal decision to discontinue treatment (n = 2; one patient had worsening ascites and the other decided to take an alternative medicine). In the second cohort (dose level II), one patient had worsening ascites and clinical deterioration, and in the third cohort (dose level III), one patient had worsening malignant pleural effusion.

Table 7 shows the evaluation of tumor response using Response Evaluation Criteria in Solid Tumors (RECIST) version (v.)1.0, Choi,²⁰ and modified international PET criteria in the mITT population. In the overall cohort, the median tumor burden was 32.6×10^9 cells; the range in tumor burden was wide across patients, with a minimum of 5.0×10^9 cells and a maximum of 115.5×10^9 cells. Notably, patients at dose level III had significantly larger tumor loads (52.1×10^9 cancer cells) than those in dose group 0–I or II (32.6×10^9 and 31.5×10^9 cancer cells, respectively). Patients were assigned to dose levels on a first-come first-served basis. No significant relationship was noted between estimated tumor burden and response, progression-free survival (PFS), or overall survival (OS).

By RECIST, one patient achieved a complete response (CR), two patients had a partial response (PR), and 12 had stable disease (SD). The tumor control rate (CR + PR + SD) by RECIST v.1.0 was 100% (15/15 patients). Responses were more frequent when assessed using modified international PET criteria or Choi criteria. By PET, one patient achieved a CR, 4 patients had a PR, and 10 patients had SD. By Choi, one patient had a CR, 5 had a PR, and 8 had SD. One patient

did not have a Choi analysis because the lesions were too small. By RECIST, PRs and CRs occurred only at dose levels II and III, suggesting a dose-dependent relationship between Regin-G dose and response. PFS by RECIST was 2.7, 4.0, and 5.6 months at dose levels 0–I, II, and III, respectively, suggesting a dose-dependent relationship between Regin-G dose and PFS. Kaplan-Meier analysis of PFS (**Figure 2**) in the mITT group suggested a trend toward a dose-response relationship between PFS and Regin-G dosage. It is important to note that a higher tumor burden was observed for patients in doses II and III compared with doses 0–I, providing evidence in support of Regin-G's antitumor activity.

Table 8 shows three patients with durable tumor response patterns when assessed by RECIST, Choi, and PET (patients 12, 16, and 18). Patient 12-CJP is a 56-year-old white female with status post (s/p) biliary stent placement and radiation therapy for poorly differentiated PDAC, who failed gemcitabine and had target lesions at the pancreatic head medial to the biliary stent and right liver lobe. She achieved a best response of PR by RECIST at week 4 and continued in PR through week 36 (**Figure 3A**). She had definitive disease progression at week 60. Patient 016-JLM is a 59-year-old white female with poorly differentiated PDAC, who had failed four chemotherapy regimens, including gemcitabine, 5FU, oxaliplatin, and capecitabine, with target lesions at the head of pancreas and an aortocaval mass by the right hepatic lobe. She achieved a best response of PR by RECIST at weeks 4, 6, 12, 24, and 36 (**Figure 3B**). She discontinued treatment with Regin-G on week 42 due to an AE (bile duct obstruction). She withdrew from the study due to symptomatic progression without a confirmatory CT scan.

Patient 18 is a white female (72 years of age at study entry) who had been initially diagnosed with non-metastatic, poorly differentiated adenocarcinoma of the pancreas; she underwent a Whipple's resection with postoperative radiation therapy, and she received chemotherapy with 5FU and gemcitabine for 1 year. A year later, she presented with hepatic and lymph node metastases (target lesions) and mesenteric stranding indicative of peritoneal carcinomatosis (non-target lesions) with a rising serum CA19.9 level. She was advised to receive further chemotherapy, but she decided in favor of participating in the phase I-II study using Regin-G. The patient completed a total of 17.9 months of therapy with Regin-G, and she did not achieve CR until week 36 of treatment; this patient has remained in CR at the time of study completion. Notably, when examined separately, the two target lesions were found to have different disappearance profiles. As shown in **Figure 4A**, the lymph node metastasis decreased more rapidly, starting at week 6, than the hepatic metastasis, which increased in size before ultimately completely resolving in week 36. Serum level of the tumor marker CA-19.9 decreased by 45%, from 76 to 42 U/mL (normal level is <37 U/mL) by week 19, and then it remained relatively constant thereafter (**Figure 4B**). She received no additional chemotherapy or alternative treatment after the discontinuation of Regin-G therapy, and she remains in sustained remission with no evidence of disease or late-onset AEs as of November 2018.

Table 2. Locations and Sizes of Lesions in 20 Patients with Metastatic PDAC

Subject ID	Location of Target Lesions (Size, Longest Diameter, mm)
1	left periumbilical (85)
2	preaortic node (40)
	right lobe liver, post aspect (27)
	pancreas (20)
	celiac node (11)
3	right lobe liver, upper anterior aspect (23)
	body of pancreas (22)
	anterior right lower lobe, liver (17)
	anterior right lobe liver, porta hepatis (18)
	posterior to porta hepatis (22)
4	inferior tip of right liver lobe (11)
	left liver lobe, lateral tip (32)
	left liver lobe (36)
	mid-right liver lobe (17)
5	body of pancreas (10)
	left lobe liver at splenic hilum (76)
	right lobe liver, lateral dome (22)
	liver, segment 4A (58)
	anterior omentum (42)
6	left lung, hilar (27)
	right pre-tracheal node (10)
	perihilar right ML, lung (10)
	left lobe liver, lateral segment (35)
	posterior right lobe, liver (41)
	left lobe liver, lateral segment (29)
7	lateral left lobe liver (26)
	pancreatic tail (22)
	left adrenal apex (11)
	right hepatic lobe, posterior (88)
	right hepatic lobe, upper portion (45)
8	right liver lobe posterior aspect (24)
	left liver lobe (55)
	anterior to left kidney (11)
	slightly lower to S1 (19)
	level of S1 first lesion (12)
9	level of S1 second lesion (14)
	lateral tip left liver lobe (14)
	inferior anterior margin, left liver lobe (16)
	right anterior subcapsular (23)
10	right liver lobe dome (19)
	left liver lobe anterior superficial subcapsular (10)
	RLL lung posterior subpleural upper (12)
	RLL lung posterior subpleural lower (15)

Table 2. Continued

Subject ID	Location of Target Lesions (Size, Longest Diameter, mm)
11	anterior segment 5, liver (64)
	segment 4 liver (48)
	pancreatic body, preaortic (52)
12	right liver lobe, posterior (21)
	head of pancreas medial to biliary stent (32)
13	tail of pancreas (66)
	left adrenal (10)
14	mid segment 4, liver (40)
	dome right hepatic lobe (20)
	lateral tip left hepatic lobe (18)
	head of pancreas (30)
15	nodule anterior abdominal wall to right of midline at T12 (12)
	head and body of pancreas (67)
	mesenteric mass slightly left of midline (19)
16	circular lesion posterior to porta hepatis (34)
	right hepatic lobe anterior and superior to gall bladder (36)
17	head of pancreas (90)
	aortocaval mass at right hepatic lobe (20)
18	pancreatic head (28)
	subhepatic area below anastomosis (28)
19	subperitoneal lymph node (22)
	right posterior sulcus lung (15)
	lateral left lower lobe lung (14)
B01	medial left lower lobe lung (21)
	right of hepatic art. (13)
	left periaortic lymph node (15)
	left adrenal nodule (11)

ML, middle lobe; RLL, right lower lobe; art., artery.

Of note, continuation of treatment contributed to efficacy or clinical benefit in at least five patients. These patients survived from 10.6 months to 10 years after starting Regin-G. Antitumor effects differed for individual target lesions in some patients. These data suggest that patients may benefit from extended treatment with Regin-G despite signs of apparent progression (pseudoprogression), which may result from the known mechanism of action of Regin-G: induction of apoptosis via cell cycle blockade of cancer cells, tumor vasculature, and malignant tumor-associated fibroblasts without bone marrow suppression, which may initially cause lesions to appear larger due to inflammatory or immunologic responses seen in published reports.^{20–22}

Median OS in the mITT group was calculated to be 4.3 months at dose 0–I, 9.2 months at dose II, and 9.2 months at dose III. The OS estimates in the efficacy evaluable mITT population among the combined groups of dose levels 0–I was 0% at 1 year. In contrast, OS estimates in the combined groups dose levels II–III were 33.3% at 1 year

Table 3. Total Exposure to Regin-G in 20 Patients with Metastatic PDAC

Parameter	Dose Group for Analysis			
	0-I(N = 6)	II(N = 7)	III(N = 7)	All(N = 20)
Number of all infusions				
Total for all patients	72	356	404	832
Median (minimum, maximum) per patient	9 (5, 23)	30 (11, 157)	52 (10, 151)	24 (5, 157)
Number of all completed cycles				
Total for all patients	5	27	31	63
Median (minimum, maximum) per patient	2 (1, 2)	3 (1, 13)	4 (1, 12)	3 (1, 13)
CFU of Regin-G				
Total ($\times 10^{11}$ CFU)	73	676	1212	1961
Median (minimum, maximum) ($\times 10^{11}$ CFU) per patient	9 (5, 24)	60 (22, 314)	156 (30, 453)	49 (5, 453)

and 25% at 1.5 years. The median OS in the ITT group was 2.6 months in the dose 0–I cohort versus 9.0 and 7.8 months in the dose II and III cohorts, respectively. The OS rates in the dose 0–I group was 0% at 1 year. In contrast, OS rates among the combined groups of dose II–III were 28.6% at 1 year and 21.4% at 1.5 years ($p = 0.03$) compared with dose 0–I. Kaplan-Meier analysis of OS in the ITT population suggests a dose-response relationship between OS and Regin-G dosage ($p = 0.03$; Figure 5).

Using the one-sided Fisher's test, we compared tumor control responses (TCRs by RECIST) in this advanced phase I-II study ($n = 15$; TCR 1 CR, 2 PR, and 12 SD) with those in the prior phase I study where patients received up to a total dose of 6×10^{11} CFU per cycle (TCR 1 SD and 11 progressive disease [PD]).¹⁴ With "tumor control response" designated as CR, PR, or SD at any given time during the Regin-G treatment period, the proportions are 15/15 for the current study and 1/12 in the prior study, with $p < 0.0001$ by the one-sided Fisher's test. These data indicate a dose-response relationship between TCR and Regin-G dosage across studies.

DISCUSSION

This report updates and extends a phase I-II study of safety and efficacy using Regin-G in gemcitabine-refractory PDAC with additional analysis and new mechanistic insights. The initial clinical data were previously reported on 13 patients by Chawla et al.¹⁹ Safety was established with no dose limiting toxicity (DLT) following multiple Regin-G infusions at all four dose levels, and the maximum tolerated dose (MTD) was not reached. It is important to note that there was no treatment-related loss of hair, bone marrow suppression, or organ dysfunction at all dose levels. The serious AEs experienced by these patients were due to disease-related complications and not related to Regin-G treatment, as assessed by the principal investigators. Further, there were no vector-related safety issues raised, as evidenced

Table 4. Clinically Non-significant Drug-Related Adverse Events by Dose Level and Toxicity Grade (n = 20)

MedRA System Organ Class	Preferred Term	Dose Level	Toxicity Grade
General disorders and administration site conditions	fatigue	2	1
General disorders and administration site conditions	fatigue	2	1
Musculoskeletal and connective tissue disorders	chills	2	1
Nervous system disorders	headache	2	1
General disorders and administration site conditions	fatigue	3	1
General disorders and administration site conditions	fatigue	3	1
General disorders and administration site conditions	fatigue	3	1
General disorders and administration site conditions	fatigue	3	1

by no detected anti-vector-neutralizing antibodies, antibodies to gp70, replication-competent retrovirus in PBLs, or vector integration into genomic DNA of PBLs. These data indicate the exceptional safety of Regin-G when compared to FDA-approved therapies such as nab-paclitaxel, gemcitabine, FOLFIRINOX, and erlotinib.^{3,4,6,23–32}

Regarding efficacy, we report durable response rates (3/15; 20%) lasting 36–72 weeks during Regin-G treatment. Two patients were progression-free for more than 1 year: patient 12 had a PFS of 13.8 months; patient 18 had a PFS >17.9 months. The best overall response rates (20%) noted in this study were significantly better than those reported by Galanis et al.,¹⁷ which used much lower Regin-G doses. In support of this observation, a significant dose-response relationship was shown between OS and Regin-G dose in the intent-to-treat population. Remarkably, one patient is still alive 10 years later with no evidence of PDAC; the documented eradication of cancer within the lymphatic system has compelling implications that warrant additional studies of the anaplastic *Signature (SIG) proteins* involved.³³

Other viral gene therapy approaches for PDAC include an ongoing phase 1 trial combining oncolytic adenovirus-mediated cytotoxic and interleukin (IL)-12 gene therapy with chemotherapy in metastatic PDAC (<https://www.clinicaltrials.gov/>) and a recently completed phase III randomized, controlled clinical trial of PANVAC-VF for the treatment of patients with advanced pancreatic cancer. PANVAC-VF is a vaccine regimen composed of a priming dose of recombinant vaccinia virus and booster doses of recombinant fowl pox virus expressing carcinoembryonic antigen, mucin-1, and a triad of costimulatory molecules (TRICOM), given subcutaneously, followed by injection of recombinant granulocyte-macrophage colony-stimulating factor at the vaccination site.^{34,35} However, the phase III randomized trial did not meet its primary endpoint of improving OS when compared with the physician's choice of palliative therapy.³⁶

Table 5. Clinically Non-significant, Unrelated, Grade 3 Adverse Events Reported in ≥ 2 Patients by Regin-G Dose Level

MedRA System Organ Class/Preferred Term	Dose Level					Total
	N = 6		N = 7		N = 7	
	0	I	II	III		
	N = 2	N = 4	N = 7	N = 7	N = 20	
Blood and lymphatic system disorders						
Anemia	1			1	2	
Endocrine disorders						
Hyperglycemia			1	1	2	
Hepatobiliary disorders						
Hypoalbuminemia		1		3	4	
Investigations						
Alanine aminotransferase increased	1		1	1	3	
Aspartate aminotransferase increased	1	1			2	
Blood alkaline phosphatase increased				2	2	
Metabolism and nutrition disorders						
Hypocalcemia	1		1		2	

Both viral gene therapies for PDAC involve either intratumor or subcutaneous viral vector injections. In contrast, Regin-G involves a systemically (intravenously) administered tumor-targeted gene delivery approach (Figure 1).^{10,13,14,18,19,22,37,38}

Regin-G is a potent inhibitor of the human cyclin G1 pathway (CCNG1 proto-oncogene). CCNG1 gene expression plays a powerful executive role in cell cycle regulation, exerting significant influence on critical oncogenic drivers, including the potent Mdm2 and cMyc oncoproteins and the p53 tumor suppressor protein, gatekeeper of DNA fidelity.³⁸ CCNG1 is overexpressed in over 50% of various malignancies, including pancreas, breast, prostate, ovarian, and colon cancer.³⁹ Albeit a small study in patient number, the single-agent antitumor activity of Regin-G in metastatic PDAC is evident. In addition to the single-agent efficacy observed in the oncology clinic, molecular mechanisms were histologically revealed, as repeated intravenous infusion of Regin-G induced the apoptosis of cancer cells, stromal fibroblasts, and associated tumor vasculature in biopsied tumors of Regin-G-treated patients.^{12,19,40} Conceivably, patients whose tumors overexpress CCNG1, revealing a pathological distortion in growth control pathways, will respond favorably to cyclin G1 inhibitor therapy, delivered precisely. Studies currently in progress aim to confirm that the Regin-G-induced tumor eradication observed histologically by enforced apoptosis of cancer cells, as well as supportive neo-vasculature and associated malignant fibroblasts of the TME, is the executive mechanism of Regin-G anticancer activity. Moreover, the demonstrated eradication of refractory, chemoresistant pancreatic cancer (that is, progressive eradication upon continued intravenous infusions) is certainly noteworthy and potentially important—prompting us to closely examine the anaplastic *Signature* (Sig) *proteins* with an aim toward further optimizing these pioneering aspects of tumor-targeted gene delivery in future investigations.³³

Table 6. Serious Unrelated Adverse Event Listings by Dose Level and Toxicity Grade

Patient	System Organ Class	MedDRA Preferred Term	Dose Level	Toxicity Grade
1	gastrointestinal disorders	obstruction gastric	0	3
	gastrointestinal disorders	intestinal perforation	0	3
2	neoplasms: benign, malignant, and unspecified	malignant pleural effusion	0	3
3	infection and infestations	sepsis	1	3
4	injury, poisoning, and procedural complications	overdose	1	3
5	gastrointestinal disorders	abdominal pain	1	3
6	vascular disorders	pulmonary embolism	2	3
7	metabolism and nutrition disorders	dehydration	2	3
	blood and lymphatic system disorders	thrombocytopenia	2	4
8	metabolism and nutrition disorders	hyponatremia	2	4
	gastrointestinal disorders	obstruction gastric	2	3
9	gastrointestinal disorders	obstruction gastric	2	3
	vascular disorders	pulmonary embolism	3	3
10	infection and infestations	sepsis	3	3
	gastrointestinal disorders	fecaloma	3	3
	gastrointestinal disorders	constipation	3	3
	metabolism and nutrition disorders	hyponatremia	3	4
11	endocrine disorders	hyperglycemia	3	4
	hepatobiliary disorders	cholangitis	3	3
12	psychiatric disorders	mental status changes	3	3
	gastrointestinal disorders	ascites	3	3
	nervous system disorders	altered state of consciousness	3	3
	respiratory, thoracic, and mediastinal disorders	pneumothorax	3	3
13	psychiatric disorders	delirium	3	3
	metabolism and nutrition disorders	dehydration	3	3
	investigations	aspartate aminotransferase increased	3	4

In conclusion, the clinical data gleaned from this phase I-II study of precision, tumor-targeted genetic medicine suggest that (1) Regin-G is exceptionally safe with a wide margin of safety, and (2) Regin-G exhibits dose-dependent antitumor activity in patients with gemcitabine-refractory metastatic PDAC. Based on the analysis of clinical data, Regin-G gained fast-track designation from the FDA for the conduct of a planned phase II-III study using the optimal dose level III treatment schedule of Regin-G versus physician's choice in a larger number of patients. This planned phase II-III study will include correlations of CCNG1 gene expression in tumors, along with pertinent companion diagnostics, histology, and treatment outcome parameters.

Table 7. Summary of Responses, Tumor Burden, Progression-Free Survival, and Overall Survival

Category	Dose Level ^a			
	0-I	II	III	All
mITT Patients ^b	N = 3	N = 6	N = 6	N = 15
Median tumor burden (number × 10e9 cancer cells)	13.5	37.1	38.8	ND
Best response				
RECIST v.1.0 (n = 15)	3 SD	1 PR, 5 SD	1 CR, 1 PR, 4 SD	1 CR, 2 PR, 12 SD
PET (n = 15)	1 PR, 2 SD	1 PR, 5 SD	1 CR, 2 PR, 3 SD	1 CR, 4 PR, 10 SD
Choi (n = 14)	1 PR, 2 SD	2 PR, 4 SD	1 CR, 3 PR, 1 SD	1 CR, 5 PR, 8 SD
Median PFS (month)				
RECIST	2.7	4.0	5.6	ND
PET	2.7	4.1	4.2	ND
Choi	2.7	4.1	6.9	ND
Median OS (month)				
RECIST	4.3	9.2	9.2	ND
OS (%)				
1 year	0	33.3		
1.5 years		25.0		
ITT population ^c	N = 6	N = 7	N = 7	N = 20
Median OS (month)	2.6	9.0	7.8	ND
OS (%)				
1 year	0	28.6		ND
1.5 years	0	21.4		ND
Number Alive ^d				
	0	0	1 10-year survivor in remission	1 10-year survivor in remission

ITT, intent-to-treat; mITT, modified ITT; RECIST v.1.0, Response Evaluation Criteria in Solid Tumors; PET, positron emission tomography; Choi, modified RECIST as described by Choi et al.;²⁰ PFS, progression-free survival; OS, overall survival; ND, not determined; BIW, two times a week; TIW, three times a week.
^aDose level 0 = 1×10^{11} CFU BIW, dose level I = 1×10^{11} CFU TIW, dose level II = 2×10^{11} CFU TIW, dose level III = 3×10^{11} CFU TIW.
^bmITT population defined as all patients who received at least one cycle and had a follow-up PET CT scan.
^cITT population defined as all patients who received at least one infusion of Rixin-G.
^dAs of December 1, 2018.

MATERIALS AND METHODS

Study Drug and FDA-Approved Vector Production

Rixin-G (Figure 1) is a non-replicative murine leukemia virus (MLV)-based amphotropic retrovector displaying a cryptic collagen-binding motif on its gp70 surface membrane, for targeting abnormal *Signature (SIG) proteins* in the TME,^{10,33} and encoding a dominant-negative mutant construct (dnG1) of CCNG1.¹¹ The vector also contains a *neo^r* gene, which is driven by the SV40 early promoter. The Rixin-G vector is produced by transient co-transfection of HEK293T cells. Clinical vector production and characterization have been described elsewhere.^{15–17} The final product exhibits a vector titer of 5×10^9 CFU/mL, a biologic potency of 50%–70% growth inhibitory activity in target cancer cells, less than 550 bp residual DNA, no detectable E1A or SV40 large T antigen, and no detectable replication-competent retrovirus (RCR), in compliance with FDA recommendations for retroviral vector-based gene therapy products.⁴¹ The vector formulation is stored in aliquots of 23 mL in a 30-mL glass vial or 40 mL in 150 mL cryobag and kept frozen at -70° to -90°C until used. Preparation of the Rixin-G vector for patient administration consisted of rapid thawing of the vector in

the vial or cryobag in a 34°C water bath. The vector was thawed 15–30 min prior to infusion into the patient and given intravenously at 4 mL/min.^{18,19} All personnel who handled and disposed of the vector observed Biosafety level 2 compliance in accordance with the NIH Guidelines for Research Involving Recombinant DNA molecules.

Vector-Related Testing and Biodistribution Studies

Detection of anti-vector antibodies in serum, testing for the presence of RCR, and vector DNA integration studies in patient PBLs were performed as described previously.¹⁷

Study Design

This was an open-label, single-arm, dose-seeking study that incorporated a modification of the standard Cohort of 3 design, which allowed patients to continue the study drug into phase II.^{18,19} Treatment with Rixin-G comprised 6-week cycles that encompassed 4 weeks of treatment, followed by 2 weeks of rest. Four dose levels were given, beginning at 1.0×10^{11} CFU given by intravenous (i.v.) infusion two times per week. Three patients were to be treated at each dose level with expansion to 6 patients per cohort if DLT was

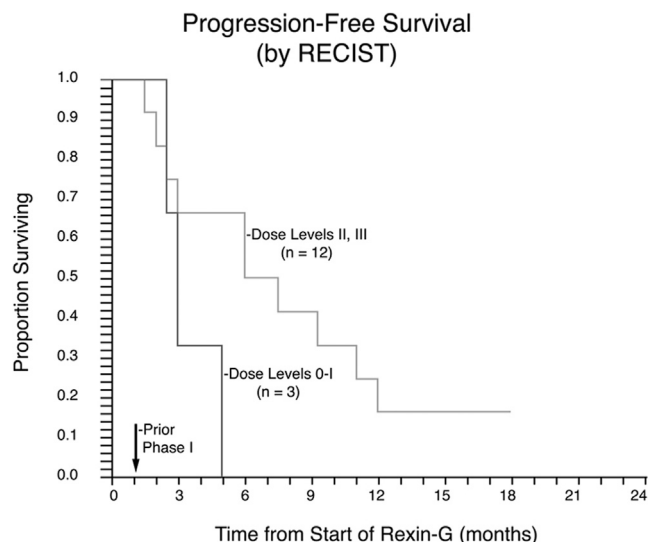


Figure 2. Kaplan-Meier Plot of Progression-free Survival in Rixin-G-Treated Gemcitabine-Resistant PDAC

Progression-free survival data for the modified intent-to-treat population are displayed. The proportion of patients surviving progression-free are plotted on the vertical axis as a function of time from the beginning of treatment, plotted on the horizontal axis.

observed in any 1 of the first 3 patients at each dose level. The MTD was defined as the highest dose in which 0 of 3 or ≤ 1 of 6 patients experienced a DLT, with the next higher dose level having at least 2 patients who experienced a DLT. A DLT was defined as any National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) grade 3, 4, or 5 AE, considered possibly, probably, or definitely related to the study drug, excluding the following: grade 3 absolute neutrophil count lasting <72 h; grade 3 alopecia; or any grade 3 or higher incident of nausea, vomiting, or diarrhea in a patient who did not receive maximal supportive care.²¹

Adaptive Design

For the phase II part of the study, patients who had no toxicity or in whom toxicity had resolved to grade 1 or less could receive additional cycles of therapy. Protocol Amendments I and II permitted an inpatient dose escalation up to dose level II for patients who had no toxicity or in whom toxicity had resolved to grade 1 or less, once safety had been established at the higher dose level in a simultaneously conducted phase I-II study for sarcoma.¹⁸ Additionally, each cohort also could be expanded to 6 or 7 patients if significant biologic activity (SD or better) was noted at each dose level. The principal investigator was allowed to recommend surgical resection or debulking after at least one treatment cycle has been completed. Response was evaluated first using the RECIST v1.0.⁴² Additional evaluations used the International PET criteria⁴³ and a modified RECIST, as described by Choi et al.²⁰ Safety and efficacy analyses were conducted by the site principal investigators (S.P.C., H.B., and M.A.M.).

Table 8. Notable Tumor Response Patterns at Each Assessment Point for Patients with Partial and Complete Responses by RECIST v.1.0 Compared to Choi and PET Criteria

Patient	Assessment Time Point (Week)	Overall Response by RECIST	Overall Response by Choi	Overall Response by PET
12	4	PR	SD	SD
	6	PR	SD	PR
	12	PR	PR	PR
	24	PR	SD	SD
	36	PR	SD	PD
	48	SD	PD	PD
	60	PD	PD	PD
16	72	PD	PD	PD
	4	PR	SD	SD
	6	PR	SD	SD
	12	PR	PR	SD
	24	PR	PR	PD
18	36	PR	PR	PD
	4	SD	SD	PD
	6	SD	PR	PD
	12	SD	SD	SD
	24	PR	PR	PR
	36	CR	CR	CR
	48	CR	CR	CR
18	60	CR	CR	CR
	72	CR	CR	CR

Patient Population and Treatment

Inclusion Criteria

Candidates included in the study had to have a histologically or cytologically confirmed pathologic diagnosis of advanced or metastatic PDAC that was resistant to gemcitabine or a gemcitabine-containing regimen; be ≥ 18 years of age; have an Eastern Cooperative Oncology Group (ECOG) performance score of 0–1; and acceptable hematologic, hepatic, and kidney functions.

Exclusion Criteria

Exclusion criteria included HIV, hepatitis B virus, or hepatitis C virus positivity; clinically significant ascites; medical or psychiatric conditions that could compromise proper adherence to the protocol; and unwillingness to employ effective contraception during treatment with Rixin-G and for 6 weeks following treatment completion.

The clinical protocol was reviewed and approved by the Western Institutional Review Board, Olympia, WA. The patients were recruited on a first-come first-served basis, and a written informed consent was obtained from each patient at the time of enrollment. All personnel who handled and disposed of the

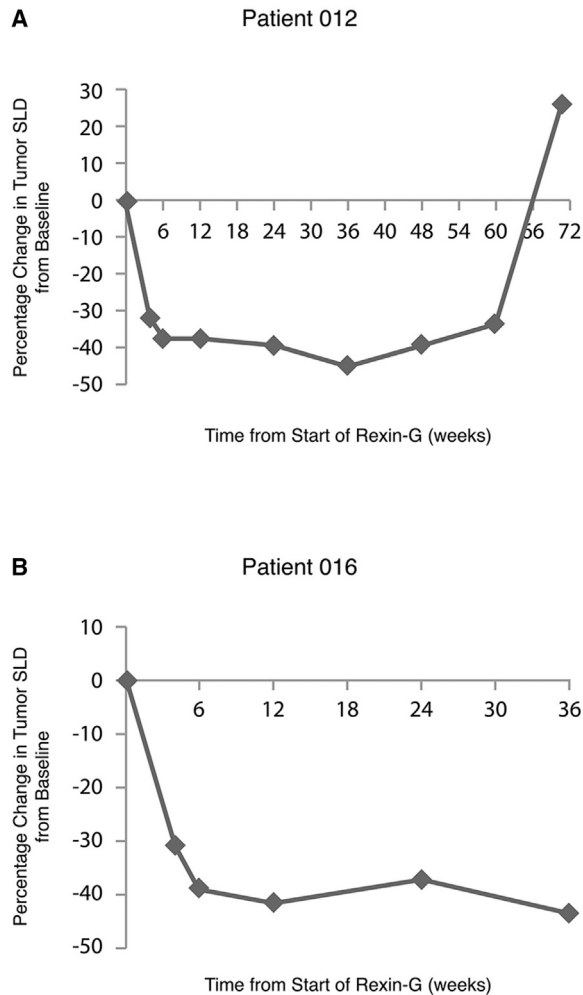


Figure 3. Tumor Regression during Treatment with Rixin-G

Patients 12 (A) and 16 (B). Percentage change in tumor size (sum of longest diameter [SLD]) is plotted on the vertical axis, as a function of time from the beginning of Rixin-G treatment, plotted on the horizontal axis.

vector observed Biosafety level 2 compliance in accordance with the NIH Guidelines for Research Involving Recombinant DNA molecules.

Evaluation of Tumor Burden

Estimated tumor burden was determined for each patient using the following formula:

$$\text{ETB(No. of cancer cells)} = [\text{Sum of Target Lesions (cm)} + \text{No. of Non-Target Lesions} + (20^*)] \times 10^9 \text{ (Assumption: } 1 \text{ cm} = 1 \times 10^9 \text{ cancer cells).}$$

*Note: 20×10^9 cancer cells for each occurrence of ascites, pleural effusion, and/or too-many-to-count non-target lesions.

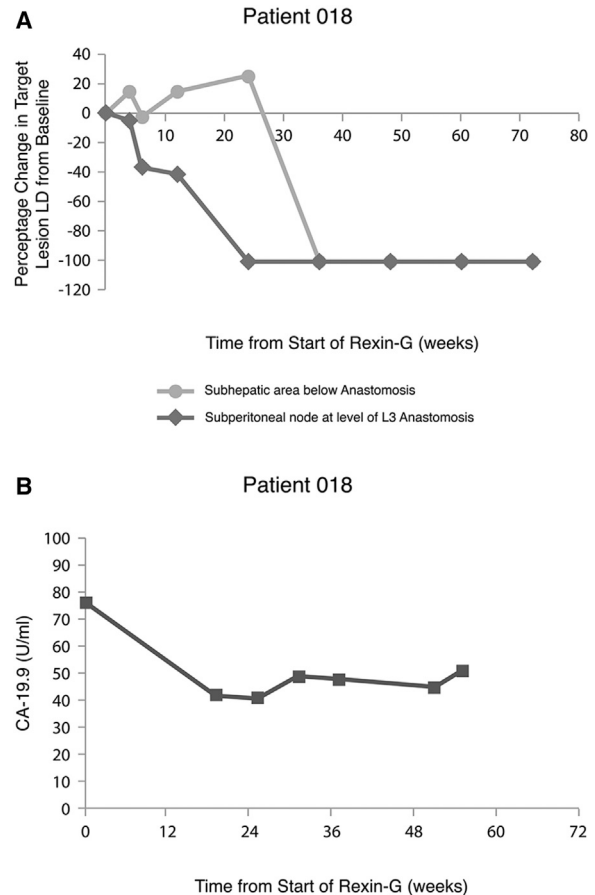


Figure 4. Tumor Regression and CA-19.9 Levels during Treatment with Rixin-G in Patient 18

(A) Percentage changes in tumor size (longest diameter [LD]) of metastatic hepatic and lymph node sub-peritoneal lesions are individually plotted on the vertical axis, as a function of time from the beginning of Rixin-G treatment, plotted on the horizontal axis. (B) Serum levels of tumor marker CA-19.9 (U/mL) are plotted on the vertical axis, as a function of time from the beginning of Rixin-G treatment, plotted on the horizontal axis.

Safety Analysis

Pretreatment evaluation included history; physical exam; complete blood count with differential and platelet count; a serum chemistry panel, including aspartate transaminase, alanine transaminase, alkaline phosphatase, creatinine, and total bilirubin; assessment of coagulation status, including prothrombin time, international normalized ratio, and activated partial thromboplastin time; and testing for HIV, hepatitis B virus, and hepatitis C virus. All patients had a complete blood count and serum chemistry panel performed weekly during treatment. Toxicity was evaluated before each vector infusion, as well as before beginning an additional treatment cycle. Toxicity was graded using NCI CTCAE version 3²¹ and MedDRA. Patients' serum was collected for the detection of vector-neutralizing antibodies and antibodies to gp70. The peripheral blood mononuclear cells were also collected to test for the presence of vector DNA integration and RCR at the

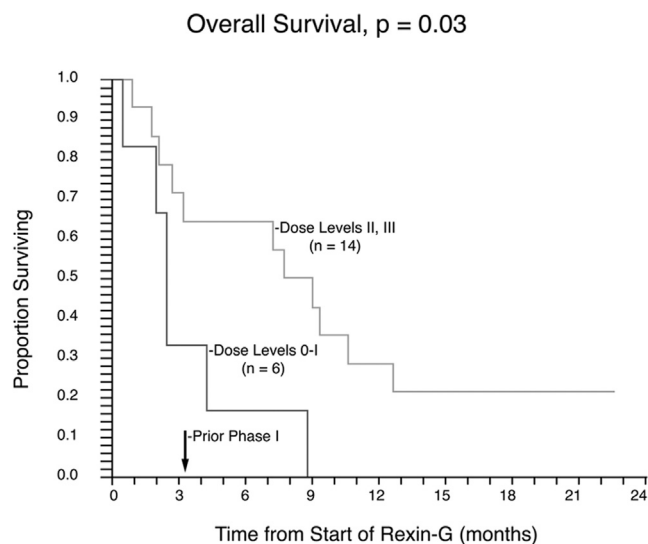


Figure 5. Kaplan-Meier Plot of Overall Survival of PDAC Patients following Regin-G Treatment at Escalating Dose Levels

Analysis of overall survival in the ITT population suggests a dose-response relationship between overall survival and Regin-G dosage ($p = 0.03$). The proportion of patients surviving are plotted on the vertical axis as a function of time from beginning of treatment, plotted on the horizontal axis.

end of 4 weeks, at 6 weeks, or before the start of a treatment cycle. Vector-related studies were performed as previously described.¹⁸ Regin-G was stored in volumes of 23 mL in 30-mL vials or 40 mL in 150-mL cryobags at -70 to -90°C . Preparation of the vector for patient administration consisted of rapid thawing in the vial in a 34°C water bath 15–30 min prior to infusion, and it was given intravenously over 5–10 min. All personnel who handled and disposed of the vector observed Biosafety level 2 compliance in accordance with the NIH Guidelines for Research Involving Recombinant DNA molecules.

Efficacy Analysis

Prior to beginning treatment, imaging evaluations such as whole-body fluorodeoxyglucose (FDG)-PET-CT scan, electrocardiography, and chest X-ray were performed. FDG-PET-CT scan was done for efficacy assessment at the end of 4 weeks, at the end of 6 weeks, or before starting an additional treatment cycle up to 12 weeks and every 12 weeks thereafter. RECIST v.1.0 criteria was used to assess the tumor responses (CR, PR, or SD).⁴² Tumor control rate was defined as the percentage of patients who had CR, PR, or SD at any time during the Regin-G treatment period. Tumor responses were also evaluated using modifications of the international PET criteria⁴³ and the Choi criteria.²⁰ The modified international PET criteria define a CR as disappearance of FDG avid uptake in target and non-target lesions with no new lesions; PR as a decrease in maximum standard uptake value of $>25\%$ from baseline with no new lesions along with no obvious progression of non-target lesions; PD as an increase in maximum standard uptake value of $>25\%$ from baseline, any new lesions, and obvious progression of non-target lesions; and SD as not meeting the criteria for CR, PR, or

PD, and no symptomatic deterioration attributed to tumor progression. The modified Choi criteria define CR as the disappearance of all disease and no new lesions; PR as a decrease in size of $\geq 10\%$ or a decrease in CT density (Hounsfield units) $\geq 15\%$ with no new lesions and no obvious progression of non-measurable disease; PD as an increase in tumor size of $>10\%$ and did not meet criteria for PR by CT density, any new lesions, including new tumor nodules in a previously cystic tumor; and SD as not meeting the criteria for CR, PR, or PD, and no symptomatic deterioration attributed to tumor progression.

AUTHOR CONTRIBUTIONS

S.P.C., H.B., and M.A.M. are the principal investigators of the clinical trial; they conducted the study; evaluated patients' tumor responses, survival, and safety; and wrote parts of the manuscript and reviewed and edited the final manuscript. E.M.G. and F.L.H. wrote the clinical protocol and informed consent, submitted the investigational new drug (IND) application to the FDA, wrote parts of the manuscript, reviewed the published literature, oversaw the clinical trial, and reviewed and edited the final manuscript. N.A. reviewed the clinical data and published literature, wrote the manuscript, and edited and reviewed the final manuscript. All authors approved the submission of the manuscript for publication.

CONFLICTS OF INTEREST

E.M.G. and F.L.H. are co-inventors of Regin-G, including its targeted gene delivery system, which was originally developed at the University of Southern California Keck School of Medicine, and they are co-founders of Delta Next-Gene, LLC. The other authors have no competing interests.

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