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The Genevieve Protocol: Phase I/II Evaluation of a Dual Targeted Approach to Cancer Gene Therapy/ Immunotherapy

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Abstract

A Phase I/II study using two tumor-targeted retro vectors (Rexin-G, encoding a cytocidal dominant negative human cyclin G1 construct, and Reximmune-C, encoding a GM-CSF gene), was conducted in chemoresistant solid malignancies and B-cell lymphoma.

Patients and Methods: Sixteen patients received Rexin-G (2 x 10e11 cfu, i.v. on D1, D3, and D5 and escalating doses of Reximmune-C (0.5 x 10e10 cfu to 2.0 x 10e10 cfu i.v.) on D3 and valacyclovir (3 gms/day p.o.) on D6-D19.

Results: No dose-limiting toxicity was observed. Post- treatment, GM-CSF serum levels were normal, and no vector neutralizing antibodies, RCR or vector integration in PBLs was detected. At Dose I, 2/5 patients had PR; 1/5 patients, SD, 2/5, PD; median PFS was 4.5 months, median OS, 21 months, one-year OS rate, 80%. At Dose II, 1/4 patients had PR, 3/4 patients, SD; median PFS was 9 months, median OS, 13 months, one-year OS rate, 50%. At Dose III, 2/7 patients had PR, 5/7 patients, SD; median PFS was 13 months, median OS, >21 months, one-year OS rate, 86%. Taken together, these data suggest that the Genevieve protocol is safe, may control tumor growth, and may prolong progression-free and overall survival in patients with chemoresistant solid malignancies and B-cell lymphoma.

Keywords: Targeted gene delivery; Cancer immunotherapy; Cell cycle control; Gene therapy

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Introduction

Recurrent or metastatic cancer is associated with approximately 90% of cancer-related deaths, according to the World Health Organization [1]. Such patients are often left without viable treatment options that have an impact on survival as resistance to these therapies eventually develop. Recently, the resurgence of immunotherapy in the oncology space has revolutionized the management of both solid and hematologic malignancies [2-9]. We hypothesized that a personalized vaccination strategy aimed specifically at enabling immune cell trafficking in the Tumor Microenvironment (TME), and evoking immune responses against a patient's own specific cancer would improve tumor control and long-term survival [10]. One approach is a dual targeted gene therapy regimen, combining (1) Rexin-G, a tumor-targeted retrovector encoding a human cyclin G1 (CCNG1) inhibitor to destroy the cancer cells and tumor vasculature, expose tumor neoantigens, impede extracellular matrix production, and enable immune cell entry into the TME [10], and (2) Reximmune-C, a tumor-targeted retrovector encoding a potent GM-CSF gene, for local paracrine secretion, polarization of M1 macrophages [11], maturation of dendritic cells, T cell activation [12-15], and recruitment of the patient's own cytotoxic T cells into the TME for in situ tumor neoantigen recognition (The Genevieve Protocol).

In this article, we report on the results of a Phase I/II evaluation of the Genevieve protocol,

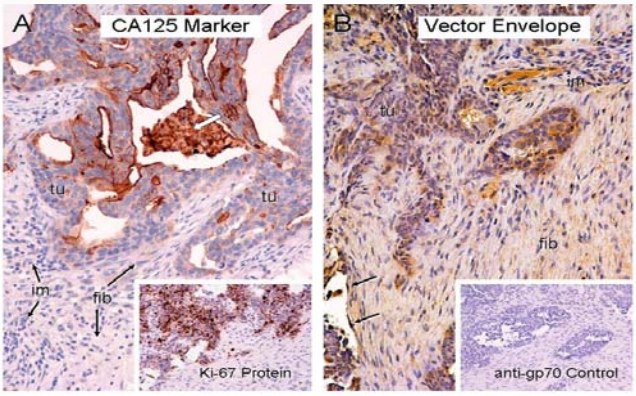

Figure 1: Vector localization in residual pelvic tumor of a patient with ovarian cancer which was resected two hours after intravenous infusion of Rexin-G. (A) Residual tumor (tu) marked by CA-125 surrounded by tumor infiltrating lymphocytes (im) and fibrosis (fib); (B) Immunoreactive Rexin-G nanoparticles in tumor nests and tumor vasculature (brown staining material).

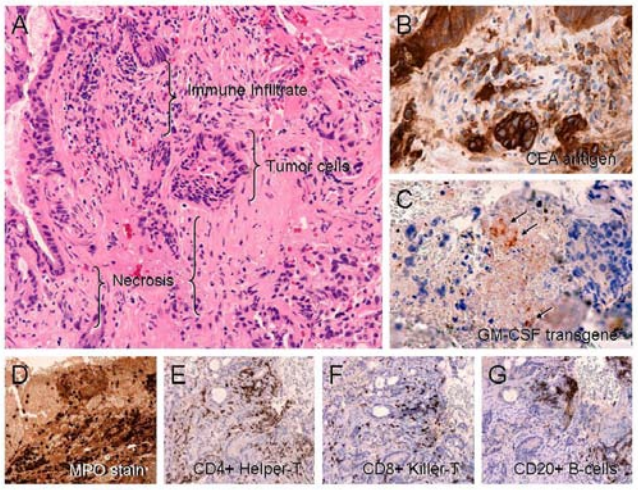

Figure 2: Expression of GM-CSF transgene and characterization of tumor infiltrating lymphocytes in resected colon cancer two days after Reximmune-C infusion. (A) H&E: areas of tumor necrosis with tumor infiltrating lymphocytes (TILs); (B) CEA+ tumor cells, (C) immunoreactive GM-CSF transgene (reddish-brown staining material) in a necrotic tumor, (D) MPO staining granulocytes; (E-G) CD4+, CD8+ and CD20+ TILs, indicating effective recruitment of patient's own immune cells into the residual tumor.

using Rexin-G and Reximmune- C tumor-targeted retrovectors, for metastatic solid malignancies and B-cell lymphoma.

Results

Patient population

Sixteen patients (10 women and 6 men) were treated. There were six white Caucasian, one African American and nine Asian subjects enrolled into this study (Table 1). Table 2 shows the patients' cancer type, stage of disease, chemotherapy resistance, ECOG Score and age. The median age was 56 years (range, 23 to 91 years), and Eastern Cooperative Group Performance Score was between 0-1 for all subjects. All 16 patients were assessable for toxicity and response. Fourteen patients (88%) had failed standard chemotherapy. All patients showed either recurrence or progressive disease within 3 months from the last chemotherapy regimen at baseline. Two patients were chemotherapy naïve but refused or were not eligible for chemotherapy due to age. Fifteen patients had metastic disease, one patient, recurrent. disease.

Analysis of safety

There was no dose-limiting toxicity at any dose level of Reximmune-C with a defined optimal dose of Rexin-G. Details regarding treatment emergent Adverse Events (AEs) are provided below.

Nonserious drug-related AEs: Related adverse events comprised of Grade 2 tumor pain (n=2; Table 3). There were no serious drug-related AEs.

Nonserious unrelated AEs: Sixteen patients experienced one or more unrelated nonserious adverse events (Table 4). The majority of unrelated adverse events were Grade 1 or 2 in severity. There were no Grade 3 nonserious unrelated adverse events that occurred in two or more patients.

Serious unrelated AEs

Seven Serious Adverse Events (SAEs) were reported in four patients. None were related to the study drug. SAEs are listed by patient in Table 5. Grade 4 sepsis occurred in two patients resulting in death, and Grade 3 anaemia occurred in two patients that required hospitalization. Grade 3 dysuria, Grade 4 pelvic haemorrhage and Grade 4 ureteral obstruction occurred in one patient each.

Deaths: None of the deaths were considered treatment-related. The cause of death was progressive disease in all but 4 patients; the causes of death in these patients were sepsis, pneumonia, sepsis and post-operative complication.

Vector-related safety parameters: No patient tested positive for any of the following: vector neutralizing antibodies, antibodies to gp70, replication-competent retrovirus in Peripheral Blood Lymphocytes (PBLs); vector integration into genomic DNA of PBLs. Further, circulating GM-CSF protein was not detected in 10 baseline and treated patient sera (GM-CSF level <6 pg/ml), while very low level circulating GM-CSF was detected in one patient in Cycle 2 (0.9 pg/ml), and in one patient wherein baseline serum GM-CSF level (6.6 pg/ml) was higher than that of the post-treatment serum (4.4 pg/ml).

Analysis of efficacy

By RECIST v1.0, 3 patients achieved PR, 9 achieved SD and 2 had PD. By bone scan, one patient had PR, and by PET scan, one patient had PR. Median PFS by RECIST, bone scan or PET scan were 4.5, 9.0, and 13.0 months at Dose Levels I, II, and III respectively, suggesting a trend towards a dose-response relationship between PFS and Reximmune-C. Overall Survival (OS) was calculated from the start of Rexin-G to time of death. Median OS was 17, 13 and >21 months at Dose Levels I, II, III respectively. A summary of responses, PFS and OS of enrolled patients are shown in Table 6. Histopathologic

Table 1: Patients enrolled according to race and gender.

Gender	White, not of Hispanic Origin	Black, not of Hispanic Origin	Asian, or Pacific Islander	Total
Male	3	1	2	6
Female	3	-	7	10
Total	6	1	9	16

Table 2: Patients enrolled according to cancer type, stage of disease, chemotherapy resistance, ECOG score and age.

Type of Cancer	No. of Patients n=16	% of Patients	Stage of Disease	No. Chemotherapy Resistant	ECOG Score	Age Years
Breast CA	3	19	Metastatic	3 of 3	0	48,53,64
Colorectal CA	3	19	Metastatic	3 of 3	0	26,35,64
Ovarian CA	2	13	Metastatic	2 of 2	0	41,60
Prostate CA	2	13	Metastatic	1 of 2*	0	65,90
Ewing sarcoma	1	6	Metastatic	1 of 1	0	36
Liposarcoma	1	6	Metastatic	1 of 1	0	48
Osteosarcoma	1	6	Recurrent	0 of 1	0	25
Pancreatic CA	1	6	Metastatic	1 of 1*	1	78
B-cell Lymphoma	1	6	Metastatic	1 of 1	0	59
Renal Cell CA	1	6	Metastatic	1 of 1	0	68

*Chemo-naive

Table 3: Non-serious drug-related adverse events.

MedDRA System Organ Class	Preferred Term	Dose Level	Toxicity Grade
Neoplasms benign, malignant and unspecified	Tumor pain	II	2
Neoplasms benign, malignant and unspecified	Tumor pain	III	2

Notes: all drug-related AEs were nonserious; toxicity grade according to NCI-CTCAE.
Notes: Numbers shown are the number of patients who experienced the indicated event at the indicated Reximmune-C dose level

examination of resected tumors showed vector localization in tumors (Figure 1) in a patient with ovarian cancer, and paracrine secretion of GM-CSF, tumor necrosis, reparative fibrosis, and tumor infiltrating lymphocytes indicating a local immunologic response (Figure 2) in a patient with metastatic chemo-refractory colorectal cancer.

Discussion

To date, four Phase I/II clinical trials using Rexin-G for sarcoma, pancreatic cancer and breast cancer one Phase II study of Rexin-G for osteosarcoma have been completed in the United States, and 80 patients have been treated with Rexin-G in formal US based studies. Rexin-G has been shown to be well tolerated with no treatment related serious AEs with one-year survival rates of 33.3% in pancreatic cancer [16-18], 38.5% in sarcoma [19,20], and 60% in breast cancer (unpublished data). In 2007, Rexin-G gained accelerated approval for all chemoresistant solid malignancies in the Philippines, and Orphan Drug status for pancreatic cancer, soft tissue sarcoma and osteosarcoma, as well as and fast track designation for a Phase 2/3 pivotal trial for pancreatic cancer in the United States in 2009 [16,18-23].

To improve treatment outcome parameters of patients with chemoresistant cancer, we introduced a second tumor-targeted retrovector, Reximmune-C, that encoded a GM-CSF gene. GM-CSF is a powerful cytokine that stimulates the differentiation of myeloid cells, but also has potent effects on the immune system including polarization of M1 macrophages [11], activation of T cells, maturation of dendritic cells, which process and present tumor antigens for the priming of antitumor cytotoxic T lymphocytes, and promoting both humoral and cell-mediated responses [12-15]. We hypothesized that rapid, strategic, and individualized vaccination of each patient against his or her specific cancer could be achieved by this dual targeted gene transfer strategy, which combines (1) the targeted vector encoding a cytocidal cyclin G1 construct, Rexin-G, with (2) a targeted vector encoding an immune response gene, Reximmune-C. Rexin-G was given first to kill the cancer cells and expose the neoantigens in the tumor compartment, followed by Reximmune-C to recruit the body's immune cells to the tumor microenvironment for neoantigen recognization. To prevent or reduce an exaggerated immune response, valacyclovir was given four days later to destroy the GM-CSF expressing cancer cells. The guiding hypothesis is that exposure of tumor neoantigens from the cytocidal activity of Rexin-G to host immune cells recruited to the tumor site by Reximmune-C will induce long lasting anti-tumor immunity.

The results of this study suggest that the Genevieve protocol is safe and well-tolerated with minimal systemic toxicity. The tumor control rate of 88% and one-year survival rate of 86% in patients receiving the higher doses of Reximmune-C suggest that the combination regimen, Rexin-G plus Reximmune-C, has substantial anti-tumor activity in patients with metastatic solid malignancies and B-cell lymphoma. The better responses and longer progression-free survival at Dose Levels II and III compared to Dose Level I suggest a trend towards a dose-response relationship between overall response/progression-free survival and Reximmune-C dosage. Histopathologic examination of biopsied or resected tumors of patients treated in this protocol showed vector localization in residual tumor (Figure 1), and GM-CSF transgene expression in necrotic (Figure 2). Progressive tumor regression was seen on serial bone scans obtained over 20 months in a patient with chemo-resistant ductal carcinoma of breast [21,24,25]. At least three patients who participated in the study are alive 9 years after completion of treatment with the Genevieve protocol, with two patients having no evidence of active disease (osteosarcoma n=1; B-cell lymphoma n=1).

In summary, Rexin-G is cytocidal to cancer cells which exposes tumor neoantigens within the TME for recognition by their own cytotoxic T cells. Rexin-G, by itself prepares the TME for immune cell entry, as evidenced by presence of tumor infiltrating lymphocytes post Rexin-G treatment [10]. However, the cytotoxic immune responses induced by Rexin-G may be inadequate in overcoming the suppressive signals from regulatory T cells that are also recruited into the TME. Therefore, Reximmune-C was also given. GM-CSF plays a critical role in development and maturation of dendritic cells, and proliferation and activation of T cells, linking the innate and acquired

Table 4: Nonserious, Unrelated Adverse Events, by Reximmune-C Dose Level and Severity**.**

		Reximmune-C Dose Level (I-III) and CTCAE Toxicity Grade (1-3)									
		Dose I			Dose II			Dose III			
MedRA System Organ Class/ Preferred Term		**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**Total**
Investigations	Bld. alkaline phosphatase inc.	1				1		2			4
	White blood cell count Inc.	2	1		1				1		5
	White blood cell count dec.	1						2			3
	Blood triglycerides inc.		1						1	1	3
	Blood urea inc.				1			1			2
	Aspartate aminotransferase inc.				1			2			3
	Alanine aminotransferase inc.					1					1
	Blood creatinine inc.							2		1	3
	Weight decreased							1			1
	Sputum abnormal (blood-streaked)				1						1
	Protein total dec.							1			1
	Blood bilirubin inc.							1			1
Metabolic and nutrition disorders	Hypercholesterolemia	2						1	1	1	5
	Hyponatraemia				1						1
Endocrine disorders	Hyperglycemia	2							1		3
Blood and lymphatic system disorders	Anaemia	2	1		1	1	1	3			9
	Lymphadenopathy	1									1
Urinary and renal disorders	Proteinuria	1									1
	Pyelocaliectasis				1			1	1		3
	Pyuria				1						1
	Dysuria		1			1					2
	Nephrolithiasis							1			1
	Hematuria				1						1
Hepatobiliary disorders	Ascites	1									1
	Pneumobilia	1									1
	Hypoalbuminemia		1			1					2
	Cholelithiasis								1		1
Respiratory, thoracic, mediastinal disorders	Pleural effusion	1			1			1			3
	Pulmonary nodules	1									1
	Cough				1			1			2
General disorders and administration site conditions	Oedema, peripheral	1				1		1			3
	Pyrexia	1						1			2
	Swelling (jaw, leg)							1	2		3
	Nodule				1						1
Gastrointestinal disorders	Abdominal pain	1			1						2
	Diarrhea	1									1
	Nausea				1						1
	Constipation				1						1
	Gastroesophageal reflux ds.				1						1
	Stasis syndrome							1			1
Musculoskeletal and connective tissue dis.	Back pain				1	1		2			4
	Chest and back pain							1			1
	Lumbar pain							1			1
	Bone pain				1						1

		Reximmune-C Dose Level (I-III) and CTCAE Toxicity Grade (1-3)									
		Dose I			Dose II			Dose III			
MedRA System Organ Class/ Preferred Term		1	2	3	1	2	3	1	2	3	Total
	Muscle weakness		1								1
	Hip pain							1			1
Eye disorders	Orbital oedema							1			1
Vascular disorders	Epistaxis							1			1
	Mouth haemorrhage								1		1
Infections and infestations	Urinary tract infection								1		1
Surgical and medical procedures	Nephrostomy tube placement							1			1

Table 5: Serious Unrelated Adverse Event Listings.

MedRA System Organ Class	MedDRA Preferred Term	Dose Level	Severity Level
Infections and infestations	Sepsis	I	4
Blood and lymphatic system disorders	Anaemia	I	3
Blood and lymphatic system disorders	Anaemia	II	3
Urinary and renal disorders	Dysuria	II	3
Reproductive and breast disorders	Pelvic hemorrhage	II	4
Infections and infestations	Sepsis	II	4
Infections and infestations	Pneumonia	II	4
Urinary and renal disorders	Ureteral obstruction	III	4

Table 6: Summary of Responses, Progression-free Survival and Overall Survival.

Category	Dose I[a]	Dose II[a]	Dose III[a]	All
All Patients	N = 5	N = 4	N = 7	N = 16
Best Overall Response				
RECIST	1PR, 1SD, 2PD	1PR, 3SD	1PR, 5SD	3PR, 9SD, 2PD
Bone Scan	IPR			1PR
PET Scan			1PR	1PR
Median PFS (mo)	4.5	9	13	ND
Median OS (mo)	17	13	>21	ND
Overall Survival Rate (%)				
12 months	80	50	86	72
# Alive[b] (9 years)	0	0	2	2

Abbreviations: RECIST: Response Evaluation Criteria in Solid Tumors; PET: Positron Emission Tomography; PFS: Progression-Free Survival; OS: Overall Survival; Cum: Cumulative; mo: month; nd: Not Determined

[a] Rexin-G, 2 x 10e11 cfu, on Days 1, 3, 5, 8, 10 and 12 plus Reximmune-C, 0.5, or 1.0, 2.0 x 10e10 cfu on Day 3 (Dose I, II, III respectively), and valacyclovir at 1 gm, p. o. 3 times a day on Days 6-19, comprising one cycle

[b] As of September, 2018.

immune response [12]. The activated T cells then recognize the tumor neoantigens in the TME for cell killing, which can induce further tumor regression and in situ vaccination of long-lasting antitumor immunity. Taken together, these data suggest that the Genevieve protocol, consisting of a strategic combination of Rexin-G and Reximmune-C/valacyclovir, is safe and well-tolerated, may control tumor growth, evoke anti-tumor immunity, and prolong overall survival time-advancing personalized cancer vaccination as a realistic goal.

Patients and Methods

Study design

This is an open label, single center, single arm, dose-seeking study that incorporates a modification of the standard Cohort of 3 designs combined with a Phase II efficacy component by adaptive design [16,19,26]. For the Phase 1 part of the study, treatment with Rexin-G was administered at a previously confirmed effective and safe dosage (2.0 × 10e11cfu, i.v.) on Days 1, 3, and 5, followed by Reximmune-C at three escalating doses (0.5 or 1.0, 2.0 × 10e10 cfui.v.) on Day 3. Valacyclovir, 3 grams/day orally was given on Days 6-19 comprising one treatment cycle. For the Phase II part, patients who had grade 1 or less toxicity received additional cycles of Rexin-G and Reximmune-C for a total of 6 months.

Clinical objectives/end points

The primary objective of this study was to evaluate the safety of Rexin-G i.v., combined with escalating doses of Reximmune-C i.v. The secondary objectives included (i) evaluation of the potential of Rexin-G plus Reximmune-C for evoking vector neutralizing

antibodies, recombination events and/or unwanted vector integration in Peripheral Blood Lymphocytes (PBLs) as non-target organs, and (ii) identification of an antitumor response to Rexin-G plus Reximmune-C.

Patient population

The phase I/II study included patient's ≥ 18 years of age, with histologically or cytologically proven locally advanced, unresectable, or metastatic cancer. Patients were required to have an Eastern Cooperative Group (ECOG) performance score of 0-1, with adequate hematologic, hepatic, and kidney function and an estimated survival of at least 3 months. Patients with Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HBC) positivity, clinically significant ascites, medical or psychiatric conditions that could compromise successful adherence to the protocol, and unwillingness to employ effective contraception during the vector infusion period and for six weeks following treatment completion were excluded from this study.

Pretreatment evaluation included history, physical exam, complete blood count with differential and platelet count, a serum chemistry panel including Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (AlkPhos), creatinine, and total bilirubin, assessment of coagulation status including Prothrombin Time (PT), International Normalized Ratio (INR), and Activated Partial Thromboplastin Time (PTT), testing for HIV, HBV or HCV, imaging evaluation to include a whole body FDG/PET-CT scan, electrocardiography, and chest X-ray. All patients had a complete blood count and serum chemistry panel performed weekly during treatment.

Patient recruitment and assignment

The phase I/II clinical trial using Rexin-G and Reximmune-C for chemoresistant cancers was approved by the Philippine Bureau of Food and Drugs, and the Institutional Review Board. Written informed consent was obtained from each patient at the time of enrollment and prior to the performance of any screening procedures. This was an open label study using Rexin-G and escalating doses of Reximmune-C. Of the 16 enrolled and treated patients, 5 patients were treated at Dose I, 4 were treated at Dose II and 7 were treated at Dose III. All sixteen patients received at least two cycles of treatment and a two-week rest period lasting 8 weeks in total and had a follow-up CT scan, bone scan or PET-CT scan, and were considered evaluable per protocol for best response, progression-free survival (PFS) and overall survival (OS).

Treatment

The clinical vectors, Rexin-G and Reximmune-C were supplied as 23 ml vector per vial. (Stock dose: 5 × 10e9 cfu/ml for Rexin-G and 1 × 10e10 cfu/ml for Reximmune-C). Vector preparation: The vector was thawed in a 34°C water bath, 15 mins to 30 mins prior to infusion into the patient, and given i.v. or through a central venous line over 5 mins to 10 mins. All personnel who handled and disposed of the vector observed Biosafety Level 2 compliance in accordance with the National Institutes of Health Guidelines for Research Involving Recombinant DNA molecules [16,19,26,29].

Safety analysis

Toxicity was assessed before each vector infusion, and before beginning an additional treatment cycle. Toxicity was graded using NCI CT-CAE version 3.0 [27]. Patient's serum was collected for vector-specific antibody detection, and for replication competent retrovirus and vector DNA integration in peripheral blood mononuclear cells, at baseline and at the end of 4 weeks or before the start of a treatment cycle.

Detection of anti-vector antibodies in serum, testing for presence of RCR and vector DNA integration studies in patient's peripheral blood lymphocytes, were performed as previously described [16,19,26].

Efficacy analysis

Efficacy assessment with FDG PET-CT scan was performed 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. All PET-CT images were performed and reviewed by independent radiologists of the Asian Hospital and Medical Center, Ayala Alabang, Philippines, who are experts at nuclear and PET imaging, and who were blinded to the Reximmune-C dose levels. Tumor responses were evaluated using RECIST v1.0 [28].

Correlative analysis

The principal and sub-investigators assessed tumor responses using modifications of the International PET criteria [14,16,19,26,29]. The modified International PET Criteria defines 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 and no obvious progression of nontarget lesions; SD as not meeting the criteria for CR, PR, or PD, and no symptomatic deterioration attributed to tumor progression; and PD as an increase in maximum standard uptake value of >25% from baseline, any new lesions, and obvious progression of non-target lesions.

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Authors' Contributions and Approval

JGI, FS, RAM and SPC are clinical investigators of the study, conducted the study and evaluated patients' tumor responses, survival and evaluated safety, wrote sections of the manuscript, reviewed and edited the final manuscript. EMG and FLH wrote the clinical protocol and informed consent, submitted the IND to the PhFDA, wrote parts of the manuscript, reviewed the published literature, oversaw the clinical trial, reviewed and edited the final manuscript. ESN, LK and JT reviewed the clinical data and published literature, wrote sections of the manuscript, edited and reviewed the final manuscript. All authors approve the submission of this manuscript for publication.

Ethics approval and consent to participate: The clinical protocol was approved by the Philippine FDA, IRB, and the Institutional Biosafety Committee of the Civic Place Clinical Research Unit. A written informed consent was obtained from each patient prior to treatment with Rexin-G.

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