# Pathotropic nanoparticles for cancer gene therapy Rexin-G™ IV: Three-year clinical experience

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**Abstract.** Metastatic cancer is a life-threatening illness with a predictably fatal outcome, thereby representing a major unmet medical need. In 2003, Rexin-G™ became the world's first targeted injectable vector approved for clinical trials in the treatment of intractable metastatic disease. Uniquely suited, by design, to function within the context of the human circulatory system, Rexin-G is a pathotropic (disease-seeking) gene delivery system bearing a designer killer gene; in essence, a targeted nanoparticle that seeks out and selectively accumulates in metastatic sites upon intravenous infusion. The targeted delivery of the cytocidal gene to primary tumors and metastatic foci, in effective local concentrations, compels both cancer cells and tumor-associated neovasculature to self-destruct, without causing untoward collateral damage to non-target organs. In this study: i) we report the results of three distinctive clinical studies which demonstrate the initial proofs of concept, safety, and efficacy of Rexin-G when used as a single agent for advanced or metastatic cancer, ii) we introduce the quantitative foundations of an innovative personalized treatment regimen, designated the 'Calculus of Parity', based on a patient's calculated tumor burden, iii) we propose a refinement of surrogate end-points commonly used for defining success in cancer therapy, and iv) we map out a strategic plan for the accelerated approval of Rexin-G based on the oncologic Threshold of Credibility paradigm being developed by the Food and Drug Administration.

## Introduction

Advanced or metastatic cancer is generally associated with a fatal outcome, which eludes the impact of conventional radiation and chemotherapy. Therefore, novel and more-effective therapeutic approaches are urgently needed to address this problem, which constitutes a major unmet medical need. For pancreatic cancer, the fourth leading cause of cancer death in the United States, complete surgical resection of the primary tumor offers the only effective treatment (1,2). Unfortunately, curative resection is only possible in 10-15% of patients with pancreatic cancer, and the median survival time is only 6-10 months for patients with locally advanced pancreatic cancer and 3-6 months for metastatic disease, respectively (3,4). Recently, gemcitabine, a deoxycytidine analogue, has been shown to improve the quality-of-life of patients with locally advanced pancreatic cancer (4,5) and with metastatic disease (6); however, the median survival was extended in the latter case by only 1-2 months, as compared to 5-fluorouracil (5-FU). Nonetheless, gemcitabine is now considered the first-line standard of care in treating patients with pancreatic cancer.

Surgical resection is also the primary treatment modality for patients with colorectal cancer, which is the second leading cause of cancer death in the United States. Additional chemotherapy and radiation treatments have helped to reduce the recurrence of colorectal cancer in patients with early-stage disease (7). However, the effect of these treatments on locally advanced tumors has been less satisfactory (8). Currently, the 5-year survival rate for colorectal cancer patients treated with surgical resection is ~90% for stage I, 70% for stage II, 50% for stage III, and <5% for stage IV. While chemotherapy for colon cancer remains a useful palliative option, which may, at times, even extend to down-staging, the majority of patients with colon cancer exhaust the benefits from standard treatment within 18 months. Moreover, there appears to be a consensus among leading clinical oncologists that targeted 'biologic therapies' hold the greatest promise in terms of future clinical development for both pancreatic and colon cancer.

Of the biologic therapies currently in development as alternatives to traditional chemotherapeutics, angiogenesis inhibitors, cancer immunotherapy, and cancer gene therapy strategies are undergoing active clinical investigations (9-11). Indeed, ~70% of all the initial gene therapy protocols prior to the year 2000 were aimed at treating metastatic cancer (11).

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Unfortunately, none of the initial studies employing non-targeted vectors demonstrated a clear clinical benefit, leading to the conclusion that the major problem that has hindered the development and deployment of effective cancer gene therapy is that of inefficient gene delivery to target cells *in vivo* (9,12-15), a technological problem that at once obviates and precludes many direct gene therapy approaches (10). Thus, genetic medicine at the turn of the century stood poised yet stalled at the threshold of clinical history. In this regard, the development of tumor-targeted Rexin-G represents a quantum step forward in both safety and efficacy in this preferred pathway of developing biologic therapies.

The advent of 'pathotropic (or disease-seeking) targeting' introduces a new paradigm in cancer gene therapy - a new vehicle, in terms of medical delivery - and a new technology which ushers modern medicine across this threshold. Uniquely suited, by design, to function within the human vascular system, this targeted delivery system embodied in the molecular structure of Rexin-G directs therapeutic nanoparticles select-ively to areas of severe pathology (i.e., pathotropic targeting), which enables preferential gene delivery to significant vascular lesions (16,17), to areas of active tumor-associated angio-genesis, and to metastatic cancer nodules (18,19) with high efficiency *in vivo*. These targeted, injectable pathotropic nano-particles, or vectors, incorporate a physiological surveillance function derived from the extracellular matrix-binding domains of von Willebrand (blood coagulation) factor by molecular engineering of the retroviral envelope protein (17). This surveillance function serves to facilitate vector accumulation in tumors and tumor-associated neovasculature wherein certain collagenous proteins are exposed and/or deposited as a result of tumor invasion and tumor-associated angiogenesis (18-22). When injected intravenously, these circulating pathotropic nanoparticles accumulate in metastatic deposits within minutes (19) and enable efficient gene delivery to remote metastatic sites (18,19).

Therefore, by targeting the distinctive histopathology of the tumor microenvironment - rather than the diversity of cancer cells *per se* - effective vector concentrations at metastatic sites can be optimized, and both the safety and the efficacy of the circulating pathotropic nanoparticles can be increased dramatically. With safety features further enhanced by the inherent properties of the retroviral vector itself, which selectively transduces dividing cells only (23), and the strategic specificity of a modified cell cycle control gene, which limits its function to tumoricidal and anti-angiogenic activities (19), the preclinical and clinical performance of these pathotropic nanoparticles establishes a critical proof-of-principle: the potential for systemic delivery of surveillant genetic medicine in the treatment of primary, metastatic, and occult cancers. As the unique ability of Rexin-G, with its pathotropic gene delivery system, is formally demonstrated to reach and safely impact metastatic disease in the clinic, it continues to lead the field of targeted genetic medicine as an enabling technology platform.

**Materials and methods**

*Product description (Rexin-G for intravenous use)*. Rexin-G is a pathotropic (extracellular matrix or ECM-targeted) retroviral-based nanoparticle/gene delivery vector, encoding a dominant negative mutant construct of the human *cyclin G1* gene expressed under the control of a hybrid LTR promoter (24). The vector also contains the neomycin resistance gene, which is driven by the SV40 early promoter and is utilized for the determination of vector titer. The Rexin-G targeted injectable vector is produced by transient co-transfection of three separate plasmids in 293T cells (human kidney 293 cells transformed with the SV40 large T antigen) maintained as a fully validated master cell bank. Producer cell growth medium is comprised of a DMEM base supplemented with 4 g per l glucose, 3 g per l sodium bicarbonate, and 10% gamma irradiated fetal bovine serum (Hyclone). The serum was obtained exclusively from USA sources, and has been tested to be free of bovine viruses in compliance with USDA regulations. The production, suspension, and collection of therapeutic nanoparticles are performed in the absence of bovine serum in a final formulation of proprietary medium, which is processed by sequential clarification, filtration and final fill into cryobags using a sterile closed loop system. The resulting C-type retroviral particles, with an average diameter of 100 nm, are devoid of all viral genes, and are fully replication defective. The titers of the clinical lots range from $3x10e^7$ to $1x10e^9$ gene transfer units (U)/ml, and each lot is validated for requisite purity and biological potency.

*Shipping, storage, preparation, and infusion of Rexin-G*. Processed clinical-grade materials in sealed cryobags are stored in a -70±10°C freezer prior to shipment. Each lot of validated and released cryobags containing the Rexin-G vector is shipped on dry ice to the clinical site where the vector is stored in a -70±10°C freezer until used. Fifteen minutes before intravenous infusion, the vector is rapidly thawed in a 37°C water bath and immediately infused or transported on ice in a dedicated tray or cooler to the patient's room or clinical site for immediate use. Patients receive the infusion of Rexin-G via a peripheral vein or a central IV line. Various dosing regimens were used, as described in clinical studies A, B and C (below); however, a maximum volume of 8 ml/kg/dose is given once a day. Each bag of Rexin-G is infused over 10-30 min at a rate of 4 ml/min.

*Clinical studies*. Three clinical trials involving seventeen patients were conducted using intravenous infusions of Rexin-G for treating metastatic pancreatic cancer and other solid tumors; these clinical trials are listed in Table I and are referred to as Studies A, B and C.

Clinical Study A includes Phase I/II or single-use protocols investigating intravenous infusions of Rexin-G for locally advanced or metastatic pancreatic cancer following approval by the Philippine Bureau of Food and Drugs (BFAD) or by the United States Food Drug Administration (FDA), and the Institutional Review Board or Hospital Ethics Committee (24). The objectives of the study were: i) to determine the safety/toxicity of daily intravenous infusions of Rexin-G, and ii) to assess potential anti-tumor responses to intravenous infusions of Rexin-G. The protocol was designed for patients with an estimated survival time of at least 3 months. After informed consent was obtained, six patients with locally advanced unresectable or metastatic pancreatic cancer

Table I. Summary of three clinical studies using Rexin-G.

| Study | Phase | n | Patient population |
|-------|-------|---|--------------------|
| A | I/II | 6 | Locally advanced or metastatic pancreatic cancer |
| B | I/II | 11 | Metastatic cancer, various types |
| C | Expanded Access | 3 | Metastatic pancreatic and colon cancer |

were treated with repeated infusions of Rexin-G. Five of the six patients had failed standard chemotherapy; three of which completed the intra-patient dose escalation protocol in Manila, Philippines and/or in Brooklyn, NY, USA, as follows: days 1 and 2, $3.8 \times 10e^9$ U; days 3 and 4, $7.5 \times 10e^9$ U; days 5 and 6, $1.1 \times 10e^{10}$ U; days 7-10, $1.5 \times 10e^{10}$ U; rest one week; days 18-27, $1.5 \times 10e^{10}$ U. Two patients received 1 additional cycle, and one patient received 7 additional cycles. The sixth patient who presented with unresectable stage IV pancreatic cancer, received combination therapy as a first-line treatment, consisting of 6 days of IV Rexin-G ($3.8 \times 10e^9$ U/day) followed by gemcitabine (1000 mg/m$^2$) weekly for 8 weeks. For Clinical Study A, the Rexin-G preparation had a potency of $3 \times 10e^7$ U/ml.

Clinical Study B represents an expansion of Clinical Study A. Based on the encouraging results of the initial clinical experiences with Rexin-G, the Phase I/II study was expanded to further determine the safety and potential efficacy of a higher dose of Rexin-G, to extend the clinical indication to all advanced or metastatic solid tumors that are refractory to standard chemotherapy, and to adjust the treatment schedule and protocol to enable outpatient treatment. The objectives of this study were: i) to determine the safety/toxicity of daily intravenous infusions of Rexin-G, and ii) to assess potential anti-tumor responses to intravenous infusions of Rexin-G at a higher dose level. The protocol was designed for patients with an estimated survival time of at least 3 months. After informed consent was obtained, ten patients with metastatic cancer originating from either the ectoderm (melanoma, 1; squamous cell CA of larynx, 1), the mesoderm (leiomyosarcoma, 1) or the endoderm (pancreas, 2; breast, 2; uterus, 1; colon, 2), and one newly diagnosed previously untreated patient with metastatic pancreatic cancer who had refused chemotherapy (total no. of patients, 11), received intravenous Rexin-G as a single agent at a dose of $3.0 \times 10e^{10}$ U per day for a total of 20 days, according to the following treatment schedule: days 1-5, 8-12, 15-19, and 22-26; Monday to Friday with weekend rest period. An improved GMP manufacturing and bioprocessing protocol enabled the production of Rexin-G at substantially higher titers, such that the preparations used for Clinical Study B exhibited a vector potency of $7 \times 10e^8$ U/ml.

Clinical Study C involves a small group of patients who participated in an Expanded Access Program for Rexin-G for all solid tumors, a provisional program which was recently approved by the Philippine BFAD. The innovative protocol was designed to address (i.e., to reduce or eradicate) a given patient's total tumor burden as quickly, yet, as safely as possible in order to prevent or forestall 'catch up' tumor growth, and thereby minimize this confounding parameter. The estimated total dosage to be utilized was determined by an empiric calculation, referred to herein as 'The Calculus of Parity' (defined as a method of equality, as in amount, or functional equivalence). The basic formula takes into consideration the overall tumor burden, estimated from imaging studies (1 cm = $\sim 1 \times 10e^9$ cancer cells), an empiric performance coefficient ($\phi$) or physiological multiplicity of infection (P-MOI, in the terms of virology) for the targeted vector system (the P-MOI for a non-targeted vector system is essentially infinite), and the potency of the clinical-grade formulation (in U/ml). Tumor burden was measured as the sum of the longest diameters of the tumor nodules, in centimeters, multiplied by $1 \times 10e^9$ and expressed as the total number of cancer cells. An 'operationally defined' performance coefficient ($\phi$) or physiological MOI (P-MOI) of 100 for Rexin-G was based on quantitative demonstrations of enhanced transduction efficiency of the targeted gene delivery system documented in a wide variety of preclinical studies, and upon the dose-dependent performance of Rexin-G observed in the crucible of the initial clinical trials. Importantly, the generation of a high-potency Rexin-G product ($\sim 1.0 \times 10e^9$ Units/ml) enabled the administration of calculated optimal doses of Rexin-G to be delivered intravenously without the risk of volume overload. After completion of the first 20 days of Rexin-G infusions, two patients with metastatic pancreatic cancer and one patient with metastatic colon cancer opted (with additional informed consent) to continue to receive intravenous Rexin-G infusions up to a total dose of $\sim 2.5 \times 10e^{12}$ U over 6 weeks (one patient) and 16 weeks (two patients), respectively. This provided a Calculus of Parity which roughly paralleled the patients' estimated tumor burden based on CAT scan or MRI.

*Evaluation of safety and efficacy of Rexin-G.* Adverse events were graded according to the NIH Common Toxicity Criteria (CTCAE version 2 or 3) (25). To evaluate the clinical efficacy of Rexin-G, we took into consideration the general cytocidal and anti-angiogenic activities of the agent (18,19), as well as the dynamic sequestration of the pathotropic nanoparticles into metastatic lesions (19) that would affect the biodistribution or bioavailability of the targeted nanoparticles during the course of the treatment. Since the vector will accumulate more readily in certain cancerous lesions, depending on the degree of tumor invasiveness and angiogenesis, it is not expected to be distributed evenly to the rest of the tumor nodules, particularly in patients with large tumor burdens. This would predictably induce a mixed tumor response wherein some tumors may decrease in size while other tumor nodules may become bigger and/or new lesions may appear. Thereafter, with the normalization or decline of the overall tumor burden, the pathotropic surveillance function would distribute the circulating nanoparticles somewhat more uniformly. Additionally, the treated lesions may initially become larger in size due to the inflammatory reactions or cystic changes induced by the necrotic tumor. Therefore, two additional measures were used in the evaluation of objective tumor responses to Rexin-G treatment, aside from the standard

Table II. Objective tumor response, progression-free survival, and overall survival of participants in Clinical Study A.

| Patient no. Age | Objective tumor response | Progression-free survival | Status/survival after Rexin-G treatment | Overall survival from Dx |
|---|---|---|---|---|
| A1 46 years | Partial response: necrosis of primary tumor with 24% decrease in tumor size; 33-62% decrease in size supra-clavicular lymph nodes. Symptomatic relief of pain | 3.5 months | Expired 10 months | 23 months |
| A2 55 years | Partial response (RECIST): 47% decrease in primary tumor volume, followed by complete disappearance of the tumor. Symptomatic relief of pain | 9 months | Expired 13 months | 25 months |
| A3 45 years | Partial response (RECIST): 47% decrease in primary tumor volume; disappearance of 6 of 8 liver nodules; apoptosis and necrosis of liver nodules in biopsied liver. Symptomatic relief of pain | 4 months | Expired 9 months | 19 months |
| A4 64 years | Partial response/stable disease: disappearance of 5 of 11 liver nodules; stable primary | 2 months | Expired 8 months | 48 months |
| A5 53 years | Stable disease: no change in primary tumor; one of 3 liver nodules disappeared | 2 months | Expired 10 months | 30 months |
| A6 46 years | Partial response (RECIST): 30% decrease in primary tumor volume; disappearance of 13 of 18 liver nodules | 5 months | Expired 7 months | 7 months |

Response Evaluation Criteria in Solid Tumors (RECIST; 26): i) O'Reilly's *et al* formula for estimation of tumor volume: L x W$^2$ x 0.52 (27), and ii) the induction of necrosis or cystic changes in tumors during the treatment period. Thus, a decrease in the tumor volume of a target lesion of ≥30%, or the induction of necrosis or cystic changes within the tumor were considered partial responses (PR) or positive effects of treatment.

*Statistical analysis*. For Clinical Study A, the one-sided exact test was used to determine the significance of differences between the PRs of patients treated with Rexin-G and historical controls with an expected 5% PR.

## Results

*Clinical Study A*. This initial Phase I/II study examines the safety and potential efficacy of an intra-patient dose escalation protocol. As shown in Table II, partial responses (PR) of varying degrees were noted in five out of six patients treated with Rexin-G while stable disease was observed in the remaining patient. Three of six (50%) patients had a ≥30% decrease in tumor size by RECIST or by tumor volume measurement, and two of six (33%) patients had necrosis of either the primary tumor or metastatic nodules by biopsy and/or by follow-up MRI/CAT scan. Further analysis of one particular patient (A3), in whom 6 of 8 liver tumor nodules disappeared by CT scan, was facilitated by means of a liver biopsy, which revealed an increased incidence of apoptosis,

necrosis, and fibrosis within the tumor nodules similar to that observed in preclinical studies (18,19), along with the observation of numerous tumor infiltrating lymphocytes in the residual liver tumors of the biopsied liver (Figs. 1-3). The presence of immunoreactive T and B lymphocytes infiltrating the residual liver tumors (Fig. 3) indicates that Rexin-G does not suppress local immune responses. Progression-free survival was greater than 3 months in four of six (67%) patients. Median survival after Rexin-G treatment in chemotherapy-resistant patients was 10 months, and median survival after diagnosis was 25 months. In contrast, the reported median survival of patients with pancreatic cancer who received either gemcitabine or 5-FU (standard treatments) as a first-line drug was 5.65 and 4.41 months after diagnosis, respectively (28). Using the one-sided exact test, the significance level of partial responses in Rexin-G-treated patients was <0.025 when compared to the PR rates of historical controls. These initial findings, albeit documented in a relatively small number of patients, are sufficient to indicate that Rexin-G is clinically effective, even in modest doses, is clearly superior to no medical treatment, and may be superior to gemcitabine when used as a single agent for the treatment of patients with advanced or metastatic pancreatic cancer.

All six patients tolerated the Rexin-G infusions well with no associated nausea or vomiting, diarrhea, mucositis, hair loss, or neuropathy. Three of six (50%) patients had symptomatic relief of pain. There was no significant alteration in hemodynamic function, bone marrow suppression, liver,
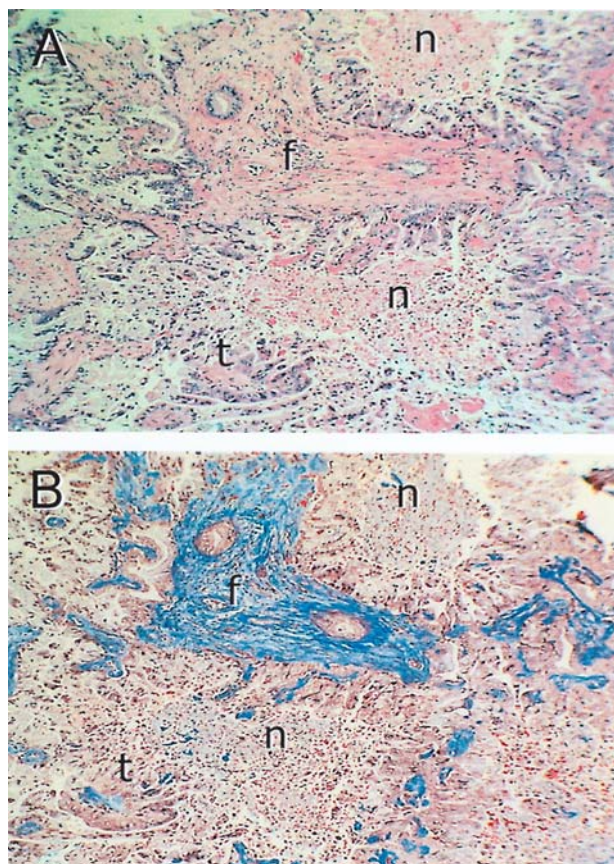
Figure 1. Intravenous Rexin-G induces necrosis and fibrosis in metastatic tumor nodules, as observed in surgically excised liver sections from a patient with Stage IV pancreatic cancer (Patient A3). (A) Representative hematoxylin and eosin-stained tissue section of a tumor nodule in biopsied liver; t, tumor cells; n, necrosis; f, fibrosis. (B) Trichrome stain of a tissue section of the same tumor nodule. Blue-staining material indicates presence of collagenous proteins in fibrotic areas.
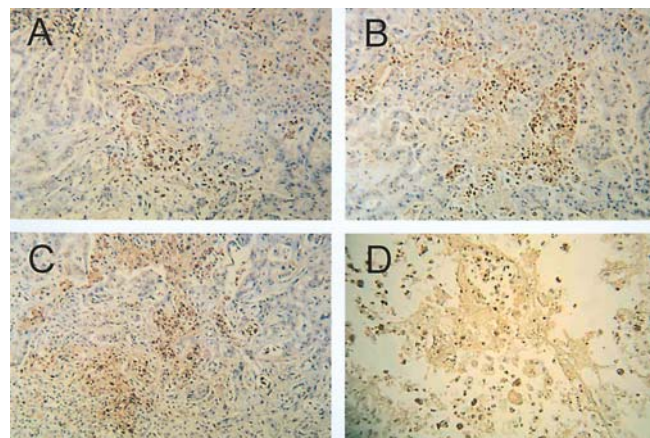


Figure 2. Intravenous Rexin-G induces overt apoptosis in metastatic tumor nodules in a patient with pancreatic cancer (Patient A3). (A-D) Representative immunostained tissue sections of tumor nodules from biopsied liver indicating an appreciable incidence of Tunel-positive apoptotic nuclei (brown-staining material).
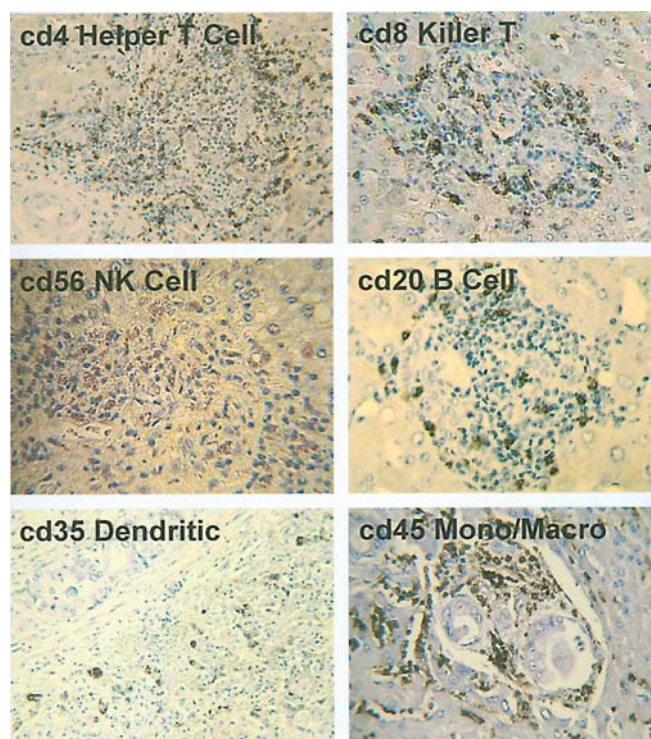


Figure 3. Immunohistochemical characterization of tumor infiltrating lymphocytes (TILs) in metastatic tumor nodules excised from a Rexin-G-treated patient with pancreatic cancer (Patient A3). Representative tissue sections of residual tumor nodules within the biopsied liver show significant TIL infiltration with a functional complement of immunoreactive T and B cells. Clockwise from upper left: helper T cells (CD4+), killer T cells (CD8+), B cells (CD20+), monocyte/macrophages (CD45+), dendritic cells (CD35+), and natural killer cells (CD56+). Note, the presence (i.e., migration) of a cadre of TILs that function in the context of cell-mediated and humoral immunity, suggesting the potential for cancer immunization in an immune competent host.

kidney or any organ dysfunction that was related to the investigational agent. The only adverse events that were attributed as definitely related to the investigational agent were generalized rash and urticaria in two of six patients (grade 1-2), and those attributed as possibly related were chills and fever in two of six patients (grade I). The limited number of treatment-emergent adverse events observed in this study suggests that Rexin-G administered intravenously at these escalating doses is a relatively safe therapy.

*Clinical Study B.* This study extends the initial Phase I/II pancreatic cancer protocols with dose intensification and expanded clinical application to all solid tumors. As shown in Table III, partial responses of varying degrees of either the primary tumor or the metastatic nodules were noted in seven of eleven (64%) patients. Five of eleven (45%) patients developed necrosis and apoptosis of the primary tumors and/or metastatic nodules by either biopsy or CT scan, and five of eleven (45%) patients had >30% reduction in the size of the primary tumor or metastatic nodules by RECIST or tumor volume measurement. Two of eleven patients had stable disease, one patient with massive tumor burden had a mixed tumor response and one patient with a large tumor burden (~50 liver nodules) had progressive disease.

Progressive reduction of cancerous lymph nodes with repeated infusions of Rexin-G was consistently observed in patients with pancreatic cancer, and again in patients with

Table III. Objective tumor response, progression-free survival, and overall survival of participants in Clinical Study B.

| Patient no., age, and Dx | Overall tumor response (symptomatic relief, Caliper, CT scan and MRI) | Progression-free survival | Status/survival after Rexin-G treatment | Overall survival from diagnosis |
|---|---|---|---|---|
| B1 53 years Breast cancer | Partial response (RECIST): apoptosis and necrosis of tumor nodule by biopsy; 50% decrease in supra-clavicular node by PET/CT scan | 3 months | Alive >13 months | >6.6 years |
| B2 58 years Uterine cancer | Partial response: necrosis of supraclavicular lymph nodes by CT scan; 33% decrease in cervical lymph node by calipers. Symptomatic relief from nerve pain | 3 months | Expired 4 months | 2 years 4 months |
| B3 52 years Breast cancer | Stable disease: no interval change in pulmonary nodules. Symptomatic relief from coughing and bone pain | 2 months | Alive >7 months | >3 years 5 months |
| B4 41 years Melanoma | Partial response: necrosis and apoptosis of biopsied tumor nodules; 50% decrease in tumor volume by CT scan | 3 months | Alive >6 months | >15 months |
| B5 53 years Pancreatic cancer | Progressive disease. Symptomatic relief from pain | N.A. | Alive >6 months | >11 months |
| B6 48 years Squamous cell carcinoma, larynx | Partial response (RECIST): 300% increase in upper air-way diameter; stable lung nodules. Regained voice | 3 months | Alive >6 months | >24 months |
| B7 34 years Colon cancer | Mixed tumor response: 20% decrease in cervical lymph node, decrease in size of lung nodules, decrease in number of lung nodules in upper lung, increase in number of nodules in lower lung, no interval change in retroperitoneal lymph nodes | 2 months | Alive >6 months | >3 years |
| B8 64 years Leiomyosarcoma | Partial response: necrosis by CT scan and 7% decrease in size of thigh tumor mass with softening. Symptomatic relief: disappearance of throbbing sensation due to external compression of femoral artery | 2 months | Expired 4.5 months | 13 years |
| B9 67 years Colon cancer | Stable disease. Symptomatic relief from pain | >6 months | Alive >6 months | >1 year 6 months |
| B10 60 years Pancreatic cancer | Partial response: necrosis and cystic conversion of primary tumor and cystic conversion or disappearance of liver nodules. Symptomatic relief from pain | 3 months | Expired 3 months | 9 months |
| B11 68 years Pancreatic cancer | Partial response (RECIST): 49% decrease in primary tumor volume, decrease in number (from 26 to 12 nodules) and size of metastatic lung nodules. Symptomatic relief from pain | >6 months | Alive >6 months | >6 months |

uterine cancer, colon cancer, breast cancer and malignant melanoma, which is remarkable and meaningful in terms of understanding the pertinent pharmacodynamics. While it is

well known that sentinel lymph node(s), the first lymph node(s) to which cancer is likely to spread from a primary tumor, are of considerable importance to our understanding
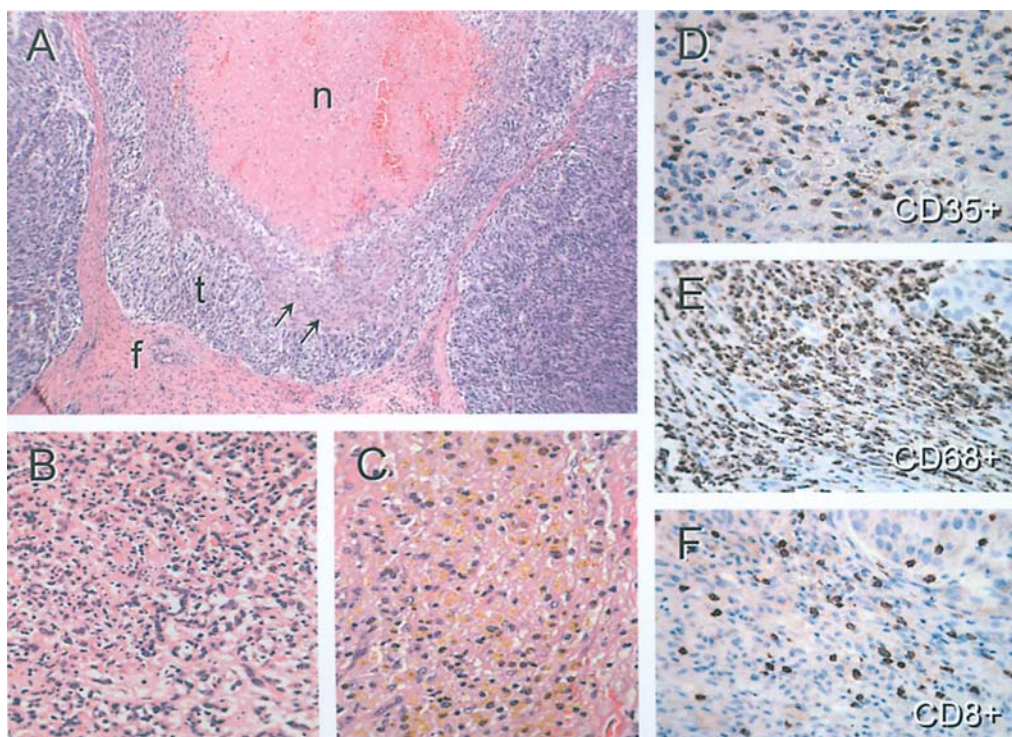
Figure 4. Intravenous Rexin-G induces necrosis, apoptosis and fibrosis in a cancerous lymph node of a patient with malignant melanoma (Patient B4). (A) H&E-stained tissue sections of inguinal lymph node revealing extensive necrosis (n), apoptosis (indicated by arrows) and fibrosis (f) of cancer cells with a rim of viable tumor cells in the periphery (t). (B) Higher magnification (x100) of sections of A showing numerous cells undergoing apoptosis indicated by small cells with pyknotic or fragmented nuclei. (C) Higher magnification (x100) of A revealing golden-yellow hemosiderin-laden macrophages. (D) Representative tissue sections of inguinal lymph node showing significant infiltration with immunoreactive CD35^{+} dendritic cells, (E) CD68^{+} macrophages and (F) CD8^{+} killer T cells.

of the pathogenesis, diagnosis, and prospective treatment of metastatic disease, the conspicuous penetrance of Rexin-G into both regional and distant lymph nodes is both striking and auspicious (Tables II and III). The clinical significance of the finding that the pathotropic nanoparticles in Rexin-G retain their bioactivity as they circulate throughout the body, not only accumulating in primary and metastatic lesions but also draining into lymph nodes with therapeutic impact, cannot be overstated. As shown in Fig. 4, a surgical biopsy of a cancerous lymph node from the inguinal region of a patient with malignant melanoma showed substantial necrosis (Fig. 4A), large areas of overt apoptosis, (Fig. 4B), and zones wherein hemosiderin-laden macrophages (Fig. 4C) are evacuating tumor debris. Moreover, immunohistochemical staining revealed significant mononuclear infiltrations with CD35^{+} dendritic cells (Fig. 4D), CD68^{+} macrophages (Fig. 4E), CD8^{+} killer T cells (Fig. 4F), and CD4^{+} helper T cells (not shown). The realization that the gene delivery function (i.e., cytocidal activity) of pathotropic nanoparticles remains active as it penetrates metastatic disease within sentinel lymph nodes, and does not disrupt but appears to work in concert with the immune system, reaffirms the potentiality of future cancer vaccinations *in situ*, using this targeted gene delivery system bearing a cytokine gene.

In another patient with squamous cell carcinoma of the larynx, a dramatic re-opening of the upper airway was documented by neck MRI (Fig. 5), which correlated with the patient regaining her voice. Progression-free survival ranged from 1 to >5 months. Median survival time was >6 months
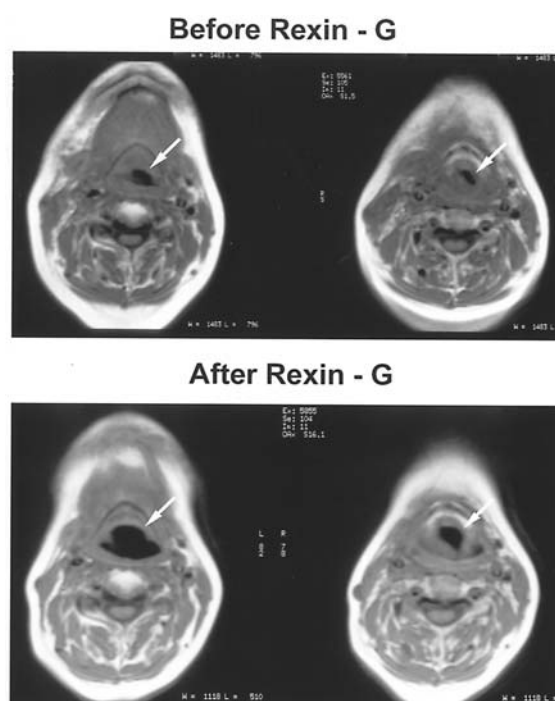


Figure 5. Evidence of tumor regression in a patient with squamous cell carcinoma of the larynx (Patient B6). MRI images of the neck region obtained before (upper panel) and after (lower panel) Rexin-G treatment. Measurement of the diameters of serial sections of the upper airway shows a dramatic (~300%) increase in the upper airway diameters after repeated infusions of Rexin-G when compared to sections obtained prior to treatment (indicated by white arrows). The increased patency of the airway corresponded to regression of the surrounding tumor mass, and a return of vocal capabilities.
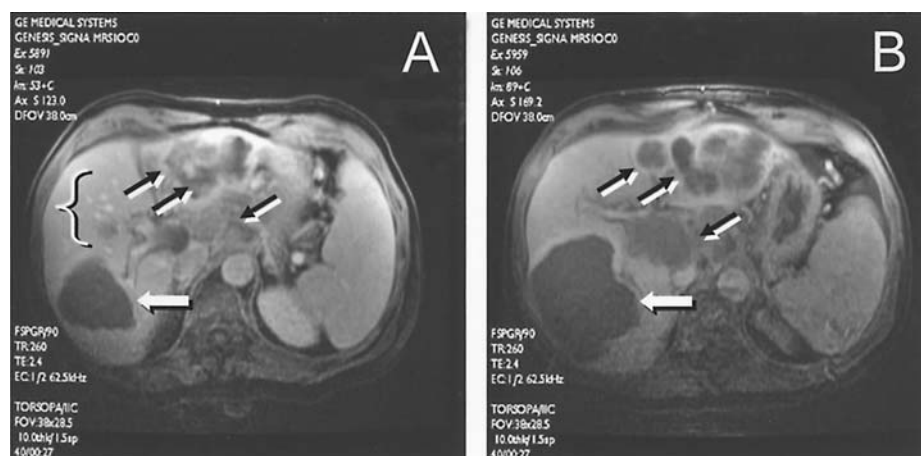
Figure 6. Effects of Rexin G infusions on the number and quality of hepatic metastatic lesions observed in a pancreatic cancer patient exhibiting a massive tumor burden (Patient C1). Abdominal MRI obtained (A) before treatment and (B) after treatment with calculated (Calculus of Parity) dose-dense infusions of Rexin-G. Note the complete eradication of numerous small dense tumor nodules in the upper left quadrant of the image (bracketed), as well as cystic conversion of established liver nodules (black arrows). Subsequent aspiration of the enlarged liver cyst (white arrow) followed by cytological analysis confirmed the complete absence of cancer cells in the aspirates following the treatment.

from the start of Rexin-G treatment, and >24 months from diagnosis. Eight of eleven (72%) patients lived/are alive >6-13 months after treatment with Rexin-G. Taken together, Rexin-G appears to have single-agent activity in a broad spectrum of resistant tumor types. Further, it was noted that sustained therapeutic benefit was observed in the majority of the patients despite the brevity of the treatment.

All eleven patients tolerated the vector infusions well with no associated nausea or vomiting, diarrhea, mucositis, hair loss or neuropathy. Eight of eleven (73%) had symptomatic relief of pain, bloating, throbbing, hoarseness, and fatigue. There was no significant alteration in hemodynamic function, bone marrow suppression, liver, kidney or any organ dysfunction that was related to the investigational agent. The absence of treatment-related adverse events further suggests that, even in increased vector doses, Rexin-G is a relatively safe therapy. At this point, the absence of dose limiting toxicity, combined with compelling indications of single-agent efficacy in a variety of different tumor types and the recent availability of higher potency formulations of Rexin-G encouraged the advancement and regulatory approval of clinical trials designed to focus on increased clinical efficacy and the optimization of treatment protocols.

*Clinical Study C*. This study represents the initial report of clinical experience in an Expanded Access Program for Rexin-G for treating all solid tumors, introducing an innovative personalized dose-dense regimen referred to as the Calculus of Parity. In this preliminary yet important interim analysis, dramatic responses were noted in all three patients, each with an extensive tumor burden. In one patient (C1), the Calculus of Parity (or functional equivalence) approximated a cumulative dosage that led to liquefaction necrosis and cystic conversion of the unresectable pancreatic tumor and either cystic conversion or disappearance of all metastatic liver nodules on follow-up MRI (Fig. 6). Aspiration of one cystic tumor nodule was negative for malignant cells. In the second patient (C2), suffering from stage IV colon cancer, a cumulative dosage

approaching the predetermined Calculus of Parity was effective in reducing the bulk of the metastatic disease: 84% necrosis observed in the liver tumor nodules was documented by image analysis. In the third patient (C3), a significant decrease in the primary pancreatic tumor and in the number (from 28 to 12 lung nodules) and the size of pulmonary nodules were noted by CT scan. Progression-free survival and overall survival was >6 months after Rexin-G treatment in two patients. These findings provide preliminary evidence to support the hypothesis that the Calculus of Parity may be used to determine the total cumulative dose of Rexin-G that would be needed to address a given patient's tumor burden, and thereby comprise an optimal induction regimen.

All three patients tolerated the vector infusions well with no associated nausea or vomiting, diarrhea, mucositis, hair loss or neuropathy. There were no acute alterations in hemodynamic function, bone marrow suppression, liver, kidney or any organ dysfunction that was related to the investigational agent. Two patients did develop anemia requiring red cell transfusion (grade 3), which was attributed as possibly related to subsequent bleeding into the necrotic tumors. One patient developed sporadic episodes of thrombocytopenia (grade 1-2) which was attributed as possibly related to the investigational agent. One patient died of acute fulminant staph epidermidis septicemia 3 months after Rexin-G treatment, which was not attributed to the investigational agent. The results of this patient's autopsy showed almost complete necrosis of the residual pancreatic tumor, and 75-95% necrosis of the metastatic tumors remaining in the liver and abdominal mesentery, with normal histology recorded in the bone marrow, heart, and brain. The lack of systemic toxicity associated with Rexin-G administration underscores the potential advantages of Rexin-G over standard chemotherapy in terms of efficacy in managing metastatic cancer, as well as other quality-of-life measures. In each case, the extent of the overall tumor destruction was impressive. The demonstration that a dose-dense regimen of Rexin-G, specifically tailored to overcome a patient's tumor burden, is capable of achieving

these levels of efficacy underscores the need to further refine the Calculus of Parity, to define the optimal rate(s) of tumor eradication, and to discern the optimal supportive care for a patient undergoing post-tumoricidal wound healing.

## Discussion

Targeted biologic therapies in general, and targeted gene delivery systems, in particular, are expected to change the way cancer therapy will be administered, evaluated, and regulated in the future. The advent of pathotropic targeting, a disease-seeking technology platform embodied in the intricate design of Rexin-G has ushered genetic medicine, as well as medical nanotechnology, across the threshold of history, with unprecedented demonstrations of single-agent efficacy. Although the total numbers of patients treated to date with this pioneering genetic medicine are still quite small, the high percentage of objective clinical responses enables tentative conclusions to be made. Critical analysis of the initial Phase I/II studies cautiously evaluated general safety considerations, while gradually escalating doses (intra-patient) to a point at which efficacy was observed in pancreatic cancer (24). Expanding the dose-escalation protocols to include higher cumulative doses, as well as additional tumor types provided additional evidence of the overall safety and efficacy of the treatment. Rapid and systematic sharing of comprehensive information and records with all regulatory authorities in both the USA and the Philippines facilitated the critical analysis and expedited the advancement of the clinical trials. Based on the initial demonstrations of safety and efficacy of Rexin-G in pancreatic cancer, the United States FDA granted Orphan Drug status to Rexin-G as an effective treatment for pancreatic cancer, while the Philippine BFAD approved limited commercialization of Rexin-G through an Expanded Access program for all solid tumors considered resistant to standard chemotherapy.

The recent availability of a very high-potency preparation $(1-5 \times 10^9$ U/ml) of Rexin-G has overcome the problems of infusion volume and dosing limitations to a remarkable extent, and enabled the advancement of the investigations to include strategic dose-dense regimens defined as the Calculus of Parity. Taken as a whole, the ongoing clinical studies presented in this report serve to validate the clinical utility of pathotropic targeting, the relative safety of intravenous Rexin-G, and the dose-dependent efficacy of this targeted genetic medicine in the treatment of incurable metastatic cancer. Moreover, as analysis of each clinical experience benefits the next, these studies provide new insights regarding strategic dosing, patient monitoring, critical evaluation of tumor responses, and a general appreciation of the need for expedited clinical development. After all, it is apparent from our studies that Rexin-G positively impacts overall survival and the quality-of-life of patients with advanced metastatic cancer.

*Tumor kinetics and the 'Calculus of Parity' for personalized cancer medicine.* In cancer therapy, a critical factor influencing the efficacy of an investigational agent is the extent of the tumor burden. Often, the margin of safety of a test drug is too narrow because dose-limiting toxicity is reached prior to gaining tumor control. Thus, the development of a cancer drug that can actually address the tumor burden without eliciting dose-limiting side effects or organ damage represents a significant milestone and advancement in cancer treatment. Another important problem is the natural kinetics of cancer growth, which requires an appropriate kinetic solution. Historic models of tumor growth are now considered overly simplistic (29,30), yet these simplistic models greatly influenced the development of standards of cancer treatment that are still enforced today; including the use of drugs in combination, and in equally spaced cycles of equal intensity. While the prediction that tumor shrinkage is correlated with improved prognosis is certainly true, the prediction that giving conventional drugs long enough would lead to tumor eradication has been proved false (31). Appreciation of a more complex kinetics, as described by Benjamin Gompertz and formalized as the Norton-Simon model, takes into account the dynamics of metastasis and the quantitative relationship between tumor burden and metastatic potential in its predictions. Thus, the concept of dose-dense chemotherapies emerged, which emphasized the optimal doses of drugs that cause regression of the tumor over shorter time intervals and favored sequential rather than combinatorial approaches (31,32). Subsequently, a number of clinical trials provided supportive evidence that giving drugs more densely made a significant difference in terms of optimizing cancer cell kill.

The introduction of pathotropic nanoparticles for targeted gene delivery enables a new and quantitative approach to treating metastatic cancer in a unique and strategic manner. Developed conceptually by Gordon and Hall, the Calculus of Parity described herein represents an emergent paradigm that seeks to meet and to match a given tumor burden in a highly compressed period of time; in other words, a dose-dense induction regimen based quantitatively on best estimates of total tumor burden. The Calculus of Parity assumes the following from the outset: i) that the therapeutic agent (in this case Rexin-G) is adequately targeted such that physiological barriers including dilution, turbulence, flow, diffusion barriers, filtration, inactivation, and clearance are sufficiently counteracted such that a physiological performance coefficient ($\phi$) or P-MOI can be calculated; ii) that the agent is effective at levels that do not confer restrictive dose-limiting toxicities; and iii) that the agent is available in sufficiently high concentrations to allow for intravenous administration of the personalized doses without inducing volume overload. To calculate the optimal daily dosage of Rexin-G, the following factors were taken into consideration: i) the total tumor burden based on radiologic imaging studies; ii) the physiological performance coefficient ($\phi$) of the system, which specifies the multiplicity of inducible gene transfer units needed per target cancer cell; and iii) the precise potency of the vector in terms of gene transfer units (U) per ml. The Calculus of Parity predicts that tumor control can be achieved if the dose of the targeted vector administered is equivalent to the emergent tumor burden; yet the total dosage should be administered in as short a period of time as considered safely possible, in order to prevent catch-up tumor growth while allowing time for the reticuloendothelial system to eliminate the resulting tumor debris (18). Our preliminary clinical experience with this calculus (see Study C) is limited to three patients, each with relatively large tumor burdens; however, the dramatic responses

observed in two patients who failed standard chemotherapy and in one patient who refused standard chemotherapy (100% response rate) underscores both the potential utility and the urgent need for further studies of the quantitative approach.

*To RECIST or not to RECIST*. The advent of targeted therapies, including targeted gene therapy, is also changing the way tumor responses to a cancer drug are being evaluated. The guiding principle in cancer therapy has been that the therapeutic benefit gained from a prospective chemotherapeutic agent must outweigh the risk of serious or fatal systemic toxicity induced by the drug candidate. To this end, the Response Evaluation Criteria in Solid Tumors (RECIST) was developed by the National Cancer Institute (NCI), Bethesda, MD, USA, and has been employed by most, if not all, academic institutions as the universal standard for tumor response evaluations (27). Specifically, an objective tumor response (OTR) has, until recently, been considered the golden standard of success in evaluating cancer therapy for solid tumors. An OTR consists of at least a 30% reduction in the size of target lesions and/or complete disappearance of metastatic foci or non-target lesions. However, many biologic response modifiers of cancer are, in fact, not associated with tumor shrinkage, but have been shown to prolong progression-free survival (PFS), and overall survival (OS) (33). Hence, the response to effective biologic agents is often physiologic and RECIST may no longer be the appropriate standard for evaluation of tumor response to biologic therapies. Thus, alternative surrogate end-points such as measurements of tumor density (an index of necrosis), blood flow and glucose utilization in tumors, and other refinements of imaging methods used to evaluate the mechanisms of tumor response are required.

Understanding the disease process, as well as the intended mechanisms of action of the proposed intervention is, therefore, critical in predicting the effect of the treatment on a given clinical end-point. In the case of tumor responses to Rexin-G, wherein the primary mechanism of action is the induction of apoptosis in proliferative tumor cells and attendant angiogenic vasculature, necrosis and cystic changes within the tumor often occur. This is due to the targeted disruption of a tumor's blood supply which starves the tumor, resulting in subsequent necrosis within the tumor. In tumors of Rexin-G-treated patients, wherein apoptosis is a predominant feature, the tumors simply shrink and disappear in follow-up imaging studies. However, in tumors wherein necrosis is a prominent feature, the size of the tumors may actually become larger after Rexin-G treatment, due to the inflammatory reaction evoked by the necrotic tumor and cystic conversion of the tumor. In this case, an increase in the size of tumor nodules on CT scan, PET scan or MRI does not necessarily indicate disease progression. Therefore, additional concomitant evaluations that reflect the histological quality of the treated tumors are needed to more accurately determine the extent of necrosis or cystic changes induced by Rexin-G treatment.

The tumor responses observed in Clinical Study C further strengthen these arguments. In the two patients who received an optimal dose of Rexin-G based on the 'Calculus of Parity', an aggregate increase in the size of the tumor nodules was noted. However, examination of the nature of the lesions showed >95% liquefaction necrosis in all the tumor nodules

of Patient C1 by MRI, while 84% necrosis within the tumor nodules of Patient C2 was noted by PET scan. In Patient C3, there was a significant decrease in both the number and size of the primary tumor and metastatic foci two weeks after completion of Rexin-G therapy, indicating that apoptosis was a prominent feature in Patient C3's tumor response to Rexin-G. Thus, careful attention to the quality of the treated tumors in Patients C1 and C2 resulted in a more accurate evaluation of tumor response which influenced the decision to continue, rather than discontinue, treatment with Rexin-G. Conversely, failure to consider the necrotic and cystic changes within the tumor would have resulted in an erroneous diagnosis of progressive disease and an imprecise conclusion of lack of Rexin-G activity in Patient C1's and C2's tumors. In summary, the understanding of events that precede and accompany tumor eradication by Rexin-G, i.e., apoptosis, necrosis, tumor lymphocytic infiltration, cystic changes, and reactive fibrosis and stroma formation, first seen in preclinical studies (18,19,34) and now documented in clinical trials, indicate that the historical RECIST criteria may be inadequate at best and, at times, misleading. Finally, while it would be desirable to deliver Rexin-G much earlier in the course of the disease process, well before the metastatic tumor burden has reached such massive proportions, it is important to note that these determinations of clinical efficacy were performed in the most resistant types of cancer.

*The oncologic 'threshold of credibility' paradigm developed by the FDA for accelerated approval of investigational cancer agents*. Finally, the development and advancement of targeted therapies, including pathotropic nanoparticle-mediated gene delivery systems, will hopefully impact the way these agents are regulated and approved by the United States FDA and other regulatory agencies worldwide (35,36). In recent years, the FDA approved certain small molecule agents and biologic response modifiers for the treatment of advanced cancer based on surrogate end-points that showed a meaningful clinical benefit, such as enhanced quality-of-life, longer progression-free survival, and overall survival (28,33). According to the FDA's 'The Pink Sheet' for prescription pharmaceuticals and biotechnology products (35), oncologic approval could be based on surpassing a threshold of credibility rather than demonstrating statistical benefit. The threshold of credibility would be achieved when relatively small numbers (20-30 patients) demonstrate a meaningful clinical effect in a single arm confirmatory clinical trial. The oncologic threshold of credibility paradigm was developed in an attempt to stream-line the approval process for highly active entities by reducing the size of the trials and the complexity of statistical analysis. This approach is particularly effective in allowing seminal clinical trials to expand the scope of potential applications, as in the case of Rexin-G for all solid tumors, while advancing the investigational agent in an orderly manner.

Based on preliminary yet consistent observations of overall safety and efficacy in the management of incurable metastatic disease, Rexin-G merits expedited evaluation. Moving forward with the clinical development of Rexin-G as a potentially important treatment for metastatic pancreatic cancer, the present strategy is to conduct a Phase II single arm study in a limited number of patients who have failed standard

chemotherapy. The development plan we have chosen addresses the most difficult clinical problems in cancer therapy, as these tumors are the most resistant and universally recognized refractory tumors. The initial demonstrations of both benefit and safety in these most difficult patients 'raises the bar' for biologic therapy. Based on our recent human experience with Rexin-G, we anticipate that the clinical protocol will be guided by the Calculus of Parity and will consist of a dose-dense induction regimen wherein optimal doses of Rexin-G will be given over a relatively short period of time (4-8 weeks) to be followed by a maintenance regimen based on estimates of residual tumor burden. The efficacy endpoints of the planned Phase II trial would involve a thorough assessment of quality-of-life parameters, measurement of tumor volume and tumor density in target lesions, identification of necrosis, inflammatory infiltrations, and/or cystic changes in tumors, in addition to the standard methods of evaluating tumor responses, including OTR, PFS and OS. As the number of treated patients increases, evaluations will be performed categorically, in each individual tumor type, as well as collectively in aggregate.

In conclusion, we have reported the results of three clinical studies using Rexin-G, a new biologic agent that has been developed for the treatment of metastatic cancer. Based on these studies, the following conclusions are presented: i) the functionality of the gene delivery system is profound, ii) the genetic construct exhibits broad spectrum activity in many resistant tumor types, and iii) the targeted genetic medicine is exceptionally safe. The lack of systemic toxicity, taken together with the reduction of tumor burden and the enhanced quality-of-life experienced by patients receiving Rexin-G infusions, constitute meaningful clinical benefits that underscore the need for the expedited development of Rexin-G for pancreatic cancer and, potentially, for all solid tumors.

## Acknowledgements

## References

1. Kuvshinoff BW and Bryer MP: Treatment of respectable and locally advanced pancreatic cancer. Cancer Control 7: 428-436, 2000.
2. Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Fay MP, Feuer EJ and Edwards BK (eds): In: SEER Cancer Statistics Review, National Cancer Institute, Bethesda, MD, Tables I-1, XXI-5, 1975-2000.
3. Van Riel JM, Giaccone G and Pinedo HM: Pancreaticobiliary cancer: the future aspects of medical oncology. Ann Oncol 10: 296-299, 1999.
4. El Kamar FG and Grossbard ML and Kozuch PS: Metastatic pancreatic cancer: emerging strategies in chemotherapy and palliative care. Oncologist 8: 18-34, 2003.
5. Rosemurgy AS and Serafini FM: New directions in systemic therapy of pancreatic cancer. Cancer Control 7: 437-444, 2000.
6. Kosuri K, Muscarella P and Bekaii-Saab TS: Updated and controversies in the treatment of pancreatic cancer. Clin Adv Heamatol Oncol 4: 47-54, 2006.
7. Macdonald JS: Clinical overview: adjuvant therapy of gastro-intestinal cancer. Cancer Chemother Pharmacol 54: S4-S11, 2004.
8. Czito BG, Bendell J and Willett CG: Radiation therapy for resectable colon cancer. Is there a role in the modern chemo-therapy era? Oncology 20: 1787, 2006.
9. Lieberman SM, Horig H and Kaufman HL: Innovative treatments for pancreatic cancer. Surg Clin North Am 81: 715-739, 2001.
10. Tseng JF and Mulligan RC: Gene therapy for pancreatic cancer. Surg Oncol Clin North Am 11: 537-569, 2002.
11. Zwiebel JA: Cancer gene and oncolytic virus therapy. Semin Oncol 28: 336-343, 2001.
12. Friedmann T: The road toward human gene therapy - a 25-year perspective. Ann Med 29: 575-577, 1997.
13. Verma I and Somia N: Gene therapy: promises, problems and prospects. Nature 389: 239-242, 1997.
14. Anderson WF: Human gene therapy. Nature 392: 25-30, 1998.
15. Peng KW and Russell SJ: Viral vector targeting. Curr Opin Biotechnol: 454-457, 1999.
16. Hall FL, Gordon EM, Wu L, Zhu NL, Skotzko MJ, Starnes VA and Anderson WF: Targeting retroviral vectors to vascular lesions by genetic engineering of the MoMuLV gp70 envelope protein. Hum Gene Ther 8: 2183-2192, 1997.
17. Hall FL, Liu L, Zhu NL, Stapfer M, Anderson WF, Beart RW and Gordon EM: Molecular engineering of matrix-targeted retroviral vectors incorporating a surveillance function inherent in von Willebrand factor. Hum Gene Ther 11: 983-993, 2000.
18. Gordon EM, Liu PX, Zhen ZH, Liu L, Whitley MD, Gee C, Groshen S, Hinton DR, Beart RW and Hall FL: Inhibition of metastatic tumor growth in nude mice by portal vein infusions of matrix-targeted retroviral vectors bearing a cytocidal cyclin G1 construct. Cancer Res 60: 3343-3347, 2000.
19. Gordon EM, Liu PX, Chen ZH, Liu L, Whitley MD, Liu L, Wei D, Groshen S, Hinton DR, Beart RW, Anderson WF and Hall FL: Systemic administration of a matrix-targeted retroviral vector is efficacious for cancer gene therapy in mice. Hum Gene Ther 12: 193-204, 2001.
20. Fidler IJ: Modulation of the organ microenvironment for treatment of cancer metastasis. J Natl Cancer Inst 87: 1588-1592, 1995.
21. Hanahan D and Folkman J: Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353-364, 1996.
22. Stromblad S and Cheresh DA: Cell adhesion and angiogenesis. Trends Cell Biol 64: 462-467, 1996.
23. Miller DG, Adam MA and Miller AD: Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 10L: 4239-4242, 1990.
24. Gordon EM, Cornelio GH, Lorenzo CC, Levy JP, Reed RA, Liu L and Hall FL: First clinical experience Using a 'Pathotropic' injectable retroviral vector (Rexin-G) as intervention for Stage IV pancreatic cancer. Int J Oncol 24: 177-185, 2004.
25. Common Toxicity Criteria Version 2.0. Cancer Therapy Evaluation Program. DCTD, NCI, NIH, DHHS, March, 1998.
26. Therasse P, Arbuck S, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 92: 205-216, 2000.
27. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR and Folkman J: Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 88: 277-285, 1997.
28. Burris HA, Moore JM, Anderson J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarasoff P, Nelson R, Dorr FA, Stephens CD and von Hoof DD: Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 15: 2403-2413, 1997.
29. Heitjan DF: Generalized Norton-Simon models of tumour growth. Stat Med 10: 1075-1088, 1991.

30. Norton L: Conceptual and practical implications of breast tissue geometry: toward a more effective, less toxic therapy. Oncologist 10: 370-381, 2005.
31. Norton L: Use of dose-dense chemotherapy in the management of breast cancer. Clin Adv Hematol Oncol 4: 36-37, 2006.
32. Fornier M and Norton L: Dose-dense adjuvant chemotherapy for primary breast cancer. Breast Cancer Res 7: 64-69, 2005.
33. Abeloff MD: Perspective: new end-points needed in targeted Rx trials. Oncol News Int 15: 2-16, 2006.
34. Chen DS, Zhu NL, Hung G, Skotzko MJ, Hinton D, Tolo V, Hall FL, Anderson WF and Gordon EM: Retroviral vector-mediated transfer of an antisense cyclin G1 construct inhibits osteosarcoma tumor growth in nude mice. Hum Gene Ther 8: 1679-1686, 1997.
35. The FDA 'The Pink Sheet' for prescription pharmaceuticals and biotechnology, 2004.
36. Hirschfeld A and Pazdur R: Oncology drug development: United States Food and Drug Administration perspective. Crit Rev Oncol Hematol 42: 137-143, 2002.