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Critical Stages in the Development of the First Targeted, Injectable Molecular-Genetic Medicine for Cancer

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1. Introduction

Quietly, cautiously, and steadily, the field of modern medicine recently progressed across a new threshold with the development of Rexin-G, the first and so far, only, targeted injectable molecular genetic medicine to be validated in the clinic. Designed to function within the context and complexities of the human circulatory system, the "smart," "stealth," "highly-selective" nanoparticles embodied in Rexin-G travel beyond the reach of the most gifted surgeons, beyond the horizons of the finest catheters, to seek out the biochemical hallmarks of invasive cancers, and to deliver a lethal genetic payload where it is needed most—i.e., targeting the histopathology of the tumor microenvironment. In this invited review, we elaborate upon the critical stages of scientific discovery, molecular-genetic target validation, preclinical studies, pathotropic (disease-seeking) platform development, clinical trial design, molecular pharmacology, regulatory considerations, and GMP production & bioprocessing that, taken together, define the advancement of this tumor-targeted genetic medicine for cancer. In the course of delineating the developmental trajectory of Rexin-G into a series of logical and discrete stages, the authors have endeavored to extract, abstract, and represent a host of molecular biotechnological innovations in an accessible manner, providing (i) a useful overview of the converging fields of applied genetics, nanotechnology, and molecular biotechnology, and (ii) a conceptual basis for advancing new *pipeline* products in the emerging field of pathotropic medicine.

2. Setting the stage – targeting metastasis, one of the gravest medical needs

The problem of managing metastatic cancer, with its accompanying progression to evermore aggressive forms of the primary tumor cell (Bacac and Stamenkovic, 2008; Wong et al., 2009), remains one of the most daunting problems of modern medicine, thereby defining an unmet medical need. While many primary tumors can be eradicated by surgery if detected early in the course of disease progression, the appearance of metastatic disease is associated with a poor prognosis that worsens with the development of resistance to conventional chemotherapies (O'Day and Gorlick, 2009; Box et al., 2010; Verma et al., 2011). In the past ten years, there has been a frustrating lack of clinical advancements in the treatment of metastatic cancers (Di Marco et al., 2010, Stathis and Moore, 2010, Nieto et al., 2008). Once metastatic disease develops in pediatric sarcomas or in breast cancer, for

example, the possibility of a cure is very limited or practically nonexistent (Krishnan et al., 2005; Gonzalez-Angulo et al., 2007). Moreover, this decade-long frustration has resulted in regulators, clinical investigators, and practicing oncologists effectively lowering their standards and expectations with regard to clinical trials and patient outcomes (Nieto et al., 2008; Allen et al., 2010; Verma et al., 2011). It is in this context that the call for innovative molecular targeted therapies emerged (Cappetta et al., 2011); it is in this context that the theoretical capability of tumor-targeted nanotechnology advanced (Gordon and Hall, 2005; Zolnick et al., 2010; Shapira et al., 2011); and it is in this context that the promise and potential of genetic medicine became apparent (Gordon et al., 2008; Sreeramoju and Libutti, 2010; Gordon and Hall, 2010).

At the turn of the 21st century, the advent of targeted genetic medicine faced three major challenges: (i) ***undeveloped biotechnology***—specifically, the problem of inefficient gene delivery *in vivo*; (ii) ***institutional incredulity***—regarding the feasibility of achieving tumor-targeting under physiological conditions; and (iii) ***scientific skepticism***—concerning the seemingly overwhelming bio-mathematics of the applied nanotechnologies required for effective tumor control. The first challenge was elucidated by the pharmaceutical industry, as it systematically withdrew from the field of genetic medicine, stating: "*From the beginning, the therapy's main difficulty has been a logistical one: how to deliver enough healthy genes to the appropriate site and get them to stay there long enough to cure or alleviate a disease....*" the consensus opinion being, "*improved gene delivery methods are needed in order to give human trials a better chance of success*" (Langreth and Moore, 1999). The second challenge was exemplified by a National Cancer Institute grant reviewer, circa 2000 (privileged communication), who stated that "*One couldn't possibly imagine that the systemic delivery of an extracellular matrix (ECM)-targeted gene delivery vector would accumulate appreciably inside a tumor nodule; more likely, the gene delivery would be restricted to the superficial 'stromal' layers, much like the peeling of an onion skin.*" The third challenge, that of insurmountable biomathematics, was addressed in a scholarly debate following a keynote presentation by the authors at the Cold Spring Harbor Laboratory 2001 meeting on "*Vector Targeting Strategies for Gene Therapy*." In view of the first preclinical demonstrations of efficient, tumor-targeted gene delivery achieved under physiological conditions *in vivo*, there still remained considerable doubt concerning the feasibility of overcoming the rigors of physiology—dilution, filtration, immunological inactivation, fluid dynamics, and shear forces—to the extent needed to deliver a sufficient number of vector particles via the systemic circulation without utilizing infectious (self-replicating) viral components. Taken together, these formal challenges that prevailed at the cusp of the 21st century represented a formidable technological *barrier to entry* into the field of targeted genetic medicine, in general, and to the advancement of cancer gene therapy, in particular.

One by one, these imposing biopharmaceutical challenges were addressed and overcome during the course of a decade of scientific discovery, biotechnological innovation, translational research, and clinical development: a decade which may, in retrospect, be appropriately regarded as the *decennium mirabilis* of targeted genetic medicine—that "remarkable decade" wherein the clinical promise and potential of cancer gene therapy was ushered across the threshold of history. In technical scope, these challenges ranged from basic and applied molecular-genetics and virology, to medical nanotechnology, to the biophysics of tumor targeting and the constructs of therapeutic gene-delivery, to the advent of pathotropic (disease-seeking) medicine and the advancement of precision-targeted retrovectors, through a series of "proof-of-concept"preclinical studies and rigorous clinical

trials, leading ultimately to the GMP bio-production and scale-up of the first fully-validated tumor-targeted gene delivery vector (i.e., Rexin-G) in accordance with the most exacting (U.S. FDA) demands of a Phase III/commercial oncology product. For the purposes of this invited review, these individual technological steps in the origination and development of Rexin-G for metastatic cancer are subdivided into discrete steps or *critical stages of development* that constitute the definitive biopharmacological foundations of a new field of medicine.

3. The pioneering stage – basic molecular-genetic research and discovery

"*To heal him, we must touch something other than the coverlet of his bed!*" exclaimed Ambroise Paré in 1569, extolling those physicians and surgeons in attendance that M. le Marquis de Auret was not yet beyond hope, but they would have to dig somewhat deeper into the fabric of nature to uncover a cure. This paternal challenge from one of the great pioneers of experimental medicine still echoes through the ages, compelling would-be healers to look beyond the superficiality and the plasticity of cellular signal transduction unto the final common pathways that function as prime *executive regulatory mechanisms* governing cell growth and viability. Indeed, after years of disappointing studies of experimental treatments for vascular proliferative disorders based on the disruption of cell receptor-mediated events, there emerged the appeal to look deeper—through an epoch of molecular-genetic research and discovery—for the executive enzymatic components of cellular growth control and those final common, highly-conserved biochemical pathways that physically execute the orderly progression of the mammalian cell division cycle (Siriam and Patterson, 2001; Ferguson and Patterson, 2003). It is in this deeper mechanistic understanding of the executive 'enzymatic engines' of the mammalian cell division cycle (Schwartz and Shah, 2005; Marretta and Ales, 2010), that the drug targets of a more effectual apothecary for both vascular proliferative disorders (Charron et al., 2006) and otherwise intractable cancers can be found (Johnson and Shapiro, 2010; Krystof and Uldrijan, 2010).

Located at the headwaters of oncogenesis, where growth-promoting proto-oncogenes meet and physically inactivate the predominant endogenous tumor suppressor proteins (Sherr and McCormick, 2002), a class of inducible regulatory proteins called "Cyclins" reside—along with their Cyclin-dependent, proline-directed protein kinase (CDK) partners (Hall and Vulliet, 1991; Pines, 1995) and their respective polypeptide CDK inhibitors, which themselves represent a potent form of physiological growth inhibition/tumor suppression (Viallard, et al., 2001; Wesierska-Gadek et al., 2011). In the course of the mammalian cell division cycle, the Cyclins appear in a sequential manner, in accordance with the progressive stages of the cell cycle [*note, the alphabetical designation Cyclins A through G denotes the order of scientific discovery/cloning, rather than the temporal induction of the gene product per se*], participating directly in the enzymatic activation of one or more cognate CDKs (Gerard and Goldbetter, 2009) while physically guiding the activated protein kinase complexes to specific substrates (first demonstrated by Peeper et al., 1993) and subcellular locations (Morgan, 1997). The operational result of progressive Cyclin expression and resulting CDK activation can be viewed conceptually as "feed-forward" regulatory control, overriding endogenous growth/tumor suppressor proteins and advancing the cell cycle beyond the limiting biochemical checkpoint(s). The reported incidence of specific Cyclin over-expression, gene amplification, and/or viral subversion of these Cyclin-dependent pathways, in association with the molecular mechanisms of carcinogenesis, are sufficient to

warrant the formal designation of proto-oncogene to describe the growth-promoting role of these executive regulatory elements.

4. The mechanistic stage – molecular-genetic target validation

Among the so-called Cyclins, the *CYCG1* gene encoding human Cyclin G1 (Wu et al., 1994) is of particular bio-pharmaceutical importance; for it represents the molecular target of Rexin-G, the first targeted, injectable genetic medicine developed for diverse cancer indications. Indeed, recent clinical demonstrations of the broad-spectrum, single-agent, anti-tumor activity of Rexin-G have generated intense medical and scientific interest in the regulatory biology of Cyclin G1, as well as the molecular mechanisms-of-action of Rexin-G. In the course of its pharmaceutical development, it should be noted that the advanced molecular-genetic "knockout" construct embodied in Rexin-G is not dependent on the mechanisms of antisense-mediated gene suppression—which could possibly be overcome by the plasticity and the redundancy inherent in the Cyclin/CDK control elements as structurally- and functionally-related families of genes (Malumbres, 2005; Santamaria and Ortega, 2006; Satyanarayana and Kaldis, 2009)—but rather Rexin-G enforces the expression of a dominant-negative construct, i.e., a mutant form of the Cyclin-G1 protein, which effectively "blocks" the *executive biochemical pathways* governed by Cyclin G1, in the presence of a veritable "sea" of wild-type protein expression (see Xu et al., 2001). It is the blockade of these *executive biochemical pathways* (shown diagrammatically in Figure 1) that is invariably cytocidal to cancer cells and their associated proliferative neovasculature (Gordon et al., 2000, 2001; Gordon and Hall, 2010).

Mechanistically, the Cyclin G1 proto-oncogene is appreciably over-expressed in numerous cancers; its enforced expression promotes cell growth and effectively shortens the cell cycle (Smith et al., 1997), while its blockade by either antisense oligonucleotides or dominant-negative mutant constructs is decidedly lethal—via the active mechanisms of apoptosis—to both proliferative vascular cells (Zhu et al., 1997; Xu et al., 2001) and to a broad spectrum of cancer cells derived from all three germ layers (Skotzko et al., 1995; Chen et al., 1997; Hung et al., 1997; Gordon et al., 2001; Gordon et al., 2007; Gordon and Hall, 2010). Notably, in Cyclin G1-deficient mice, there is a significant reduction in the observed incidence of chemically-induced tumorigenesis (Jensen et al., 2003), which is consistent with a loss of antagonistic Cyclin G1-mediated effects on the levels and activities of the p53 tumor suppressor protein. As shown in Figure 1 (below), there is an intimate "negative-feedback" relationship between Cyclin G1 and at least two of the most prominent tumor suppressor proteins characterized to date: that is, the Retinoblastoma (Rb) tumor suppressor protein, which is genetically dysfunctional in many types of cancer, and the p53 tumor suppressor, which governs both apoptosis and senescence, in addition to regulating pivotal biochemical checkpoints involving DNA damage and enzymatic repair. In the first case, it appears that Cyclin G1, which is capable of forming complexes with multiple CDK partners (Piscopo and Hinds, 2008), participates in the inactivation of the Rb protein by directing site-specific protein phosphorylation events that ultimately enable cell cycle progression. In the second case, it appears that Cyclin G1 inactivates p53 (Ohtsuka et al., 2003, 2004), at least in part, by activating the MDM2 oncoprotein, which initiates the ubiquitin-mediated protein degradation of the p53 protein (Kimura and Nojima, 2002; Feng et al., 2011). In this case, it is important to note that both the Cyclin G1 effector and the MDM2 oncoprotein are transcriptionally activated (induced) by p53-dependent mechanisms (Okamoto and Beach,

1994; Moll and Petrenko, 2003), yet both of these regulatory proteins clearly function through additional biochemical pathways that are entirely independent of p53 (Zhang et al., 2005; Rayburn et al., 2009), which is genetically altered and/or inactivated in a large number of cancers.

Perhaps the most important aspect of target validation that can be discerned for Cyclin G1 as a drug target—and thus Rexin-G as an anti-cancer agent—is the recent finding that nature itself has seen fit to target this executive regulatory locus through endogenous microRNAs (Feng et al., 2011; Huang and He, 2011) that exert stringent control of Cyclin G1 expression. A series of high-throughput screens investigating the role of microRNAs in the pathogenesis of human hepatocellular carcinoma (HCC) identified miR-122 as the preeminent species of microRNA that is either missing or severely down-regulated in approximately 70% of HCC cancers and in all of the HCC-derived cancer cell lines (Gramantieri et al., 2007). Importantly, these studies identified Cyclin G1 as a gene target of miR-122, further validating the inverse correlation between miR-122 and Cyclin G1 expression that exists in primary liver carcinomas. Assuredly, the biochemical mechanism by which miR-122 expression decreased the viability of liver cancer cells was determined to be via apoptosis (Wu et al,. 2009); and enforced expression of miR-122 by adenoviral vectors has been shown to induce both cell cycle arrest and apoptosis in a number of different cancer cell lines (Ma et al., 2010) .

It was further determined that the pathological loss of miR-122 expression in liver cancer was not only correlated with an increased proliferative potential of the cancer cells, but with the extent of disease progression and metastasis (Coulouarn et al, 2009); while the re-expression of miR-122 was demonstrated to inhibit both the tumorigenic properties (Bai et al., 2009) and the metastatic potential (Tsai et al., 2009). In terms of molecular-genetic mechanisms of action, it was confirmed that, by modulating Cyclin G1 expression, miR-122 influences the stability and the transcriptional activity of p53, as it reduces the metastatic *invasiveness* of HCC-derived cell lines (Fornari et al., 2009). Moreover, the inhibitory effect of experimentally-restored miR-122 expression on Cyclin G1 levels serves to increase the sensitivity of HCC cells to doxorubicin-induced apoptosis, thereby establishing a mechanistic basis for the future development of combined chemotherapy and RNA-based cancer therapies. Taken together with the emerging molecular biology of Cyclin G1 (see Figure 1), it now appears that the biopharmaceutical agent, Rexin-G (a RNA-based genetic medicine), essentially restores a natural *tumor suppressor function* that is inherent in a normally-abundant species of microRNA—a species of microRNA that is lost with the pathogenesis of cancer, particularly that of invasive metastatic cancer.

Fig. 1 legend: As a mitotically-activated (or transformed) cell becomes 'competent' to proliferate and passes through the sequential phases of cell growth and DNA synthesis on to cell division, Cyclin G1 plays a pivotal role in governing the executive enzymatic activities of key regulatory components, including the checkpoints sensing DNA damage and repair. Normally held tightly in check (by microRNA-122), the growth-associated Cyclin G1 stands at the headwaters of cell cycle progression: advancing the cell cycle (arrows) through a myriad of enzymatic complexes, (i) by regulating site-directed protein phosphorylation by cyclin-dependent protein kinases (CDKs), which phosphorylate and inactivate the Rb tumor suppressor protein (blunted arrows), and (ii) by activating the cellular oncoprotein MDM2, which in turn inactivates the p53 tumor suppressor protein (blunted arrows) by initiating its destruction. Cyclin G1 governs the enzymatic activities of such key regulatory enzymes by transcriptional control of downstream elements, enzymatic

activation/inactivation of the executive enzymes, and by directing the regulatory complexes to specific substrates and/or specific subcellular locations.

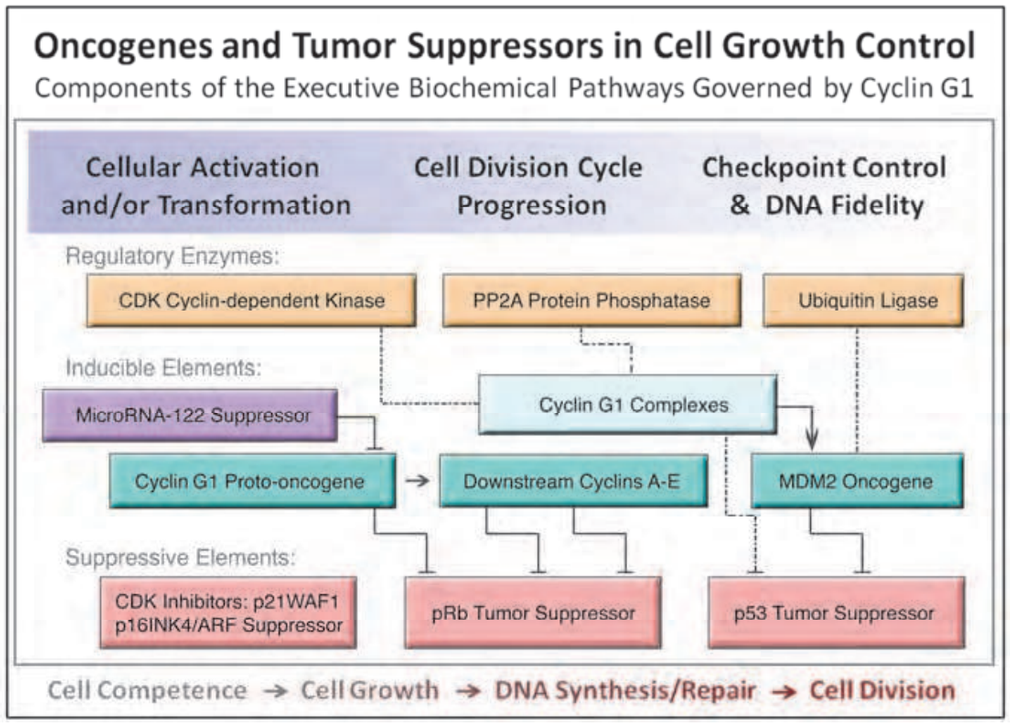


Fig. 1. Diagram depicting the major enzymatic activities, proto-oncogenes, and tumor suppressor proteins operating in the executive biochemical pathways governed by Cyclin G1.

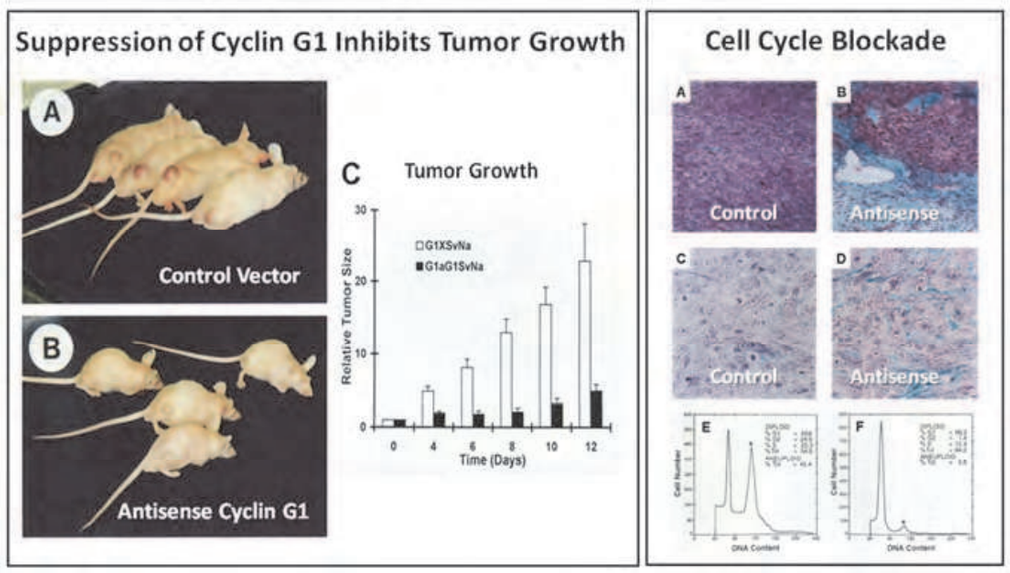


Fig. 2. Retroviral vector-mediated gene transfer of an antisense Cyclin G1 construct inhibits osteosarcoma growth in nude mice.

Following the identification of Cyclin G1 as a strategic therapeutic locus, a series of preclinical studies provided the initial *proofs-of-concept* (Skotzko et al., 1995; Chen et al., 1997; Gordon and Hall, 2010), thereby validating the therapeutic potential of cyclin G1 "knockout" constructs as anti-cancer agents by direct injection of therapeutic gene-delivery vehicles, or *vectors*, into tumor xenografts in experimental animals. As shown in Figure 2, both the reduction of tumor growth and the accompanying blockade of cell cycle progression are readily apparent. However, the more pressing technological challenge to reach beyond the obvious accessibility of localized primary tumors, and to address the fundamental problem of metastatic disease—i.e., to deliver the therapeutic potential of Cyclin-G1 knockout constructs where they are needed most—remained to be accomplished by a separate and distinct stage of scientific innovation.

Fig. 2 legend: Down-regulation of Cyclin G1 expression inhibits the proliferation of human osteosarcoma cells, as shown in rapidly growing subcutaneous tumors in athymic nude mice (Left Panel) treated by direct injection of either a Control (A) or Antisense Cyclin G1 vector (B); comparative tumor growth over time is shown in C. Histological analysis of tumor nodules (Right Panel) from Control (A,C,E) versus Antisense Cyclin G1 treated animals (B,D,F) demonstrates a significant reduction in the mitotic index in Antisense Cyclin G1-treated animals, which is confirmed by FACS analysis (E vs F), revealing a decrease in the number of cells in S and G2/M phases of the cell cycle (Chen et al., 1997). Cytocidal activity is confirmed by an increased incidence of apoptotic nuclei in the Antisense Cyclin G1-treated osteosarcoma cells (Skotzko et al., 1995).

5. The advent of pathotropic targeting – an enabling therapeutic gene delivery platform

In the course of scientific research and development it is often "The Road Not Taken," that is, the conscious decision to take the *road less traveled by*, that turns out to make the most significant difference in terms of historical outcome. In the case of targeted gene delivery, it was the conscious decision to target a *common* histopathological property of the metastatic process, rather than the unique and ever-changing surface features (ligands, receptors, etc) of the individual cancer cells, that made all the difference in terms of enhancing the efficiency of tumor-targeting under the most demanding of physiological conditions. Indeed, in the process of metastasis and metastatic tumor formation, both nascent and underlying extracellular matrix (ECM) proteins are characteristically exposed; and it is this characteristic exposure of one particular class of ECM proteins, the ubiquitous and determinative collagens (i.e., *collagen patefacio,* from Gordon and Hall, 2009), that now forms the basis for *disease-seeking* (or Pathotropic) tumor targeting. By conceptually grasping the *physiological surveillance function* that is inherent in the von Willebrand blood coagulation factor (vWF), which normally guides platelets to the sites of vascular injuries, and then physicochemically transposing a synthetic derivative of this *physiological surveillance function*, via genetic engineering, onto the surface of a nanoparticle-sized gene delivery vector (Hall et al., 2000; Gordon et al., 2000, 2002), the fields of molecular biotechnology and nanotechnology converged to enable the medical oncologist to reach beyond the mere coverlets of the proverbial bedside and to expose the very fabric of the nature of the metastatic disease process (Gordon and Hall, 2005, 2007). It is the advent of pathotropic targeting which would ultimately serve as the enabling biotechnological platform for therapeutic gene delivery *in vivo,* enabling the development of tumor-targeted gene therapy vectors that could be administered systemically, which would then seek-out sites of

cancerous histopathology and accumulate to high levels in primary tumors and in the remote, occult, and otherwise inaccessible lesions of cancer metastasis.

6. The definitive proofs of principle - targeting metastasis *in vivo* in pertinent models of cancer

Comprised of (i) a structural multi-lamellar capsule (or retrovector core), (ii) a genetic payload of various designs, and (iii) a pathotropic envelope protein, the first tumor-targeted gene delivery vectors were initially designed to carry unique "marker genes," in the place of a therapeutic RNA construct, in order to study the kinetics and biodistribution of the circulating nanoparticles under well-defined experimental conditions, as well as the overall efficiency of the resulting gene transfer events. As shown in Figure 3 (below), which depicts a classic subcutaneous tumor xenograft model in which human tumor cells are flagrantly grafted into the flanks of athymic mice, the tumor-targeted vector is subsequently introduced into the systemic circulation through the tiny tail vein of the sleeping animal. The tumor-targeted nanoparticle must not only withstand the intense turbulence and dilution of the general circulation in this model, it must transit the heart, pass through the extensive filtering networks of the lungs, and transit the heart once again, before it is pumped through the aortic arch and a mere fraction of the blood flow is distributed to the flanks on the first pass. Nevertheless, the targeted nanoparticles are demonstrably partitioned into the tumor xenografts within a matter of minutes with intense avidity, where they can be seen to leave the fenestrated circulation within the tumors and begin to spread throughout the tumor nodules, much like a particulate dye accumulates from solution (by high affinity) into a natural sponge. With this constant pathotropic partitioning and resulting accumulation of vector particles in high local concentrations within the tumor nodules, it is clear that the targeted nanoparticles are highly active in terms of effectuating marker gene delivery to the proliferative cancer cells, as evidenced by the quantitative efficiency of the resulting transgene expression.

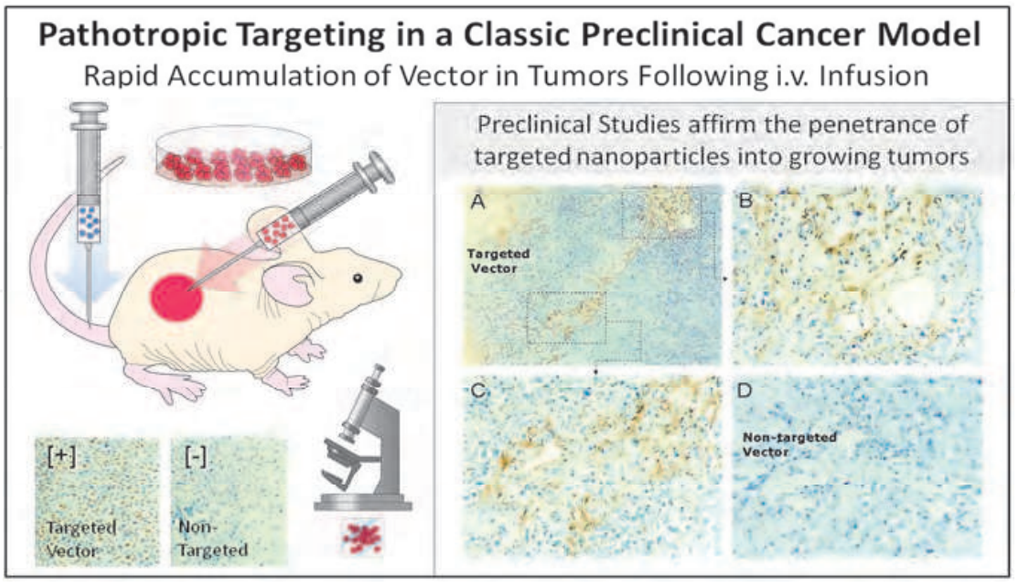


Fig. 3. Redistribution and gene transfer activity of a tumor-targeted retrovector bearing a marker gene into subcutaneously-implanted tumor xenografts in athymic mice.

Fig. 3 legend: Human pancreatic cancer cells were implanted in this classic cancer model (Left Diagram) followed by intravenous infusions of a tumor-targeted gene transfer vector (+) or non-targeted control vector (-). Immunohistochemical staining for the retrovector envelope protein (Right Panel) demonstrated appreciable accumulation of the targeted vector within 60 minutes of infusion (brown stain, A), which can be seen spreading out from the vasculature into the interstitial matrices of the tumor nodule (higher magnification, B, and C), in comparison with the non-targeted control vector where little if any accumulation can be found. Immunohistochemical staining for the β-galactosidase marker gene (Lower Left) confirmed high levels of transgene expression with the targeted vector.

Moving on to a somewhat more pertinent model of metastatic pancreatic cancer, where metastasis to the liver is all too common, additional characterization of the *physiological surveillance function* of these tumor-targeted nanoparticles was revealed, along with a striking demonstration of the high degree of selectivity of this targeted gene delivery platform for tumor cells and their associated neovasculature, while sparing normal liver cells in the immediate vicinity. In the model of metastatic pancreatic cancer shown in Figure 4 (below), the tumor-targeted retrovector bearing the designated marker gene was instilled at certain stages following the development of the liver metastasis. In the earliest stages of metastasis, where small groups and clusters of cancers cells invade the liver—before a distinctive tumor nodule is apparent – the vector demonstrates a striking ability to follow the submicroscopic biochemistries of tumor cell invasion, tracking the path of the invasive cancer cells, and delivering its transgene payload (marker gene) selectively to the invasive tumor cells while sparing the normal liver parenchyma. With the onset of neoangiogenesis, it becomes clear that, in addition to delivering the marker gene to the proliferative tumor cells, the pathotropically-targeted vector efficiently targets the vasculature of these aggressive tumors as well; thus the major focus of the transgene gene delivery is restricted to metastatic cancer cells and their attendant blood supply. These findings further indicate that an appropriate therapeutic (i.e., cytocidal) payload would exhibit significant anti-angiogenic properties, as well as anti-tumor activities.

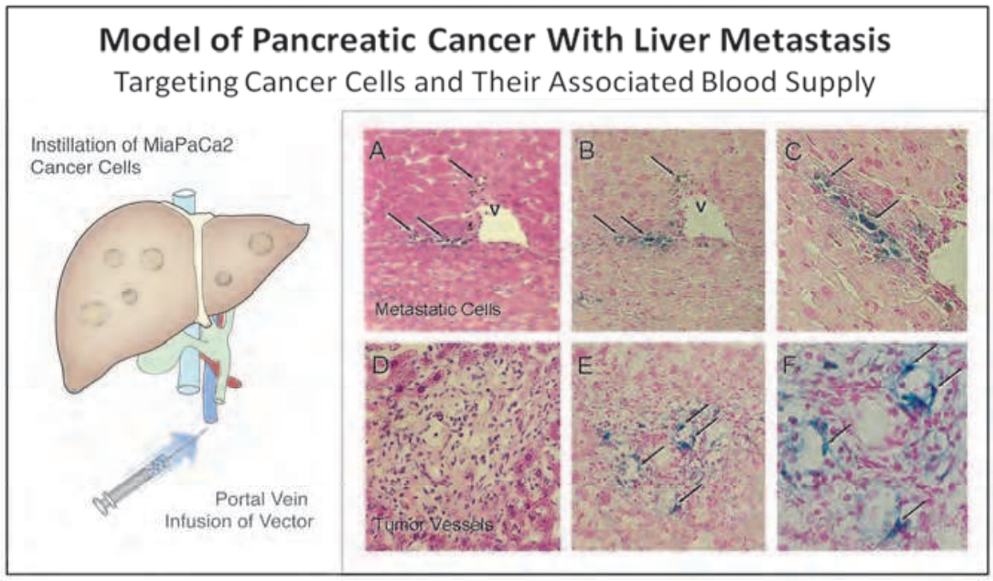


Fig. 4. Pathotropic vector bearing a marker gene identifies cellular targets for gene transfer in a murine model of pancreatic cancer metastatic to the liver.

Fig. 4 legend: Human pancreatic cancer cells were infused via the portal vein into the liver (Left Panel) followed, three days later, by portal vein infusions of a tumor-targeted gene transfer vector bearing a β-galactosidase marker gene at various stages of tumor formation (Right Panel). H&E staining of the earliest stage of metastasis (A) shows a small group of pancreatic cancer cells exiting a hepatic vein and migrating into the liver parenchyma. Histochemical staining for the marker gene expression (blue-green stain in B, enlarged in C) demonstrates efficient and selective gene delivery to the cancer cells. Following the establishment of the liver tumors with onset of neoangiogenesis (H&E stain of vessels shown in D), transfer of the β-galactosidase marker gene is seen in the proliferative endothelial cells of the tumor vessels (E and F).

Replacing the marker gene cassette with a cytocidal dominant-negative Cyclin G1 construct resulted in the development of the therapeutic anti-cancer agent designated Rexin-G, an acronym that conveys its molecular engineering roots: *Retroviral expression vector bearing an inhibitory construct of the Cyclin G1 gene*. Pioneering studies in the aforementioned preclinical cancer models with this *killer gene* as the genetic payload, resulted in the first demonstrations of clinical efficacy for targeted gene delivery *in vivo*: (i) corroborating the high-efficiency of tumor-targeted gene delivery with evidence of clinical efficacy, (ii) establishing the initial dose-response curves of an emergent pharmacology, (iii) confirming the fundamental biochemical mechanisms-of-action as enforced apoptosis, and (iv) revealing the characteristic hallmarks of tumor destruction and regression under the onslaught of this targeted cytocidal genetic medicine. As shown in Figure 5, repeated intravenous infusions of Rexin-G administered in a tumor xenograft model induced significant inhibition of tumor growth while altering the entire histology of the residual tumor nodules: as areas of focal vascular destruction (anti-angiogenesis) and massive tumor necrosis are observed among distinctive zones of overt cellular degeneration and reparative fibrosis.

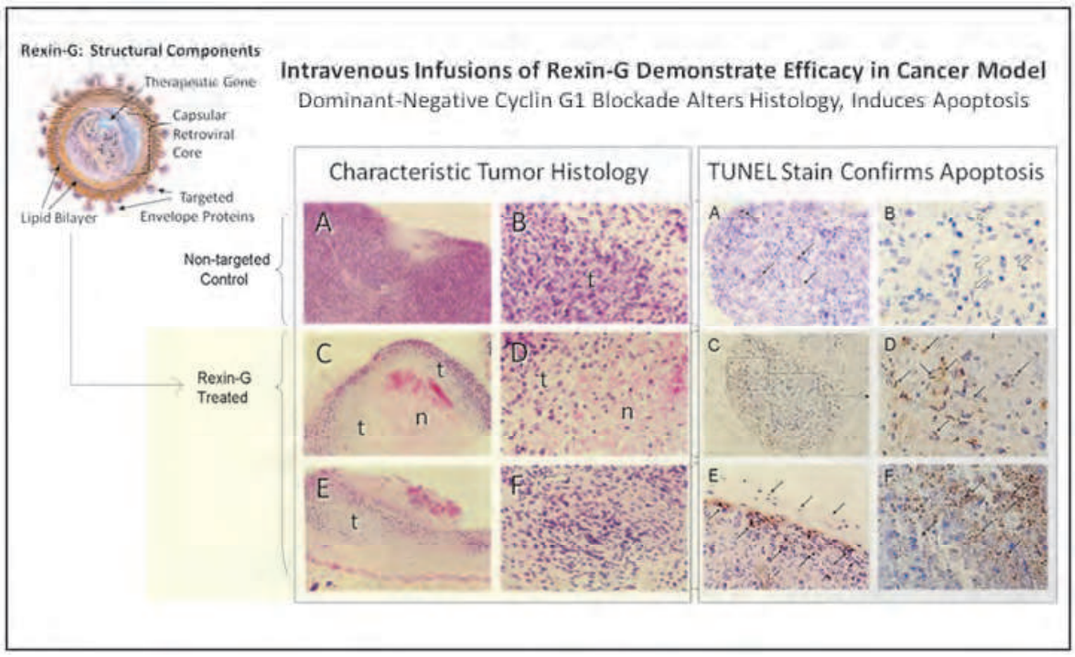


Fig. 5. Repeated intravenous infusions of Rexin-G abate the growth and alter the histology of pancreatic cancer xenografts in athymic mice.

Further histological analysis confirms that the major mechanism-of-action responsible for the observed tumor destruction is the induction of apoptosis by Rexin-G, which is evident in both the cancer cells and the endothelial cells of the associated tumor neovasculature. In addition to these histological indications of tumor destruction attributed to Rexin-G (Gordon et al., 2000, 2001) is the recruitment of tumor infiltrating lymphocytes to clean up the resultant tumor debris (see Figure 6).

Fig. 5 legend: Human pancreatic cancer cells were established as subcutaneous xenografts in the flanks of the experimental animals, followed one week later, by tail vein infusions of a tumor-targeted Rexin-G or a non-targeted vector (as indicated). Histological analysis of tumors from control vector- (A,B) versus Rexin-G vector-(C-F) treated animals (Left Panel) showed massive and focal necrosis (n) of tumor cells (t), along with zones of vascular disruption (C), and fibrosis (F). TUNEL staining for the detection of DNA fragmentation (Right Panel) confirmed the primary mechanism-of-action to be apoptosis (brown stain, arrows), which is rare in control tumors (A) with their robust vascular beds (B), but is readily evident in the disrupted vasculature (C,D) and in the dying tumor cells (E,F) of the Rexin-G treated animals.

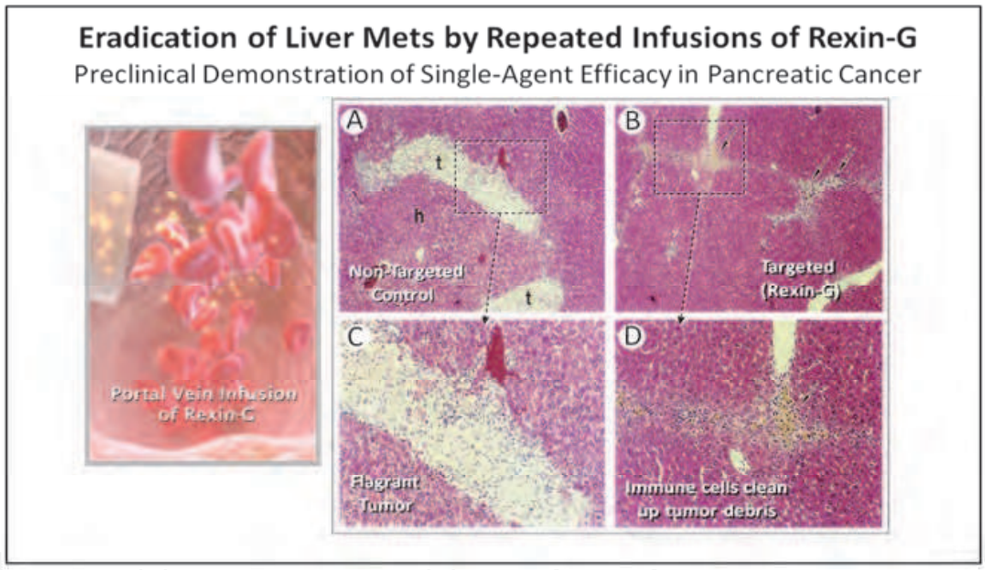


Fig. 6. Complete eradication of liver metastases by repeated infusions of Rexin-G retrovector in a murine model of metastatic pancreatic cancer.

Fig. 6 legend: Flagrant tumors are shown in control animals (A, enlarged in C) vs no evidence of active tumor cells in Rexin-G –treated mice (B, enlarged in D). Note: Resident Kupffer cells (macrophages in the liver) are observed to be engorged with hemosiderin indicative of phagocytosis of tumor debris. Analysis of dose-response (Gordon et al., 2000), in relation to inhibition of tumor growth, formed the basis of a more-predictive clinical pharmacology.

7. The 1st clinical stage – phase I studies establish clinical feasibility and overall safety

The definitive demonstrations of selective tumor targeting, predictable mechanisms-of-action, and single-agent efficacy in preclinical cancer models provided compelling impetus

for expedient clinical development, which necessitated further evaluations of general safety, dose-response relationships, bio-distribution, pharmacokinetics, and monitoring of gene transfer in a series of scientific studies that ranged from mice, to rats, to rabbits, to larger animals (pigs). Taken as a whole, the resulting compilation of scientific evidence served to provide the *requisite documentation of general safety* and the *reasonable expectation of clinical benefit* that was critically analyzed by the NIH Recombinant DNA Advisory Committee (RAC), which was formed in 1974 in response to public concerns regarding the safety of manipulating genetic material through the use of recombinant DNA techniques. In accordance with its role as a federal advisory committee, the RAC forwarded its recommendations to the Director of the Office of Biotechnology Activities, in line with the General NIH Guidelines for basic and clinical research involving recombinant DNA molecules and human gene transfer trials, respectively. Critical review and analysis of scientific, safety, and ethical considerations by the RAC pertaining to the clinical utility and administration of Rexin-G in humans, was conducted in 2000 (Lenz et al., 2002) at which time, the appointed reviewers stated that the platform targeting biotechnologies embodied in Rexin-G were both elegant and important (Russell, RAC Transcript, 2000).

While it was initially envisioned that Rexin-G would be administered to human cancer patients regionally at first, via hepatic arterial infusions (Lenz et al., 2002), it so happened that a series of formal requests for Compassionate Use applications of Rexin-G in Stage IV metastatic pancreas cancer took precedent—in compliance with both U.S. FDA permissions and Philippine BFAD/FDA regulations—which served to propel the clinical advancement of Rexin-G. In terms of time, it served to validate the tumor-targeted gene delivery platform as a systemically administered agent. With federal allowances for such commendable international collaboration in place, Rexin-G was first deployed in the clinic in the Philippines in 2002, with the tacit acknowledgement that Epeius Biotechnologies would advance its clinical development program in the USA "as soon as practicable" (U.S. FDA Communications, 2002).

These pioneering clinical studies of Rexin-G in chemotherapy-resistant pancreatic cancer (Gordon et al., 2004) stand as the seminal foundations of targeted genetic medicine by (i) demonstrating the safety and single-agent anti-tumor activity of repeated intravenous infusions, (ii) affirming predicted dose-response relationships astutely extrapolated from pertinent preclinical data, and (iii) validating the tumoricidal mechanisms-of-action of Rexin-G, along with the now-classical hallmarks of tumor destruction (see Figure 7).

Fig. 7 legend: Combined PET-CT scan (Left Plate) shows central necrosis in 5 out of 6 visible lesions in one patient with chemo-resistant pancreatic cancer. An opportunistic surgical biopsy of a liver lesion after Rexin-G treatment (Right Plate) in another patient with chemo-resistant metastatic pancreatic cancer reveals characteristic zones of anti-angiogenesis (A), along with focal necrosis of tumor cells (A,B), overt apoptosis, verified by TUNEL stain (D,E), reparative fibrosis (A-f, Mason's Trichrome in C), and recruitment of tumor-infiltrating lymphocytes (F), including CD4+ helper (stained in F) and CD8+ killer T-cells (not shown).

The authors' report of unprecedented single-agent anti-tumor activity of Rexin-G observed in 3 out of 3 pancreatic cancer patients who were treated in the Philippines with increasing weekly doses of Rexin-G in an innovative *intra-patient dose-escalation regimen*—where careful analysis of drug safety was verified before escalating to progressively higher doses—gained Orphan Drug Designation for pancreatic cancer by the U.S. FDA in 2003, and federal funding from the FDA Orphan Products Development program in 2006. The first U.S.-based Phase I study established the overall safety of repeated infusions of Rexin-G and the lower

rungs of the pharmacological dose-response curve for Stage IV pancreatic cancer (Galanis et al., 2008) compared to the higher, more-effective doses shown in succeeding advanced Phase I/II studies (shown in Figure 11; Chawla et al., 2010). Meanwhile, clinical development of Rexin-G advanced in the Philippines through a series of Phase I/II studies and an Expanded Access program, which extended the scope of clinical applications to a wider variety of cancers, including breast cancer, melanoma, and laryngeal CA (Gordon et al., 2006, 2007).

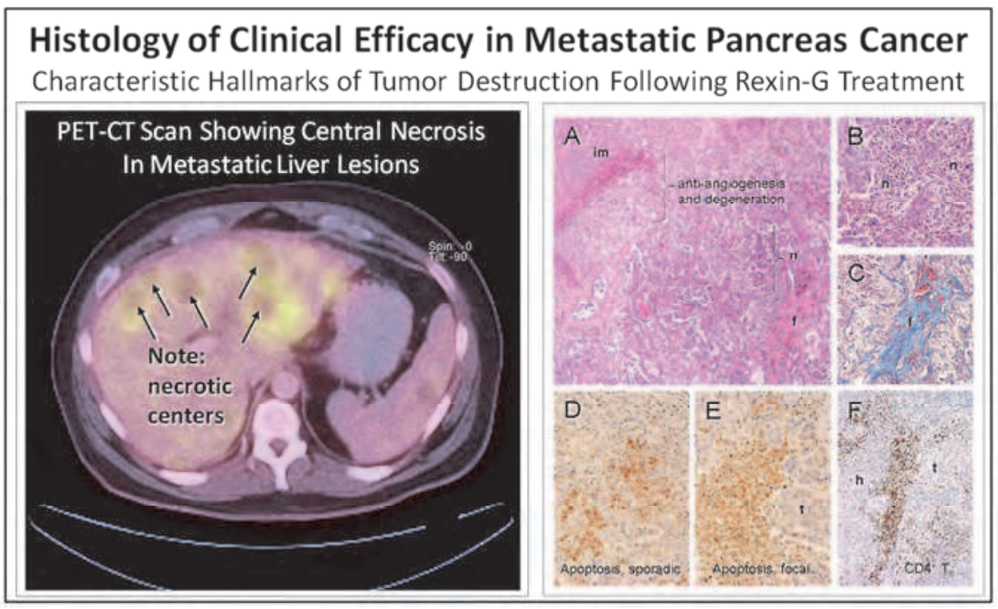


Fig. 7. Radiological and histological evidence of anti-tumor activity of Rexin-G.

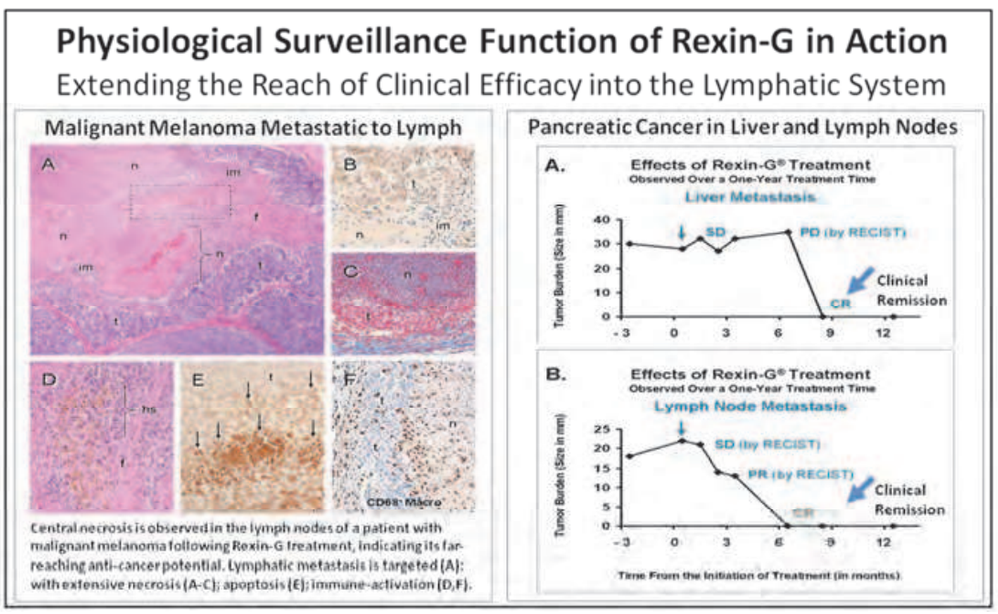


Fig. 8. Tumor-targeted nanoparticles extend physiological reach and clinical efficacy into the lymphatic system.

Among the most important insights gained from these pioneering clinical studies—insights which were not immediately apparent from the preclinical models—was the finding that the physiological surveillance function inherent in the Rexin-G nanoparticles was not only capable of targeting tumors by successive excursions through the general circulation, but was also capable of penetrating and eliminating cancer metastases in the lymphatic system (see Figure 8).

Fig. 8 legend: Biopsy of a surgically excised lymph node (Left Plate) in a patient with metastatic malignant melanoma exhibits the characteristic hallmarks of Rexin-G mediated tumor destruction along with its apoptotic mechanisms-of-action (legend). Monitoring stabilization of disease (SD), along with the observed decrease in the size and extent of lymph node metastasis (B) in a Stage IVb pancreatic cancer patient (Right Plate) encouraged clinical oncologists to "hold the course" of Rexin-G treatment, even in the face of slight progressive disease (PD) seen in the liver (A); an astute clinical decision that resulted in a clinical remission, initially observed after 9 months of Rexin-G treatment. Note: remission/survival ongoing > 2 yrs.

8. The 2nd clinical stage – phase II studies establish the molecular pharmacology

With the clinical feasibility and general safety of the Rexin-G retrovector formally established in the clinical setting, the U.S. FDA approved the stepwise escalation of Rexin-G dosage in a series of three *adaptive* and *advanced* Phase I/II studies for metastatic chemotherapy-resistant pancreas cancer, sarcoma, and breast cancer. The *adaptive* study designs were intended to further refine the analysis of Rexin-G bioactivity, in terms of pertinent tumor response criteria, while these inter-patient dose escalation studies were *advanced*, in as much as a Phase II evaluation of clinical efficacy was incorporated in each of the study designs. These studies demonstrated that Rexin-G was well tolerated, with no evidence of dose-limiting toxicities (DLT), and that Rexin-G exhibited dose-dependent anti-tumor activity when administered as a stand-alone therapy for pancreatic cancer and sarcoma (Chawla et al., 2009, 2010). Of particular importance was the availability of surgical specimens obtained during the course of Rexin-G treatment, where Rexin-G was permitted by study design to serve as both neoadjuvant therapy, to bring the cancer under control, and as post-surgical adjuvant therapy, to help prevent recurrence. The availability of such germane and opportune histology served to validate the predictable molecular and histological mechanisms of Rexin-G action (Hall et al., 2010; see Figure 9 below).

Fig. 9 legend: Immunostaining for the vector nanoparticles (Left Plate) demonstrates the generalized accumulation of vector particles within the tumor (light brown staining material A, versus Control, with no primary antibody B); moreover, the natural propensity/targeting of the retrovector envelope for the phosphate transporters that are abundant on proliferative cells results in targeting of tumor cells and associated vasculature (C-E). Right Plate: Immunostaining for Keratin identifies the islands tumor cells (A, insert) amidst extensive fibrosis (B, Trichrome stain), while TUNEL stain verifies active cancer cell death by apoptosis (C, D versus Control E). It is relevant to note that this patient with metastatic pancreatic cancer has enjoyed a sustained *surgical remission* with adjuvant Rexin-G therapy for over 2 years.

In the course of these advanced, adaptive Phase I/II studies, the clinical responses to Rexin-G were examined in a comprehensive manner, which included an analysis of tumor size (RECIST criteria), metabolic activity (International PET criteria), and changes in tumor

density (CHOI criteria). From this, it was possible (i) to discern early tumor responses to Rexin-G treatment (recall Figure 7) in relation to its tumor-targeted mechanisms-of-action, and (ii) to refine the evaluation of clinical efficacy (previously established as RECIST for cytotoxic chemotherapy) and to apply more pertinent evaluation criteria to the emerging field of targeted biologics. In the case of an osteosarcoma patient, for example, the use of

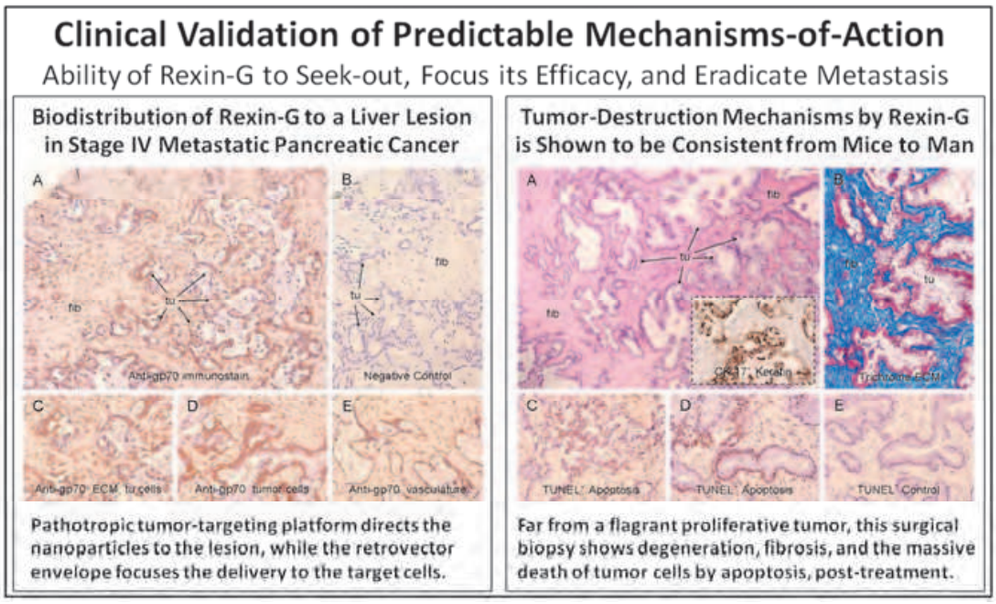


Fig. 9. Immunohistochemical staining of sections of a biopsied liver nodule obtained during Rexin-G treatment, revealing tumor-targeting and tumor-destroying mechanisms-of-action.

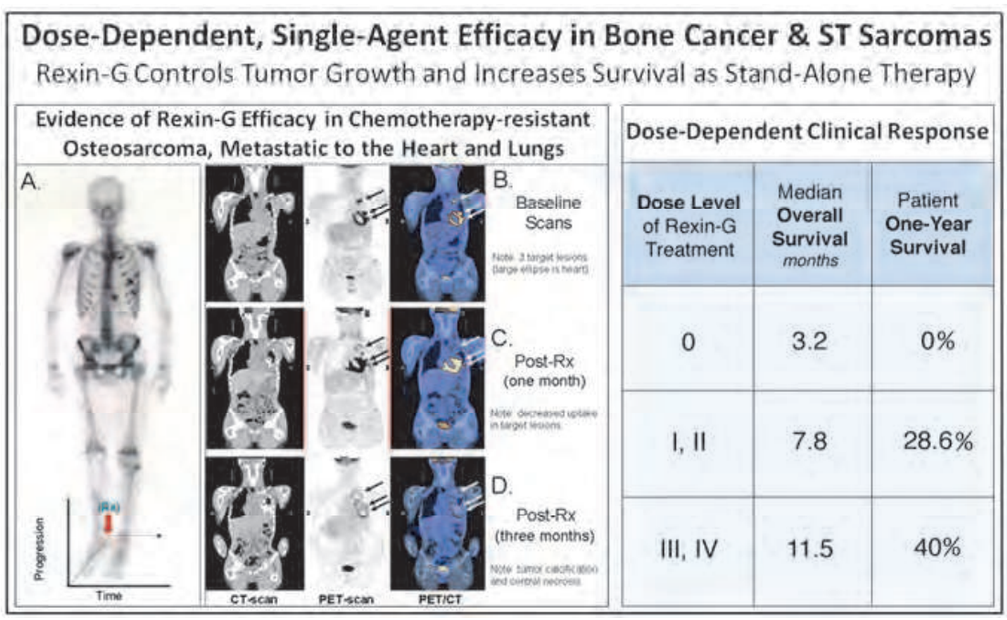


Fig. 10. Demonstration of dramatic anti-tumor activity of Rexin-G as monotherapy for osteosarcoma (by PET/CT scans) and dose-dependent overall survival time by analysis of a Phase I/II clinical study of Rexin-G in bone and soft tissue sarcomas.

RECIST criteria for evaluating tumor response was not reliable, since clinical efficacy was characterized more appropriately by the reduction in tumor metabolic activity (by PET scan), accompanied by extensive calcification of tumor nodules (see Figure 10).

Based on these findings, the U.S. FDA approved a Phase II study for osteosarcoma to be conducted concomitantly with the ongoing Phase I/II study for bone and soft tissue sarcomas (STS) that used the PET scan as the primary imaging tool for the evaluation of clinical efficacy. Aggregate analysis of these study results confirmed the overall safety of Rexin-G (with no DLT), its anti-tumor activity, and the positive dose-dependent impact on patient survival parameters (Figure 10) which, after all, represents a "gold standard" for the evaluation of clinical responses for a prospective anti-cancer agent.

Fig. 10 legend: Left Panel: Radiological examination of a 17-year old male patient after primary tumor excision and limb salvage surgery (A) reveals rapidly progressing metastases (insert) to lung and adrenal gland. Rexin-G infusions halted tumor progression, as evidenced by no new lesions, a significant (48%) reduction in tumor metabolic activity and overt calcification of the target lesions shown in follow-up PET-CT scans during Rexin-G treatment (baseline B, versus C and D). Right Panel: Analysis of the Phase I/II study data demonstrates a dose-dependent increase in patient survival parameters.

In these Phase I/II and Phase II studies of clinical safety and efficacy, it became increasingly evident that the observed dose-response phenomenology, as well as the predicted Calculus of Parity (Gordon et al., 2006; Gordon and Hall, 2007), had served to establish the basic foundations of an emerging clinical pharmacology for Rexin-G, providing estimates of optimal weekly doses, by determining the quantitative *threshold for bioactivity* for both pancreas cancer and sarcomas; and providing a practical estimate of the actual numbers of nanoparticles needed on a weekly basis to match an aggressive, chemotherapy-resistant tumor burden and, thus, to impact the fatal course of metastatic disease. This emergent clinical pharmacology has several important implications: First, it serves to address and to resolve the problem of exponential tumor cell growth, with the conclusion that it is indeed difficult, but not impossible, to provide sufficient numbers of targeted nanoparticles needed to meet and match a given tumor burden. Second, it affirms that chemo-resistant cancer patients do indeed die, as predicted, in the absence of sufficient quantitative intervention, in accordance with the results of the Rexin-G low-dose safety studies and the historical controls (see Figures 10 and 11). Most importantly, by establishing the quantitative pharmacology of Rexin-G action in end- or late-stage chemo-resistant cancer, these seminal Phase I/II dose-escalation studies serve to establish critical analytical parameters and meaningful benchmarks for further clinical studies—studies that will extend the utility of Rexin-G to additional types of solid tumors, to surgical oncologists who will employ it as neoadjuvant/adjuvant therapy to effectuate a curative surgery, to clinical oncologists who will deploy it in combination with other useful anticancer agents, and eventually to many cancer patients at much earlier stages of the disease, where the respective tumor burden could be matched and can no longer be considered either unreachable or insurmountable.

9. Regulatory considerations – efficacy & safety as the basis of pharmacology / toxicology

The conduct and the progression of clinical trials for an investigational new drug (IND) are carried out under stringent oversight by the clinical investigators, the medical and scientific authorities of the corporate sponsor, and the local and federal regulatory agencies, the latter of which provide an additional level of critical analysis and assurance that adequate safety

considerations have been achieved prior to granting approval for a given cadre of patients to be treated at the next higher dose level, as specified in the clinical study design. In this regard, it is important to note that Rexin-G exhibited an exemplary safety profile in the adaptive Phase I/II studies sufficient to warrant an expedited dose escalation. As represented graphically in Figure 11, the enrollment of chemotherapy-resistant sarcoma patients in the Phase I/II study happened to outpace the enrollment of patients in the Phase I/II study for pancreatic cancer; and, as such, the scheduled dose-escalations proceeded to higher, more-effective doses in a shorter period of time. However, in view of (i) the documented safety of each preceding dose level, (ii) the evidence of dose-dependent tumor control, and (iii) the survival benefits achieved in the sarcoma study, the U.S. FDA granted permission for an "*across-the-board*" dose escalation for all three ongoing Phase I/II trials in an effort to improve cancer control and patient outcome. An examination of the resulting Kaplan-Meier survival curves (see Figure 11, below) affirms the astuteness of this regulatory approval, whereby the critical analysis of the bone and soft tissue sarcoma patients served, in effect, to extend the lives of the pancreatic cancer patients. In this context, the constructive philosophical adage, first introduced in the FDA Centennial Book chapter "A Primer on Pathotropic Medicine"(Gordon and Hall, 2007), is all the more compelling: "*When the pathotropic medication is of broad spectrum utility, as in the case of Rexin-G, it behooves the clinical investigator to expand the scope of the clinical applications to include a broad spectrum of different intractable metastatic cancers, with the realization that – given the appropriate interim analysis – each new patient's experience may benefit the next, and that additional penetrating insights can be gained upon extensive critical analysis performed in aggregate.*" Hence, it is important to consider that the *safety* and *efficacy* data obtained from a relatively small number of patients in clinical trials of this investigational new drug are, in actuality, the prime scientific building blocks upon which the *toxicology*, the *pharmacology,* and ultimately the *praxis* of targeted genetic medicine will be constructed.

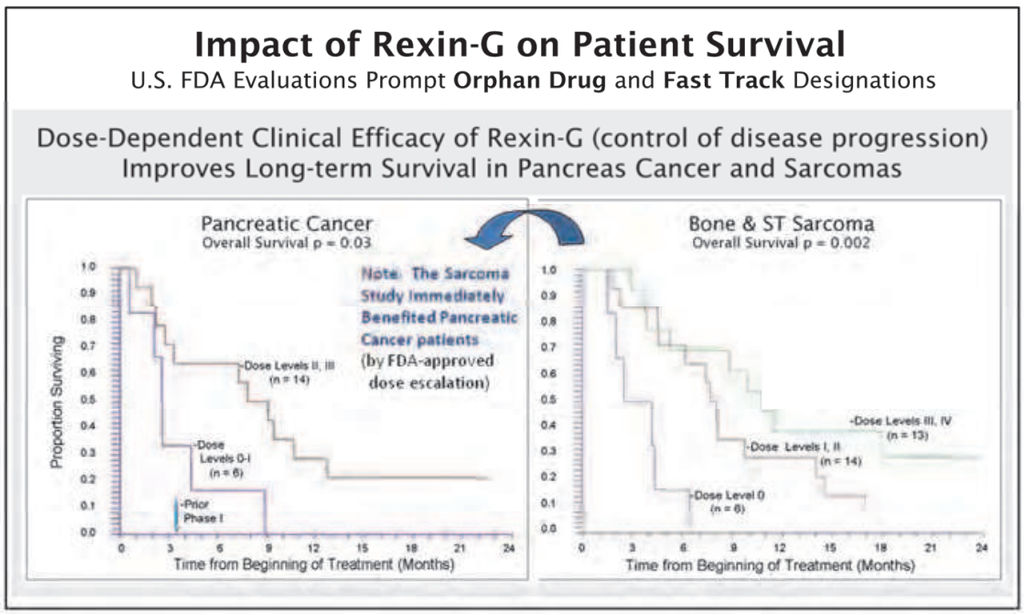


Fig. 11. Kaplan-Meier survival curves for two concomitant Phase I/II studies of Rexin-G administered as monotherapy to metastatic cancer patients who had previously failed standard chemotherapies.

Fig. 11 legend: Left Panel: Overall survival of pancreatic cancer patients is increased significantly from the previous Phase I safety studies (arrow) by successive dose escalation, which was expedited by critical analysis of an ongoing Phase I/II study of bone and soft tissue sarcoma (Right Panel) and subsequent allowance of an *across-the-board* dose escalation to effective clinical doses. Note, the dismal survival times observed in the lowest dose groups is in agreement with historical control data for each of these respective chemo-resistant cancers; while dose-dependent gains in overall survival are clinically, statistically significant.

In the Philippines, where the clinical development of Rexin-G proceeded to advance with similar regulatory oversight, data analysis, and guidance, the clinical studies were facilitated by the federal approval of an Expanded Access Program, wherein a larger and more diverse set of metastatic cancers – cancers originating from all three germ layers – were determined to be responsive to the anti-cancer bioactivity of Rexin-G. At this point, it became exceedingly important to standardize the GMP bio-manufacturing of Rexin-G to an even greater extent, and to undertake a program of research and development aimed at characterizing the *stability* of the drug product under conditions of long-term storage, which is measured in years. Following extensive analysis of product safety, efficacy, composition, purity, and long-term stability, Rexin-G was granted Accelerated Approval in 2007, receiving a Certificate of Product Registration from the Philippine Bureau of Food and Drugs (BFAD/Philippine FDA) enabling its commercialization for the treatment of all solid tumors that are determined to be resistant to standard chemotherapies. Thus, it is reasonable to expect that Rexin-G will follow a similar course of clinical development in the United States and worldwide, where clinical studies and regulatory oversight are comparable.

Following the registration of Rexin-G for all solid chemo-resistant tumors in the Philippines, and the attainment of its first Orphan Drug Designation for pancreatic cancer in the U.S, the clinical development of Rexin-G advanced steadily in the USA with the successful completion of three adaptive Phase I/II studies for sarcoma, breast cancer and pancreatic cancer, and a Phase II study for osteosarcoma (Chawla et al., 2009, 2010); in each of these studies, both primary and secondary endpoints were achieved. Formal critical evaluation of the U.S. safety and efficacy data, with due consideration for the unmet medical need, resulted in the granting of two additional *Orphan Drug Designations* for Rexin-G – with its implicit market protections and clinical development priorities for soft tissue sarcoma and osteosarcoma. In mid-2009, Rexin-G gained *Fast Track Product* designation from the U.S. FDA as 2nd-line treatment for pancreatic cancer, to expedite the development and validation of the tumor-targeted gene delivery platform and its therapeutic "payload."

10. The commercial product – GMP production, bio-processing, QA testing, and scale-up

As Rexin-G approaches the cusp of Phase III clinical trials in the USA for both pancreatic cancer and sarcomas, it is important to note that the clinical development of Rexin-G to date is a function of the multiple levels of safety and efficacy embodied in its design engineering, as is the observed broad-spectrum anti-cancer activity. Indeed, the molecular-genetic components of the Rexin-G retrovector are enhanced by the set of virtues and limitations inherent in the biotechnology platform, which work together to provide *four distinctive levels of safety* in coordination with *three distinctive levels of efficacy* (see Gordon and Hall, 2010). In

terms of Safety: (i) the stealth vector platform allows repeated infusions without untoward immunologic reactions; (ii) the limitations of the retroviral core becomes a virtue, as the vector is capable of enforcing gene expression in proliferative / dividing cells only; (iii) the growth-associated designer gene is active against cancer cells and proliferative vasculature but not against normal non-dividing cells; and (iv) the pathotropic accumulation in cancerous tissues sequesters the vector away from non-target organs. In terms of Efficacy: (i) the cell cycle gene knockout provides for broad-spectrum anti-cancer activity, while (ii) the anti-angiogenic activity destroys tumor-associated vasculature, and (iii) the pathotropic targeting leads to effective drug accumulation where it is needed most, i.e., in cancerous lesions in the vicinity of target cancer cells. In terms of the therapeutic "designer gene" it bears reiteration that recent studies of functional genomics using high-throughput screening methodologies have served to validate the Cyclin G1 locus as a critical gene target for the key tumor-suppressive microRNA, miR-122 (Gramanteri et al., 2007; Fornari et al., 2009), thereby linking the loss of this natural endogenous molecular regulation (of the Cyclin G1 locus) to both the mechanisms of carcinogenesis (Bai et al., 2009) and the cytological progression of metastatic disease (Coulouarn et al., 2009). In light of such scientific validation of the drug target, it is increasingly apparent that, by inhibiting the executive oncogenic Cyclin G1 pathway in a highly selective manner, the molecular-genetic construct delivered by Rexin-G serves to restore a natural *tumor-suppressive function* that is lost or disabled with the onset of many cancers. In anticipation of the calculated need for higher-potency Rexin-G formulations, along with the need to produce much larger quantities of the Phase III clinical grade product—which essentially becomes the commercial product—coordinated research and development activities have focused on the large-scale biopharmaceutical manufacturing of Rexin-G under stringent GMP conditions, where an uncompromising effort was made to preserve the integrity of each and all of the structural and enzymatic components that constitute the functionality of the tumor-targeted nanoparticle, and to preserve the fidelity of the therapeutic transgene.

Physicochemically, the Rexin-G nanoparticle is assembled from a certified bank of *producer cells* under the instructive directions of three separate gene cassettes (called plasmids): 1. Gag (structural), 2. Pol (enzymatic), and 3. Envelope (cell recognition and entry), which come together in a process called transfection to determine the unique properties of the synthetic retrovector particles. Namely, the nano-sized particles are *replication incompetent*, packaging only the therapeutic gene of interest; they are *stealth* in terms of their low immunogenicity, enabling repeated intravenous infusions; they are *selective*, capable of delivering the therapeutic transgene to dividing cells only; and they are *pathotropically-targeted,* physically capable of seeking-out and accumulating in diseased tissues under physiological conditions. The use of the 'Split Genome' elements in biopharmaceutical manufacturing—i.e., the partitioning of the retrovector elements into three separate and distinct plasmids—renders the synthetic nanoparticles certifiably replication incompetent, while successive generations of R&D and improvements in plasmid structure and performance elevated the industry to a new *state-of-the-art* (see Gordon et al., 2008 for a review). The strategic utilization of a transient co-transfection system for the bioproduction of each large-scale batch of Rexin-G—as opposed to stable packaging or producer cell lines, which are susceptible and prone to genetic drift—is purposeful, in that it maintains all the refinements in retrovector design engineering in the final clinical product.

While the myriad details of biopharmaceutical GMP protocols, optimizations of cell factories, post-production bio-processing procedures, qualification of bioassays, product identity assessments, product purity testing, and sterility certification, along with the

multitude of quality control and quality assurance issues and documentation that accompany these highly-standardized procedures is beyond the scope of this review, it should be emphasized nonetheless that these are among the most meticulously-prepared and thoroughly-documented records that are subjected to critical analysis by the FDA, under the auspices of Process Analytical Technology (PAT) and Chemistry, Manufacturing and Control (CMC) reviews. Suffice it to say that the developers of Rexin-G are in complete accord with the U.S. FDA's current perspective on modern drug quality systems, which states, "*…quality cannot be tested into products; it should be built-in or should be by design.*"

By addressing and resolving a number of serious GMP and CMC issues that had previously plagued the biopharmaceutical industry, in terms of the consistency, variability, and industrial scale-up of complex biologics to the point of commercial feasibility, the developers of Rexin-G demonstrated the practical utility of applied research: improving the overall safety, efficiency, productivity, purity, scalability, economy-of-scale, and ultimately the affordability of the clinical grade biologic product for the benefit of cancer patients, the biopharmaceutical industry, and society.

In the context of this communication, it bears mention that the relative purity of Rexin-G, over that which was originally approved for use in humans and employed in years of clinical trials, has been increased more than 400X. In other words, the clinical product (now at 1 x 10e10 cfu/ml) is well over 99.7% more pure in terms of allowable excipients per dose. For example, a 500ml dose of Rexin-G at 2.5 x 10e7cfu/ml is now administered as 1.25 ml.

In January 2011, the U.S. FDA granted Phase 3 status for Rexin-G. What this means, in terms of clinical development, is that the Rexin-G product, with its advanced GMP manufacturing, bio-processing, and final formulation, meets rigorous FDA standards for obtaining a marketing license in the future; and that the developers can now proceed with its strategic, diversified Phase 3 drug development programs for osteosarcoma, soft tissue sarcoma, and pancreatic cancer where it has received Fast Track Designation and Orphan drug priorities.

11. The 3rd clinical stage – multiple phase III studies for U.S. product registration

Despite the enlightened intentions of the U.S. FDA Accelerated Approval program to shorten the development times of promising new drugs for serious medical illness – that is, to grant accelerated approval for new molecular entities on the basis of compelling Phase II trial data, followed by confirmatory post-approval trials – there has been a discernable reversion, in recent years, for the U.S. FDA to restrict Phase II efficacy endpoints and to encourage sponsors to design accelerated approval applications on the basis of interim analyses of protracted Phase III trials (Richey et al., 2009). Moreover, a number of adverse instances in the drug approval process have raised legitimate concerns by the FDA Oncology Drugs Advisory Committee (ODAC), which has essentially created new hurdles, for both clinically effective and ineffective agents alike, and has increased the focus on post-marketing studies (Goozner, 2010, 2011). Therefore, it cannot be considered either expedient or logical to undertake a program of clinical development for a new and potentially important oncology agent based solely on acceleration of its marketing approval. Rather, the heightened and politically-charged regulatory climate encourages the responsible sponsor to undertake a robust and long-term program of clinical development, which (i) strengthens and improves clinical validation of safety and efficacy, (ii) mitigates the risk that is inherent in the conduct of a single clinical trial, (iii) broadens clinical utility (and potential market

share) by expanding clinical indications, and (iv) serves to inspire confidence and lasting support from regulatory bodies in the fullness of time.

Commensurate with the fundamental principles of basic science—the step-wise demonstrations of physiological tumor-targeting, predictable mechanisms-of-action, pharmacological safety, and single-agent anti-tumor activity, including dose-dependent survival benefits—is the strident recommendation of the primary inventors that the high-value biotechnology platform embodied in Rexin-G merits the implementation of a comprehensive, progressive, and diversified program of clinical development: a program of clinical development that not only meets but far exceeds the minimal requisites for federal regulatory approval. After all, the cancer genetics, the molecular biotechnologies, the functional genomics, and the medicinal nanotechnologies embodied within Rexin-G represent a *nano-architectural* triumph of modern medicine. It is within the most stringent guidelines and guidance of the U.S. FDA that Phase II and Phase III pivotal studies of Rexin-G are currently being designed: as first-line therapy in combination with anti-metabolites, as stand-alone therapy for second-line indications, and as neoadjuvant/adjuvant therapy in combination with surgical procedures.

With the clinical validation of the tumor-targeted gene delivery platform accomplished, and the biopharmaceutical evaluation of Rexin-G as a Phase 3 (i.e., commercial) product achieved, the end of one particular *Product Development Stage* (that of Rexin-G) signals the beginning of the next (introducing Reximmune-C). In light of the potential "pipeline" of pathotropically targeted anti-cancer agents, it was first reasoned, and then proven in preclinical studies, that the same bio-technological platform developed for the targeted delivery of the cytocidal *designer gene* could just as readily deliver an immune-stimulating cytokine gene directly to the same cancerous lesions, which would provide a highly-localized and personalized form of cancer vaccination (Gordon et al., 2007, 2008; Zolnik et al., 2010). This two-tier complementary approach—termed the *GeneVieve (Genes for Life) Protocol*—aimed at both tumor eradication and cancer vaccination, was evaluated in a limited number of patients who benefited from previous Rexin-G therapy (see Figure 12 below). The sequential delivery of Rexin-G followed by Reximmune-C, which bears a controllable construct of the granulocyte-macrophage colony-stimulating factor (GM-CSF) transgene, induced substantial tumor necrosis and recruitment of tumor-infiltrating lymphocytes in cancerous lesions without raising the baseline levels of the powerful cytokine in the patient's blood; thereby affirming both general safety and an effective Reximmune-C dose range (Cornelio et al., 2010). In the subsequent follow-up of the first 9 patients receiving the tumor-targeted cancer vaccination in a Phase I/II study of the GeneVieve Protocol, a large percentage (> 70%) of these otherwise poor prognosis patients exhibited an overall survival beyond one year (Ignacio et al., 2010). By meeting both primary and secondary study endpoints—defining a safe and effective dose range—these findings indicate that this strategic combination of two pathotropic medicines (Rexin-G plus Reximmune-C) is safe and well-tolerated, and may help control tumor growth and prolong survival, thus advancing the protocol and the molecular biotechnologies of personalized cancer vaccination as a feasible and promising approach.

Fig. 12 legend: Rationale: "Pathotropic" targeting of therapeutic gene delivery enables personalized cancer vaccinations *in situ* by means of simple intravenous infusions. Rexin-G and Reximmune-C are tumor-targeted retrovectors bearing a cytocidal cyclin G1 'knockout'

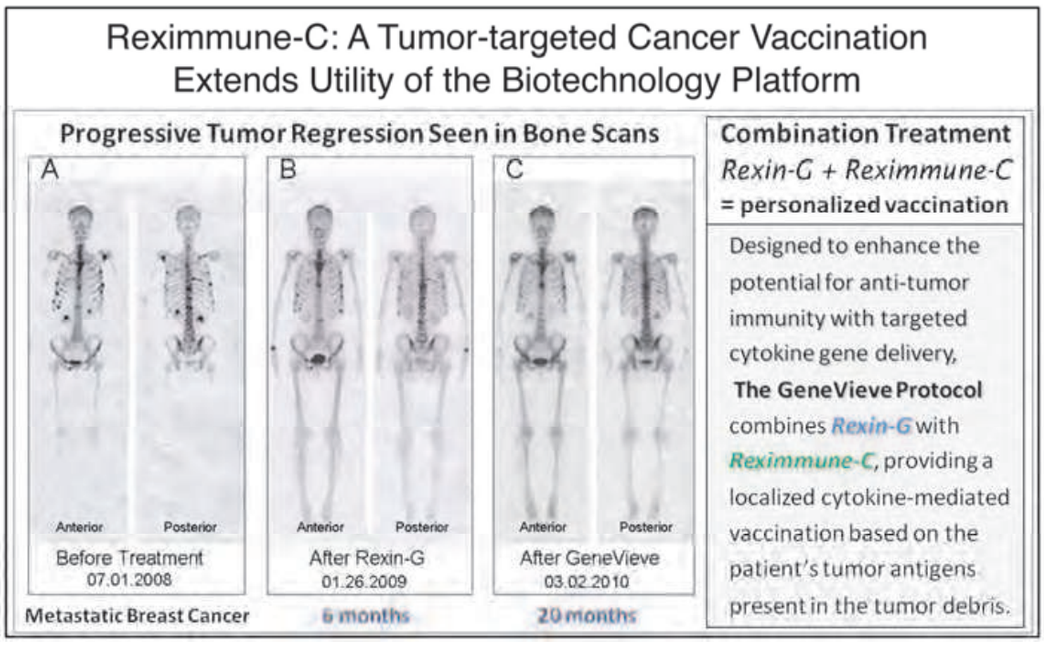


Fig. 12. Bone scans of a patient with metastatic breast cancer obtained at intervals following treatment with Rexin-G followed by a two-tier cancer vaccination with Rexin-G plus Reximmune-C (The GeneVieve Protocol).

construct and a controllable GM-CSF expression construct, respectively. The working hypothesis for this bipartite tumor-targeted cancer vaccination strategy is that the personalized vaccination of a patient against his/her own specific cancer type can be achieved by combining (1) a targeted vector bearing a tumoricidal payload, i.e. Rexin-G with (2) a targeted vector bearing a potent immuno-stimulatory gene, i.e. Reximmune-C. First, Rexin-G is administered to control tumor growth and to expose neoantigens within the tumor microenvironment, followed by defined pulses of Reximmune-C, which recruits the patient's immune cells into the lesions, thereby prompting immunologic activation, recognition of tumor neoantigens, and induction of an antitumor immunity. Baseline (A); post Rexin-G (B); post Rexin-G plus Reximmune C (C).

12. Looking back and then forward – reflections on proper values and valuations

Looking back over the past decade of scientific and medical achievement in the emerging field of targeted genetic medicine, the term *decennium mirabilis* seems all the more appropriate. The technological challenges that once stymied and precluded therapeutic gene delivery *in vivo* have all been overcome. The triad of forbidding challenges of *undeveloped biotechnology*, *institutional incredulity*, and *scientific skepticism* have been confronted and allayed by a significant amount of scientific and clinical data that heralded the advent of pathotropic targeting as an enabling biotechnological platform with a series of sound conclusions: (i) it is no longer impossible to reach the fabric of the nature of malignant disease itself (i.e., *collagen patefacio,* from Gordon and Hall, 2009); (ii) it is no longer impossible to deliver a sufficient number of therapeutic genes to the appropriate site and get them to stay there long enough to impact and reverse the course of metastatic disease

(Gordon and Hall, 2010; Hall et al., 2010); (iii) it is no longer appropriate to deny the mathematical potentiality of a targeted genetic medicine, in light of the quantitative demonstrations of single agent dose-dependent anti-tumor activity, along with the recent advances in biopharmaceutical vector production that have raised the potency of the clinical-grade vectors more than two orders of magnitude (Gordon and Hall, 2009). Indeed, as the development and validation of the world's first (Waehler et al., 2007; Gordon and Hall, 2010), but no longer only (see Reximmune-C; Gordon et al., 2007, 2008), tumor-targeted genetic medicine is recognized, the promise and potential of the platform have begun to percolate into the general medical literature, impacting the practice of clinical oncology (Hughes, 2009), medical imaging (Bjojani et al, 2010), medicinal nanotechnology (Peach et al., 2009), and gene therapy (Sverdlov, 2009), as well as the discussions of bedside bioethics (Toh, 2011) and the practical applications of tumor immunology (Zolnik et al., 2010).

Looking forward into the future, it is only a matter of *time* (see Gordon and Hall, 2009) when the progression of metastatic disease is no longer considered to be intractable, and the poor prognosis of chemotherapy-resistant cancer is summarily improved. It is also only a matter of *time*, when the potentially "disruptive" biotechnology is eventually viewed as enabling platform for further research and development, and the potential of the resulting "pipeline" becomes a value-added resource for the biopharmaceutical industry. As the Development Stage of this leading genetic medicine comes to a close, and the resources of the academic institutions and the idealistic enterprises that initially supported its advancement are expended in the process of serving such unmet medical needs, it would be expected that the pioneering inventors and the visionary business builders are eventually replaced by professional financial institutions and pharmaceutical conglomerates that are capable of supporting vast expenditures required for the progressive, diversified programs of late-stage clinical studies that will expand the clinical applications, optimize the protocols for a multitude of new treatment combinations, and ultimately extend the reach of pathotropically-targeted gene delivery into the evolving praxis of modern and post-modern medicine. One can only hope that the abiding values of inspiration and compassion that once fueled the hearts and minds of the physicians and scientists who carried this platform thus far for the benefit of the cancer patient, will not be lost entirely in the valuations to come.

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14. References

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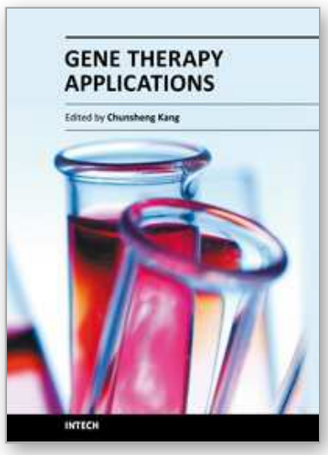
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The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.

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