

Technology evaluation: Rexin-G, Epeius Biotechnologies

Michael Morse

Address

Duke University Medical Center
Division of Medical Oncology
Box 3233 Room 3803
Red Zone
Duke South Clinics
Durham
NC 27710
USA
Email: m.morse@cgct.duke.edu

Current Opinion in Molecular Therapeutics 2005 7(2):164-169
© The Thomson Corporation ISSN 1464-8431

Rexin-G is a 'pathotropic', tumor-targeted, injectable retroviral vector carrying a mutant form of the cyclin G1 gene, under development by Epeius Biotechnologies for the potential treatment of metastatic cancer. The therapy is currently undergoing phase I/II clinical trials.

Introduction

Advanced solid cancers such as those of the colon and pancreas have poor prognoses. The median survival time for a sufferer of colon cancer is 21 months [585535] and of pancreatic cancer, only 6 months [582703]. Although therapy of colon cancer has evolved with the availability of the monoclonal antibodies bevacizumab and cetuximab [585536], the chemotherapeutic agent gemcitabine remains the standard for pancreatic cancer [582703]. Numerous novel therapies are in development, although none have consistently improved patient outcome by more than a small margin [582707].

Among the major pathway derangements in pancreas and colon cancers are abnormalities in cell-cycle control, and numerous drugs in clinical trials are focusing on cell-cycle inhibition, for example the Cdk-inhibitor flavopiridol (National Cancer Institute), the non-specific kinase inhibitor UCN-01 (Kyowa Hakko Kogyo Co Ltd/National Cancer Institute), and the now discontinued bryostatin-1, an inhibitor of protein kinase C. Cyclin G1, a transcriptional target of *p53* that is induced by DNA damage, is another potential target for a cell-cycle intervention strategy. Cyclin G1 plays an important role in regulation of the cell cycle and is upregulated in many malignancies [585537]. The major activity of cyclin G1 is thought to involve arresting DNA damaged cells in G₂/M-phase, thus allowing recovery from damage and continued cell proliferation [582898], [582902].

Blockade of cyclin G1 activity by antisense or mutant constructs inhibited the proliferation of cancer cells *in vitro* [228193], and intratumoral injection of an antisense cyclin G1 vector inhibited tumor growth in *in vivo* tumor models [572356], [572357]. As described below, the expression of a deletion mutant cyclin G1 construct significantly inhibited metastatic tumor growth in a mouse model [572353]. Rexin-G (denoting retroviral expression vectors bearing inhibitory genes) is a retroviral vector carrying a dominant negative (dn) mutant form of the cyclin G1 gene, which is under development by Epeius Biotechnologies Corp for the potential treatment of metastatic cancers [501651], including pancreatic cancer [572354].

Originator Epeius Biotechnologies Corp

Status Phase I/II Clinical

Actions Angiogenesis inhibitor, Anticancer, Retrovirus-based gene therapy

Indications Colorectal tumor, Metastasis, Pancreas tumor

Biotechnology Virus (recombinant)

Synonyms cyclin G1 gene therapy (cancer), Mx-dnG1

While a variety of vectors are available for delivering genes to tissues, retroviral vectors have the advantage of targeting dividing cells [585538] (which comprise a significant proportion of those in tumors), and stably integrate into the genome of a transfected cell so that gene expression is maintained. Unfortunately, retroviral vectors do not selectively target tumors and thus must be delivered to each cancer cell. This could lead to significant inefficiency in tumor transduction and could potentially result in toxicity if normal cells are transduced. The vector must also be exposed to the tumor mass for a prolonged period of time, over which different cells enter the cell cycle. Rexin-G preferentially targets the tumor matrix by incorporating the collagen-binding portion of von Willebrand factor (vWF) on the viral surface. vWF, a glycoprotein necessary for platelet binding to damaged stroma, is frequently expressed at the site of damaged tissues and is upregulated in response to the pro-angiogenic molecules vascular endothelial growth factor and basic fibroblast growth factor [585540]. Exploiting the targeting mechanism of vWF therefore permits delivery of the retrovirus to the site of a tumor where angiogenesis and collagen matrix exposure are occurring [582905], [582907].

Synthesis and SAR

Rexin-G is a matrix (collagen)-targeted retroviral vector encoding an N-terminal deletion mutant form of human cyclin G1 under the control of a hybrid long-terminal repeat/cytomegalovirus (CMV) promoter [572352]; in some literature it is referred to as Mx-dnG1 (matrix-targeted vector encoding cytotoxic mutant cyclin G1) [572355]. In addition, the vector contains the neomycin resistance gene, driven by the SV40 early promoter, which allows selection of virus-producing cells during manufacture. The Rexin-G vector is produced by transient co-transfection of 293T cells (HEK293 cells transformed with the SV40 large T antigen) with three plasmids simultaneously: the pdnG1/C-REX therapeutic plasmid construct contains the deletion mutant of the human cyclin G1 gene (amino acids 41 to 249) driven by the CMV immediate-early promoter, packaging sequences, and the bacterial neomycin resistance gene under the control of an internal SV40 early promoter; the Mx (Bv1/pCAEP) plasmid, which encodes the collagen-targeting viral envelope component of the Moloney murine leukemia virus (MuLV), contains a CMV-driven modified amphotropic 4070A envelope protein wherein the collagen-

binding portion of the vWF peptide was inserted into an engineered *Pst*I site in the N-terminal region of the 4070A envelope coding sequence; and the third plasmid, pCgpn, contains the MuLV *gag-pol* elements driven by the CMV immediate-early promoter [572352].

Information on the formulation of Rexin-G is provided in the publication of the first phase I study [572354]. The vector was produced in accordance with current good manufacturing practices by transient transfection of human 293T cells. The vector (at a viral titer of 3×10^7 cfu/ml) was stored in volumes of 150 ml at 80°C. The biological potency was measured as a 65 to 70% growth inhibition of human breast, colon and pancreatic cancer cells. The product exhibited a uniform particle size of approximately 100 nm with no viral aggregation, < 550 bp residual DNA, no detectable E1A or SV40 large T antigen, and no detectable replication-competent retrovirus. This final product was sterile, with low endotoxin levels and no mycoplasma or adventitious viruses [572354]. The method of vector purification, if any, and stability of the vector are not mentioned in reference [572354].

Preclinical Development

Two major streams of preclinical development have contributed to Rexin-G, the technology for targeting a vector to the collagen matrix, and the technology for inhibiting cell cycling by targeting cyclin G1. Frederick Hall, who originally conceived these studies and co-founded Epeius Biotechnologies, described the insertion of the collagen-binding motif (WREPSFMALS, derived from the D2 domain of vWF) into the retroviral envelope protein [582905], [582907]. This motif is displayed on the N-terminal region of the amphotropic 4070A envelope [582907]. The resulting single-enveloped vectors maintained their wild-type infectivity but also had collagen-binding affinity, as demonstrated by localization of targeted, but not wild-type, virions to the exposed collagen of mouse aorta *ex vivo* and injured rabbit artery *in vivo* [582905].

As regards to targeting the cell cycle, in initial preclinical studies, a retroviral vector encoding antisense cyclin G1 was tested. Antisense strategies suppress the production of the target protein (in this case cyclin G1) by causing degradation of the mRNA encoding that protein. The first publication detailed the construction of this vector and the initial demonstration of cytostatic activity in MG63 osteogenic sarcoma cells [228193]. In subsequent studies, the antisense cyclin G1 vector inhibited proliferation of cell lines derived from colon cancer (HT29, KM12C4A, KM12C and DHDDK12), Ewing's sarcoma (EW1), C6 glioma, undifferentiated carcinoma (VX2), pancreatic cancer (BxPc3 and MiaPaca2) and osteosarcoma (MG63 and MNNG/HOS). *In vitro* testing of a number of cancer cell lines and normal cells showed that transduction efficiencies generally ranged from 5 to 85%, with pancreatic cancer (MiaPaca2) and osteosarcoma cells exhibiting the highest transduction levels (~ 80%). Most normal human primary cells studied (endothelial, stromal or hepatic fibroblast cells) were not inhibited, although cytostatic effects were observed in keloid and dermal fibroblasts and keratocytes [572357]. Comparable results were achieved in an earlier study in 2- to

5-day VX2 cell cultures, in which proliferation was inhibited and fluorescence-activated cell sorting analysis confirmed blockade of the cell cycle at G₁ phase [572358].

In vivo, in an athymic nude mouse VX2 carcinoma xenograft model (1×10^7 cells injected subcutaneously and grown for 5 weeks), tumor growth was reduced after an intratumoral injection of the antisense cyclin G1 construct (1×10^4 cfu/day for 2 weeks), but not a control vector. Gross tumor size and cell densities decreased in treated animals, but residual cells in treated tumors indicated that a substantial population of cells were not transduced [572357]. A study in an MNNG/HOS osteosarcoma cell xenograft model showed that treatment with this antisense cyclin G1 construct (1×10^4 cfu/day for 10 days) significantly inhibited tumor growth compared with control vector-treated tumors, which increased 3-fold in size [572356].

Following these studies, an improvement in the vector was made by substituting the antisense component for a mutant human cyclin G1 (dnG1), created with a deletion in the cyclin box, which is important for cyclin-Cdk (cyclin-dependent kinase) associations that induce Cdk activation [572353]. In other words, instead of using the vector to degrade mRNA encoding cyclin G1, the vector is now used to deliver a mutated cyclin G1 gene (dnG1) which encodes a protein that may act to inhibit the function of wild-type cyclin G1 or form inactive complexes with the target Cdk molecules [572353].

In vitro analyses and studies in animal models were subsequently performed using the dnG1 vector [572353]. This vector reportedly contains a vWF-derived matrix-targeting motif that appears to be identical to that used in the final formulation of Rexin-G. The transduction efficiency of the retroviral vector in a human undifferentiated pancreatic cancer cell line (MiaPaca2) was 26 to 85%, and correlated with the multiplicity of infection used (4 and 250). The cytotoxic or cytostatic effects of mutant and antisense cyclin G1 retroviral vectors were then demonstrated in transduced cancer cells. Based on the increased cytotoxic activity of the Mx-dnG1 vector in transduced MiaPaca2 cancer cells, the Mx-dnG1 vector was used in subsequent *in vivo* efficacy studies. In a nude mouse model of liver metastasis (7×10^5 MiaPaca2 cells administered via the portal vein), intravenous infusions of either a low- or high-dose Mx-dnG1 vector (titers of 6×10^2 and 1.8×10^5 cfu/day, respectively) were given for a total of 9 days. Gene delivery by portal vein infusions ranged from 1 to 3% to $\geq 50\%$ in focal areas. This significant ($\geq 50\%$) transduction of tumor-associated stromal and endothelial cells was observed in some tumor foci undergoing active angiogenesis, whereas in neighboring hepatocytes (that are not actively dividing) there was no evidence of transduction. Treatment produced reductions in the sizes of tumor foci, which were enhanced by increasing the number of vector infusions. There was no evidence of hepatocyte injury, vessel thrombosis or cholestatic changes in tissue sections from Mx-dnG1 vector-treated animals [572353].

To determine the effectiveness of systemic vector administration, the Mx-dnG1 was administered

intravenously in a murine MiaPaca2 cell xenograft model, and compared with a non-targeted dnG1-expressing vector (CAE-dnG1) and a control matrix-targeted vector bearing a marker gene (Mx-nBg) [572355]. Seven daily vector infusions of approximately 8×10^6 cfu/dose were given, and tumor size was measured 1 day later. The Mx-dnG1-encoding vector induced efficient transduction of tumor nodules (35.7%) that correlated with therapeutic efficacy without associated toxicity. Tumors regressed by a mean of 6.1% in animals treated with Mx-dnG1, but enlarged in other treatment groups by 4.1, 6.3 and 12% in the CAE-dnG1, Mx-nBg and placebo groups, respectively. Kaplan-Meier analysis confirmed that the probability of long-term control of tumor growth was significantly greater with Mx-dnG1.

Metabolism and Pharmacokinetics

In nude mouse (MiaPaca2 cell) xenograft models of gastrointestinal or liver metastasis, the vector (5.6×10^7 or 1.6×10^8 cfu of Mx-dnG1, or 4.8×10^6 or 1.1×10^9 cfu of modified Mx-dnG1, MxV-dnG1 or control vector) was injected through the tail vein. Using a realtime PCR assay, low-level positive signals were detected in the liver, lung and spleen of animals treated with both vector doses. No signal was detected in the testes, brain or heart of vector-treated animals [572352].

Toxicity

The vectors Mx-dnG1 or MxV-dnG1 were administered without significant toxicity and caused no mortality or morbidity in the above studies. No bone marrow suppression or significant abnormalities in liver or kidney function were observed in studies in mice, rats and pigs [572354]. In mice, the only pathological observations on histopathological examination included portal vein phlebitis, and pyelonephritis with focal myocarditis in two animals, which was attributed to the use of in-dwelling catheters in the absence of antibiotic prophylaxis. Mild increases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that did not exceed the normal range for mice were observed in the Mx-dnG1-treated animals. At over 7 weeks of follow-up, no anti-vector neutralizing antibodies were detected [572352].

Clinical Development

Phase I/II

The first phase I study of Rexin-G was performed in three stage IV pancreatic cancer patients [572354]. An inpatient dose escalation of Rexin-G vector (administered daily as an intravenous infusion over 1 to 3 h) was performed as follows: 4.5×10^9 , 9.0×10^9 and 1.4×10^{10} cfu were administered on days 1 to 6, 7 to 8, and 9 to 10, respectively. Patients were given a week to rest before receiving 1.4×10^{10} cfu on days 18 to 27. The first patient, who had experienced recurrence of previously resected pancreatic cancer despite prior gemcitabine treatment, entered the study with disease at the original primary site and metastases to the supraclavicular and abdominal lymph nodes. The patient received a total of three 10-day treatment cycles of Rexin-G (a cumulative dose of 3×10^{11} cfu), each separated by 1 week. The volumes of the two supraclavicular lymph node tumors eventually decreased by 33 and 62%. An abdominal magnetic resonance imaging (MRI) scan at day 28

demonstrated 40 to 50% necrosis of the primary tumor, and a significant decrease in the size of a para-aortic lymph node. An MRI scan on day 54 showed no further change. The level of serum carbohydrate antigen 19-9 (CA19-9; a diagnostic marker for pancreatic cancer) decreased from 1200 to 584 U. Unfortunately, a computed tomography (CT) scan on day 101 showed a significant increase in the size of the primary tumor and the supraclavicular lymph nodes. The patient was alive with progressive disease on day 189 [572354].

The second patient experienced progressive, locally advanced pancreatic cancer despite prior radiotherapy and chemotherapy with fluorouracil and gemcitabine [572354]. The patient received two treatment cycles of Rexin-G, a total cumulative dose of 1.8×10^{11} cfu. An abdominal CT scan on day 28 showed a 47% decrease in tumor volume, and a follow-up scan on day 103 showed no change. The patient then received monthly gemcitabine. The patient was alive with stable disease on day 154. The third patient presented with metastatic pancreatic cancer with numerous metastases to the liver. Rexin-G was administered at a dose of 4.5×10^9 cfu/day for 6 days (total cumulative dose of 2.7×10^{10} cfu), followed by 8 weekly doses of gemcitabine (1000 mg/m^2). An abdominal CT scan on day 62 demonstrated a 30% decrease in primary tumor volume, and an 89% regression in the volume of the largest liver nodule. The number of liver nodules decreased from 18 to 5. The patient was alive with stable disease on day 133 [572354].

A second phase I study is ongoing in patients with metastatic colorectal cancer, who will receive a hepatic arterial infusion of the Rexin-G retroviral vector once a day on days 1 to 5 at doses of 3×10^9 , 6×10^9 and 1×10^{10} cfu [572352]. The objectives are to evaluate the safety/toxicity of hepatic arterial administration of Rexin-G, evaluate the pharmacodynamics of hepatic arterial infusion of Rexin-G administered as hepatic arterial infusion, to obtain preliminary data on molecular markers of tumor response, and to identify an antitumor response to hepatic artery-administered Rexin-G.

Side Effects and Contraindications

The three patients who received Rexin-G in the above phase I trial experienced no significant toxicity. No bone marrow suppression, significant alterations in liver and kidney function, nausea and vomiting, mucositis or hair loss were observed. Brief febrile episodes were the only adverse events associated with the vector infusions [572352].

Patent Summary

A series of patents supporting the development of Rexin-G are held by the University of Southern California (USC), all of which include the named inventors FL Hall and EM Gordon, the founders of Epeius Biotechnologies Corp. The most recent applications were published in the name of the company itself. The earliest of the former is WO-09716209, published as a PTC application in May 1997, which claims methods of inhibiting cyclic G1 protein for the treatment of tumors. In particular, the use of antisense oligonucleotides or antibodies for the treatment of tumor cells is disclosed, but, relevant to Rexin-G, the use of retroviral vectors to induce production of such agents or the cyclin G1 protein itself within target cells is also proposed.

The PTC application WO-09955893 is the first document claiming the use of modified retroviral envelope proteins to target the vector to bind to a specified molecule on target cells. The wide range of potential targeting mechanisms and sequences are claimed, including antibodies, cytokines, growth factors and other ligands. The collagen-binding domain of vWF is first claimed in this document, and is specifically claimed in WO-00006195 (published by USC in February 2000), in which the collagen-binding domain would be fused to an angiogenesis-modulating molecule, for the potential treatment of cardiovascular disease, ulcerative and inflammatory lesions and arthritis, as well as tumors. Similarly, WO-00107059 (published in February 2001) describes a fusion polypeptide of a collagen-binding domain of vWF and an epithelial cell proliferation-modulating agent. The May 2001 application WO-00131036 makes more specific claims covering vectors with modified viral surface proteins to target and destroy endothelial cells in tumor vasculature. The pharmaceutical composition of a targeted retroviral vector and a cytotoxic gene for inhibiting cancer was further claimed in WO-00244394 (June 2002), an application that provides data from mouse xenograft studies of targeted and non-targeted vectors.

The concept of using a dominant negative cyclin G1 construct appears in WO-00164870, published by USC in September 2001. This application claims a novel method of treating or preventing abnormal cell proliferation such as tumors or cancers by administering a mutated cyclin G1 protein or a gene construct encoding this protein that is a dominant negative inhibitor of wild-type cyclin G1. Claims include a viral expression vehicle such as a retroviral or adenoviral expression vehicle comprising the gene construct. In support of this claim are data demonstrating that treatment of tumors in mice with the Mx-dnG1 cytotoxic vector resulted in a 3-fold reduction in tumor growth rate.

Most recently, WO-2004093810 (the first application published by Epeius Biotechnologies, in November 2004) details methods and compositions for producing and administering targeted delivery vectors (preferably retroviral particles) for use in treating neoplastic disorders. Production of the targeted delivery vector involves transiently infecting a producer cell with plasmids containing sequences encoding a modified 4070A amphotropic protein, a viral Gag-Pol polypeptide and a polypeptide that confers drug resistance on the producer cell, and a diagnostic or therapeutic polypeptide, specifically Rexin-G. In support of this claim, data describe the efficacy of Mx-dnG1 (now known as Rexin-G) in a nude mouse model of liver metastasis.

Current Opinion

Rexin-G takes advantage of several unique features to induce antitumor responses. Firstly, it is targeted to the sites of tumor growth, as determined by angiogenesis or tumor invasion that exposes the collagen matrix. Secondly, it permits integration into the proliferating tumor cell, and thirdly, it inhibits cyclin G1 function, which is important for cell-cycle progression and cell proliferation. Results from animal models clearly demonstrate that the vector can be delivered to the tumor site and can induce apoptosis in a significant population of cells.

The need for effective, low toxicity agents for advanced cancers, particularly poor-prognosis pancreatic cancers, is great and Rexin-G appears to have clinical activity, albeit demonstrated so far in a small number of patients. In the phase I study, all three patients had an initial clinical regression of disease, followed by a period of disease stabilization. Looking to the future, it is not clear what Epeius' developmental strategy is, although the ability to combine this agent with gemcitabine would suggest the possibility of a study of gemcitabine plus Rexin-G versus gemcitabine alone for metastatic pancreatic cancer. Significant challenges that still exist to the development of Rexin-G include ensuring the high specificity of vector targeting, and management of the regulatory hurdles faced by all gene therapies and in particular retroviral vectors. The recent description of T-cell leukemias in two children with severe combined immunodeficiency who received T-cell precursors modified to express the ADA gene suggests that retroviral integration could potentially have safety issues. However, because the dnG1 gene delivered by Rexin-G is actually antiproliferative, it may be considered less likely to lead to the transformation of cells. Another consideration is how best to combine this drug with other effective therapies for pancreatic cancer. While it appears appropriate to combine it with chemotherapy that induces apoptosis, combination with anti-angiogenic therapies may be inappropriate because such therapy requires targeting to areas with ongoing angiogenesis.

Therapies that could potentially compete with Rexin-G include other cell-cycle inhibitors such as the Cdk inhibitor flavopiridol, which is in phase II trials by the National Cancer Institute. The major advantage of a small-molecule approach to cell-cycle inhibition is the established ability of small molecules to reach their tumor target, whereas the efficacy of gene therapies for inhibiting target molecules *in vivo* has still to be convincingly demonstrated.

Development history

By February 2003, Rexin-G was in phase I/II trials for pancreatic cancer [501606], and by April 2002 a US phase I trial evaluating the safety of hepatic arterial infusion of Rexin-G for colorectal cancer that has metastasized to the liver had been approved by the FDA [501609]. In September 2003, Rexin-G was granted Orphan Drug status for pancreatic cancer [504355].

Developer	Country	Status	Indication	Date	Reference
Epeius Biotechnologies Corp	Philippines	Phase I/II	Pancreas tumor	14-FEB-03	501606
Epeius Biotechnologies Corp	US	Discovery	Metastasis	14-FEB-03	501606
Epeius Biotechnologies Corp	US	Discovery	Colorectal tumor	20-AUG-03	501609

Literature classifications

Key references relating to the technology are classified according to a set of standard headings to provide a quick guide to the bibliography. These are as follows:

Chemistry: References which discuss synthesis and structure-activity relationships.

Biology: References which disclose aspects of the drug's pharmacology in animals.

Metabolism: References which discuss metabolism, pharmacokinetics and toxicity.

Clinical: Reports of clinical phase studies in volunteers providing, where available, data on the following: whether the experiment is placebo-controlled or double- or single-blind; number of patients; dosage.

Chemistry

Study Type	Result	Reference
SAR.	Rexin-G is a matrix (collagen)-targeted retroviral vector encoding an N-terminal deletion mutant form of human cyclin G1 construct. This vector is produced by transient co-transfection of 293T cells with three plasmids, pdnG1/C-REX (the deletion mutant of the human cyclin G1), Mx (Bv1/pCAEP; encoding the viral envelope component of MuLV modified with the vWF collagen-targeting sequence) and pCgpn (containing the MuLV <i>gag-pol</i> elements), all driven by the CMV immediate-early promoter.	572352

Biology

Study Type	Effect Studied	Experimental Model	Result	Reference
<i>In vitro</i>	Activity.	Human undifferentiated cancer cell line of pancreatic origin (MiaPaca2) treated with vectors expressing inhibitory antisense or mutant cyclin G1.	Transduction efficiency of the retroviral vector was 26 to 85% and correlated with the multiplicity of infection used (4 and 250). The cytotoxic or cytostatic effects of mutant and antisense cyclin G1 retroviral vectors were observed in transduced cancer cells.	572353
<i>In vivo</i>	Efficacy.	Nude mouse model of liver metastasis (MiaPaca2 cells administered via the portal vein). Mice received infusions of low- or high-dose Rexin-G (titers of 6×10^2 and 1×10^5 cfu/day, respectively) for 9 days.	Gene delivery ranged from 1 to 3% in most cells to $\geq 50\%$ in focal areas, where tumor-associated stromal and endothelial cells undergoing active angiogenesis were injected, while neighboring cells were not. Reductions were observed in the sizes of tumor foci, which were enhanced by increasing the number of vector infusions. There was no evidence of hepatocyte injury, vessel thrombosis or cholestatic changes in tissue sections from dnG1 vector-treated animals.	572353
<i>In vivo</i>	Efficacy.	Mouse MiaPaca2 cell xenograft model, administered Mx-dnG1, CAE-dnG1 or Mx-nBg. Seven daily vector infusions of $\sim 8 \times 10^6$ cfu/dose were administered and tumor size measured the following day.	The Mx-dnG1-encoding vector induced efficient transduction of tumor nodules (35.7%) that correlated with therapeutic efficacy, without associated toxicity. Tumors regressed by a mean 6.1% in animals treated with Mx-dnG1, but enlarged in other treatment groups by 4.1, 6.3 and 12% in the CAE-dnG1, Mx-nBg and placebo groups, respectively. Kaplan-Meier analysis confirmed that the probability of long-term control of tumor growth was significantly greater with the matrix-targeted Mx-dnG1.	572355

Metabolism

Study Type	Effect Studied	Model Used	Result	Reference
<i>In vivo</i>	Vector distribution.	Nude mouse (MiaPaca2 cell xenograft) models injected with 5.6×10^7 or 1.6×10^9 cfu of Mx-dnG1, or 4.8×10^6 or 1.1×10^9 cfu of modified MxV-dnG1 or control vector.	Using a realtime PCR assay, low-level positive signals were detected in the liver, lung and spleen of animals treated with both vector doses. No signal was detected in the testes, brain or heart of vector-treated animals.	572352

Clinical

Effect Studied	Model Used	Result	Reference
Safety and efficacy.	Phase I study in stage IV pancreatic cancer patients (n = 3). An inpatient dose escalation of Rexin-G (administered as a 1 to 3 h intravenous infusion) was performed; 4.5×10^9 , 9.0×10^9 and 1.4×10^{10} cfu were administered on days 1 to 6, 7 to 8, and 9 to 10, respectively. Patients were given a week to rest before receiving 1.4×10^{10} cfu on day 18 to 27.	The first patient experienced 40 to 50% necrosis of the primary tumor at day 28, and 33 and 62% regressions of two metastatic lymph node tumors. At day 101, a significant increase in the size of the primary and metastatic tumors was noted, however, the patient was alive with progressive disease on day 189. In the second patient, a 47% decrease in primary tumor volume was noted at day 28, and with monthly gemcitabine treatment the patient was alive with stable disease on day 154. The third patient demonstrated a 30% decrease in primary tumor volume and an 89% regression in the volume of the largest liver nodule on day 62. The number of liver nodules decreased from 18 to 5, and the patient was alive with stable disease on day 133. Rexin-G was well tolerated at total cumulative doses of up to 3×10^{11} cfu.	572354

Associated patent

Title Mutated cyclin G1 protein.

Assignee University of Southern California

Publication WO-00164870 07-SEP-01

Priority US-20000517832 02-MAR-00

Inventors Gordon EM, Hall FL.

Associated references

- of special interest

228193 **Retroviral vector-mediated gene transfer of antisense cyclin G1 (CYCG1) inhibits proliferation of human osteogenic sarcoma cells.** Skotzko M, Wu L, Anderson WF, Gordon EM, Hall FL *CANCER RES* 1995 **55** 23 5493-5498

501606 **Press release.** Epeius Biotechnologies Corp *PRESS RELEASE* 2003 June 03

501609 **Clinical trials.** Epeius Biotechnologies Corp *COMPANY WORLD WIDE WEB SITE* 2003 August 20

501651 **'Pathotropic' targeting for cancer gene therapy.** Hall FL *CANCER GENE THER* 2003 **10** Suppl 1 Abs S19

504355 **Rexin-G gets orphan status for pancreatic cancer.** Epeius Biotechnologies Corp *PRESS RELEASE* 2003 September 08

572352 **Clinical protocol. Tumor site specific phase I evaluation of safety and efficacy of hepatic arterial infusion of a matrix-targeted retroviral vector bearing a dominant negative cyclin G1 construct as intervention for colorectal carcinoma metastatic to liver.** Lenz HJ, Anderson WF, Hall FL, Gordon EM *HUM GENE THER* 2002 **13** 12 1515-1537

572353 **Inhibition of metastatic tumor growth in nude mice by portal vein infusions of matrix-targeted retroviral vectors bearing a cytotoxic cyclin G1 construct.** Gordon EM, Liu PX, Chen ZH, Liu L, Whitley MD, Gee C, Groshen S, Hinton DR, Beart RW, Hall FL *CANCER RES* 2000 **60** 13 3343-3347

- A detailed description of the *in vitro* cytotoxicity and *in vivo* efficacy of Rexin-G in a metastatic cancer animal model. Details of the vector construction are provided.

572354 **First clinical experience using a 'pathotropic' injectable retroviral vector (Rexin-G) as intervention for stage IV pancreatic cancer.** Gordon EM, Cornelio GH, Lorenzo CC, Levy LJP, Reed RA, Liu L, Hall FL *INT J ONCOL* 2004 **24** 1 177-185

572355 **Systemic administration of a matrix-targeted retroviral vector is efficacious for cancer gene therapy in mice.** Gordon EM, Chen ZH, Liu L, Whitley M, Liu L, Wei D, Groshen S, Hinton DR, Anderson WF, Beart RW, Hall FL *HUM GENE THER* 2001 **12** 2 193-204

- A detailed report of an *in vivo* model for systemic delivery of Rexin-G. Results are compared for vectors encoding Rexin-G, a non-targeted dominant negative cyclin G1-encoding vector and matrix-targeting construct without the cyclin G1 gene, or placebo. Tumors regressed by a mean of 6.1% in animals treated with Rexin-G, but were respectively enlarged by 4.1, 6.3 and 12% in other treatment groups.

572356 **Retroviral vector-mediated transfer of an antisense cyclin G1 construct inhibits osteosarcoma tumor growth in nude mice.** Chen DS, Zhu NL, Hung G, Skotzko MJ, Hinton DR, Tolo V, Hall FL, Anderson WF, Gordon EM *HUM GENE THER* 1997 **8** 14 1667-1674

572357 **Intratumoral injection of a concentrated antisense cyclin G1 retroviral vector inhibits growth of undifferentiated carcinoma in nude mice.** Hung G, Skotzko MJ, Chang M, Parekh D, Stain SC, Hall FL, Gordon EM, French AW *INT J PED HEMATOL/ONCOL* 1997 **4** 4 317-325

572358 **Cytostatic and cytotoxic effects of an antisense cyclin G1 expression vector on undifferentiated carcinoma cells after retroviral-mediated gene transfer.** Hung G, Skotzko MJ, Parekh D, Hall FL, Anderson WF, Gordon EM *PROC AM ASSOC CANCER RES* 1996 **37** Abs 344

582703 **Pancreatic cancer today.** Safioleas MC, Moulakakis KG *HEPATO-GASTROENTEROLOGY* 2004 **51** 57 862-868

582707 **Pancreatic cancer: Future outlook, promising trials, newer systemic agents, and strategies from the Gastrointestinal Intergroup Pancreatic Cancer Task Force.** Jafari M, Abbruzzese JL *SURG ONCOL CLIN N AM* 2004 **13** 4 751-760

582898 **The p53-regulated cyclin G gene promotes cell growth: p53 downstream effectors cyclin G and Gadd45 exert different effects on cisplatin sensitivity.** Smith ML, Kontny HU, Bortnick R, Fornace AJ, Jr *EXP CELL RES* 1997 **230** 1 61-68

582902 **Cyclin G1 is involved in G₂/M arrest in response to DNA damage and in growth control after damage recovery.** Kimura SH, Ikawa M, Ito A, Okabe M, Nojima H *ONCOGENE* 2001 **20** 25 3290-3300

582905 **Targeting retroviral vectors to vascular lesions by genetic engineering of the MoMuLV gp70 envelope protein.** Hall FL, Gordon EM, Wu L, Zhu NL, Skotzko MJ, Starnes VA, Anderson WF *HUM GENE THER* 1997 **8** 18 2183-2192

582907 **Molecular engineering of matrix-targeted retroviral vectors incorporating a surveillance function inherent in von Willebrand factor.** Hall FL, Liu L, Zhu NL, Stapfer M, Anderson WF, Beart RW, Gordon EM *HUM GENE THER* 2000 **11** 7 983-993

585535 **Survival of patients with advanced colorectal cancer improves with the availability of fluorouracil-leucovorin, irinotecan, and oxaliplatin in the course of treatment.** Grothey A, Sargent D, Goldberg RM, Schmoll HJ *J CLIN ONCOL* 2004 **22** 7 1209-1214

585536 **Systemic therapy for colorectal cancer.** Meyerhardt JA, Mayer RJ *N Engl J Med* 2005 **352** 5 476-487

585537 **Cell-cycle targeted therapies.** Swanton C *LANCET ONCOL* 2004 **5** 1 27-36

585538 **Retroviral vectors: New applications for an old tool.** Barquinero J, Eixarch H, Perez-Melgosa M *GENE THER* 2004 **11** Suppl 1 S3-S9

585540 **Expression of von Willebrand factor, an endothelial cell marker, is up-regulated by angiogenesis factors: A potential method for objective assessment of tumor angiogenesis.** Zanetta L, Marcus SG, Vasile J, Dobryansky M, Cohen H, Eng K, Shamamian P, Mignatti P *INT J CANCER* 2000 **85** 2 281-288