The Rogosin Institute
505 East 70th Street
New York, NY 10021

An Open-Label, Phase II Efficacy Trial of the Implantation of Mouse Renal Adenocarcinoma Cell-Containing Agarose-Agarose Macrobeads in the Treatment of Patients with Treatment-Resistant, Metastatic Pancreatic Adenocarcinoma or Colorectal Cancer

Clinical Study Protocol Number: 0911010739

IND Number: BB-IND 10091

November 12th 2014
Amendment 11

SIGNATURE PAGE

THE ROGOSIN INSTITUTE
APPROVAL PAGE

Protocol: An Open-Label, Phase II Efficacy Trial of the Implantation of Mouse Renal Adenocarcinoma Cell-Containing Agarose-Agarose Macrobeads in the Treatment of Patients with Treatment-Resistant, Metastatic Pancreatic Adenocarcinoma or Colorectal Cancer

Amendment: 11

Investigators' Agreement:

I have read the protocol and agree to conduct the study as outlined herein.

Signature: _____ **Date:** 3/17/14

Name (print): Thomas J. Fahey, M.D.

PROTOCOL SYNOPSIS	
TITLE OF STUDY	An Open-Label, Phase II Efficacy Trial of the Implantation of Mouse Renal Adenocarcinoma Cell-Containing Agarose-Agarose Macrobeads in the Treatment of Patients with Treatment-Resistant, Metastatic Pancreatic Adenocarcinoma or Colorectal Cancer
Investigators/ Study Centers:	Thomas J. Fahey,III, MD Nataniel Berman, MD Weill Cornell Medicine/The Rogosin Institute 505/520 East 70th Street. New York, NY 10021
Objectives:	The primary efficacy outcome for colorectal cancer is post-implantation all-cause mortality, where time to death is defined as the time from the first scan showing disease progression after completion of prior treatment (time of origin, T_0) to death from any cause. The primary objective for pancreatic cancer is to determine the Response Rate (RR), at 2 weeks, 4 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months or longer as possible, of subjects treated with macrobeads after they have failed standard chemotherapeutic regimens or have decided not to pursue standard or experimental chemotherapy for treatment-resistant, metastatic pancreatic adenocarcinoma. The secondary objectives for both pancreatic and colorectal cancer are outlined in Section 3 of this protocol.
Rationale for Dosage:	Based on the animal studies conducted to date, as well as the Phase I human trial that has enrolled 31 subjects to date, a reasonable dosage range (number of beads to be implanted) is 8 macrobeads per kilogram of body weight. We know that 16 macrobeads per kilogram were also well-tolerated in the Phase I human trial, but, at least with a single dose, this number was not seen to be any more effective than 8 macrobeads per kilogram. As it is planned to carry out up to four implants (over approximately 12 months) in this Phase II trial, each implantation will be carried out at the 8 macrobeads per kilogram dosage.
Study Design:	This is an open-label clinical trial. The clinical trial will have duration of 12 months and involve a potential total of four macrobead implants, each no less than three months apart. A total of 74 subjects will be treated. After the formal phase of the trial is completed, subjects will be followed for life. The response of each subject to the intraperitoneal implantation of the RENCA agarose-agarose macrobeads will be measured as outlined in Section 3 of this protocol. Each implant will be considered as Day 0. Post implant follow-up visits will be done in reference to the corresponding Implantations i.e. Days 14, 30, 60 and 90. Because much of the testing will be objective in terms of

PROTOCOL SYNOPSIS	
	quantifiable clinical laboratory, bias on the part of subjects, investigators, and analysts should be minimized.
Planned Sample Size:	The study will enroll a total of 116 evaluable subjects with advanced pancreatic cancer and colorectal cancer, and implant 74 eligible patients. An evaluable subject is defined as a subject who completes the first implantation of the RENCA agarose-agarose macrobeads. If a subject signs an Informed Consent and is not implanted, the subjects will not be classified as evaluable and will be replaced. These subjects will be classified as screen failures
Inclusion Criteria:	1. Histologically-confirmed adenocarcinoma of the pancreas, colon, or rectum. 2. Radiographic evidence of metastatic cancer of the colon or rectum. 3. The subject has pancreatic cancer that is unresectable or already metastatic or colorectal cancer that has failed available treatment modalities. 4. For the pancreas subjects, he/she may be accepted without prior chemotherapy or with multiple therapies that have failed. The colon and rectal cancer subjects must have failed available chemotherapy/targeted regimens. There is no limit to the number of prior chemotherapeutic regimens. 5. The subject must have evidence of progressive disease defined as at least one of the following: a. Progressive measurable disease: using conventional solid tumor criteria b. Increasing tumor markers and/or activity on PET-CT SUV measurement 6. All clinically significant toxic effects (excluding alopecia) of prior surgery, radiotherapy, or hormonal therapy have resolved to ≤ Grade 1 based on the NCI-CTCAE v 3.0, with the exception of peripheral neuropathy, which must have resolved to Grade ≤ 2. 7. The subject has an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-2. 8. The subject has adequate hematologic function (minimum requirements: absolute neutrophil count [ANC] ≥ 1500/mL, hemoglobin ≥ 9 g/dL, and platelets ≥ 100,000/mL). 9. The subject has adequate hepatic function (bilirubin ≤ 1.5 times the upper limit of normal (ULN)], aspartate transaminase [AST] and alanine transaminase [ALT] ≤ 3 times the ULN, or ≤ 5 times the ULN, if liver metastases are present). 10. The subject has adequate renal function (creatinine ≤ 2.0 mg/dL). 11. The subject has adequate coagulation function (an international normalized ratio [INR] ≤ 1.5 and a partial thromboplastin time [PTT] ≤ 5 seconds above the ULN [unless on oral anticoagulant therapy]). Subjects receiving full-dose anticoagulation therapy are eligible provided they meet all other criteria, are on a stable dose of oral anticoagulant or low molecular weight heparin (and if on warfarin have

PROTOCOL SYNOPSIS	
	a therapeutic INR between 2 and 3). 12. The subject has a life expectancy of 6 weeks. 13. The subject is male or female and at least 18 years of age. For females, a negative pregnancy test is required. 14. The subject agrees to use contraceptives while on study, if sexually active. 15. The subject has provided signed informed consent.
Exclusion Criteria:	1. Any condition (cardiovascular or other), making subject an unacceptably high anesthetic or surgical risk based on current anesthesia/general surgery standards. 2. Positive testing for HIV. 3. Cognitive impairment sufficient to render the subject incapable of giving informed consent. 4. Hypersensitivity reaction that, in the opinion of the investigators, poses an increased risk of an allergy to the macrobeads, particularly any known allergy to murine antigens or body tissues. 5. Surgical treatment or chemotherapy within three weeks of scheduled macrobead implantation or within four weeks of bevacizumab (or similar drugs), or radiation therapy within four weeks of scheduled macrobead implantation. 6. Investigational medications for their respective tumors within one month of baseline evaluation. 7. The subject has inadequate hematologic function (absolute neutrophil count [ANC] < 1500/mL, hemoglobin < 9 g/dL, and platelets < 100,000/mL). 8. The subject has inadequate hepatic function [bilirubin > 1.5 times the upper limit of normal (ULN)], aspartate transaminase [AST] and alanine transaminase [ALT] > 3 times the ULN, or > 5 times the ULN, if liver metastases are present). 9. The subject has inadequate renal function (serum creatinine > 2.0 mg/dL). 10. The subject has inadequate coagulation function (an international normalized ratio [INR] > 1.5 and a partial thromboplastin time [PTT] > 5 seconds above the ULN [unless on oral anticoagulant therapy]). Subjects receiving full-dose anticoagulation therapy are eligible provided they meet all other criteria, are on a stable dose of oral anticoagulant or low molecular weight heparin (and if on warfarin have a therapeutic INR between 2 and 3). 11. Hepatic blood flow abnormalities: portal vein hypertension and thrombosis; and/or large volume of ascites. 12. Concurrent cancer of any other type except skin cancer (excluding melanoma). 13. History of allergic reactions to mouse antigen. 14. The subject has an ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, serious cardiac

PROTOCOL SYNOPSIS	
	arrhythmias, (well-controlled atrial fibrillation, is permitted,) psychiatric illness/social situations that could interfere with the subject's ability to participate in the protocol, active bleeding. 15. As a result of the medical history, examination or blood testing, the investigator considers the subject unfit for the study.
Efficacy Variables:	The response of each subject to the intraperitoneal implantation of the RENCA agarose-agarose macrobeads will be measured after each implantation with respect to changes in: • Primary tumor size • Size or number of secondary/metastatic tumors • Tumor marker (such as CEA, CA 19-9 and CA-125) or other relevant biochemical parameters such as liver enzymes, alkaline phosphatase. lactic dehydrogenase • Cellular and humoral immune status • Global clinical status or rating • Activities of daily living (ADL) rating • Symptom rating/severity (including narcotic analgesic consumption' visual analog pain scale; • Quality of life- EORTC • ECOG and Karnofsky Performance Status • Abdominal MRI/PET/CT scan with other imaging studies as appropriate for the subject's tumor type (primary and metastatic) and condition
Safety Variables:	The following safety variables will be monitored to insure the safety of the participants in the study: • Complete physical examination with all vital signs • Weight • Global Clinical Assessment • EKG • Biochemical profile including appropriate tumor markers • Amylase, lipase • Complete blood count (CBC) with differential WBC • Erythrocyte sedimentation rate • C-reactive protein • Standard biochemical profile • Liver function (AST, ALT, LD, alkaline phosphatase, GGT) • Tumor markers as appropriate and available • Urinalysis • Coagulation profile (PT, INR, PTT) • Hepatitis B, C, E • HIV • Immunoglobulin levels (IgA, IgG, IgE, IgM) • Skin testing with murine epithelial antigen preparation, with positive

PROTOCOL SYNOPSIS	
	and negative controls • Cellular and humoral immune function o T cells o Cytokine production o B cells o Antibodies to common bacteria and viruses (such as diphtheria, tetanus, mumps, rubella) o Quantitative IgG subclass determinations o NK cells count (CD16) Analysis of circulating tumor cells. • Ecotropic murine leukemia virus (e-MuLV) (PCR-based assay) • Ultrasound examination, if medically indicated, will include views of the hepatic veins and inferior vena cava to be certain that they are free of clots and that there is free peritoneal drainage
Principles of Statistical Analysis:	The critical parameters of this Phase II trial are those related to efficacy and safety. Various markers, such as CEA, CA 19-9, CA125 and C-reactive protein, amylase, liver function tests, cell counts, and cytokine levels, will be sampled and analyzed in relationship to their baseline values. Given the study design, simple population comparisons designed for small samples (non-normal distribution) as well as simple trend analysis for selected parameters will be performed. The data from all subjects, including those who withdraw or are withdrawn from the study will be analyzed. All data will be analyzed using SAS software.

TABLE OF CONTENTS

1 LIST OF ABBREVIATIONS

ADL	Activities of daily living
AE	Adverse event
Alk Phos	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Beta cells	The predominant type of cell in the islets.
BB-IND	Bureau of Biologics,Investigational New Drug
CA 125	Cancer antigen, marker for ovarian cancer
CA 19-9	Cancer antigen 19-9, specific marker for certain gastrointestinal tumors
CBC	Complete blood count
Cc	Cubic centimeter
cc/min	Cubic centimeter per minute
CD133+	Some melanoma cells contain subset of cells expressing CD133+, a surface antigen
CD44+	Multispecific cell adhesion molecule
Cd16	A cluster of differentiation found on the surface of natural killer cells
CEA	Carcinoembryonal antigen
CT scan	Computerized tomography
CTCAE v3.0	Common Terminology Criteria for Adverse Events
DLT	Dose limiting toxicity
DNA	Deoxyribosenucleic acid
DSMB	Data Safety Monitoring Board
ECOG	Eastern Collaborative Oncology Group
EGFR	Epidermal growth factor receptor/gene
EKG	Electrocardiogram
e-MuLV DNA	Ecotropic murine leukemia virus envelope protein
EORTC	European Organization for Research and Treatment of Cancer
FDA	Food and Drug Administration
G1-S	Stage in the cell cycle at the boundary between the G1 phase and the S phase.
G2-M	Checks for damaged DNA and unreplicated DNA
GADD45	A p53-regulated stress protein
GGT	Gamma glutamyl transpeptidase
GI	Gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HAMA	Anti-mouse antibodies
HER-2	Human epidermal growth factor receptor
HIV	Human immunodeficiency virus
IgA	Immunoglobin A
IgE	Immunoglobin E

IgG	Immunoglobin G
IgM	Immunoglobin M
IND	Investigational New Drug
INR	International Normalization Ratio
IRB	Institutional Review Board
IV	Intravenous
kD	KiloDalton
Kg	Kilogram
Ki67	Cell-cycle related nuclear protein; proliferation marker.
LD	Lactic dehydrogenase
mg/dl	Milligram per deciliter
μM	Micromole
MuLV	Murine leukemia virus
Mm	Millimeter
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
mTor	Coordinates several upstream signaling pathways in renal tumor cells.
MTT assay	Laboratory test for measuring the activity of enzymes
NCI	National Cancer Institute
NK	Natural killer cell
OS	Overall survival
p27	Gene which lies on chromosome 12 in humans
PCR DNA	Method by which a few fragments of DNA can be duplicated
PET scans	Positron emission tomography
PFS	Progression-free survival
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RENCA	Renal adenocarcinoma
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious adverse event
T cells	A type of blood cell that protects the body from infection.
TPN	Total parenteral nutrition
USPHS	United States Public Health Service
VEGF	Vascular endothelial growth factor

2 BACKGROUND

Treatment for cancer has traditionally consisted of three modalities: surgery, radiation therapy and chemotherapy. Advances with all three modalities over the years have produced some long-term remissions and/or cures in certain types of cancer such as the leukemias, and prolonged survival for many other patients. The advent of targeted, often more "biological" therapies such as tyrosine kinase inhibitors, inhibitors of angiogenesis, inhibitors of, and antibodies to, specific receptors such as mTor, HER-2, VEGF, and EGFR, has further changed the face of anti-cancer therapy. Much remains to be accomplished, however, especially with respect to the treatment of solid tumors, including some of the most common and deadly cancers such as those of the lung, colon, breast, ovary, prostate, pancreas, and kidney. New types of less toxic, less debilitating and more effective therapies are needed.

Pancreatic cancer:

Carcinoma of the pancreas has had a markedly increased incidence during the past several decades and ranks as the fourth leading cause of cancer death in the United States (American Cancer Society, 2009; Greenlee et al, 2000). Despite the high mortality rate associated with pancreatic cancer, its etiology is poorly understood. Cancer of the exocrine pancreas is rarely curable and has an overall survival (OS) rate of less than 4%[Greenlee et al, 2000]. The highest cure rate occurs if the tumor is truly localized to the pancreas; however, this stage of the disease accounts for fewer than 20% of cases. For those patients with localized disease and small cancers (<2 cm) with no lymph node metastases and no extension beyond the capsule of the pancreas, complete surgical resection can yield actuarial 5-year survival rates of 18% to 24%. (Yeo et al, 1997). Improvements in imaging technology, including spiral computed tomographic scans, magnetic resonance imaging scans, positron emission tomographic scans, endoscopic ultrasound examination, and laparoscopic staging can aid in the diagnosis and the identification of patients with disease that is not amenable to resection. For patients with advanced cancers, the OS rate of all stages is less than 1% at 5 years with most patients dying within 1 year.

No tumor-specific markers exist for pancreatic cancer; markers such as serum CA 19-9 have low specificity. Most patients with pancreatic cancer will have an elevated CA 19-9 at diagnosis. Following or during definitive therapy, the increase of CA 19-9 levels may identify patients with progressive tumor growth. The presence of a normal CA 19-9, however, does not preclude recurrence.

According to the National Cancer Institute (www.cancer.gov/cancerinfo/types/pancreatic), patients with any stage of pancreatic cancer can appropriately be considered candidates for clinical trials because of the poor response to chemotherapy, radiation therapy, and surgery as conventionally used. One such trial is the gemcitabine, docetaxel, capecitabine (GTX) therapy being utilized at Columbia University by Dr. Robert Fine and his colleagues (Phase II).

Colorectal cancer:

In contrast to pancreatic cancer, cancer of the colon is a highly treatable and often curable disease when localized to the bowel. Surgery is the primary form of treatment and results in cure in approximately 50% of the patients. Recurrence following surgery is a major problem and is often the ultimate cause of death.

The prognosis of patients with colon cancer is clearly related to the degree of penetration of the tumor through the bowel wall, the presence or absence of nodal involvement, and the presence or absence of distant metastases. Beyond those characteristics, elevated pretreatment serum levels of carcinoembryonic antigen (CEA) have a negative prognostic significance. The fact is, however, that, even with good prognostic factors and aggressive chemotherapy with regimens such as FOLFOX and FOLFIRI, with or without the addition of bevacizumab and cetuximab (the latter for $KRAS^{+}$ patients), many patients become resistant to available chemotherapies and targeted biological therapies. In addition, the other surgical and ablative techniques may no longer be applicable. With liver and lung metastases common problems, and brain metastases less common, but potentially devastating, there clearly is a need for new, more effective therapeutic measures, especially for those patients who do have metastatic spread of their tumors

Among the therapeutic possibilities currently being explored for pancreatic and colorectal cancer, as well as other solid tumors, those that involve cellular biological control mechanisms seem both promising and attractive. Many such modalities-- such as induction of terminal differentiation, enhancement of growth-inhibitory (negative) feedback, selective programmed cell death (apoptosis), targeted insertion of viral or other genes into the proliferating cancer cell, and growth arrest at either the G1-S or G2-M checkpoints of the cell cycle -- all seem to be feasible goals with significant potential for clinical use. Furthermore, recent data suggest that a subpopulation of cells within a tumor, i.e., the so-called cancer stem or progenitor cells, which have been described and characterized in certain tumor types such as those of the brain (glioma series), colon, and breast, may, in fact, be responsible for tumor survival, progression, resistance, and metastasis. Should these observations be verified, these cell populations represent a novel and fundamental target for anti-neoplastic therapy.

The development of truly new and more effective therapeutic approaches to the treatment of neoplastic disease requires a far better understanding of the nature of cancer than we currently have. It is increasingly clear that cancer is not simply the result of a rogue, mutated cell or clone of cells exhibiting unrestricted proliferative and metastatic behavior. Rather, cancer is itself a biological system, in a sense a kind of (undesirable) organ and/or organ system. Furthermore, cancer is not a entirely separate entity within the host, but rather dependent on complex interactions with the host as a whole and its own microenvironment, just as a normal organ is. The local microenvironment may, in fact, aid and abet the neoplastic cells, providing them with blood flow and nutrition, for example. The "normal" host cells in the microenvironment may not be normal at all, but may become incorporated into the structure and workings of the tumor. In other words, the tumor is a heterogeneous collection of interdependent cells, the least desirable of which may be what we think of as the neoplastic cells. Those cells, however, are only part of the story.

The fact that cancer is, in effect, an alternative organ system, suggests that it should be subject to at least some of the same regulatory processes that govern normal, physiologic system function. One such process, the control of proliferation in a normal organ, is quite strict. Although it has long been thought that cancer cells and the tumors they form are not subject to the same regulatory growth-control feedback mechanisms as are normal cells and organs, increasing evidence suggests that they are, in fact, subject to such regulation. Not surprisingly, an important signal in the growth-regulatory process for tumor cells is the mass of tumor present. Tumor growth slows as the mass of both primary and metastatic tumors increase (7-12). Surgeons, for example, have observed that surgical excision of part of a tumor mass can be associated with rapid re-growth of the remaining tumor and/or distant metastases (13,14). De Wys (15) and Fisher et al (16) have demonstrated the same phenomenon in animal models of tumors. We have been able to confirm this ourselves in a mouse tumor model involving the injection of mouse renal adenocarcinoma cells under the renal capsule. In these studies, removal of the primary tumor at an early stage of development of the malignancy has resulted in the appearance of dramatically greater numbers of distal metastases. Taking these various findings into account, it is not unreasonable to argue, as Prehn has done (17), that a promising therapeutic approach to the biological control of tumor growth could consist of "fooling" tumors into sensing that their mass is greater than it actually is, thereby slowing or halting tumor growth.

The proposed cancer treatment to be tested in the Phase II clinical trial outlined in this protocol is based, at least in part, on the concept that tumor growth

can be controlled by tumor mass or signals that indicate that such mass is present. In this case, however, the induction of such signals is brought about not by true tumor mass, but rather by placing cancer cells in a proliferation-restrictive hydrophilic matrix composed of agarose. In brief, cancer cells (in this case, mouse renal adenocarcinoma cells) placed in such a growth-restrictive matrix contained within the structure of a 6-8mm "macrobead' are induced to produce and release signals that can inhibit the proliferation of free cancer cells of the same and different types (and without species specificity) both *in vitro* and *in vivo.* Translated into practical clinical terms, the release of such inhibitory signals from cancer cells in a proliferation-restrictive environment could be useful in the treatment of human cancers.

Based on our continued laboratory and clinical studies with the macrobead approach to the control of the cancerous growth, we can say that the mechanisms by which this is achieved are more complex than the model advocated by Prehn. In fact, the cells that survive to function in the bead have at least some of the properties of cancer stem or progenitor cells. These colonies are comprised of cells that secrete inhibitory factor(s), which, in turn, regulate a variety of processes in the freely growing cancer cells. These affected processes include regulation of the cell-cycle check points (G1S and G2M), DNA synthesis/replication, cell-cell interactions, angiogenesis, cellular metabolism and cytoskeletal function more generally. In other words, the macrobeads themselves constitute an altered, constrained neoplastic system with the capability of influencing the behavior of other, non-constrained neoplastic systems. In vivo, these changes are achieved without any evident impairment to date of normal body functions, including that of the immune system. In fact, data from our Phase I clinical trial show that the human immune system is of normal to increased activity after the beads have been placed.

The heterogeneous renal adenocarcinoma cell population initially placed in the agarose matrix of the macrobead undergoes dramatic depopulation followed by dedifferentiation of the surviving cells. Specifically, colonies emerge that contain both cells with stem cell-like properties and cells with other as yet undefined properties. The latter group has features suggestive of the tumor "niches" that have now been described in a range of tumors. These niches represent the bodies that are now postulated to be responsible for tumor generation and survival/perpetuation. They are likely also to be the source of the cells that can break loose and form both local and distant metastases. In vivo, niches are composed of cells morphologically and molecularly similar to those seen in the macrobeads, but have the additional feature of being surrounded by an outer coat of fine blood vessels that both nourishes and protects them from host defenses, as well as possibly external therapies. Quite apart from their biological importance in neoplasia, the niches represent a unique endpoint for assessing the anti-neoplastic efficacy of the macrobeads.

While the properties of the cancer stem cells, “niches”, and the macrobeads that contain them are of significant scientific interest, their precise mechanism of anti-tumor action has yet to be fully explored. Genomic studies of the cells within the colonies in the macrobeads show striking up-regulation of certain genes such as CHOPP and GADD45. One or more, or all, of the proteins produced by these and other genes, which can be measured by PCR, can be released into the medium and serve as the postulated signals to the freely-growing cancer cells. Whatever the outcome of this important line of research, however, there is solid evidence in preclinical studies that the macrobeads may yield clinically important results. Preclinical studies in mouse tumor models, both in vivo and in vitro, have indicated statistically significant activity of the macrobeads with respect to suppression of tumor growth. Studies in 45 dogs and cats with the above macrobeads in the treatment of a variety of spontaneous tumors including gastrointestinal lymphoma, prostate cancer and hepatocellular tumors have shown a tumor response rate of at least 50%. Increased activity levels and weight gain have also characterized these animals after RENCA macrobead implantation consistent with an improved quality of life.

New and ongoing in vitro laboratory studies with human tumor cell lines including those with an androgen-independent, docetaxel-resistant human prostate cell line have shown that the macrobeads can slow or stop the proliferation of these cells when no other agents are effective. These in vitro studies, plus in vivo tests with the same tumor line in an immunodeficient mouse tumor model, add further, preclinical support to the trial proposed here. If the macrobeads continue to be effective after cancer cells have developed resistance to all currently available agents, then they could have a place in the clinical treatment of advanced cancers, let alone those at an earlier stage.

The Phase I trial of the cancer macrobeads in the initially targeted 13 subjects with a variety of Stage IV, end-stage, treatment-resistant epithelial-derived tumors has enrolled and implanted 31 subjects to date, 21 more than the original goal of 10. It has demonstrated that the macrobeads are well tolerated when implanted in the human abdominal cavity for periods of up to 24 months. Tumor types evaluated in the safety/toxicity trial have included colorectal carcinoma, gall bladder cancer (cholangiocarcinoma), gastric carcinoma (schirrous type), pancreatic carcinoma, ovarian carcinoma, and non-small cell lung carcinoma. There was no pattern of any consistent adverse effect attributable to the macrobeads. Rather, the problems that did occur were attributable to the disease process itself. Phase I study is closed for the subject accrual and is only open for data analysis.

3 OBJECTIVES

All study objectives will be assessed in each of the two study groups to be enrolled in this clinical trial: subjects with advanced pancreatic cancer and subjects with advanced colorectal cancer.

3.1 Objectives for the advanced colorectal cancer

The objectives for the advanced colorectal cancer, as defined in the Statistical Analysis Plan (SAP), are:

3.1.1 Efficacy

The primary efficacy outcome is post-implantation all-cause mortality, where time to death is defined as the time from the first scan showing disease progression after completion of prior treatment (time of origin, T_0) to death from any cause.

Secondary efficacy outcomes are (if feasible):

- Time from first implantation to death.
- Time from disease Stage IV diagnosis to death.
- Changes from baseline based on Magnetic Resonance Imaging (MRI), computerized tomography (CT) or positron emission tomography (PET)-CT scan, and other appropriate imaging techniques (e.g., sonography, bone scans, other x-rays) as indicated, if feasible.
  - In primary and/or secondary tumor size (volume, area) and
  - In state (necrosis, vascularization) on MRI, CT or PET-CT scan, and other appropriate imaging techniques (e.g., sonography, bone scans, other x-rays) as indicated.
- Change from baseline in tumor markers (CEA, CA19-9)
- Changes from baseline in CA125 (as a marker of inflammation)
- Changes from baseline in
  - Clinician Global Clinical Assessment
  - Activities of daily living
  - Symptom rating
  - Quality of life (QCQ-C30)

- Pain scale
- ECOG

- Additional exploratory efficacy outcomes may be analyzed if feasible and will be defined in the CSR.

3.1.2 Safety

Safety outcomes will include:

- Reason for study discontinuation (coming off protocol).
- Incidence of adverse events (AEs).
- Changes from baseline in physical examination parameters, including weight, vital signs, and electrocardiogram (ECG).
- Changes from baseline in laboratory parameters (see section 5.6 of the protocol).
- Changes from baseline in murine antigens skin test.
- Changes from baseline in status of murine leukemia virus as detected by polymerase chain reaction (PCR).

3.1.3 Exploratory

Exploratory outcomes will include presence or absence of circulating tumor cells. An addition to the endpoints for assessing efficacy will be, where possible, when a biopsy is indicated, a careful search for, and identification of, the niches of cancer stem and other cells within the niche bodies of the tumors the macrobeads are being used to treat. Markers of the niche used to identify these cells and study their properties (numbers, integrity of surrounding blood vessels, degree of stress) will include HLA, CD44+, CD133+.

3.2 Objectives for the advanced pancreatic cancer

3.2.1 Primary Objective

To determine the Response Rate (RR), at 2 weeks, 4 weeks, 8 weeks, 3 months, 6 months, 9 months, 12 months or longer as possible, of subjects treated with macrobeads after they have failed standard chemotherapeutic regimens or have decided not to pursue standard or experimental chemotherapy for treatment-resistant, metastatic pancreatic adenocarcinoma, where RR is defined as:

- Evidence of change in primary or secondary tumor size (volume, area) as well as state (necrosis, vascularization, metabolic rate) on PET-CT, as well as MRI and/or CT as appropriate
- Decrease in tumor markers (including CEA and CA 19-9) and specific biochemical parameter levels in plasma/serum
- Stable or improved quality of life as measured by EORTC QLQ-C30, weight, and pain control
- Stable or improved global clinical rating of subject by physician and/or nurse
- Increase in LDH consistent with tumor necrosis (significance of LDH changes will be determined in relation to imaging changes indicative of necrosis)

3.2.2 Secondary Objectives

1. To determine the safety and tolerability of the implantation of the RENCA cancer-cell-containing macrobeads in advanced pancreatic cancer subjects with respect to:

- The peritoneum: irritation, inflammation, mechanical effects (over a period of at least 3 months);
- Other intraperitoneal organs (liver, spleen, large and small intestines, kidney, bladder, omentum, etc.);
- Local and systemic immune response (T and B cell/cellular, antibody responses);
- Bacterial, viral zoonotic infection (clinical and hematological examination; PCR DNA analysis for viral DNA; specific viral antibodies);
- Hypersensitivity reactions to murine antigens.

2. To determine progression, defined as the time from the first implantation until the first occurrence of any of the following events
    a. Death due to any cause, or
    b. Progression as defined by the presence of the occurrence of any three of the five following events:
        i. An increase in the levels of tumor markers (e.g., CEA, CA19-9) of 50% from baseline determinations
        ii. Radiographic progression including increase in primary or metastatic tumor size/volume; increasing metabolic activity of the presenting tumor or of new metastatic lesions on a PET/CT scan after the administration of radio-labeled fluorodeoxyglucose (FDG); and evidence of new metastatic lesions.

iii. A decrease in the EORTC QLQ-C30 quality of life scale as compared to baseline of more than 25%;
(Above data will also be analyzed with censorship of deaths to further evaluate progression)

3. Determine overall survival (OS)

4. Circulating tumor cells will also be measured

3.2.3 Exploratory objectives

An addition to the endpoints for assessing efficacy will be, where possible, when a biopsy is indicated, a careful search for, and identification of, the niches of cancer stem and other cells within the niche bodies of the tumors the macrobeads are being used to treat. Markers of the niche used to identify these cells and study their properties (numbers, integrity of surrounding blood vessels, degree of stress) will include HLA, CD44+, CD133+.

3.3 Rationale for Study Dosages

Based on the animal studies conducted to date, as well as the Phase I human trial that has been completed to date, a reasonable dosage range (number of beads to be implanted) is 8 macrobeads per kilogram of body weight. We know that 16 macrobeads per kilogram were also well-tolerated in the Phase I human trial, but, at least with a single dose, this number was not seen to be any more effective than 8 macrobeads per kilogram. As it is planned to carry out up to four implants (over 12 months) in this Phase II trial, each implantation will be carried out with the 8 macrobeads per kilogram dosage.

4 INVESTIGATIONAL PLAN

4.1 Description of Overall Study Design and Plan

This is an open-label clinical trial. The clinical trial will have duration of approximately 12 months and involve a potential total of four macrobead implants, each no less than three months apart.

Each subject will serve as his own control. This means that changes from baseline to various post-treatment time points will be analyzed for some variables (such as tumor markers, for example). The confidence intervals for these changes will be calculated. Of course, it is not planned for the survival analysis.

Seventy-four (74) male and female subjects with advanced pancreatic cancer or metastatic, treatment-resistant colorectal cancer meeting all the above inclusion criteria will be recruited and treated for the study. Baseline measurements will include tumor burden and size, tumor history and pathological diagnosis and grade, global clinical state, activities of daily living, symptom checklist including tumor-related symptoms, a sense-of-well-being scale, routine clinical biochemistry and any appropriate tumor markers as available, and an assessment of cellular and humoral immune status.

The primary tumor histopathology will be reviewed and will be evaluated with special staining to identify, if possible, the presence or absence of cancer stem cells and niche bodies.

The response of each subject to the intraperitoneal implantation of the RENCA agarose-agarose macrobeads will then be measured over the ensuing 3 months (and a 6, 9, and 12 months, if possible) with respect to changes in:

- Primary tumor size
- Size or number of secondary/metastatic tumors
- Tumor marker or relevant biochemical parameters such as liver enzymes, alkaline phosphatase, lactic dehydrogenase
- Cellular or humoral immune status
- Global clinical status or rating
- Performance status (Karnofsky PS and ECOG PS)
- Activities of daily living (ADL) rating
- Symptom rating/severity (including use of pain medications)
- Quality of life scale
- Circulating tumor cells (when possible)

After each successive implant, follow-up will be at Days 14, 30, 60, and 90. Because much of the testing will be objective in terms of quantifiable clinical laboratory and imaging testing, bias on the part of subjects, investigator, and analysts should be minimized.

Each implant will be considered as Day 0. Post implant follow-up visits will be done in reference to the corresponding implantations i.e. Days 14, 30, 60 and 90 for each implantation.

4.2 Study Device/Biological Product

In this study, the study device/drug is the RENCA macrobead. The cancer cell-containing agarose-agarose macrobeads that are to be used for the Phase II clinical trial are composed of two concentric spherical agarose layers. The inner layer, which is also the layer in which the neoplastic cells (in this case, mouse renal adenocarcinoma cells) are embedded, is made up of 1.0% agarose. The outer layer is composed of 5% agarose. 150,000 mouse renal adenocarcinoma (RENCA) cells are placed in each bead. The diameter of the macrobead is 6,000-8,000 μM or 6-8 mm. Its structure is such that molecules above 80,000 kD are largely excluded from either entering or leaving the bead. Importantly, the cancer cells, although they proliferate in the inner layer of agarose, do not invade the outer layer or escape from an intact bead. In addition, peritoneal or immune system cells from the host cannot enter into the macrobead as well. The macrobeads produce the inhibitory effect for at least 3 years *in vitro* and up to 6 months *in vivo* in animal studies. Data from the Phase I human clinical trial indicate the macrobeads have a functional longevity of from 3 to 4 months.

The cancer-cell containing macrobead is derived from previous and currently ongoing work with a porcine-islet containing agarose-agarose macrobead, an IND for the human Phase I testing of which has been submitted to the FDA (IND # 9672, originally submitted April 9, 2001 and now continuing with further submissions to address the outstanding “clinical hold” issues). This islet macrobead is described in detail in that IND and also in publications from our laboratory (Jain et al, 1995, 1999; Gazda, 2003, Gazda et al, 2005, Vinerean et al, 2008 contained in that IND).

The overall process of preparing the RENCA-cell-containing macrobead is described in the IND documents. As for the islet macrobeads, the cancer macrobeads are placed in the peritoneal cavity in humans. In the peritoneal cavity the beads should remain free (floating) in the intraperitoneal space. They do not become vascularized and thus will remain as implants rather than true grafts.

It is important to emphasize that the macrobeads must be allowed to "mature" *in vitro* prior to their implantation into animals or humans willing to participate in the Phase II trial. Although there is an early proliferation-inhibitory effect related to the apoptotic death of cancer cells in the macrobead, the effect of interest and therapeutic effectiveness is seen after approximately three weeks increasing to a maximum by approximately 8-10 weeks. The appearance of the inhibitory effect coincides with dramatic changes in the tumors cells in the 1% agarose matrix.

The use of immunohistological and gene chip microarray techniques indicates that the cancer cells in the macrobeads undergo dramatic changes over the first several weeks of their encapsulation. After 14-21 days, the cells begin to form ovoid colonies, which are characterized by proliferation (as indicated by a proliferation marker, Ki67) and growth inhibition (as indicated by the appearance of p27, a cyclin-dependent kinase inhibitor, which acts to inhibit cell proliferation at the G1-S checkpoint of the cell cycle). Changes in the expression of genes involved in signal transduction and stem cell pathways are confirmatory of these dramatic changes in the cell populations within the bead. For example, they show decreased expression of genes related to DNA synthesis and angiogenesis (VEGF).

In sum, the colonies formed by the RENCA cells thus emerge following a process marked by both proliferation and apoptotic cell death. The appearance of the cell-proliferation inhibitory effect coincides with the formation of the colonies. It should be emphasized again that RENCA cells removed from the colonies in the macrobeads after periods of up to 3 years return quickly to their prior malignant cell growth patterns both *in vitro* and *in vivo*.

The cellular products of the cancer-cell-containing macrobeads are beginning to be resolved by a variety of techniques including most importantly, the gene arrays already described, as well as Western blot analysis. The products indicating the greatest changes include osteocalcin, bone morphogenic protein-4, GADD45, connexin 43, and aldehyde dehydrogenase. It would appear from the data we have that there are likely to be multiple signals. Based on the physical constraints of the 5% outer layer of agarose, the active product(s) must be less than 80 kD in molecular weight, but may include proteins, peptides, and lipids of many different types. It is possible that small tumor antigens are also released, but it does not appear that these are one and the same with the signaling molecules. A recent advance has been the ability to maintain the macrobeads in serum-free medium. Analyses of the serum-free conditioned media by gel electrophoresis indicate that there are approximately 20 bands. These are being analyzed now to determine which are the signals producing the inhibitory effect on the growth of non-constrained tumor cells.

4.3 Selection of Study Population

4.3.1 Inclusion Criteria:

1. Histologically-confirmed adenocarcinoma of the pancreas, colon, or rectum.
2. Radiographic evidence of metastatic cancer of the colon or rectum
3. The subject has pancreatic cancer that is unresectable or already metastatic or colorectal cancer that has failed available treatment modalities.
4. For the pancreas subjects, he/she may be accepted without prior chemotherapy or with multiple therapies that have failed. The colon and rectal cancer subjects must have failed available chemotherapy/targeted regimens. There is no limit to the number of prior chemotherapeutic regimens.
5. The subject must have evidence of progressive disease defined as at least one of the following:
   a. Progressive measurable disease: using conventional solid tumor criteria
   b. Increasing tumor markers and/or activity on PET-CT SUV measurement
6. All clinically significant toxic effects (excluding alopecia) of prior surgery, radiotherapy, or hormonal therapy have resolved to ≤ Grade 1 based on the NCI-CTCAE v 3.0, with the exception of peripheral neuropathy, which must have resolved to Grade ≤ 2.
7. The subject has an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0-2.
8. The subject has adequate hematologic function (minimum requirements: absolute neutrophil count [ANC] ≥ 1500/mL, hemoglobin ≥ 9 g/dL, and platelets ≥ 100,000/mL).
9. The subject has adequate hepatic function (bilirubin ≤ 1.5 times the upper limit of normal (ULN)], aspartate transaminase [AST] and alanine transaminase [ALT] ≤ 3 times the ULN, or ≤ 5 times the ULN, if liver metastases are present).
10. The subject has adequate renal function (creatinine ≤ 2.0 mg/dL)
11. The subject has adequate coagulation function (an international normalized ratio [INR] ≤ 1.5 and a partial thromboplastin time [PTT] ≤ 5 seconds above the ULN [unless on oral anticoagulant therapy]). Subjects receiving full-dose anticoagulation therapy are eligible provided they meet all other criteria, are on a stable dose of oral anticoagulant or low molecular weight heparin (and if on warfarin have a therapeutic INR between 2 and 3).
12. The subject has a life expectancy of 6 weeks.
13. The subject is male or female and at least 18 years of age. For females of childbearing potential, a negative pregnancy test is required.
14. The subject agrees to use contraceptives (barrier method) while on study, if sexually active.
15. The subject has provided signed informed consent.

4.3.2 Exclusion Criteria:

1. Any condition (cardiovascular or other), making subject an unacceptably high anesthetic or surgical risk based on current anesthesia/general surgery standards.
2. Positive testing for HIV.
3. Cognitive impairment sufficient to render the subject incapable of giving informed consent.
4. Hypersensitivity reaction that, in the opinion of the investigators, poses an increased risk of an allergy to the macrobeads, particularly any known allergy to murine antigens or body tissues.
5. Surgical treatment or chemotherapy within three weeks of scheduled macrobead implantation or within four weeks of bevacizumab (or similar drugs), or radiation therapy within four weeks of scheduled macrobead implantation.
6. Investigational medications for their respective tumors within one month of baseline evaluation.
7. The subject has inadequate hematologic function (absolute neutrophil count [ANC] <1500/mL, hemoglobin <9 g/dL, and platelets <100,000/mL).
8. The subject has inadequate hepatic function [bilirubin >1.5 times the upper limit of normal (ULN)], aspartate transaminase [AST] and alanine transaminase [ALT] > 3 times the ULN, or > 5 times the ULN, if liver metastases are present).
9. The subject has inadequate renal function (serum creatinine > 2.0 mg/dL).
10. The subject has inadequate coagulation function (an international normalized ratio [INR] > 1.5 and a partial thromboplastin time [PTT] > 5 seconds above the ULN [unless on oral anticoagulant therapy]). Subjects receiving full-dose anticoagulation therapy are eligible provided they meet all other criteria, are on a stable dose of oral anticoagulant or low molecular weight heparin (and if on warfarin have a therapeutic INR between 2 and 3).
11. Hepatic blood flow abnormalities: portal vein hypertension and thrombosis; and/or large volume of ascites.
12. Concurrent cancer of any other type except skin cancer (excluding melanoma).
13. History of allergic reactions to mouse antigen.
14. The subject has an ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, serious cardiac arrhythmias, (well-controlled atrial fibrillation, is permitted,) psychiatric illness/social situations that could interfere with the subject's ability to participate in the protocol, active bleeding.
15. As a result of the medical history, examination or blood testing, the investigator considers the subject unfit for the study.

4.4 Screening to Exclude Subjects with Allergic Reactions to Mouse Antigens

All subjects eligible for this protocol will be screened by history of allergy to mouse antigens and with an available mouse allergen extract using skin testing initially to determine if a wheal and flare reaction is present. This will be carried out with Greer Laboratories, Inc. product # E2 (mouse epithelia). A positive reaction in the skin test will exclude the subject from this protocol. Positive control with histamine, and negative control with saline or glycerin will be done along with each murine skin test.

Baseline and follow-up Human Anti-Mouse Antibody (HAMA) levels will not be followed in this Phase 2 trial in as much as the experience in the Phase I trial indicated that HAMA is not a reliable indicator of reaction to mouse antigens. False positives are a major problem.

4.5 Treatments

4.5.1 Dosage Determination

Based on the animal studies conducted to date, as well as the Phase I human trial, a reasonable dosage range (number of beads implanted) is 8 macrobeads per kilogram of body weight. We know that 16 macrobeads per kilogram was also well tolerated in the Phase I human trial, but, at least with a single dose, this number was not seen to be any more effective than 8 macrobeads per kilogram. Given the fact, it is planned to carry out up to four implants (over approximately 12 months) in this Phase II trial, each implantation will be carried out at the 8 macrobeads per kilogram dosage.

4.5.2 Drug Labeling

Labeling will be defined as in BB-IND 10092.

4.6 Assessments

The objective of the proposed Phase II study of the RENCA macrobeads is, as stated above, to determine the human clinical efficacy of the cancer macrobeads in advanced pancreatic and colorectal cancer. Responses will be

assessed according to the usual categories of complete or partial response (with specification of percentage), and none/progression (with specification of percentage). Response assessment will be measured in terms of changes in volume of primary tumor and number of metastases as assessed by CT or MRI; tumor marker PSA, as well as the physician global clinical assessment; performance rating scale (Karnofsky or similar); quality of subject life as measured by the EORTC Quality of Life Scale. Alkaline phosphatase and lactic dehydrogenase will also be followed.

Other laboratory determinations to monitor subject response to the implantation of the macrobeads will include detection of inflammatory responses (CBC with differential WBC and platelet count, erythrocyte sedimentation rate, C-reactive protein, amylase, immunological changes, and cytokine production), RT-PCR (polymerase chain reaction) specific to e-MuLV DNA, plasma viremia, and antibodies to normal murine antigens.

A critical and unique addition to the endpoints for assessing efficacy will be, where possible, a careful search for and identification of the niches of cancer stem and other cells within the niche bodies of the tumors the macrobeads are being used to treat. Markers of the niche bodies used to identify them and study their state (numbers, integrity of surround blood vessels, degree of stress) will include HLA, CD44+, CD133+, p27, Ki67, and the Tunel assay for apoptosis, Determination of the presence or absence of circulating tumor cells will also be made. When possible/available, pathology specimens will be examined for cancer stem cell niches.

5 STUDY CONDUCT

5.1 Schedule of Assessments

Clinical procedures, laboratory tests, and other measures to be utilized (and their order and flow) in the trial are indicated in Appendix 2 (section 9.2), and below:

5.1.1 Study Days -28 to -1:

1. Pretreatment evaluations used to determine the subject's study eligibility must be completed within 28 days prior to implantation unless otherwise specified for particular individuals and circumstances
2. Written informed consent must be obtained prior to performing to any study-specific evaluations. Results of all pretreatment evaluations must be reviewed by the Principal Investigator or his/her designee to ensure that all eligibility criteria have been satisfied prior to subject implantation. There will be a complete review of the subject's cancer history and reports of tumor biopsies obtained. A series of clinical tests and procedures are to be performed at different intervals throughout the study.
3. All subjects must undergo the following pretreatment evaluations:
    - Medical history (including an assessment of baseline conditions, global clinical assessment, symptoms rating/severity assessment, quality of life scale [EORTC], pain scale assessment and activity of daily living rating)
    - Physical examination (including neurological examination, height and weight measurements)
    - Recording ECOG Performance Status and Karnofsky Performance Status
    - Vital signs (including temperature, pulse rate, respiration rate and blood pressure)
    - Concomitant medication assessment (including those medications taken within 30-days prior to implantation)
    - Hematology profile (CBC with differential WBC and platelet count)
    - Coagulation profile (PT, PTT and INR)
    - Erythrocyte sedimentation rate
    - Comprehensive chemistry profile (CO_2, sodium, potassium, chloride, creatinine, BUN, glucose, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, AST and ALT), glucose, GGT, LD
    - Urinalysis, and if clinically indicated, a 24-hour urine collection
    - Amylase, lipase
    - Serum direct bilirubin

- Immunoglobulin levels
- Tumor markers: CEA, CA 19-9, CA 125.
- Circulating Tumor Cell levels (if possible)
- Baseline ECG within 28 days of implantation
- PA/lateral chest x-ray (within 28 days of implantation). If chest x-ray results indicate potential thoracic cancer-related abnormalities, then perform a non-contrast chest CT (within 21 days of implantation).
- PET-CT of whole body or MRI of the abdomen and pelvis (within 21 days prior to receiving first implant)
- Radionuclide bone scan (only if medically indicated)
- Disease assessment (baseline tumor assessment from x-rays, CT/MRI, and bone scans)
- C-reactive protein
- Hepatitis B, C, E
- HIV
- Cellular immune function
    - T cells
    - Cytokine production
    - B cells
    - Antibodies to common bacteria and viruses (diphtheria, tetanus, mumps, rubella)
    - Quantitative IgG subclass determinations
    - NK cells count (CD16)
- Ecotropic murine leukemia virus (e-MuLV) (PCR-based assay)
- Murine allergen skin test, including positive and negative controls
- Pregnancy test for females of childbearing potential

5.1.2 Decision to Proceed with First Implantation

The medical/surgical decision to proceed to the first implantation will be made based on the overall clinical assessment (physical examination, vital signs, EKG, routine blood work, status of the subject's cancer, availability of alternate treatment modalities, and the absence of evidence of allergic reaction to mouse antigens). Much of the above testing, such as that for immunological function, will not be available by Day -1, but the samples for testing will be obtained prior to therapy and the results of the testing is intended to establish a reliable baseline for research purposes and would not have an impact on the clinical decision-making process.

5.1.3 Study Day -28 to Study Day -1:

From Study Day -28 to Study Day -1, all test results that are available will be collected and reviewed. If the data indicate that there are no contraindications to proceeding, on Day 0 (Day of Implantation), RENCA macrobeads (8 per kg body weight) will be implanted intraperitoneally.

5.1.4 Visit 1 / Study Day 0:

The day of implantation will be protocol Implant 1 / Study Day 0. RENCA macrobeads (8 per kg body weight) will be implanted intraperitoneally. The subject will receive a small abdominal incision(s) utilizing laparoscopy, when possible, under general or local anesthesia, as indicated, and under sterile conditions in the operating rooms of New York Presbyterian Hospital. The location of the incision(s) will be at the surgeon's discretion. Standard procedures call for the administration of 1 gm of cefazolin just prior to surgery as antibiotic prophylaxis, unless otherwise indicated by the subject's medical history (allergies or other condition).

Standard surgical procedures and three-layer closure of the abdominal wound, if possible, will be carried out. Vital signs will be monitored as per routine for a post-laparoscopy subject and according to any special needs individual subjects may have. Assuming that the subject's post-surgical condition is stable (as defined by standard post-operative criteria) and that the subject is taking oral fluids and soft foods at a minimum, the subject will be discharged from surgical recovery room to either home care or hospital confinement as soon as medically indicated which may be same day as implantation. Any other blood drawing or procedures will be those dictated by optimal clinical care.

If deemed medically and surgically safe by the operating surgeon, one or more biopsies of tumor mass will be taken during this procedure. Fresh frozen tissue and fixative-treated tissue will be sent to the laboratory for immunohistochemical and gene array analysis to determine baseline tumor histopathology and gene expression patterns.

5.1.5 Home Care:

Post-surgical home care requirements are expected to be minimal. Medical and wound care regimens will be carried out as individually prescribed by the investigators and appropriate physicians directly involved in the subject's care. The subject will complete a daily log of activity (ADL) and a general symptom checklist (defined subjectively), but with special emphasis on appetite, abdominal symptoms such as pain or cramping, bowel movements, urination (frequency,

dysuria), and pain medication requirements. Both the subject and her/his family members will be trained in the simple vital sign measurements and the filling in of the activity log/assessment and symptom checklist. Vital signs will be measured twice daily at 8am and 8pm for the first month after implantation, then once a day for the next two months. The investigators may modify this schedule, if the clinical circumstances demand such modification. Subject and family training will take place prior to macrobead implantation, with a review prior to discharge after implantation.

A critical safety parameter will be the determination of the presence or absence of antibody to murine viruses, including mouse leukemia virus (MuLV), as well as viral titers for murine viruses known to be infective for humans. The published literature and the co-culture experiments carried out in our laboratory do not indicate that MuLV can infect human cells. Furthermore, the RENCA cells used to prepare the cancer macrobead have tested negative for other known murine viruses (Charles River panel of 18 murine viruses). In addition, none of the thirty-one (31) subjects treated to date in the Phase I study have shown any evidence of viral infection associated with the macrobeads. Nonetheless, this protocol includes laboratory and clinical monitoring for any such viral transmission. If there is any laboratory or clinical evidence indicating or suggesting a viral infection with MuLV, all family members having direct physical contact with that subject will also be assessed for the identified murine virus or for any clinical or laboratory evidence of a known or unknown viral infection. Virological monitoring of all staff in contact with the subject will be carried out at that time as well. It should be emphasized again that there has been no evidence of such transmission or infection in the Phase I trial.

5.1.6 Visit 2, Study Day 14 ± 3 days:

A brief examination including the parameters listed below will be performed. Assuming that the wound examination is satisfactory, all sutures/staples will be removed by the surgeon who performed the laparoscopy or appropriate surrogate. In addition, the Investigator will also examine the subject.

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin

- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.7 Visit 3, Study Day 30 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.8 Visit 4, Study Day 60 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125

- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.9 Visit 5, Study Day 90 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus (immunology, PCR) tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate
- MRI if needed
- PET-CT scan of whole body
- Pregnancy test, females of childbearing potential only
- Urinalysis
- EKG
- CXR
- Cellular immune function [T cells, B cells, Antibodies to common bacteria and viruses (diphtheria, tetanus, mumps, rubella), Quantitative IgG subclass determinations, NK cells count (CD16)]
- Immunoglobulin Levels (IgA, IgG, IgE, IgM)
- Murine allergen skin test, including positive and negative controls

Decision to Proceed with Second Implantation

All available information from the imaging studies, tumor marker and other laboratory findings, as well as physical examination findings and quality of life measurements from Visit 5 will be reviewed to determine if a subject is cleared for the proposed anesthesia and second implantation of macrobeads. Assuming the subject is cleared, the second implantation will proceed as soon as possible.

5.1.10 Visit 6, Implant 2 / Study Day 0:

Beginning 3 months after the first implant, the subject will be scheduled for a second implant of the cancer macrobeads at a dosage of 8 macrobeads per kilogram body weight. Assuming that the subject's post-surgical condition is stable (as defined by standard post-operative criteria) and that the subject is taking oral fluids and soft foods at a minimum, the subject will be discharged to home care as soon as medically indicated which may be the same day as implantation.

If deemed medically and surgically safe by the operating surgeon, one or more biopsies of tumor mass will be taken during this procedure. Fresh frozen tissue and fixative-treated tissue will be sent to the laboratory for immunohistochemical and gene array analysis to determine any changes in tumor state that may have been the result of exposure to the cancer macrobeads.

5.1.11 Visit 7, Implant 2 / Study Day 14 ± 3 days:

A brief examination including the parameters listed below will be performed. Assuming that the wound examination is satisfactory, all sutures/staples will be removed by the surgeon who performed the laparoscopy or appropriate surrogate. In addition, the Investigator will also examine the subject.

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin

- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.12 Visit 8, Implant 2 / Study Day 30 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.13 Visit 9, Implant 2 / Study Day 60 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125

- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.14 Visit 10, Implant 2 / Study Day 90 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus (immunology, PCR) tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Symptoms rating/severity assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate
- MRI if needed
- PET-CT scan of whole body
- Pregnancy test, females of childbearing potential only
- Urinalysis
- EKG
- CXR
- Cellular immune function [T cells, B cells, Antibodies to common bacteria and viruses (diphtheria, tetanus, mumps, rubella), Quantitative IgG subclass determinations, NK cells count (CD16)]
- Immunoglobulin Levels (IgA, IgG, IgE, IgM)
- Murine allergen skin test, including positive and negative controls

Decision to Proceed with Third Implantation

All available information from the imaging studies, tumor marker and other laboratory findings, as well as physical examination findings and quality of life measurements from Visit 10 will be reviewed to determine if a subject is cleared for the proposed anesthesia and third implantation of macrobeads. Assuming the subject is cleared, the third implantation will proceed as soon as possible.

5.1.15 Visit 11, Implant 3 / Study Day 0:

Beginning 3 months after the second implant, the subject will be scheduled for a third implant of the cancer macrobeads at a dosage of 8 macrobeads per kilogram body weight. Assuming that the subject's post-surgical condition is stable (as defined by standard post-operative criteria) and that the subject is taking oral fluids and soft foods at a minimum, the subject will be discharged to home care as soon as medically indicated which may be same day as implantation.

If deemed medically and surgically safe by the operating surgeon, one or more biopsies of tumor mass will be taken during this procedure. Fresh frozen tissue and fixative-treated tissue will be sent to the laboratory for immunohistochemical and gene array analysis to determine any changes in tumor state that may have been the result of exposure to the cancer macrobeads.

5.1.16 Visit 12, Implant 3 / Study Day 14 ± 3 days:

A brief examination including the parameters listed below will be performed. Assuming that the wound examination is satisfactory, all sutures/staples will be removed by the surgeon who performed the laparoscopy or appropriate surrogate. In addition, the Investigator will also examine the subject.

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125

- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.17 Visit 13, Implant 3 / Study Day 30 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.18 Visit 14, Implant 3 / Study Day 60 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications

- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.19 Visit 15, Implant 3 / Study Day 90 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus (immunology, PCR) tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate
- MRI if needed
- PET-CT scan of whole body
- Pregnancy test, females of childbearing potential only
- Urinalysis
- CXR
- EKG
- Cellular immune function [T cells, B cells, Antibodies to common bacteria and viruses (diphtheria, tetanus, mumps, rubella), Quantitative IgG subclass determinations, NK cells count (CD16)]
- Murine allergen skin test, including positive and negative controls
- Immunoglobulin Levels (IgA, IgG, IgE, IgM)

Decision to Proceed with Fourth Implantation

All available information from the imaging studies, tumor marker and other laboratory findings, as well as physical examination findings and quality of life measurements from Visit 15 will be reviewed to determine if a subject is cleared

for the proposed anesthesia and fourth implantation of macrobeads. Assuming the subject is cleared, the fourth implantation will proceed as soon as possible.

5.1.20 Visit 16, Implant 4 / Study Day 0:

Beginning 3 months after the third implant, the subject will be scheduled for a fourth implant of the cancer macrobeads at a dosage of 8 macrobeads per kilogram body weight. Assuming that the subject's post-surgical condition is stable (as defined by standard post-operative criteria) and that the subject is taking oral fluids and soft foods at a minimum, the subject will be discharged to home care as soon as medically indicated which may be same day as implantation.

If deemed medically and surgically safe by the operating surgeon, one or more biopsies of tumor mass will be taken during this procedure. Fresh frozen tissue and fixative-treated tissue will be sent to the laboratory for immunohistochemical and gene array analysis to determine any changes in tumor state that may have been the result of exposure to the cancer macrobeads.

5.1.21 Visit 17, Implant 4 / Study Day 14 ± 3 days:

A brief examination including the parameters listed below will be performed. Assuming that the wound examination is satisfactory, all sutures/staples will be removed by the surgeon who performed the laparoscopy or appropriate surrogate. In addition, the Investigator will also examine the subject.

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment

- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.22 Visit 18, Implant 4 / Study Day 30 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.23 Visit 19, Implant 4 / Study Day 60 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate

5.1.24 Visit 20, Implant 4 / Study Day 90 ± 5 days:

The following data will be obtained:

- Vital signs with weight
- Physical examination with brief neurological exam
- CBC with differential WBC, platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine panel
- CEA, CA 19-9, CA 125
- Circulating Tumor Cell Levels
- Ecotropic murine leukemia virus (immunology, PCR) tests
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- Quality of life scale
- ECOG and Karnofsky Performance status rating
- Pain scale, if appropriate
- Urinalysis

5.1.25 Visit 21, Implant 4 / Study Day 120 ± 5 days:

This will be the last formal protocol visit. The following data will be obtained:

- Physical examination with brief neurological exam
- Vital signs with weight
- CBC with differential WBC and platelet count
- Coagulation profile (PT, PTT and INR)
- Erythrocyte sedimentation rate
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- Cytokine levels
- Ecotropic murine leukemia virus tests
- CEA, CA 19-9, CA 125
- Circulating tumor cells
- Monitoring of adverse events and concomitant medications
- Global clinical assessment
- ECOG and Karnofsky Performance status rating
- Quality of life scale

- ECOG performance scale rating
- MRI as needed, PET-CT scan of whole body
- Pain scale, if appropriate

After Day 120 of the fourth implantation, all examinations and further treatment will be guided by optimal patient care principles and the decisions of the physicians providing overall health care to the subject. It should be noted that all subjects entered into this trial will be followed at appropriate intervals (not less than yearly) for the rest of their lives in accord with USPHS regulations and guidelines for any procedures or treatments involving xenotransplantation.

With regard to the question of whether the macrobeads can or should remain implanted or be removed, the safety data in the laboratory animals and the veterinary patients (cats and dogs) have indicated that the beads are well tolerated for at least one year, four years being the longest that any of the RENCA macrobeads have been followed in vivo to date. Some chronic peritoneal inflammation has been seen with RENCA macrobeads in those animals where the RENCA macrobeads have been in place for more than 3 months, although no clinical evidence of functional problems or signs or symptoms resulting from this reaction has been documented.

In human subjects in the Phase I trial, there has been no evidence that the macrobeads have caused any adverse clinical effects and thus far have been well-tolerated for up to 24 months. This has been true for subjects receiving the 8 macrobeads per kg or 16 macrobeads per kg doses. Four subjects also received a second implant at the 8 macrobeads per kg dose and tolerated that well. Post-mortem results in those subjects consenting to such examination have revealed some fibrinous collections around some of the beads, but no clinically significant peritonitis and no alterations in organ structure or function, specifically bowel function. There have been no requests for or attempts at macrobead removal.

Unfortunately, macrobeads removed at post-mortem examination are not suitable for any testing other than formalin fixation and possibly histology because of the time from death to actual performance of the autopsy. Should any beads be removed in the course of one of the subsequent implantations, they will be sent for histology, MTT testing, inhibition testing, and routine microbiology screening.

The study will continue to follow the subject regardless of macrobead function. Further examinations will be at approximately 6-month intervals for 2 years and then yearly thereafter for the life of the subject. Per PI's discretion, these long-term examinations may include:

- General Physical Examination with Brief neurological Exam;

- Vital signs with weight;
- PET-CT of whole body;
- MRI scans as needed;
- CBC with differential WBC and platelet count;
- Coagulation Panel;
- Erythrocyte sedimentation rate;
- Comprehensive Metabolic Panel with LD, GGT, CRP, direct bilirubin
- CEA, CA 19-9, CA 125;
- Cytokine panel;
- Ecotropic murine leukemia virus tests
- All subjects will be monitored for adverse events and concomitant medications;
- Global clinical assessment;
- Activities of daily living rating;
- Symptoms rating/severity assessment;
- Quality of life scale;
- ECOG and Karnofsky Performance status rating;
- Pain scale, if appropriate;

Should the subject die, and the subject and/or his/her family have consented to it, a postmortem examination will be carried out. Permission for such an examination is requested in the consent form, although the subject's decision cannot be binding on his/her family in the event of the subject's death. The subject is also free to change his/her own decision at any time during or after the study. Samples of tumor tissue (primary and metastatic sites), peritoneum, internal organ serosa and underlying tissue may be taken at any postmortem examination to evaluate tumor state and any inflammatory or connective tissue reaction to the RENCA macrobeads.

5.2 Number of Clinical Sites

One clinical site will be utilized.

5.3 Safety and Tolerability Assessments

All subjects will be monitored for safety at every visit and with telephone follow-ups at regular intervals between visits. At every scheduled study visits, this monitoring will include routine checks of the subject's clinical status, measuring of vital signs, physical examination including a neurological examination, clinical laboratory evaluations including clinical chemistries, hematology, urinalysis, liver functions tests, monitoring of tumor markers, CT/MRI scans as appropriate, and

the monitoring of adverse events, as well as global clinical assessments, quality of life, performance and pain, if any. At unscheduled visits, evaluations will be done at the PI's discretion.

5.4 Medical History

A complete medical history will be collected from each subject. This will include an evaluation of the subject's malignancy, a recording of the results of previous diagnostic tests and therapies. This evaluation will include an assessment of possible therapies that can be used for the management of the malignant condition. The medical/surgical decision to proceed to the first implantation will be made based on the overall clinical assessment. This overall clinical assessment will include an evaluation of the medical history, physical examination, vital signs, EKG, routine blood work, and the absence of evidence of allergic reaction to mouse antigens.

5.5 Physical Examination

All subjects will undergo a complete physical examination including vital signs, with measurement of height and weight.

- Complete physical examination with all vital signs
- Weight
- Global Clinical Assessment

5.6 Clinical Laboratory Parameters

The following clinical laboratory parameters will be tested in all subjects (the schedule is summary in Appendix 2 – Section 9.2):

- EKG
- Biochemical/immune system profile including
- Complete blood count (CBC) with differential WBC and platelet count
- Erythrocyte sedimentation rate
- C-reactive protein
- Biochemical profile as outlined above with amylase, lipase at screening, and thereafter as medically necessary
- Liver function (AST, ALT, LD, alkaline phosphatase, GGT)
- Urinalysis
- Coagulation profile (PT, INR, PTT)

- Hepatitis B, C, E
- HIV
- CEA, CA 19-9, CA 125
- Immunoglobulin levels (IgA, IgG, IgE, IgM)
- Skin testing with murine epithelial antigen preparation, along with positive and negative controls
- Cellular immune function
    - T cells
    - Cytokine production
    - B cells
    - Antibodies to common bacteria and viruses (diphtheria, pertussis, tetanus, mumps, rubella)
    - Quantitative IgG subclass determinations
    - NK cells count (CD16)
- Ecotropic murine leukemia virus (e-MuLV) (PCR-based assay)
- Abdominal/whole body MRI/CT scan with other imaging studies as appropriate for the subject's tumor (primary and metastatic) and condition. Ultrasound examination, if medically indicated, will include views of the hepatic veins and inferior vena cava to be certain that they are free of clots and that there is free peritoneal drainage.

5.7 Appropriateness of Measurements and Assessments

In the evaluation of a therapeutic entity for the treatment of a malignant condition such as advanced pancreatic or colon cancer, the following measurements are appropriate. The response of each subject to the intraperitoneal implantation of RENCA agarose-agarose macrobeads will be measured with respect to:

- primary tumor size
- response: complete, partial, stable disease, progression
- progression as defined in Section 3 of the protocol
- size or number of secondary/metastatic tumors; tumor markers or relevant biochemical parameters such as liver enzymes
- tumor metabolic activity as measured by ^{19}F-fluorodeoxyglucose uptake (if applicable)
- cellular or humoral immune status
- cytokines
- inflammatory response
- global clinical assessment
- activities of daily living (ADL) rating/performance status
- symptom rating/ severity (including use of pain medications)

- quality of life
- neuropsychological status/cognitive function
- pain rating scale, if appropriate

5.8 Progression of Disease During the Study

In addition to the subject's overall response to the implantation of the RENCA agarose-agrose macrobeads, subjects will be monitored for evidence of progression during the study. Evidence of local or metastatic disease progression will be based on clinical signs and symptoms, imaging studies and biochemical parameters as appropriate after a minimum period of eight weeks after each RENCA macrobead implantation will initiate a formal, documented discussion of the Principal Investigator, subject's attending or primary physician, if any, other consultants if appropriate, and the subject and his/her family regarding consideration of the use of other available standard or experimental therapies. The decision to employ another therapy may be made jointly or by the subject himself/herself.

Where the evidence of such progression is questionable or unclear, review of the subject's condition and laboratory or imaging data (after the minimum period of nine weeks) as defined by the protocol and discussion with the subject and his/her primary physician/consultant(s) will be carried out with careful documentation of the discussions and any decisions or uncertainties. New bone lesions detected on bone scans at the end of the initial 90 days after implantation) may reflect pretreatment disease activity, and may not necessarily constitute disease progression unless confirmed by additional lesions developing subsequently. Hence, subjects will be evaluated for progression based on the following criteria: 1. Tumor progression with PET-CT/MRI assessments after every cycle). 2. Evidence of progression demonstrating the appearance of ≥ 2 new lesions. New lesions detected at the end of the initial implantation period and confirmed confirmation ≥ 6 weeks later. 3. Symptomatic progression (for subjects without measurable disease). Deterioration in ECOG PS of ≥ 2 units compared to baseline attributable to cancer in the opinion of the investigator. 4. Weight loss ≥ 20% (NCI-CTCAE v 3.0, Grade 3) of the initial body weight attributable to cancer in the opinion of the investigator. 5. Other clinical events attributable to the cancer that in the opinion of the investigator require major interventions, such as surgical intervention for pain control. 6. Death from any cause. If in the opinion of the investigator, the subject has symptomatic improvement as measured by improvement in pain scales or narcotic analgesic consumption and there is evidence of bone scan or biochemical progression, he may be continued on treatment at the investigators discretion.

5.9 Rescue Program

During the time period that a subject is participating in this clinical study, the need may arise for additional oncologic therapy. If an alternative or additional therapy other than the macrobeads becomes indicated, because of disease progression, this type of therapy would be considered Rescue Therapy. This would include, for example, chemotherapy, radiation, surgery, or a different experimental protocol. This rescue program would be instituted and would not require the suject to be discontinued from the RENCA macrobead protocol. While undergoing these treatments, the subject will continue to be closely monitored for disease status as well as toxicity, as specified in this protocol. Should the clinical situation develop, the principal investigator and subject, with appropriate consultant input, could restart treatment with the macrobeads. A treatment "holiday" of at least three weeks (for chemotherapy) and four weeks (for radiation) would be required before commencing with this phase II protocol.

6 SAFETY EVALUATIONS

6.1 Adverse Events

Adverse events mean any sign, symptom, syndrome or illness that occurs or worsens during the use of the study device/drug, regardless of causality. Symptoms of abnormal laboratory values and clinically significant asymptomatic laboratory values will constitute adverse events. A medical condition that is present when the subject enters the study is not defined as an adverse event, unless this medical condition worsens after the study drug has been administered.

The Investigator, the safety physician of The Rogosin Institute, and the Safety Data Monitoring Board will be responsible for monitoring the safety parameters that are collected in the study. As of their review of the safety/toxicity date from Phase I in June 2009, they found no adverse events attributable to the macrobeads. The Safety Data Monitoring Board will independently review all safety parameters and safety results in the study.

The description of the adverse events will use the CTCAE v3.0 definitions (issued on December 12, 2003) to define the severity or intensity of the adverse events and clinical laboratory abnormalities. The coding of the adverse events will use the most current version of the Medical Dictionary for Regulatory Agencies (MedDRA). The coding will include reported term, preferred term, and classification of the System, Organ, Class (SOC). The coding of concomitant medications will use the latest version of the WHO Drug Dictionary (WHODD).

6.2 Classification of Adverse Events by Intensity

If the intensity of the adverse event is not described in the CTCAE v3.0, the Investigator will rate the intensity of the adverse event according to the following scale.

Grade 1 **Mild AE.** The adverse event is transient and easily tolerated by the subject.

Grade 2: **Moderate AE.** The adverse event causes the subject discomfort and interrupts the subject's normal activities.

Grade 3: **Severe AE.** The adverse event causes considerable interference with the subject's normal activities.

Grade 4: **Life-threatening or Disabling AE.**

Grade 5: **Death**

6.3 Classification of Adverse Events by Relationship to Study Drug

The Investigator will also assess the relationship of the adverse event to the RENCA macrobead according to the following definitions:

Not related:
The adverse event does not follow a reasonable temporal sequence from administration of the study drug, or that can be reasonably explained by other factors, including underlying disease, complications, concomitant drugs or concurrent treatment. Even if the investigator feels there is no relationship to the study drug, the adverse event must be reported.

Remote:
The adverse event follows an improbable temporal sequence to study drug administration and in which underlying disease, complications, concomitant drugs or concurrent treatment offers a plausible explanation.

Possibly related:
The adverse event follows a reasonable temporal sequence from administration of the study drug (including the course after withdrawal of the study drug), that can not exclude the possibility of the study drug involvement, although other factors such as underlying disease, complications, concomitant drugs or concurrent treatment are presumable.

Probably related:
The adverse event follows a reasonable temporal sequence from administration of the drug (including the course after withdrawal of the drug), and that can exclude the possibilities of other factors, such as underlying disease, complications, concomitant drugs or concurrent treatment, other than the study drug.

These events will be reported to the IRB and FDA according to their requirements.

6.4 Serious Adverse Events

A serious adverse event is any untoward medical occurrence, regardless of relationship to the study drug and intensity, at any dose that:

- Results in death
- Is life-threatening – (which is defined as an event in which the patient or subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event – (which is defined as a medical event(s) that may not be immediately life-threatening or results in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent any of the other serious outcome listed in the definition above. Examples of such event include, but are not limited to, intensive treatment in an emergency room or at home or allergic bronchospasm; blood dyscrasias, or convulsions that do not result in hospitalization

Adverse events classified as “serious” require expeditious handling and reporting to the product’s sponsor to comply with regulatory requirements. Any SAE must be reported as quickly as possible after learning of the event by telephone or by a facsimile transmission of a Serious Adverse Event Form. The reporting requirements are outlined in The Rogosin Institute Clinical Research Program Operating Procedure.

Serious adverse events (i.e., any adverse event that meets the FDA definition and has a CTCAE Grade of 3-5) in subjects receiving the RENCA macrobeads will be reported as soon as possible to:

Stuart Saal, MD
Attending Physician
The Rogosin Institute
505 East 70th Street
New York, NY 10021

Office: (212) 746-6117
Service: (212) 535-1120
Fax: (212) 288-8370

If only limited information is initially available, follow-up reports will be required, and must be submitted to the study contact named above no greater than fifteen (15) days of receipt of additional information. Copies of SAE reports are to be forwarded to the DSMB as per Weill Cornell Medical College DSMB/IRB procedures and to the IND as per FDA current reporting procedures.

All adverse events will also be evaluated for intensity using the CTCAE v3.0.

Any serious adverse events 120 days after the last implantation, not related to the RENCA macrobeads will not need to be reported to the sponsor.

6.5 Stopping Rule for an Individual Subject

If the sponsor, investigator, study monitor, Data Safety Monitoring Board (DSMB) or officials from the Food and Drug Administration (FDA) discover conditions arising during the study that indicate that the study should be halted or that the study center should be terminated, this action may be taken after appropriate consultation between the sponsor and investigator. Conditions that may warrant termination of the study include, but are not limited to, the following:

- The discovery of an unexpected, serious, or unacceptable risk to the patients enrolled in the study
- A decision on the part of the sponsor to suspend or discontinue testing, evaluation, or development of the product

6.5.1 Maximum Tolerated Dose/Dose Limiting Toxicity

In this protocol, there will be four implantations of macrobeads. The single dose (i.e., 8 macrobeads per kilogram) was previously shown to be well tolerated. In this study, the dose limiting toxicity (DLT) is most likely to be related to the number of implantations rather than the number of macrobeads implanted at each implantation.

Dose limiting toxicity (DLT) will be defined as

1. Any Grade 2 allergic reaction of generalized urticaria or any other grade $\geq$ 3 allergic reaction;
2. Any Grade $\geq$ 3 infection;
3. Any grade $\geq$ 3 local (intraperitoneal) reaction including peritoneal irritation or interference with intraperitoneal organ function; and
4. Any grade $\geq$ 3 hematologic or non-hematologic reaction.

This definition of DLT is in accord with the NCI Common Terminology Criteria for Adverse Events v3.0 (CTCAE).

6.6 Premature Termination of the Study

This protocol provides for four doses of the RENCA macrobeads. The dosage to be used at each implantation will be 8 RENCA macrobeads per kg of subject body weight. The Safety Committee, as well as the investigators, will review each instance of Grade 5 adverse event. In order to protect subjects from excessive toxicities possibly associated with the study treatment, the following stopping rule will be utilized: if 3 or more subjects experience a grade 5 adverse event deemed possibly related to treatment, accrual will be halted and a thorough investigation will take place before the re-opening of accrual may be considered.

6.7 Potential Risks

Animal and human subjects studies to date have defined the parameters of the safety of the RENCA cancer macrobeads. In summary, the RENCA macrobeads have been well tolerated in murine tumor models, in cats and dogs with naturally occurring tumors of various types, where the RENCA macrobeads have now been implanted for periods of up to four years. Furthermore, in 13 humans with end-stage cancer implanted with the macrobeads (up to 17 months), no serious adverse events have been recorded which can be attributed to the marcobeads. Typically, the animals undergoing implantation have exhibited improved appetite, weight gain, and increased activity levels (the latter have been remarkable in at least one half of the animals treated). Adverse reactions observed to date have been 1) mild-to-moderate intraperitoneal chronic inflammatory reaction evident after 3 months in cats and dogs, but not mice; and 2) entrapment of some macrobeads in the feline and canine omentum. In the humans the reactions have been mild as well. Some subjects have experienced mild, intermittent fevers after implantation (generally resolved by four to six weeks after implant).

A mild-to-moderate inflammatory reaction to the intraperitoneal placement of the macrobeads has been noted in humans and veterinary patients, but not mice. In the cats and dogs, where the RENCA macrobeads have been implanted for periods of up to four years, this reaction becomes apparent beyond three months. It consists of a minimal to moderate increase in inflammatory markers, with no disruption of normal physiological functions, such as those of intestinal peristalsis or biliary or pancreatic drainage/secretion. Neither has it interfered with subsequent re-implantations of the RENCA macrobeads when indicated. In humans, implantation has led to an increase in inflammatory markers, with intermittent, self- limiting, low grade fevers in some cases. However, as observed in animals, the reactions were mild and not accompanied by clinical limitations.

Comparison of the reaction to the RENCA macrobeads with that seen with the porcine islet agarose-agarose macrobeads implanted in mice, cats and dogs described in IND BB-IND 9762 submitted April 9, 2001, is useful. In these experiments porcine islet-containing macrobeads were implanted in mice, Wistar-Furth rats, cats, and dogs with both streptozotocin-induced and naturally occurring insulin-dependent diabetes and in a large series of normal (non-diabetic) Sprague-Dawley rats. Beads have remained in some of the animals for long periods of time (in rats, 1 year; in cats for up to 6 years; and in dogs, over 3 years.) One subject in the Phase I clinical trial had the macrobeads in place for 24 months without any evidence of adverse effects. No significant toxicity, other than the chronic inflammatory reaction, has occurred as documented by clinical veterinary observations, laboratory blood chemistry results, or histopathological analysis of recipient organs and tissues after sacrifice by euthanasia.

Beyond macrobead removal (likely to be only partial (50-70%), but also not needed in any of the subjects treated with the macrobeads to date), whatever medical or surgical treatments are in the best interests of the subject and consistent with optimal medical care will be carried out with subject and/or family consent at any stage of the formal sixteen month protocol period and for the lifetime of the subject as the protocol indicates

A second potential adverse effect of the implantation of the RENCA macrobeads is the transmission of a murine virus to a human host. Theoretically, this could involve transmission of the virus to the subject without infection; clinically significant infection; and infection of a third party via exposure to the beads or to the potentially infected subject. Such risks are greatly reduced by the fact that the RENCA cell line has been maintained in our laboratory over many years and has been screened for known murine viruses, including those that present a possible risk of infection for humans. Although the RENCA cell line does require passing through Balb/c mice approximately three times a year to maintain its tumorigenicity, the mice used for this passing are from Charles River Laboratories, are pathogen-free to the most stringent possible standards, and are

maintained under strictly controlled animal care conditions. Furthermore, the passing process itself is carried out in the Rogosin laboratories under sterile conditions. The RENCA cells are routinely tested for microbiological contaminants during their maintenance in culture as well as after their incorporation into the agarose-agarose macrobeads so that the risk of their carrying any known infectious agent is eliminated. Clinical monitoring of the approximately 500 mice, cats and dogs treated to date, furthermore, has given no indication of any viral or other infection related to the implantation of the macrobeads.

The only virus identified to date in the RENCA cell line is the ecotropic (non-xenotropic) variant of the murine leukemia virus (MLV), an endogenous retrovirus that is not known to infect human cells. Literature reports (National Research Council, 1991) of its lack of ability to infect human cells have been confirmed in our laboratory using a standard co-culture protocol (for up to 30 days) with human 293, an embryonic kidney cell line. Thus, there is no evidence that this murine retrovirus variant poses any threat to humans. In the 30 subjects in the Phase I clinical trial there has been no evidence to date of the transmission of or infection with the MuLV.

Risks associated with the surgical procedure (including anesthesia) required for the implantation of the RENCA macrobeads will be those normally expected for such a procedure involving a simple abdominal incision. They will be dependent, of course, on individual subject risk factors, but no subjects with unacceptable risks for such a procedure will be admitted to this study. The performance of the abdominal incision and the placement of the macrobeads in the peritoneal cavity are both simple and minimally invasive.

Allergic reaction to mouse antigens is a potential risk. With respect to this risk the following procedures will be in place:

All subjects eligible for this protocol will be screened with available mouse allergen extracts using intradermal skin testing (Greer Diagnostics) initially to determine if a wheal and flare reaction is present, indicating the presence of human anti-mouse antibody. Positive and negative skin test controls will be done at the same time.

Data from repeated implantations of mouse RENCA macrobeads in dogs and cats with spontaneously occurring tumors of various types have not provided any clinical or laboratory evidence of sensitization to mouse proteins over periods of up to four years. It should also be noted that, over the period of 4 to 6 months after a given implant, the RENCA cells in the macrobeads die so that there is not an ongoing production of antigenic substances by the mouse cells after this time. The agarose itself has been well tolerated (except for local foreign body reaction) for periods of up to several years in studies with porcine islet macrobeads and for

up four years (the longest the veterinary patients have been followed to date) with the RENCA cancer macrobeads implanted up to five times. The beads have been tolerated without evidence of adverse reaction in humans now for up to 24 months. There has also been no evidence of recreation to mouse antigen skin testing in any of the subjects in the Phase I clinical trial.

Should a subject in the protocol develop evidence of sensitization to mouse proteins, appropriate local and systemic therapy (antihistamines, steroids) will be given. If the reaction is medically manageable, it should not be necessary to remove the RENCA macrobeads, given the gradual loss of cells within these beads. Should the reaction not be amenable to medical treatment removal of the beads will be performed via an open procedure, although complete retrieval may not be surgically possible (see below).

The total amount of blood drawn over the first 180 days of the study will be approximately 300 cc. This amount will hold constant for the subsequent study periods of the same length. The risk of mild anemia secondary to this blood withdrawal is low, but will be appropriately treated if present. The exact amount of blood to be drawn, as the study progresses past 6 months will depend on the subject's clinical status and the need for tests to monitor the patient's clinical status. As noted, the protocol blood drawing amounts will be constant in each of the 180-day study periods. In addition, the placement of an intravenous catheter involves the risk of bleeding around the needle site, local inflammation, pain or infection.

6.7.1 Procedures to minimize risks

In all cases, the Investigators will minimize risks by carefully screening subjects prior to admission to the study and by carefully monitoring their clinical status during the course of the study. The RENCA macrobeads themselves will be monitored carefully with rigorous GMP and GLP standards as defined in the IND application. The lack of adverse events attributable to the macrobeads evident in the Phase I trial is reassuring, especially with regard to the potential problems of peritonitis, viral transmission (eMuLV) problems interference with abdominal organ function, seeding of mouse tumor cells in the human peritoneal cavity and sensitivity to mouse antigens.

For phlebotomies and intravenous line placements, the usual sterile technique will be used and adequate pressure applied when the indwelling catheters are removed. Confidentiality will be observed for all data obtained from clinical or laboratory evaluations.

6.8 Escape Arm (Withdrawal with/without Bead Removal)

The RENCA macrobeads will be left in place in the peritoneal cavity for the life of the subject unless consideration of the removal of the macrobeads is occasioned by:

- Subject request;
- Principal Investigator, attending or consulting physician decision, and/or Safety Board/IRB/FDA decision that such removal is in the best medical interests of the subject.

The events or findings that would initiate such considerations/discussion include, but are not limited to:

- Any finding of Dose Limiting Toxicity (DLT) as defined above;

- Evidence of active e-MuLV viral infection in subject's cells/serum/plasma (i.e., transmission of MuLV from the mouse cells to the subject with active infection productive of a viral load in the subject);

- Chronic peritonitis reaction $\geq$ grade 3. Although not specifically defined per se in the NCI Common Terminology Criteria for Adverse Events v3.0, several of the subcategories under the Gastrointestinal section are applicable. The GI-Other sub-category will be used: Peritonitis/Chronic Inflammatory Reaction.

  Grade 1: Asymptomatic; radiographic (flat plate, CT, or MRI) or ultrasound imaging of peritoneal reaction.

  Grade 2: Symptomatic: abdominal pain or tenderness or pain on palpation (direct, rebound); altered dietary habits (i.e., appetite, fluid intake); requirement or IV fluids <24 hours indicated; altered bowel function (mild constipation or diarrhea).

  Grade 3: Symptomatic and severely altered GI function (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated $\geq$ 24hours.

  Grade 4: Life-threatening consequences.

Any toxicity of Grade 2 or above as defined above in relation to the NCI CTCAE v3.0 or DLT, as defined immediately above, will initiate discussion of the possibility/ desirability/ necessity of the removal of the RENCA macrobeads. Any such discussion will necessarily involve multiple considerations including the

advisability of a surgical procedure in the subject and the best interests of that subject from a medical point of view. As such, all discussions will involve, at a minimum, the Principal Investigator, the subject's primary or attending physician, relevant consultants, and the Safety Board as appropriate, with notification of the IRB and the FDA. It should be noted and emphasized that it will not be possible to remove all of the macrobeads. 50 to 80% removal is likely to be possible, but the exact level of removal of macrobeads that is achievable will depend on many factors, most importantly the subject's overall health status and best medical interests. Again, the removal of any macrobeads has not been warranted in any of the humans implanted with them thus far.

6.9 Treatment of Adverse Effects

Should any subject in this Phase II trial of the RENCA experience an adverse event directly related to the Macrobeads or study-related procedures, all appropriate medical care will be provided at no cost to the subject.

7 DATA MANAGEMENT AND STATISTICAL ANALYSIS

The critical parameters of this Phase II trial are those related to efficacy. Various markers, such as C-reactive protein, amylase, liver function tests, cell counts, and cytokine levels, will be sampled and analyzed. Given the study design, we will undertake simple population comparisons designed for small samples (non-normal distribution) as well as simple trend analysis for selected parameters. The data from all subjects, including those who withdraw or are withdrawn from the study will be analyzed. All data will be analyzed using SAS software.

7.1 Determination of Sample Size

Seventy-four (74) male and female subjects with advanced pancreatic or metastatic, treatment-resistant colorectal cancer meeting the protocol inclusion criteria will be sought for this study. Each subject will serve as his/her own control with measurements obtained and evaluated in an unbiased manner. This means that changes from baseline to various post-treatment time points will be analyzed for some variables (such as tumor markers, for example). The confidence intervals for these changes will be calculated. Of course, it is not planned for the survival analysis which will use a retrospective patient population comparison as defined in the Statiscal Analysis Plan (a copy of which is attached). The colorectal patient data from this study has already been used to plan a controlled Phase IIb protocol for colorectal cancer. Data from the pancreatic patients, when completed, will be utilized to plan a phase IIb controlled protocol for the pancreatic patients.

7.2 Determination of Response

All subjects will be evaluated for response. The Evaluation Team will be composed of the Investigators, and one outside physician. All of the clinical tests will be evaluated independently by each member of the Team for changes against the baseline findings. This evaluation will take place prior to each implantation or when the subject leaves the study. The individual subject's baseline established prior to the first implantation will be used for the entire study. A table will be constructed using the changes from baseline.

Outline of Response Rate				
TESTS	**Baseline Prior to First Implantation**	**Results Prior to Second Implantation**	**Results Prior to Third Implantation**	**Results Prior to Fourth Implantation**
Clinical Findings				
Symptoms				
Global Clinical Assessment				
Quality of Life				
Clinical Laboratory Tests (Abnormalities)				
Tumor Markers (Decrease)				
Imaging Studies (Evidence of Change, e.g., volume or area)				
CT				
PET				
MRI				
Rating Scale				
Major Improvement: 3				
Improvement: 2				
No Change: 1				

8 STUDY MANAGEMENT

8.1 Recruitment and Consent Procedures

Subjects will be recruited by physician referral. Full informed consent will be obtained prior to admission by one of the physician investigators. The hospital and state guidelines for obtaining consent prior to HIV testing will be followed.

8.2 Regulatory Guidelines

The study will be performed in accordance with United States IND regulations (21 CFR Part 312), the guidelines of the International Conference on Harmonization (ICH), Good Clinical Practice (GCP) Guidelines, and the most recent guidelines of the Declaration of Helsinki. These Guidelines will be on file in the Investigator's office and in The Rogosin Institute Clinical Research Program Operating Procedure Manual and, in addition, are available on the FDA's Web Site.

8.3 Institutional Review Board

Conduct of the study must be approved by an appropriately constituted Institutional Review Board (IRB). Approval is required for the study protocol, protocol amendments, informed consent forms, subject information sheets, and advertising materials (if any). No study drug will be shipped to the study center until written IRB authorization has been received by the Investigator or his or her representative.

8.4 Informed Consent

For each subject enrolled into the study, written informed consent will be obtained prior to any protocol-related activities. As part of this procedure, the principal investigator or appropriate personnel must explain orally and in writing the nature, duration, and purpose of the study, and the action of the study drug in such a manner that the subject is aware of the potential risks, inconveniences, or adverse effects that may occur. Subjects should be informed that they could withdraw from the study at any time. If the subject decides to withdraw from the

study, he or she will be asked if they would agree to continue to be monitored. They will receive all information that is required by federal regulations and ICH guidelines. The Principal Investigator will have on file a copy of the IRB-approved Informed Consent form prior to the start of the study.

8.5 Data Monitoring and Safety Board

The Data Safety and Monitoring Board will be provided with detailed safety information from the trial. This information will include information on adverse events, clinical laboratory results, and the results of all imaging studies. They are responsible along with the Investigator and The Rogosin Institute's' Medical Monitor for providing oversight and monitoring of the safety of the participants in the study.

8.6 Study Documentation

By signing a copy of Form FDA 1572, the Principal Investigator acknowledges that he or she has a copy of the Investigator's Brochure and has reviewed the latest version. In addition, he or she will comply with the protocol and the provisions stated in Form FDA 1572. No changes in this protocol can be made without the written approval of the IRB and the FDA, unless the change is to protect the immediate safety of a subject. If changes are made to protect the safety of a subject, approval of the IRB and the FDA will be obtained as soon as possible.

The investigator will have the following forms and documents on file:

- Original, signed Form FDA 1572
- Curricula vitae for all investigators listed on Form FDA 1572
- Copy of principal investigator's state medical license
- Signed protocol signature page
- List of IRB members and their occupations/affiliations or multiple assurance number
- Letter indicating IRB approval to conduct the protocol
- Copy of IRB-approved informed consent form
- Laboratory certification records and reference ranges
- A statement of financial disclosure

A Case Report Form will be used to collect the data from the ongoing clinical study. This Case Report Form will be compiled in relationship to the protocol. In addition, to the pages for the collection of the results of all evaluations,

there will be sections for the entry of text to describe the progress of the subject and any significant events.

8.7 Study Monitoring and Auditing

This study will be monitored as outlined in 21CFR§312.50 and 21CFR§312.56, the Guidelines of Good Clinical Practice (ICH-E6), and The Rogosin Institute Clinical Research Program Operating Procedures. Monitoring will include personal visits and telephone communication to assure that the investigation is conducted according to the protocol and Guidelines of Good Clinical Practice (ICH-E6). Review of Case Report Forms (CRF) at the study center will include a review of printed or electronic forms for completeness and clarity, and consistency with source documents available for each subject. Note that a variety of original documents, data, and records will be considered as source documents in this trial. The CRF itself is not to be used as a source document under any circumstances, although some of the original source documents, such as those reporting laboratory values/data will themselves be electronic. Physician and nursing records will also be only electronic and so will form original source documents. The electronic records will be recorded in MIQS, a clinical data collection system that is compliant with all FDA-mandated regulatory requirements.

8.8 HIPAA

This study will be conducted in compliance with the protocol, FDA regulations, ICH, and GCP guidelines, and all other applicable federal, state, and local laws, rules, regulations, ordinances and guidelines, all other relevant professional standards, all requirements of the host institution or facility, and the Statement of Investigation, FDA Form 1572, as described in 21CFR§312.53, which the investigator has completed and signed, in the performance and documentation of the study. Without in any way limiting the foregoing, the investigator shall obtain valid advance written informed consent and advance written authorization for use and disclosure of protected health information from each of the subjects participating in the study and shall retain such documentation after completion of the study.

8.9 Retention of Records

In accordance with 21CFR§312.62, the investigator must arrange for retention of study records at the study center for 2 years after the study drug's

New Drug Application is approved or the IND is withdrawn, as required by FDA regulations. The investigator should take measures to prevent accidental or premature destruction of these documents.

APPENDICES

Appendix 1

Name, Address, and Qualifications of Each Investigator

Principal Investigator:

Thomas J. Fahey III, M.D.
Professor of Surgery
Section Chief of Endocrine Surgery
Weill Cornell Medical Center / New York Presbyterian Hospital
520 East 70th Street, Starr 8
New York, NY 10021
Phone: 212 746-5130
Fax: 212 746-8771

Sub-Investigators

Nathaniel Berman, MD
Assistant Attending Physician
Assistant Professor of Medicine
The Rogosin Institute
505 E. 70th Street
New York, NY 10021
Phone: 212-746-1551
Fax: 212-288-8370

Barry H. Smith MD, PhD
Director/President/CEO
The Rogosin Institute
505 E. 70th Street
New York, NY 10021
Phone: 212-746-1551 or 212-750-5075
Fax: 212-371-2776

Allyson Ocean, M.D.
Medical Oncologist and Attending Physician
Gastrointestional Oncology
Weill Cornell Medical Center / New York Presbyterian Hospital
1305 York Avenue
New York, NY 10021
Phone: 212 746-2844
Fax: 212 746-0416

Zoe P. Andrada, NP
The Rogosin Institute
505 E. 70th Street
New York, NY 10021
Phone: 212-746-9233

Cytokines/Genomics

Daniel Levine, PhD
Thomas Parker, PhD

The Rogosin Institute
505 E. 70th Street
New York, NY 10021
Tel: 212-570-3252
212-570-3044
Fax: 212-570-3083

Appendix 2

Schedule of assessments (see pages 67-68)

	Screening	Visit 1 / Day 0	Visit 2 / Day 14	Visit 3 / Day 30	Visit 4 / Day 60	Visit 5 / Day 90	Visit 6 / Implant 2	Visit 7 / Day 14	Visit 8 / Day 30	Visit 9 / Day 60	Visit 10 / Day 90	Visit 11 / Implant 3	Visit 12 / Day 14	Visit 13 / Day 30	Visit 14 / Day 60	Visit 15 / Day 90	Visit 16 / Implant 4	Visit 17 / Day 14	Visit 18 / Day 30	Visit 19 / Day 60	Visit 20 / Day 90	Visit 21 / Day 120	Follow-Up[12]
Informed Consent	X																						
PE / VS / Medical History	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
ConMed / AE Review	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Global Clinical Assessment Questionnaire	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Karnofsky Performance Status	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Quality of Life Scale	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Pain Scale Assessment	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
ECOG Performance Scale	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Neurological Examination	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
12-Lead ECG	X					X					X					X							
Urinalysis	X					X					X					X							
Tumors Markers (CEA, CA19-9, CA125)	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Hematology Profile[1]	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Coagulation Panel[2]	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Erythrocyte sedimentation rate	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Comprehensive Metabolic Panel[3]	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
GGT[4]	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Amylase[5]	X																						
Lipase[5]	X																						
Lactate Dehydrogenase	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
C-Reactive Protein	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Bilirubin, Direct	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Hepatitis Panel[6]	X																						
HIV	X																						
Serum Pregnancy (Qualitative)[7]	X					X					X					X							
Immunoglobulin Levels[8]	X					X					X					X							
Cytokine Panel[9]	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	X
Cellular Immune Function[10]	X					X					X					X							
Ecotropic Murine Leukemia Virus	X			X		X			X		X			X		X			X			X	X

	Screening	Visit 1 / Day 0	Visit 2 / Day 14	Visit 3 / Day 30	Visit 4 / Day 60	Visit 5 / Day 90	Visit 6 / Implant 2	Visit 7 / Day 14	Visit 8 / Day 30	Visit 9 / Day 60	Visit 10 / Day 90	Visit 11 / Implant 3	Visit 12 / Day 14	Visit 13 / Day 30	Visit 14 / Day 60	Visit 15 / Day 90	Visit 16 / Implant 4	Visit 17 / Day 14	Visit 18 / Day 30	Visit 19 / Day 60	Visit 20 / Day 90	Visit 21 / Day 120	Follow-Up[12]
Immunohistochemical & Gene Array Analysis (when indicated)		X					X					X					X						
Murine Allergen Skin Test with positive and negative controls	X					X					X					X							
Circulating Tumor Cells	X		X	X	X	X		X	X	X	X		X	X	X	X		X	X	X	X	X	
Macrobeads Implantation (8 per kg body wt)		X					X					X					X						
Cefazolin (Prophylaxis – 1 gm)		X					X					X					X						
Chest X-Ray (PA/lateral)	X					X					X					X							
CT Scan Chest (Non-Contrast)[11]	X																						
MRI of Abdomen[11]	X					X					X					X						X	X
MRI of Pelvis[11]	X					X					X					X						X	X
Tumor Mass Biopsy (where indicated)		X					X					X					X						
PET-CT Scan Whole Body	X					X					X					X						X	X[11]
Radionuclide Bone Scan[11]																							

1 Hematology Profile: WBC, RBC, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets, automated differential WBC
2 Coagulation Panel: PT, PTT, INR
3 Comprehensive Metabolic Panel: CO_2, sodium, potassium, chloride, creatinine, BUN, calcium, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, glucose
4 GGT: gamma glutamyl-transpeptidase
5 Amylase and lipase are done at baseline, then as medically necessary for individual subjects
6 Hepatitis Panel: Hepatitis B, C and E
7 Serum pregnancy only for females of childbearing potential
8 Immunoglobulin Levels: IgA, IgG, IgE and IgM
9 Cytokine Panel: IL-6, TNF-α, TNF receptors p55, TNF receptors p75
10 Cellular Immune Function: T cells; B cells; antibodies to diphtheria, tetanus, mumps, rubella; quantitative IgG subclasses, NK cells count (CD16)
11 Only if clinically indicated
12 Long-term follow-up visits will consist of a telephone contact every 6 months for 2 years, then every year thereafter until death to determine overall survival

Appendix 3

Statistical considerations

1. Study design

Study 0911010739 is an open-label, non-randomized, single-center Phase II trial of RENCA agarose-agarose macrobeads. The study is designed to assess the efficacy and safety of this new treatment for subjects with treatment-resistant pancreatic adenocarcinoma or colorectal cancer. The study will enroll a total of 116 evaluable subjects with advanced pancreatic cancer and colorectal cancer, and implant 74 eligible patients. An evaluable subject is defined as a subject who completes the first implantation of the RENCA agarose-agarose macrobeads. If a subject signs an Informed Consent and is not implanted, the subjects will not be classified as evaluable and will be replaced. These subjects will be classified as screen failures. All efficacy and safety analyses will be by cancer-type, that is, pancreatic and colorectal subjects will be evaluated separately with no formal between group comparisons. Analyses will be performed with a focus on an estimation of specific clinically important parameters for use in the planning of larger subsequent Phase IIb and/or Phase III trials designed to fully assess efficacy and safety.

There will be no formal interim statistical analysis. The amount and nature of missing data will be characterized and no method of imputation will be used for missing data. All statistical analyses will be carried out using SAS version 9.2 (or higher) statistical software.

2. Study objectives

All study objectives, as detailed in Section 3 of the protocol, will be assessed in each of the two study groups to be enrolled in this clinical trial: subjects with advanced pancreatic cancer and subjects with advanced colorectal cancer.

3. Accrual

The projected accrual is approximately 3 subjects per month and therefore recruitment is expected to be completed at about 12 months following the study starting point.

4. Analysis populations

Efficacy/Safety Population

The efficacy/safety population is defined as all evaluable subjects regardless of subject compliance with protocol procedures. This population will be used in the primary analysis for assessment of efficacy. Efficacy sub-analyses may be performed on various subsets of subjects, such as those with no major protocol deviations or those who continued in the study for the entire treatment period (i.e., did not withdraw prematurely). One of the sub-analyses will include efficacy response after one, two, three, and/or four implantations.

All evaluable subjects enrolled in the study who complete at least one implantation procedure will be included in the safety population and considered evaluable for toxicity and safety. The efficacy and safety populations do not differ. All treatment related toxicities will be recorded and then tabulated at the end of the study. All treatment related toxicities will be recorded and then tabulated at the end of the study.

Screen Failures

All subjects who sign an Informed Consent but for any reason are not implanted will be considered screen failures. These subjects will be listed and the reasons for not being implanted listed and described.

5. Descriptive analyses

Measured outcome variables will be summarized overall and by relevant demographic and baseline variables. Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as the mean, standard deviation and range. A variety of graphical techniques will also be used to display data, for example, histograms, boxplots, scatterplots, etc.

6. Primary statistical analyses

The primary objectives are outlined in Section 3 of this protocol. The analysis will be performed as detailed in Statistical Analysis Plan (SAP).

7. Secondary statistical analyses

The secondary objectives are outlined in Section 3 of this protocol. The analysis will be performed as detailed in Statistical Analysis Plan (SAP).

8. Stopping rules

In order to protect subjects from excessive toxicities possibly associated with the study treatment the following stopping rule will be utilized: if 3 or more subjects experience a grade 5 adverse event deemed possibly related to treatment, accrual will be halted and a thorough investigation will take place before the re-opening of accrual may be considered. The probability of excising the stopping rule within this study is a function of the true unknown probability of a grade 5 adverse event possibly related to the treatment corresponding to the study population. The table below contains the probability of observing at least 3 such toxicities in the 30 subjects for a variety of values of the true probability within the population.

True probability of treatment related toxicity	5%	10%	20%	30%
Probability of stopping the study based on 30 subjects	19%	59%	96%	99.8 %

The calculations above are based on the assumption that our study sample may be treated as a random sample from the population of interest. As can be seen there does exist acceptable properties associated with the stopping rule.

9. Sample size justification

Since the primary objectives are addressed through estimation and not formal hypothesis testing, the original sample size of 20 colorectal subjects and 10 pancreatic subjects was based on the expected precision corresponding to the estimates of the progression at appropriate intervals and toxicity rates. We believe that these statistical goals will be fulfilled with the current intended sample size of 74 patients, of which, 40 patients will be pancreatic cancer type. The precision associated with these estimates is a function of the true unknown parameter value and the sample size used in the calculation. Note that a true rate of 50% represents the worst case scenario with in regards to precision and therefore we may take precision at that point to be an upper bound of expected accuracy in the estimation of study parameters. Calculations reveal that the precision associated with estimates based on the colorectal subjects will be at most 22 percentage points. In the case of pancreatic subjects, the corresponding quantity is 31 percentage points.

10. References

Brookmeyer, R. and Crowley, J.J. (1982). A confidence interval for the median survival time. Biometrics; 38:29-41.

Clopper, C.J. and Pearson, E.S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika; 26:404-413.

Hosmer, D.W. and Lemeshow, S. (2000). *Applied Logistic Regression.* Wiley and Sons, New York.

Kaplan, E.L. and Meier, P.J. (1958). Nonparametric estimation from incomplete observations. Journal of the American Statistical Association; 53:457-481.

REFERENCES

References in Introduction:

Pancreatic cancer:

American Cancer Society.: Cancer Facts and Figures 2009. Atlanta, Ga: American Cancer Society, 2009.

Greenlee RT, Murray T, Bolden S, et al.: Cancer statistics, 2000. CA Cancer J Clin 50 (1): 7-33, 2000 Jan-Feb

Yeo CJ, Abrams RA, Grochow LB, et al.: Pancreaticoduodenectomy for pancreatic adenocarcinoma: postoperative adjuvant chemoradiation improves survival. A prospective, single-institution experience. Ann Surg 225 (5): 621-33; discussion 633-6, 1997.

Lillemoe KD: Current management of pancreatic carcinoma. Ann Surg 221 (2): 133-48, 1995.

Yeo CJ: Pancreatic cancer: 1998 update. J Am Coll Surg 187 (4): 429-42, 1998.

Nitecki SS, Sarr MG, Colby TV, et al.: Long-term survival after resection for ductal adenocarcinoma of the pancreas. Is it really improving? Ann Surg 221 (1): 59-66,

1995. [• Conlon KC, Klimstra DS, Brennan MF: Long-term survival after curative resection for pancreatic ductal adenocarcinoma. Clinicopathologic analysis of 5-year survivors. Ann Surg 223 (3): 273-9, 1996. [

Willett CG, Daly WJ, Warshaw AL: CA 19-9 is an index of response to neoadjunctive chemoradiation therapy in pancreatic cancer. Am J Surg 172 (4): 350-2, 1996.

Norton, L, Simon, R., Bereton, H.D., Bogden, A.E. Predicting the course of Gompertzian growth. Nature 264: 542-45. 1976.

Norton, L. A Gompertzian model of human breast cancer growth. Cancer Res 48; 7067-71. 1988.

Norton, L. Cancer stem cells, self-seeding, and decremented exponential growth: Theoretical and clinical implications. Breast Dis 29: 27-36. 2008.

Speer, J.F., Petrosky, V.E., Retsky, M.W., Wardwell, R.H. A stochastic numerical model of breast cancer growth that simulates clinical data. Cancer Res 44:4124-30. 1984.

Laird, A.K. Dynamics of tumor growth: Comparison of growth rates and extrapolation of growth curve to one cell. *Brit J Cancer* 29:278-291. 1965.

Laird, A.K., Tyler, S.A., Barton, A.D. Dynamics of normal growth. *Growth* 29: 233-248. 1965.

De Wys, WD. Studies correlating the growth rate of a tumor and its metastases and providing evidence for tumor-related systemic growth-retarding factors. Cancer Res 32(2): 374-9, 1972.

Fisher, B. et al. Presence of a growth-stimulating factor in serum following primary tumor removal in mice. Cancer Res 49(8): 1996-2001, 1989.

Colorectal cancer:

Gleisner, A.L., Choti, M.A., Assumpcao, L., Nathan, H. Schulick, RD, Pawlik, T.M. Colorectal liver metastases: recurrence and survival following hepatic resection, radiofrequency ablation, and combined resection-radiofrequency ablation. Arch Surg 143(12):1204-12. 2008.

Ocean AJ, Parikh T, Berman N, Escalon J, Shah MA, Andrada Z. Phase I/II trial of intraperitoneal implantation of agarose-agarose macrobeads (MB) containing mouse renal adenocarcinoma cells (RENCA) in patients (pts) with advanced

colorectal cancer (CRC). American Society of Clinical Oncology (ASCO) Annual 2013 Meeting, Chicago IL (Abstract).

Onaitis, M.W., Peterson, RP, Haney, J.C., Saltz, L. Park, B. Flores, R., Bains, M.S. Dycoco J., D'Amico, T.A., Harpole, D.H., Kemeny, N., Rusch, V.W., Downey, R. Prognostic factors after pulmonary resection of colorectal cancer metastases. Ann Thorac. Surg 87(6): 1684-8. 2009.

Timmerman, R.D., Bizekis, C.S., Pass, H.I., Fong, Y., Dupuy, D.E., Dawson, L.A., Lu, D. Local surgical, ablative, and radiation treatment of metastases. CA Cancer J Clin 59(3):145-170. 2009. (Epub, April 2009).

Wolpin, B.M., Mayer, R.J. Systemic treatment of colorectal cancer. Gastroenterology 134(5): 2008 1296-1310. 2008.

Yamane, B., Weber, S. Liver-directed treatment modalities for primary and secondary hepatic tumors. Surg Clin N Amer 89(1):97-113.

Cancer General References:

Al-Hajj, M. et al, Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100(7): 3983-8, 2003.

Clark, MF, Fuller, M. Stem cells and cancer: Two faces of Eve. Cell 124: 1111-1115, 2006.

Fan, X, Matsui, W, Khaki, L, et al. Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. Cancer Res 66:7445-7452, 2007.

Fayers PM, Aaronson NK, Bjordal K, Groenvold M, Curran D, Bottomley A, on behalf of the EORTC Quality of Life Group. The EORTC QLQ-C30 Scoring Manual (3rd Edition). Published by: European Organisation for Research and Treatment of Cancer, Brussels 2001.

Gazda LS, Martis PC, Laramore MA, Bautista MA, Dudley A, Vinerean HV, Smith BH. Treatment of agarose-agarose RENCA macrobeads with docetaxel selects for OCT4+ cells with tumor-initiating capability. Cancer Biology & Therapy December 2013; 14:12,

Gilbertson, RJ, Rich, JN. Making a tumour's bed: Glioblastoma stem cells and the vascular niche. Nat Rev Cancer 7:733-736, 2007.

Hanahan, D, Weinberg, RA. The hallmarks of cancer. Cell 100: 57-70, 2000.

Karnofsky DA, Burchenal JH. The Clinical Evaluation of Chemotherapeutic Agents in Cancer. In: MacLeod CM (Ed), Evaluation of Chemotherapeutic Agents. Columbia Univ Press 1949. page 196.

Keir CH, Ocean AJ, Fahey TJ, Berman N, Wadke A, Kelly-Rossini L, Goldstein MJ, Leeser DB, Michelassi F, Smith BH. Treatment of advanced, epithelial-derived cancer (AEC) with intraperitoneal implantation of agarose-agarose macrobeads (MB) containing mouse renal adenocarcinoma cells (RENCA). American Society of Clinical Oncology (ASCO) Annual 2011 Meeting; J Clin Oncol 29: 2011 (suppl; abstr e13594).

Kobielak, K, Stokes, N, de la Cruz, J et al. Loss of a quiescent niche but not follicle stem cells in the absence of bone morphogenic protein signaling. Proc Nat Acad Sci USA 104: 10062-10068, 2007

Littlepage, LE, Egeblad, M, Werb, Z. Coevolution of cancer and stromal cellular responses. Cancer Cell 7:499-500, 2007.

Matsushansky, I Hernando, E, Socci, N, et al. Derivation of sarcomas from mesenchymal stem cells via inactivation of the Wnt pathway. J Clin Invest 117: 3248-3257, 2007.

Moore KA, Lemischka, IR. Stem cells and their niches. Science 311: 1880-1885, 2006.

Prehn, RT. The inhibition of tumor growth by tumor mass. Cancer Res 51(1): 2-4, 1991.

Reya, T. et al. Stem cells, cancer, and cancer stem cells. Nature 414(6859): 105-11, 2001.

Sanai, N, Alvarez-Buylla, A, Berger,MS. Neural stem cells and the origins of gliomas. N Engl J Med 353: 811-822, 2005.

Smith, BH. et al, Feedback inhibition in the regulation of malignant cell proliferation and tumor growth: the agarose-agarose mouse renal adenocarcinoma cancer macrobead. Manuscript to be submitted to Nature Oncology, 2008.

Smith BH, Gazda LS, Conn BL, Jain K, Asina S, Levine DM, et al. Hydrophilic agarose macrobead cultures select outgrowth of carcinoma cell populations that can restrict cancer growth. Cancer Res 2011;71(3):725-35. (a)

Smith BH, Gazda LS, Conn BL, et al. Three-dimensional culture of mouse renal carcinoma cells in agarose macrobeads selects for a subpopulation of cells with cancer stem cell or cancer progenitor properties. Cancer Res. 2011;71(3):716-724. (b)

Sneddon, JB, Werb, Z. Location, location, location: The cancer stem cell niche. Cell Stem Cell 1:607-611, 2007.

Tu, SM et al. Stem-cell origin of metastasis and heterogeneity in solid tumors. Lancet Oncol 3(8): 508-13, 2002

Xenotransplantation:

Bach, FH. Xenotransplantation: problems and prospects. Ann Rev Med 49: 301-310, 1998.

Boneva, RS, Folks, TM, Chapman, LE. Infectious disease issues in xenotransplantation. Clinical Microbiol Rev. 14: 1-14. 2001.

Chapman, LE, Folks, T, Salomon, D, et al. Xenotransplantation xenogenic infections. NEJM 333: 1498-1500. 1995.

Denner, J. Is porcine endogenous retrovirus transmission still relevant? Transplant Proc 40:587-589, 2008.

Fishman, J, Sachs, D, and Shaikh, R (Eds). Xenotransplantation: Scientific Frontiers and Public Policy. Ann NY Acad Sci 862: 1, 1998.

Fujimura, T, Miyagawa, S, Takahagi, Y, et al. Prevalence of porcine endogenous retroviruses in domestic, miniature, and genetically modified pigs in Japan. Transplant Proc 40:594-595, 2008.

Garkavenko, O, Wynyard, S, Nathu, D, et al. Porcine endogenous retrovirus transmission characteristics from a designated pathogen-free herd. Transplant Proc. 40:590-593, 2008.

Institute of Medicine. Xenotransplantation: Science, ethics, and public policy. Washington, DC. National Academy Press, 1996.

Lower, R, Lower, J and Kurth, R. The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. Proc Nat Acad Sci 93: 5177-5184. 1996.

Mattiuzzo, G, Scobie, L, Takeuchi, Y. Strategies to enhance the safety profile of xenotransplantation: minimizing the risk of viral zoonoses. Curr Opin Organ Transplant 13:184-188, 2008.

Platt, JL and Nagayasu, T. Current status of xenotransplantation. Clin Exp Pharm Physiol 26: 1026-1032, 1999.

Soin, B, Vial, CM, and Friend, PJ. Xenotransplantation. British J. Surg 87:138-148, 2000.

Weiss, RA. Xenografts and retroviruses. Science 285: 1221, 1999.

Weiss, RA. Xenotransplantation: Pausing to reflect. Hospital Practice, (October 15): 71-78, 2000

Murine Leukemia Virus:

National Research Council, Companion Guide to Infectious Diseases of Mice and Rats, National Academy Press, Washington, DC, 1991.

Charles River Laboratories, Testing for Xenotropic and Non-Xenotropic Murine Leukemia Virus. Protocols GP-600 and GP-V601.1 in accord with GLP regulations, Title 21 CFR Section 58.

Ganguly, K, Kalyanaraman, VS, Sarngadharan, MG. Cell surface receptors for ecotropic murine viruses are different from those for xenotropic and amphotropic viruses. Intervirology 19: 224-229. 1983.