Clinical Trial of Manganese-Enhanced MRI (MEMRI) to Assess Peri-Infarct Injury

Study Protocol and Statistical Analysis Plan

NCT02933034

December 21, 2018

TITLE: Clinical Trial phase 1-2 of **M**anganese-**E**nhanced **MR**I to Assess Myocardial Injury and Predict Future **Car**diovascular **E**vents (MEMRI-CARE)

Amendment 5.

Study Product: *EVP 1001-1 (See More™)*

IND Number: [redacted]

Amended: (1) 06/30/2015
Amended: (2) 10/14/2016
Amended: (3) 12/19/2016
Amended: (4) 03/21/2017
Amended: (5) 12/21/2018

List of Abbreviations

AA	atrial arrhythmia
ACMA	Arterial Cardiomyopathy arrhythmia
AE	adverse event
AMBMC	autologous mononuclear bone marrow cells
BMC	bone marrow cell
BSC	biologic safety cabinet
COA	Certificate of Analysis
CABG	coronary artery bypass graft
CAD	coronary artery disease
CCTRN	Cardiovascular Cell Therapy Research Network
CFR	Code of Federal Regulations
CFU-F	colony forming units – fibroblasts
CK-MB	creatinine kinase – myocardial band
CMR	Cardiac MRI
CNR	contrast to noise ratio
CPL	cell processing lab
CPQCL	Cell Processing Quality Control Lab
CRO	contract research organization
CSCs	cardiac stem cells
CVE	cardiovascular events
DAPI	4'-6-Diamidino-2-phenylindole
DCM	dilated cardiomyopathy
DEMRI	delayed enhanced MRI
DMSO	dimethyl sulfoxide
DSMB	Data and Safety Monitoring Board
EB	Evans Blue dye
EF	ejection fraction
ECG	Electrocardiogram
EPC	endothelial progenitor cells
ESR	expedited safety report
EVP	EVP 1001-1 injectable contrast agent
FBS	fetal bovine serum
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
GMP	Good Manufacturing Practice
hAMSC	Human autologous mesenchymal stem cells
HARP	Harmonic Phase
BMC	human bone marrow cell
HF	heart failure
HIPAA	Health Insurance Portability and Accountability Act
HSA	human serum albumin
HSC	hematopoietic stem cell

HTLV	human T-cell lymphotropic virus
ICAM	intracellular adhesion molecule
ICD	implantable cardioverter-defibrillator
ICF	Informed consent form
ICH	International Conference on Harmonisation of Technical Re-quirements of Pharmaceuticals for Human Use
ICM	Ischemic Cardiomyopathy
IDM	Infectious Disease Markers
IIR	Intra infarct regions
IR	Ischemia reperfusion
ISCI	Interdisciplinary Stem Cell Institute (University of Miami)
IEC	institutional ethics committee
IND	Investigational New Drug application
IRB	Institutional Review Board
I.V.	Intravenous
KDR	VEGF receptor-2
LAD	left anterior descending artery
LV	left ventricular
LVAD	left ventricular assist device
MACE	major adverse cardiac events
MEM	minimum essential medium
MEMRI	Manganese enhanced MRI
MHC	major histocompatibility complex
MI	myocardial infarction
MLHF	Minnesota Living with Heart Failure
MN	manganese
MR	magnetic resonance
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell (human)
NICM	non-ischemic cardiomyopathy
NYHA	New York Heart Association
PBS	phosphate buffered saline
PIR	Peri-infarct region
PK	Pharmacokinetics
QA	quality assurance
QC	quality control
SAE	serious adverse event
SCCT	Specialized Center for Cell-Based Therapy
SCF	stem cell factor
SDF-1	stromal cell derived factor 1
SOP	standard operating procedures
SDCC	Stanford Data Coordinating Center
SNR	Signal to noise ratio
SW	Stroke Work
TEM	Tissue Electron Microscopy
TESPI	Transendocardial Study Product Injection
TTC	triphenyltetrazolium chloride
TI	Inversion time

U.S.	United States
VEGF	vascular endothelial growth factor
Peak VO2	peak oxygen consumption
WBC	white blood count

Study Summary

Title	Clinical Trial of Manganese-Enhanced MRI (MEMRI) to Assess Peri-Infarct Injury
Short Title	*MEMRI-DEMRI*
Protocol Number	*28508*
Phase	*Clinical study phase 2*
Methodology	*Open Label*
Study Duration	i. Screening - approximately 1-2 hours including H&P, blood work and EKG. ii. Active participation - MRI study itself is approximately 1.5 hr per exam. iii. Analysis of participant data takes approximately 1 hr per exam with an additional 0.5 hr per patient for tallying clinical outcomes and AEs. iv. Total time estimated 3 days per patient. We expect the study to take approximately 12 months.
Study Center(s)	*Single*
Objectives	This is a first-in-human clinical translation of manganese-enhanced MRI (MEMRI) to assess the peri-infarct injury in patients with severe ischemic *cardiomyopathy (ICM), non-ischemic cardiomyopathy (NICM)* and *a*trial fIbrillation
Number of Subjects	*50*
Diagnosis and Main Inclusion Criteria	• Patients wiith ischemic or dilated cardiomyopathy with an EF less than or equal to 50 or patients with paroxysmal or persistent atrial fibrillation who are at least 18 and less than 80 years of age. Only males and non-pregnant females to be included.
Study Product, Dose, Route, Regimen	**EVP 1001-1 (SeeMore™) Injectable** **Manufacturer:** University of Iowa Pharmaceuticals **IND #** [redacted] **Dosage:** 0.*28* ml/kg
Duration of administration	Intravenous injectable given slowly over approximately one (1) minute
Reference therapy	Gadolinium
Statistical Methodology	Student's t-test to assess for significant difference between MEMRI and DEMRI; Pearson Correlation to assess for relationship between MEMRI and signal and cardiac function/clinical outcome

1 Introduction

This document is a protocol for a human clinical research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and institutional research policies and procedures.

1.1 Background

- This is a first-in-human clinical translation of manganese-enhanced MRI(MEMRI) to assess the peri-infarct injury in patients with patients wiith ischemic or dilated cardiomyopathy with an EF less than or equal to 50 or patients with paroxysmal or persistent atrial fibrillation
- and those who received stem cell therapy for these indications. The PI recently obtained an approval for an Investigational New Drug (IND #[redacted]) to conduct a clinical trial of MEMRI contrast agent (EVP1001-1, Eagle Vision, Downingtown, PA). Funding of this proposal will enable a clinical trial of 120 patients with ischemic or dilated cardiomyopathy with an EF less than or equal to 50 or patients with paroxysmal or persistent atrial fibrillation to evaluate the safety and efficacy of sequential acquisition of MEMRI and delayed-enhanced MRI (DEMRI) for precise tissue characterization of the peri-infarct region (PIR). This novel technique designed to integrate the biology of the viable cardiomyocytes with the anatomy of the scar will predict future cardiovascular events (CVE), in this highly morbid patient population. ICM and NICM are the primary etiology of advanced heart failure (HF) while ACM is a frequent sequela of HF. Today, HF is the leading diagnosis of hospital admission in the US. Our studies have confirmed a direct association between the presence of PIR and the occurrence of atrial and ventricular arrhythmia and remodeling, resulting in high morbidity and mortality in advanced HF. PIR has been recognized as an important substrate to trigger atrial and ventricular arrhythmias and stem cells may provide an alternative therapy for PIR. Studies employing DEMRI has identified atrial scar as the potential cause of atrial arrhythmia. This proposal contains novel hypotheses well-grounded on the following 2 innovations: (1) MEMRI for management of HF. An alternative approach is to employ manganese (Mn^{2+}), which targets the viable myocardium. Mn^{2+} is specific to the L-type calcium channels and complements the non- viable scar delineated by DEMRI. The dual MEMRI-DEMRI approach will, therefore, identify the viable and, potentially, salvageable cardiomyocytes outside the core infarct zone but inside the PIR. Our recently published study demonstrated that an overlap of DEMRI and MEMRI signal denoted a significant number of live cardiomyocytes (MEMRI-positive) within the non-viable (DEMRI- positive) region. This innovation will profoundly impact the clinical management of the advanced HF patients and AA patients including atrial fibrillation. EVP1001-1 allows highly effective intracellular targeting of viable cardiomyocytes with high level of safety and unique magnetic properties. (2) Assessment of therapeutic response using MEMRI. EVP1001-1 is rapidly transported, intra-cellularly, to mitochondria rich tissue such as heart, liver, and kidney, demonstrating significant enhancement indicative of cell metabolism and viability. High dose clinical safety studies of SeeMoreTM with safety index of 45-90 correspond more favorably than the widely used clinical imaging agents (10-20 for iodinated X-ray products and 20-60 for gadolinium). This specific, non-perfusion dependent distribution due to active intracellular uptake is one of the key attributes that offers real-time assessment of therapeutic response, using cardiac cellular imaging. A clinical safety trial in 6 patients have demonstrated negligible uptake in the intra-infarct region (IIR), reduced uptake in the PIR, and significantly higher uptake in the normal tissue to generate positive signal. In this trial, the mean LVEF was 32±?4%. The DEMRI vs. MEMRI (scar/myocardial volume) demonstrated significantly higher measurement of the scar by DEMRI in all patients (39±11%* vs. 16±3%, *$p<0.01$) These data indicate 2 important findings: 1) significant over-estimation of the scar by DEMRI and 2) quantitative difference of the scar volume between MEMRI and DEMRI delineates the PIR. This precise characterization allows accurate assessment of the efficacy of novel therapeutic agents in restoring the PIR at both cellular and tissue levels. EVP1001-1 is a new manganese (Mn)-based intravenous imaging agent being developed to enhance magnetic resonance imaging (MRI). While Mn has long been known to have desirable magnetic and kinetic properties for MRI, use in humans was not initially possible due to cardiovascular depression and electrocardiogram (ECG) changes, including prolongation of PR and QTc intervals, associated with intravenous administration. Chelation of Mn, as had been done with gadolinium for use with MRI, provided relevant safety, but sacrificed desirable magnetic and kinetic properties. EVP1001-1 provides Mn in a form that maintains the desired magnetic and kinetic properties while overcoming the cardiovascular toxicity of Mn. EVP1001 is taken up into heart cells (primarily via addition of calcium to avoid cardiotoxic effects; please refer to patent listed below). The potential to distinguish healthy heart tissue from unhealthy heart tissue based on a specific sustained pattern of enhancement provides a basis for evaluating the performance of EVP1001-1 in heart patients. MRI offers benefits over other imaging technologies. Relative to radioactive nuclear imaging procedures, MRI is 3- dimensional, provides good soft tissue discrimination, and is of high spatial and temporal resolution. These features have been reported to identify smaller defects (e.g., subendocardial infarcts) and match angiographic results more closely than other modalities such as SPECT. It may be possible to enhance the utility of MRI for heart disease further through the use of an imaging agent that is specifically taken up into heart cells. EVP1001-1 is the only cardiac-specific agent being developed for this purpose. Unlike nuclear perfusion agents, EVP1001-1 is not radioactive and does not require special handling, shielding, transport or storage. In addition, the specific pattern of enhancement achieved in the heart muscle persists

over time, offering potential benefits over the nonspecific extracellular agents currently available for MRI or X-ray/CT procedures. This feature allows full use of the high resolution of MRI, since there is not a trade-off of high spatial resolution for temporal (first-pass) resolution. It is anticipated that the features offered by EVP1001-1 along with the high resolution, three-dimensional attributes of MRI will result in higher accuracy than is available with other current modalities in practice, including stress echocardiograms, cardiac MRI using gadolinium contrast and nuclear studies such as SPECT and PET. This will be evaluated in this study and serve as the basis for pivotal registration studies. All components of EVP1001-1 are USP and are approved for use as drugs in man, orally and/or intravenously. A summary of the Phase I safety and PK (pharmacokinetics) study are provided below. The Phase I study evaluated the safety and tolerance of EVP1001-1 in humans, with special emphasis on cardiovascular safety, and assessed its PK profile. Overall, EVP1001-1 was found to be safe at all doses. There were no serious adverse events (SAE) or any events requiring medical treatment. Of special importance, no signs of cardiac depression were reported. In addition, there was no observation of prolongation of the QTc or PR intervals or other ECG-changes that had previously been reported in animals for Mn. There were no changes in hematology, serum biochemistry (including liver function tests), coagulation parameters nor in temperature, respiratory rate or blood oxygen levels. Transient increases in HR and BP were seen. These, for the most part, reflected transient changes within the normal reference range. There was no obvious dose-relationship for these responses, although their duration and magnitude were greater for the 5-minute than for the 1-minute dose administrations. Other AEs were reported, almost all which were regarded as of mild or moderate intensity. Almost all events occurred during or immediately after dosing and resolved within minutes. Over 83% of the AEs occurred during dosing and over 93% occurred within one minute of dosing. Nearly one-third of all events resolved within one minute from dosing, over 80% within 5 minutes, and 94% resolved within 1 hour. The most frequent types of AEs were injection-associated discomforts (warmth, flushing, cold, pressure, injection site changes, increased HR/BP), gastrointestinal disturbances (discomforts, nausea, vomiting, diarrhea), lacrimation and headache. Although gastrointestinal disturbances, such as nausea and occasional vomiting, resolved rapidly without treatment, these bothersome events may be avoidable through the use of a standard anti-emetic. This proved to be beneficial in humans and also in high-dose animal studies. The PK profile reflected that known for Mn in humans (based on reported radiotracer Mn studies), with a primary blood half-life under 1 minute, and less than 1% of the dose remained in the blood by 30 minutes after dosing. The performance of EVP1001-1 was as expected based on information that was in the Investigators' Brochure. Lastly, please refer to US Patent #5,980,863 regarding further details of EVP1001-1 contrast ingredients.

1.2 Investigational Agent

Using aseptic technique, transfer via syringe 10 mL of 10% Calcium Gluconate, USP and 30 mL of Sterile Water for Injection to a vial containing EVP 103. Shake gently to dissolve the powder. This prepares 40 mL of Drug. Drug should be used promptly (within 1 to 2 hours) following preparation. The calculated osmolality of Drug is 0.27 (nearly isomolar), and the pH is slightly acidic to neutral (5.5 to 7.0).

The proper dose should be specified in the protocol on a “per kilogram body weight” basis and must be given slowly over approximately one (1) minute intravenously. This same administration rate applies to flushing residual drug in connecting tubing/injection port.

Dilutents and drug project should be inspected to assure that a clear solution is present. Should any precipitate or undissolved material remain in the vial, the vial should be gently warmed and shaken gently to dissolve any visible material prior to either dilution or administration.

1.3 Preclinical Data

A reliable porcine ischemia reperfusion (IR) injury model has been developed in our lab based on 46 swine with myocardial injury created by balloon occlusion of the LAD. Subsequent validation of the peri-infarct region (PIR) and intra-infarct regions (IIR) was performed using a novel *in situ* double-staining method with 1% Evans blue dye (EB) and 1% 2,3,5-triphenyltetrazolium chloride (TTC) solution. While DEMRI is the gold-standard in identifying the non-viable myocardium, it does not allow direct measurement of myocardial viability. In fact, DEMRI is known to overestimate the area of non-viable scar due to the non-specific gadolinium distribution and the kinetics of T1 signal *in vivo*. MEMRI signal, on the other hand, is generated from the specific Mn^{2+} uptake by the viable, active cardiomyocytes. This recognition led to the development of our novel dual MEMRI-DEMRI contrast technique designed to enable rapid assessment of both the biology of viable myocardium and the anatomy of the nonviable scar, optimizing the evaluation of the heterogeneous PIR. In a porcine IR

model, the injured but viable cardiomyocytes in the PIR was identified *in vivo*. While MEMRI and DEMRI infarct volumes correlated with the *ex-vivo* TTC stain infarct volume, MEMRI infarct (defect) volume percentage was significantly lower than DEMRI. The majority of the discrepancy was found in the PIR positive for both MEMRI signal (viable) and DEMRI signal (non-viable). These regions displayed lower SNR by MEMRI than remote zones, and lower SNR by DEMRI than core infarct zones, indicating heterogeneity and significant population of viable cardiomyocytes with intact Ca^{2+}-channel function (MEMRI positive) surrounded by large amounts of surrounding necrotic tissue (DEMRI positive). This tissue heterogeneity was validated by both *in situ* double stain (EB/TTC) and tissue electron microscopy (TEM), which showed EB-negative/TTC-red area of PIR in the transmural DEMRI region. In addition, TEM demonstrated preserved cellular ultrastructure in the PIR and the non-viable ultrastructure in the intra-infarct region (IIR). TEM exhibited significantly different scores among the cells in the remote region, IIR, and PIR (inter-observer agreement 0.79, $p<0.05$). Significant difference in the structural integrity of PIR generated the following score: remote (4.7±0.3), intra-infarct (1.2±0.2), and PIR (3.1±0.4*, *$p<0.05$).

In both sub-acute and chronic porcine ischemia-reperfusion injury (IR) models, MEMRI demonstrated a consistent anteroseptal infarct pattern from the mid-LAD IR injury). MEMRI of the hAMSC group revealed discrete foci of high contrast-to-noise ratio (CNR) *within* infarct and peri-infarct zones, which were absent in control group. These hyperintense foci increased in CNR over the 3-week and 6-week time course after cell delivery in comparison to the control group. To confirm the specificity of the positive MEMRI signal for hAMSC engraftment, the hAMSCs for one sub-acute and one chronic IR swine were modified with injection of ~20% herpes simplex virus-thymidine kinase (HSV-tk) PET reporter gene (PET-RG) transduced hAMSCs. Serial cardiac PET-CT scans (day 0, day 7, and day 21 or 38 after injection) were then performed using a specific 9-[4-[F]fluoro-3-(hydroxymethyl) butyl] guanine (F-FHBG) radioisotope, which are phosphorylated and retained only by the viable cells expressing PET-RG. At each time point, apical PET signal co-localized with the site of cell injection and MEMRI signal. Quantitative assessment of PET activity from these regions indicated significantly higher radiotracer uptake compared to surrounding myocardium by approximately 25-fold. When plotted according to the AHA 16-segment model, the integrated MEMRI and PET signal within each segment showed a significant linear correlation in both the sub-acute and chronic IR models. The increased MEMRI CNR tracked the survival of hAMSCs longitudinally, suggesting that the hAMSCs engrafted *in vivo* up to 6 weeks.

1.4 Clinical Data to Date

This is a clinical translation of manganese-enhanced MRI (MEMRI) to assess the peri-infarct injury in patients with severe ischemic cardiomyopathy (ICM). The PI recently obtained an approval for an Investigational New Drug (IND #[redacted]) to conduct a clinical trial of MEMRI contrast agent (SeeMore™, Eagle Vision, Downingtown, PA). He has already completed initial pilot study of safety in 6 patients and found no adverse event. Funding of this proposal will enable a clinical trial of 60 patients with severe ICM, NICM and atrial arrhythmia (AA) and those undergoing stem cell therapy to evaluate the safety and efficacy of sequential acquisition of MEMRI and delayed-enhanced MRI (DEMRI) for precise tracking of stem cell engraftment and tissue characterization of the peri-infarct region (PIR). This novel technique designed to integrate the biology of the stem cells delivered into the myocardium and their regional effects on myocardia viability will elucidate the mechanism underlying clinical outcome in this highly morbid patient population. ICM is the primary etiology of advanced heart failure (HF), the leading diagnosis of hospital admission in the US. Our studies have confirmed a direct association between the presence of PIR and the occurrence of ventricular arrhythmia and LV remodeling, resulting in high morbidity and mortality in advanced HF. PIR has been recognized as an important substrate to trigger ventricular arrhythmias and clinical trials have demonstrated that the revascularization of the PIR result in lower incidence of CVE. In order to reliably evaluate the role of the PIR, we conducted 3 published clinical studies employing DEMRI, the current gold standard, to assess myocardial injury. These studies confirmed that the presence of ischemia, scar, and/or tissue heterogeneity in the PIR predicted future CVE. Although transmurality of DEMRI scar is used to predict functional recovery and improved survival after revascularization, DEMRI does not evaluate direct myocardial viability due to its nonspecific distribution within the extracellular space. The majority of the discrepancy lies within the heterogeneous PIR, resulting in overestimation of the non-viable infarct. To date, there is no established imaging strategy to identify the viable and, potentially, salvageable cardiomyocytes within the PIR. In order to address these issues, this proposal contains novel hypotheses well-grounded on the following 2 innovations: 1) MEMRI for management of HF. An alternative approach is to employ manganese (Mn2+), which targets the viable myocardium. Mn2+ is specific to the L-type calcium channels and complements the non- viable scar delineated by DEMRI. The dual MEMRI-DEMRI approach will, therefore, identify the viable and, potentially, salvageable cardiomyocytes outside the core infarct zone but inside the PIR. Our recently published study demonstrated that an overlap of DEMRI and MEMRI signal denoted a significant number of live cardiomyocytes (MEMRI-positive) within the non-viable (DEMRI- positive) region. This innovation will profoundly impact the clinical management of the advanced HF patients and AA patients including atrial fibrillation. EVP1001-1 allows highly effective intracellular targeting of viable cardiomyocytes with high level of safety and unique magnetic properties. 2) Assessment of therapeutic

response using MEMRI. EVP1001-1 is rapidly transported, intra-cellularly, to mitochondria rich tissue such as heart, liver, and kidney, demonstrating significant enhancement indicative of cell metabolism and viability. High dose clinical safety studies of SeeMoreTM with safety index of 45-90 correspond more favorably than the widely used clinical imaging agents (10-20 for iodinated X-ray products and 20-60 for gadolinium). This specific, non-perfusion dependent distribution due to active intracellular uptake is one of the key attributes that offers real-time assessment of therapeutic response, using cardiac cellular imaging. A clinical safety trial in 6 patients have demonstrated negligible uptake in the intra-infarct region (IIR), reduced uptake in the PIR, and significantly higher uptake in the normal tissue to generate positive signal. In this trial, the mean LVEF was 32±?4%. The DEMRI vs. MEMRI (scar/myocardial volume) demonstrated significantly higher measurement of the scar by DEMRI in all patients (39±11%* vs. 16±3%, *p<0.01).

EVP is novel manganese (Mn)-based intravenous imaging agent being developed to enhance magnetic resonance imaging (MRI). While Mn has long been known to have desirable magnetic and kinetic properties for MRI, use in humans was not initially possible due to cardiovascular depression and electrocardiogram (ECG) changes, including prolongation of PR and QTc intervals, associated with intravenous administration. Chelation of Mn, as had been done with gadolinium for use with MRI, provided relevant safety, but sacrificed desirable magnetic and kinetic properties. EVP provides Mn in a form that maintains the desired magnetic and kinetic properties while overcoming the cardiovascular toxicity of Mn. EVP is taken up into heart cells (primarily via addition of calcium to avoid cardiotoxic effects; please refer to patent listed below). The potential to distinguish healthy heart tissue from unhealthy heart tissue based on a specific sustained pattern of enhancement provides a basis for evaluating the performance of EVP in heart patients. MRI offers benefits over other imaging technologies. Relative to radioactive nuclear imaging procedures, MRI is 3-dimensional, provides good soft tissue discrimination, and is of high spatial and temporal resolution. These features have been reported to identify smaller defects (e.g., sub-endocardial infarcts) and match angiographic results more closely than other modalities such as SPECT. It may be possible to enhance the utility of MRI for heart disease further through the use of an imaging agent that is specifically taken up into heart cells. EVP is the only cardiac-specific agent being developed for this purpose. Unlike nuclear perfusion agents, EVP is not radioactive and does not require special handling, shielding, transport or storage. In addition, the specific pattern of enhancement achieved in the heart muscle persists over time, offering potential benefits over the nonspecific extracellular agents currently available for MRI or X-ray/CT procedures. This feature allows full use of the high resolution of MRI since there is no trade-off of high spatial resolution for temporal (first-pass) resolution. It is anticipated that the features offered by EVP along with the high resolution, three dimensional attributes of MRI will result in higher accuracy than is available with other current modalities in practice, including stress echocardiograms, cardiac MRI using gadolinium contrast and nuclear studies such as SPECT and PET. This will be evaluated in this study and serve as the basis for pivotal studies for routine clinical application.

All components of EVP are USP and are approved for use as drugs in man, orally and/or intravenously. A summary of the Phase I safety and PK (pharmacokinetics) study are provided below.

The Phase I study evaluated the safety and tolerance of EVP in humans, with special emphasis on cardiovascular safety, and assessed its PK profile. Overall, EVP was found to be safe at all doses. There were no serious adverse events (SAE) or any events requiring medical treatment. Of special importance, no signs of cardiac depression were reported. In addition, there was no observation of prolongation of the QTc or PR intervals or other ECG-changes that had previously been reported in animals for Mn. There were no changes in hematology, serum biochemistry (including liver function tests), coagulation parameters nor in temperature, respiratory rate or blood oxygen levels.

Transient increases in HR and BP were seen. These, for the most part, reflected transient changes within the normal reference range. There was no obvious dose-relationship for these responses, although their duration and magnitude were greater for the 5-minute than for the 1-minute dose administrations.

Other AEs were reported, almost all which were regarded as of mild or moderate intensity. Almost all events occurred during or immediately after dosing and resolved within minutes. Over 83% of the AEs occurred during dosing and over 93% occurred within one minute of dosing. Nearly one-third of all events resolved within one minute from dosing, over 80% within 5 minutes, and 94% resolved within ½ hour. The most frequent types of AEs were injection-associated discomforts (warmth, flushing, cold, pressure, injection site changes), increased HR/BP, gastrointestinal disturbances (discomforts, nausea, vomiting, diarrhea), lacrimation and headache. Although gastrointestinal disturbances, such as nausea and occasional vomiting, resolved rapidly without treatment, these bothersome events may be avoidable through the use of a standard anti-emetic. This proved to be beneficial in humans and also in high-dose animal studies.

The PK profile reflected what was known for Mn in humans (based on reported radiotracer Mn studies), with a primary blood half-life under 1 minute, and less than 1% of the dose remained in the blood by 30 minutes after dosing. The performance

of EVP was as expected based on information that was in the Investigators' Brochure. Lastly, please refer to US Patent #5,980,863 regarding further details of EVP contrast ingredients.

1.5 Dose Rationale and Risk/Benefits

The safety and tolerance of EVP1001-1 has been evaluated in healthy volunteers, in various preclinical models and in patients with a known myocardial infarction. The doses to be tested in this study demonstrated high safety factors in animal studies and were well tolerated in human studies to date. There have been no life-threatening or other unexpected AEs. There have been no clinically meaningful changes in hematology, clinical chemistry or coagulation profile, including liver function tests. Regarding the ECG, there were no consistent drug-related changes in QT or ST segment or prolongation of the PR interval. Importantly, no cardiac depression has been observed. Results in heart patients to date were similar to those in normal volunteers with no meaningful changes in blood or urine parameters and AEs that occur at the time of dosing and resolve in less than 5 minutes whose nature is that of discomforts, transient vital sign changes.

2 Study Objectives

This is a first-in-human clinical trial of combined manganese-enhanced MRI(MEMRI) and delayed-enhanced MRI(DEMRI) to determine reliable detection and clinical significance of peri-infarct injury in both the ventricles and atria. An IND approval was obtained to utilize EVP1001-1 to assess peri-infarct injury in patients with ischemic or dilated cardiomyopathy atrial fibrillation and those who undergo stem cell therapy for these indications. In preparation for a Phase I/II clinical trial, an initial safety study of 6 patients has been completed and no adverse events were observed. This proposal will enable a clinical trial of 50 patients to evaluate the safety and efficacy of MEMRI-DEMRI to characterize peri-infarct injury.

Primary Objective

Specific Aim 1 evaluates the efficacy of MEMRI-DEMRI to characterize the peri-infarct injury in the ventricles and atria.

Secondary Objective

Specific Aim 2 is a clinical follow-up to determine the occurrence of cardiovascular events.. To evaluate the safety and efficacy of MEMRI-DEMRI to characterize peri-infarct injury, in the ventricles and atria, through an innovative approach, integrating direct biological evaluation of myocardial viability and anatomical delineation of scar.

3 Study Design

3.1 General Design

Prior to entry into this study, all subjects will sign an Informed Consent and will then undergo a physical assessment including medical history, details regarding their cardiac history, prescription and over-the-counter drug review,, vital signs, electrocardiogram (ECG), evaluation of the major organ systems, hematology, serum chemistries. In addition, female subjects will undergo a serum pregnancy test.

Starting 30 minutes before the cardiac MRI scan (CMR), the subjects will take 16 mgs of ondansetron by mouth. CMR imaging will subsequently take place and then EVP1001-1 will then be administered approximately 15 minutes into the scan for contrast enhanced images. EVP1001-1 will be administered intravenously over approximately one minute. The subjects will each receive 0.28 mL/kg of EVP 1001-1. All subjects will be monitored closely from before ondansetron administration until their discharge from the imaging center. Following MEMRI, delayed-enhanced MRI(DEMRI)is performed using 0.2mmol/kg gandolinium. We will compare the two different contrast enhanced images (EVP1001-1 vs Gadolinium) in determining the viable, peri-infarct, and intra-infarct myocardial tissue.

To ensure a high-quality image, if the participant heart rate is greater than 65 bpm and regular rhythm, metoprolol will be given. If the HR is 65 and irregular, metopolol will be given if there

are no contraindications (see table 1 below)

Beta Blocker Administration Protocol

PROTOCOL	PARAMETER(s)	ACTION(s)
Assess whether ®-blockers are necessary	If pulse is < 65 bpm and regular or if pulse is < 60 bpm and irregular If pulse is > 65 bpm or if pulse is > 60 bpm and irregular	A ®-blocker is **not** given Screen for contraindications for giving ®-blocker
Screen for contraindications for ®-blockers	If any of the following conditions exist: Heart rate, < 60 bpm Systolic blood pressure, < 100 mm Hg Decompensated cardiac failure Allergy to ®-blocker Asthma or COPD on ®2-agonist inhaler Active bronchospasm Second- or third-degree atrioventricular block If the above conditions are absent	A ®-blocker is **not** given Oral metoprolol is given
Administer metoprolol	If giving **oral** metoprolol,	One 50-mg dose of metoprolol is given orally and patient is monitored over 1 hr, during which heart rate is checked every 15 min. If heart rate remains elevated, perform practice breath-hold If heart rate still remains elevated, give IV metoprolol
	If giving **IV** metoprolol	Two 2.5-mg doses of metoprolol are given 5 min apart; then, two doses of 5 mg each are given 5 min apart. Total maximum dose, 15 mg. Blood pressure and heart rate are checked before each IV dose
Administer postprocedure care	If only one dose of oral metoprolol is given	No monitoring necessary, patient can leave department after study
	If IV metoprolol is given	The patient is observed for 30 min
	If heart rate drops to < 45 bpm	Consideration is given to administering atropine
	If bronchospasm occurs	A ®-agonist inhaler is given

Note—bpm = beats per minute, COPD = chronic obstructive pulmonary disease.

Parameters to be Measured:

A standard physical assessment will be performed on the day of imaging (approximately 1-hour post dosing). Body temperature and respiration rate will be measured within 24 hours prior to dosing and at approximately 1- and 2-hours post dose. A standard 12-lead EKG will be performed both before and after the MR study to assure no significant EKG changes have occurred. Patient's hemodynamics will also be serially monitored. A baseline BP and HR will be performed before beginning the study. After EVP1001-1 contrast infusion, BP and HR will be measured every 2 minutes until 10 minutes post EVP1001-1 contrast infusion after which point a BP and HR will be measured every 5 minutes until the conclusion of the MEMRI and every 10 minutes until the conclusion of DEMRI study. There will be a 5-minute post administration observation interval outside the scanner. The patient will also have continuous EKG monitoring during this time. After the study, the patient will have another standard 12-lead EKG performed to compare with the pre-MRI 12-lead EKG. The patient's symptoms will be

reassessed 1 hour after infusion of the EVP1001-1 contrast. A differential and serum chemistry profile (with liver function test) will be performed within a month prior to administration of EVP1001-1 and within 24 hours post EVP1001-1. Adverse events (AEs) and discomforts will be recorded until discharge from the imaging center. At the 24 hour follow-up, the subject will be evaluated by telephone to determine if any AEs occurred from the time the subject left the imaging center. The subjects will have a contact number to report any AEs or to ask questions. Any clinically relevant changes from baseline in vital signs, ECG rhythm, clinical chemistries or physical condition and all AEs will be followed until resolution or until the outcome is known. Injection-associated discomforts will be recorded during administration and until imaging is completed. Primary efficacy will be based on determining whether the heart is normal or abnormal and if abnormal whether there is an infarction, ischemia or both. This provides the basis for calculating sensitivity, specificity, accuracy and predictive values. In addition, the location, size, conspicuity and associated confidence for any disease will be recorded.

The study will be stopped for any significant adverse cardiovascular event including cardiovascular death, myocardial infarction, ventricular arrhythmia, ICD firing, congestive heart failure, hospitalization, or syncope. All serious adverse events that require medical intervention and/or withdrawal of a patient will be reported to the IRB within 24 hours if it meets the unanticipated problem reporting criteria.

3.2 Primary Study Endpoints

The key endpoints of this study include the assessment of safety, tolerance and efficacy of EVP1001-1. Safety and tolerance will be evaluated based on the nature and extent of AEs and injection-associated discomforts and changes in laboratory parameters, vital signs and ECG after administration of EVP1001-1. Effects, if any, of EVP1001-1 on QT/QTc intervals will be determined by comparing the intervals before and after EVP1001-1 across the full HR range for each patient, including use of individual correction of QT by RR. All subjects who receive EVP1001-1 will be included in the analyses. Pre- and post-dose results will be compared for vital signs, clinical laboratory parameters, ECG parameters and hemodynamic parameters to identify individual changes from baseline and potential systematic shifts. The presence or absence of infarcts and/or ischemic areas relative to the current clinical standard (by echo and gadolinium-based cardiac MRI) along with comparison to patient outcomes will determine efficacy.

3.3 Secondary Study Endpoints

In addition, location, size, conspicuity, confidence, wall motion deficits and related diagnostic information will be assessed.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

All subjects to be entered must:

- be at least 18 years of age and less than 80 years
- if female, be non-pregnant as evidenced by a serum pregnancy test and using a medically-approved method of birth control, or post-menopausal or surgically sterile
- provide written informed consent after having received oral and written information about the study
- patients with ischemic or dilated cardiomyopathy with an EF less than or equal to 50 or patients with paroxysmal or persistent atrial fibrillation
- Have completed the MRI checklist and do not have exclusions to having an MRI
-

4.2 Exclusion Criteria

- have a positive pregnancy test (females)
- received an investigational drug or device within 30 days prior to administration of EVP1001-1

- have known hypersensitivity to ondansetron or other selective serotonin 5HT3 receptor blockers
- are taking a digitalis preparation or calcium channel blocker within 30 days to MRI
- Contraindication to receiving metoprolol
- have a history of torsades or prolonged QT/QTc interval (QTc > 470 msecs)
- have NYHA Grade IV heart failure or EF less than 25
- have abnormal liver function tests or a history of liver disease (2 times upper limits of normal)
- have stage 11 hypertension (Systolic Blood Pressure > 140 or Diastolic BP > 90 consistently at baseline)
- have abnormal baseline potassium or calcium (outside normal reference ranges)
- hemoglobin less than 10 g/dl

- Have a history of ventricular arrhythmia per stress test or monitoring
- No recent cardiac intervention within 90 days (CABG, Valve, PTCA)
- No recent CHF, MI or Angina within 60 days
- Conduction disease (QRSd >120 msec or Sick Sinus Syndrome.)
- No cardiac implantable device
- Weight not to exceed 300 kgs and/or chest circumference .36cms
-

4.3 Subject Recruitment and Screening

This is an open-label, baseline-controlled study to be conducted. Adult male or non-pregnant female patients who have been referred for evaluation of cardiomyopathy and atrial fibrillationand patients who have received stem cell therapy related to these indications will be recruited. An initial cohort study of 6 patients to conduct safety evaluation was conducted before proceeding with 120 additional patients. In the initial cohort, a patient was dosed based on the Phase 1 and 2 clinical trial data evaluation completed by Eagle Vision Pharmaceutical, Inc. Subjects were excluded if they had received an investigational device within 30 days prior to administration of EVP1001-1; had a history of drug abuse or alcoholism; were taking a digitalis preparation; had a history of torsades; had NYHA Grade IV heart failure; had abnormal liver function tests or a history of liver disease; had uncontrolled hypertension; had abnormal calcium, potassium or hemoglobin values at baseline; if they develop a cardiac arrhythmia prior to or during either of the exercise tests-- EVP1001-1 was not administered. The same parameters will apply to the new group of subjects.

5 Study Drug

5.1 Description

The active ingredient is EVP 103 and is provided as a sterile, non-pyrogenic powder in 50 ml vials. Please see 5.4 for preparation instructions

5.2 Treatment Regimen

The proper dose should be specified in the protocol on a "per kilogram body weight" (0.28 ml/kg) basis and must be given slowly over approximated one (1) minute intravenously. This same administration rate applies to flushing residual drug in connecting tubing/injection port.

5.3 Method for Assigning Subjects to Treatment Groups

Not Applicable

5.4 Preparation and Administration of Study Drug

The active ingredient is EVP 103 and is provided as a sterile, non-pyrogenic powder in 50 ml vials. Prior to administration, Drug will be prepared as follows: Using aseptic technique, transfer via syringe 10 ml of 10% Calcium Gluconate, USP and 30 ml of Sterile Water for Injection to a vial containing EVP 103. Shake gently to dissolve the powder. This prepares 40 ml of Drug. Drug should be used promptly (within 1 to 2 hours) following preparation. The calculated osmolality of Drug is 0.27 (nearly isomolar) and the pH is slightly acidic to neutral (5.5 to 7.0).

Dilutents and drug product should be inspected to assure that a clear solution is present. Should any precipitate or undissolved material remain in the vial, the vial should be gently warmed an shaken gently to dissolve any visible material prior to either dilution or administration

5.5 Packaging

Drug will be supplied as a non-pyrogenic powder in 50 ml vials.
• Shipped in boxes of 30 vials each
• Labeling:

EVP 103: Sterile Lot No: 11211114
CAUTION; FOR INVESTIGATIONAL USE ONLY
Contents: 1161umol of EVP 103
Store at room temperature
Stanford University Medical Center
269 Campus Drive, Stanford, CA 94305
Expiration: 0612019 Vial #'s: 0001 - 0030

5.6 Blinding of Study Drug

Not Applicable

5.7 Receiving, Storage, Dispensing and Return

5.7.1 Receipt of Drug Supplies

Drug to be delivered via Federal Express

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.7.2 Storage

The investigational drug will be in our locked cabinet and only the necessary vials will be withdrawn at the time of the study. They will be mixed by the administering physician in a sterile fashion.

5.7.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.7.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented.

6 Study Procedures

Please refer to #3 above

7 Statistical Plan

7.1 Sample Size Determination

The total number of 0 patients will power this study adequately, however as we have extended the protocol to include the atrial fibrillation participants (and extra 60 pts) the difficulty in imaging the atria, coupled with the thin atrial walls, means that we expect a difference in DEMRI volume of 40 (+ or – 20) in participants with early disease and 60 (+ or – 20) in participants with advanced disease. Accordingly, 60 participants will be needed for the additional cohort. Our published pre-clinical study in 13 swine demonstrated the infarct volume percentage difference of 9% between MEMRI and DEMRI ($p<0.001$)[17]. This difference will enable significant delineation of the changes in the peri-infarct viability between the cell therapy and control arms. The preliminary porcine data presented above demonstrated a mean of 100% increase in the CNR of the peri-infarct region and a mean of 50% decrease in the scar volume, suggesting robust MEMRI signal detection of stem cell engraftment and increased peri-infarct viability[17,98,99]. Furthermore, the clinical trial in 6 ICM patients demonstrated significant difference in the infarct volume between MEMRI defect and DEMRI enhancement. This difference between MEMRI and DEMRI represents PIR assuring reliable detection of the PIR. Employing these data, in order to detect a mean difference of 20% in MEMRI signal of stem cell engraftment and MEMRI-DEMRI signal of peri-infarct viability with a standard deviation of 5%, the t-test sample size calculation in this study has 90% power at the alpha 0.05 significance level, assuming an equal variance between the two distributions.

7.2 Statistical Methods

Student's t-test to compare MEMRI and DEMRI measurement of the infarct and viable myocardium. Pearson's correlation to generate any association between stem cell engraftment/peri-infarct viability and functional restoration and/or clinical outcome.

7.3 Subject Population(s) for Analysis

We expect to enroll 60 patients with various cardiomyopathies including NICM, ICM, and ACM (dilated cardiomyopathy, ischemic cardiomyopathy, and atrial cardiomyopathy). Our goal is to demonstrate improved sensitivity and specificity of identifying viable myocardium and PIR in the ventricles and atria in various cardiac patients to help improve clinical management

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

- <u>Unexpected in nature, severity, or frequency</u> (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- <u>Related or possibly related to participation in the research</u> (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- <u>Suggests that the research places subjects or others at greater risk of harm</u> (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Inter-current illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests

- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Pre-existing Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

As for intravenous injection of any investigational drug, competent personnel and emergency facilities must be available to address severe and possibly delayed reactions after administration of EVP1001-1 or severe responses to the stress prior to EVP1001-1 administration. Subjects will be observed for AEs starting with the pre-treatment (with ondansetron), during and for at least 1 hour after EVP1001-1 administration during which time emergency equipment will be readily available. AEs will be recorded until discharge from the imaging center. At the 24-hour follow-up, the subject will be evaluated by telephone to determine if any AE occurred from the time the subject left the imaging center. In addition, the subjects will have a contact number to report any AE or to ask questions. Any clinically relevant changes from baseline in vital signs, ECG rhythm, clinical chemistries or physical condition and all AEs will be followed until resolution or until the outcome is known. AEs, if any, will be recorded on the CRF (case report form) along with the action(s) required, time of onset, duration, intensity, and outcome. A judgment will be made by the investigator as to the causality of each event. If a subject has more than one incidence of a given AE, that AE with the greatest severity will be used for analyses. If a cluster of AEs are related, a term describing the syndrome or overall adverse experience should be captured.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the Stanford IRB and Study Sponsor

An initial cohort study of 6 patients was conducted under protocol #24224. The safety evaluations were conducted with no adverse events before proceeding with the remaining 120 patients. In this initial cohort, a patient will be dosed based on

the Phase 1 and 2 clinical trial data evaluation completed by Eagle Vision Pharmaceutical, Inc. The stopping rule will include any significant adverse cardiovascular event including cardiovascular death, myocardial infarction, ventricular arrhythmia, , congestive heart failure, hospitalization, or syncope. All serious adverse events that require medical intervention and/or withdrawal of a patient will be reported to the IRB within 24 hours. Any unexpected fatal or life-threatening suspected adverse reactions will be reported to the FDA no later than 7 calendar days after initial receipt of the information. Any serious, unexpected suspected adverse reactions, findings from other clinical, animal, or in-vitro studies that suggest significant human risk, and a clinically important increase in the rate of a serious suspected adverse reaction will be reported to the FDA and all investigators no later than 15 calendar days after determining that the information qualifies for reporting [21 CFR 312.32(c)(1)]. An annual progress report will be submitted to the FDA as required within 60 days of the anniversary of the date the IND went into effect.

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Reporting Deaths: more rapid reporting requirements
Concerning deaths that occur during the course of a research study, the following describes the more rapid reporting requirement of the Stanford IRB for specific situations:

- Report the event within 24 hours when the death is unforeseen (unexpected) and indicates participants or others are at increased risk of harm.
- Report the event within 72 hours, for all other deaths, regardless of whether the death is related to study participation.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the Stanford IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

Reporting Process:
Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study sponsor by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and facsimile to:

Phillip C. Yang, MD

Stanford University Medical Center
Center for Clinical Science Research (CCSR), 3115C
650-498-8008 (phone)
650-724-4034 (facsimile)

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor

8.3.2 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening
    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

[redacted], M.S., MSDRA: Regulatory Health Project Manager: Division of Medical Imaging Products Office of Drug Evaluation IV : Center for Drug Evaluation and Research : Food and Drug Administration
10903 New Hampshire Avenue, [redacted] : Silver Spring, MD 20993
Phone: [redacted] ·Fax: [redacted] : [redacted]@fda.hhs.gov

8.4 Stopping Rules

Those patients meeting the exclusion criteria or those patients wishing to withdraw will be terminated from the study. This will not prohibit the patient from receiving standard medical care outside the study. Should a serious adverse event occur, we will first and foremost stabilize the patient. Further administration of our contrast study will be stopped. Depending on the severity of the adverse event, standard medical care will be provided including inpatient hospitalization if deemed necessary. Patient will be overseen directly by a physician at all times during the imaging exam to ensure appropriate medical care is always provided. Emergency medications will be present and up to date at all times.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

Relevant clinical data will be linked only to the unique code. Our data will be maintained in an electronic spreadsheet. The spreadsheet has a unique identifier for each participant. The computer which the spreadsheet is maintained on is encrypted. This computer is located in a locked office in the Department of Cardiovascular Medicine at Stanford University. De-identification of data and specimen will be handled by the study personnel responsible for collection of the specimen. Only the Protocol director has the key to the code. That is also on an electronic spreadsheet that is on an encrypted computer. The only data that is transferred is the coded data. This is transferred through secure email according to the Stanford Computing guidelines.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment 1 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

Pending at this time

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All Stanford University investigators will follow the University conflict of interest policy.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 Attachments

- Sample Consent Form
- Study Procedures Flowchart/Table
- Investigational New Drugs Pharmacy Policy
- Specimen Preparation And Handling