CLINICAL STUDY PROTOCOL

Title: A Follow-Up Study to Monitor Therapeutic Response in Transthyretin Cardiac Amyloidosis Using Amyloid Reactive Peptide 124I-evuzamitide (AT01) PET/CT

Protocol Version:	2.0
Protocol Date:	October 26, 2023
ClinicalTrials.gov:	NCT05635045
Sponsor:	Columbia University Irving Medical Center Clinical Cardiovascular Research Laboratory for the Elderly (CCRLE) 21 Audubon Avenue New York, NY 10032, USA
Sponsor-Investigator:	Mathew Maurer, MD 212-932-4537
Collaborating Investigators:	Andrew Einstein, MD, PhD Columbia University Irving Medical Center Dia Smiley, DO Columbia University Irving Medical Center

STUDY SYNOPSIS

Protocol Title	Title: A Follow-Up Study to Monitor Therapeutic Response in Transthyretin Cardiac Amyloidosis Using Amyloid Reactive Peptide 124I-evuzamitide (AT01) PET/CT
Protocol Number	1.0
Design	This is a single center, prospective cohort study that is evaluating the ability of ^{124}I-evuzamitide PET scanning to serially assess and quantify TTR stabilizer and TTR silencer response in the heart in ATTR-CA. 10 subjects will be recruited from our pilot study that have a baseline 124I-evuzamitide PET/CT showing cardiac uptake, who are on TTR stabilizer and/or silencer therapy. Data generated from this study will provide amongst the first evidence of changes in myocardial amyloid load on PET/CT imaging with disease modifying therapy and provide preliminary data to support larger investigations in this arena. Consented eligible patients will undergo a single ^{124}I-evuzamitide PET scan. Clinically available demographic, clinical and phenotypic data that is collected as part of routine clinical care will be used to characterize the type, severity, and stage of ATTR-CM. Bloodwork, electrocardiograms, and echocardiograms will be performed at the time of the scan.
Study Sites	This study is to be conducted at Columbia University Irving Medical Center.
Time on Study	The duration of patient participation in this study is 31 days.
Primary Objective	To assess and quantify TTR stabilizer and TTR silencer response in the heart in ATTR-CA.
Sample Size	10 patients will be included in this study.
Inclusion and Exclusion Criteria	10 Participants from the current study on ATTR treatment will be recruited to one cohort. **Overall Inclusion Criteria**: 1. Must have given written informed consent (signed and dated) and any authorizations required by local law and be able to comply with all study requirements. 2. Were enrolled in the initial pilot using 124I-Evuzamitide, on ATTR stabilizer and/or silencer therapy. Able to understand and sign the informed consent document after the nature of the study has been fully explained. 3. TTR genotype shown to be either Val122Ile or wild type. The presence of any of the following excludes eligibility for enrollment in this study:

	1. Primary amyloidosis (AL) or secondary amyloidosis (AA). 2. Active malignancy or non-amyloid disease with expected survival of less than 1 year. 3. Heart failure, in the opinion of the investigator, primarily caused by something other than amyloidosis. 4. Ventricular assist device. 5. Impairment from stroke, injury or other medical disorder that precludes participation in the study. 6. Disabling dementia or other mental or behavioral disease. 7. Enrollment in a clinical trial not approved for co-enrollment. 8. Continuous intravenous inotropic therapy. 9. Inability or unwillingness to comply with the study requirements. 10. Chronic kidney disease requiring hemodialysis or peritoneal dialysis. 11. Patients taking heparin, or heparin derivatives (e.g. low molecular weight heparins) for anticoagulation. 12. Other reason that would make the subject inappropriate for entry into this study.
Aims	The specific aim is to determine if 124I-evuzamitide PET/CT scanning can: (1) Serially quantify TTR cardiac amyloid load as measured after 1 year of TTR stabilizer and/or silencer treatment.
Safety Assessments	The safety of study participants will be evaluated by: 1. Assessment of adverse events (AEs), including serious adverse events (SAEs). 2. Vital sign measurements (blood pressure, pulse rate, and respiratory rate). 3. Physical examinations

SCHEDULE OF ACTIVITIES

The following table summarizes the activities to be performed at each of the designated study visits. Details of the different procedures can be found in subsequent sections of this manual.

Procedure	**Screening**	**Historically Obtained Demographic and Clinical Information**	**^{124}I-evuzamitide PET scanning**	**Day 7 (+/- 2 days)**	**Day 28 (+/- 4 days)**
Informed Consent	X				
Inclusion and Exclusion Criteria	X				
Medical History		X			
Co-morbidities		X			
ATTR-CM cardiac stage[a]		X			
TTR genotype		X			
Height and Weight[†]			X		
Vitals Signs			X		
Medications		X			
Clinical Examination			X		
Adverse Events Review			X		
KCCQ Questionnaire			X		
Electrocardiogram			X		
Echocardiogram			X		
Bloodwork			X		
^{99m}Tc-PYP Scintigraphy		X			
PET scanning			X		
Follow-Up Safety Call				X	X

[a] Stage of ATTR-CM will be defined by criteria as specific in Cheng, R, JACC CardioOncol 2020 Sep;2(3):414-424.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
99mTc-PYP	99mTechnetium pyrophosphate
AE	Adverse event
SAE	Serios adverse event
TTR	Transthyretin
ATTRv	Variant transthyretin amyloidosis
ATTRwt	Wild-type Transthyretin amyloidosis
ATTR-CA	Transthyretin cardiac amyloidosis
ATTR-CM	Transthyretin amyloid cardiomyopathy
AT-01(^{124}I-evuzamitide)	Pan amyloid imaging peptide labeled with Iodine-124

1. INTRODUCTION

1.1 Background and Rationale

Transthyretin cardiac amyloidosis (ATTR-CA) is increasingly recognized due to better diagnostic imaging and enhanced clinical awareness, as well as the introduction of novel treatments [1]. Although there have been great advances in the diagnosis of ATTR-CA using nuclear imaging; it has yet to be delineated how myocardial amyloid load changes with disease-specific treatment. Specifically, it is unclear if the amount of TTR amyloid decreases, increases, or remains unchanged after TTR stabilizer and/or silencer treatment. In short, the objective monitoring of response to ATTR-CA therapy on imaging is an unmet need. We believe that monitoring response to therapy by measuring amyloid load on imaging will be complementary to cardiac biomarkers, physical battery tests and quality of life questionnaires -- and will provide essential pathophysiologic insights.

Studies have shown that echocardiography is not sensitive or specific for the diagnosis of ATTR-CA, but also cannot be used for monitoring with ATTR therapy, since parameters such as LVEF, strain, and wall thickness do not consistently change with therapy and are highly variable. Cardiac MRI can provide quantification but doesn't measure amyloid deposits directly. Technetium 99m-pyrophosphate (Tc99M-PYP) scintigraphy has no doubt revolutionized the diagnosis of ATTR-CA, but it is unclear what PYP is binding to [2,3]. Further, recent TTR silencer studies have shown that improvement in Perugini score can occur in some patients after TTR silencer treatment, but this is not accompanied by changes in cardiac structure and function. We have shown in a pilot study [NCT05635045] evaluating 124I-evuzamitide PET/CT scanning for the diagnosis of ATTR-CA, that even with an improvement in baseline Tc99M-PYP with Perugini score of 3 to a Perugini score of 1 after <2 years of silencer treatment, there was significant and diffuse cardiac uptake of 124I-evuzamitide on PET/CT and no change in structure or function on echocardiography, suggesting that Tc99M-PYP may not be reliable for monitoring response to ATTR-CA therapy (figure 1) [4].

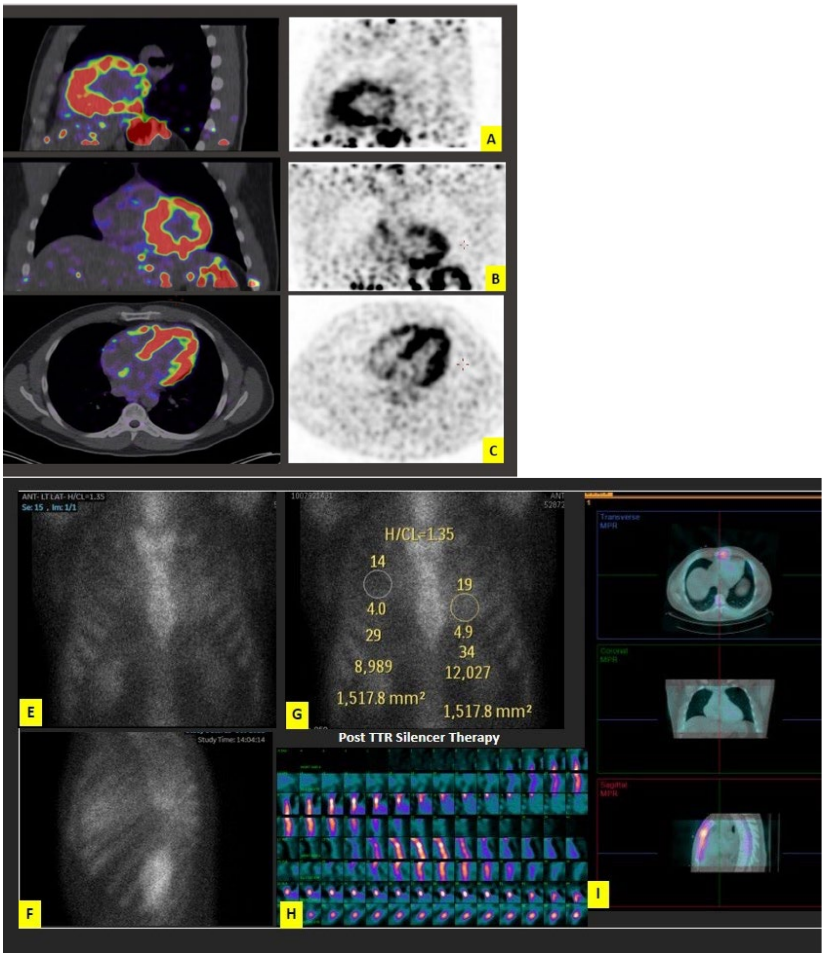

Figure 1 Post TTR gene silencer therapy 124I-evuzamitide PET-CT scan images. (A) Transaxial, (B) coronal and (C) sagittal images demonstrate 124I-evuzamitide uptake in the left and right ventricle. Images were obtained 300 minutes after administration of tracer. Tc99-PYP imaging was performed approximately 2 years after initiation of TTR gene silencer therapy. Images were obtained 3-hours post-infusion of radiotracer. Planar images demonstrate uptake over the cardiac silhouette less than rib uptake (Perugini visual grade 1), with heart to contralateral ratio (H:CL) of 1.35. (I) SPECT-CT imaging confirmed that radiotracer activity was limited to the left ventricular blood-pool, with no myocardial uptake, a significant change from the prior scan.

Results of the APOLLO-B trial, a phase 3, randomized, double-blind, placebo-controlled multicenter global study of patisiran, were presented in September 2022. In APOLLO-B, cardiac uptake of Tc99M-PYP on scintigraphy was assessed in a subset of patients (37 patisiran and 28 placebo) at baseline and 12 months after initiation of therapy. All subjects in the patisiran arm had reduced or no change in the Perugini grading scale at month 12 relative to baseline, whereas no placebo subjects demonstrated a reduction from baseline in Perugini grade at month 12. Of the evaluable patients in the patisiran arm, 37.8% demonstrated reduction of ≥1 Perugini grade, including 3 (8.1%) patients who reduced by ≥2 Perugini grades at month 12 [5]. However, as noted previously the implications of these findings regarding amyloid load are unknown.

Several recent studies suggest a promising role of PET imaging in cardiac amyloidosis, and several PET tracers are now available for in vivo detection of amyloid deposits [6-11]. PET imaging has high sensitivity and specificity for amyloid with quantitation and can identify cardiac amyloidosis earlier than Tc99M-PYP scans [11]. PET radiotracers C-11–Pittsburgh compound-B (C-11–PiB), F-18–florbetapir, F-18–florbetaben, F-18–flutemetamol have been approved by the Food and Drug Administration (FDA) for imaging Alzheimer's disease, and have been used successfully in off-label research to image light chain cardiac amyloidosis (AL-CA) and ATTR-CA. Although they are currently not being used in clinical practice, these amyloid PET radiotracers are being investigated for early detection, quantitation, and assessment of response to therapy [6-13]. Multiple studies demonstrated visual uptake of radiotracer and a higher retention index of C-11–PiB in all patients with AL and ATTR compared with control subjects but had the limitation of not distinguishing AL-CA from ATTR-CA; and due to the short half-life of 20 minutes for C-11–PiB, it requires an onsite cyclotron for production [6,14,15]. Further, reports suggested that Tc99M– DPD imaging could be negative despite cardiac amyloid deposition diagnosed by C-11–PiB in certain forms of variant (hereditary) ATTR-CA, such as with the Val30Met variant, where there is a substitution of a single amino acid valine for methionine at position 30 of the TTR gene [15].

A novel amyloid reactive peptide, 124I-evuzamitide, also known as AT01, has been shown to detect various forms of amyloidosis in the liver, spleen, kidney, and bone marrow [16- 18]. We conducted a pilot study at our institution (n=25), where we used 124I-evuzamitide for early detection of ATTR-CA, the assessment of cardiac involvement in rare TTR variants, and the detection and quantification of extra-cardiac amyloidosis [4,19]. PET with molecular imaging could provide detailed information on the extent and quantity of systemic amyloid load, which could be valuable in tracking serially in the context of emerging therapies.

Accordingly, we propose a prospective pilot study using amyloid reactive peptide 124I-evuzamitide PET/CT scans to serially assess and quantify TTR stabilizer and TTR silencer response in the heart in ATTR-CA. 10 subjects will be recruited from our pilot study that have a baseline 124I-evuzamitide PET/CT showing cardiac uptake, who are on TTR stabilizer and/or silencer therapy. Data generated from this study will provide amongst the first evidence of changes in myocardial amyloid load on PET/CT imaging with disease modifying therapy and provide preliminary data to support larger investigations in this arena.

2. STUDY OBJECTIVES & STUDY DESIGN

2.1 Primary Objective

To determine if 124I-evuzamitide PET/CT scanning can quantify and detect changes in myocardial amyloid load with stabilizers and Silencers in ATTR-CA.

2.1.1 Research Goal

The goal of our research is to obtain preliminary data on stabilizer and silencer therapy response to the treatment in ATTR-CA by quantitatively comparing cardiac uptake of 124I- evuzamitide

on PET/CT after one year of disease specific therapy.
The specific aim is to determine if 124I-evuzamitide PET/CT scanning can:

(1) Serially quantify TTR cardiac amyloid load as measured after 1 year of TTR stabilizer and/or silencer treatment.

2.2 Overview of Study Design

We will undertake a prospective cohort study among ATTR-CA subjects that have had 124I-evuzamitide PET/CT scan showing cardiac uptake. Eligible subjects, who have already had baseline scans (n=10) will undergo a repeat cardiac PET/CT scan using 124I-evuzamitide one year after the baseline scans. Additional information regarding demographic and clinical features including measures of disease severity and stage and TTR genotype will be obtained from previous recent medical encounters. The study will entail a single in-person visit for the whole-body PET scan.

Consented eligible patients will undergo a single 124I-evuzamitide PET scan. Cardiac biomarkers and basic metabolic panel, along with quality of life questtionaires, which include: KCCQ, COMPASS 31, SF-36, and EQ-5D-5Land 6-minute walk, will obtained at the time of the 1-year follow-up scan. Clinically available demographic, clinical and phenotypic data that is collected as part of routine clinical care will be used to characterize the type, severity, and stage of ATTR-CM. An echo will also be obtained at the time of the scan.

2.2.1 Methods

The subjects that will be enrolled will be of variant ATTR-CA (ATTRv) or wild-type ATTR-CA (ATTRwt). Subjects will receive < 1.5 mg of 124I-evuzamitide (1 mCi ± 10%), and this will be administered as a single slow IV bolus. PET/CT images will be acquired ~5 hours after dose, as has been demonstrated in our previous studies. 20 minutes of cardiac PET acquisition will be performed.

2.2.2 Study sites

This study is to be conducted at Columbia University Irving Medical Center.

2.2.3 Time on Study

The duration of patient participation in this study is 31 days (for one month follow-up to assess for safety and 3 days of potassium iodide administration prior to PET scanning).

2.2.4 Sample size

10 patients have already been identified who have had baseline scans to be included in this study.

2.2.5 Patient Recruitment

The subjects have been selected from among subjects who underwent 124I-evuzamitide PET/CT scanning previously. All necessary follow-up care or communication of adverse events or incidental findings will either take place as part of the clinical care of the enrolled subjects or be communicated in writing and via telephone with the physician.

2.2.6 Selection Criteria

Inclusion Criteria

1. Subjects with TTR-CA who had a 124I-evuzamitide PET/CT in our previous pilot study (table 1) with PET/CT cardiac uptake.
2. On TTR stabilizer and/or silencer therapy for 12 months between the original 124I-evuzamitide PET/CT scan, and the 124I-evuzamitide PET/CT in this study.

Table 1: Characteristics of Subjects to be scanned.

Table 1: Characteristics of Subjects to be scanned.

subject	Age	Genotype	NYHA Class	Stabilizer therapy	Silencer therapy
1	65	Val122Ile	II	X	
2	64	ALA60	I	X	X
3	81	Wild-type	I	X	X
4	69	Wild-type	I	X	
5	73	Wild-type	II	X	
6	74	Wild-type	II	X	
7	64	Thr60Ile	I		X
8	67	Thr60Ala	I		X
9	68	Val30MET	II		X
10	60	Val122Ile	II	X	

Exclusion criteria:

Primary amyloidosis (AL) or secondary amyloidosis (AA), Active malignancy or non-amyloid disease with expected survival of less than 1 year, Heart failure, in the opinion of the investigator, primarily caused by something other than amyloidosis, Ventricular assist device, impairment from stroke, injury or other medical disorder that precludes participation in the study, disabling dementia or other mental or behavioral disease, enrollment in a clinical trial not approved for co-enrollment, continuous intravenous inotropic therapy, inability or unwillingness to comply with the study requirements, chronic kidney disease with eGFR

2.2.7 Cardiac PET/CT with 124I-evuzamitide

Eligible subjects will begin therapy with potassium iodide (KI) 130 mg orally daily for 3 days prior to receiving 1 mCi 124I-evuzamitide to protect the thyroid. On the day of the scan 124I-evuzamitide will be administered via intravenous push at a proposed rate of ~1 mL per 5 seconds. This dose corresponds to <1.5 mg of AT-01 peptide and 1 mCi +/- 10%). This dosage of 124I-evuzamitide is approximately 38MBq and should be calculated based on the half-life of 96 hours and the date and time of calibration.

Vitals including blood pressure and pulse will be monitored 15 min (+/- 5 minutes) before and after the injection. Cardiac PET/CT images will be acquired at approximately 5 hours post-injection using a low-dose CT (120 kVp, 50 effective mAs). Cardiac PET images will be acquired for 20 minutes. PET data will be reconstructed using a 3DOSEM algorithm with attenuation weighting and prompt gamma correction. PET/CT images will be evaluated for positive radiotracer retention by a nuclear cardiologist blinded to the subjects' history. The retention of 124I-evuzamitide may be quantified from PET/CT images using region-of-interest (ROI) analysis employing a 2D freehand ROI placement method. The data will be expressed as Bq/cc or as standard uptake value ratios (SUVR) using the lumen of the left atrium (blood pool) as the reference tissue.

2.2.8 Statistical Considerations and Sample Size

Given the pilot nature of this investigation, normal formal power calculation was performed.

2.2.9 Data Analysis Plan

Descriptive statistics will be used to summarize subjects' demographics and clinical features. The subjects will be re-randomized for the readers and for quantification. The quantification will be performed using PMOD, and 3 different physicians will be performing quantification individually to verify reproducibility. PET scans will be interpreted by two readers blinded to the clinical information. The organ-specific radioactivity data will be expressed as the mean and standard deviation (SD) of three independent ROIs. The coefficient of variation (COV) will be expressed as (SD/mean)*100.

2.3 Limitations and Assumptions

This is a pilot study and thus generalizability to the broader population of patients who have ATTR-CA and are on stabilizer and/or silencer treatment, is unknown. Further, the patients were already on stabilizer and/or silencer therapy at the time of PET/CT scan, for varying duration.

3.PATIENT NUMBERING

Each patient will be uniquely identified in the study by a combination of the site number and screening number. The site number will be assigned by the coordinating site. The screening number will be assigned sequentially.

4. ADVERSE AND SERIOUS ADVERSE EVENTS

4.1 Adverse Events Definition

An AE is any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the subject's participation in the research.

4.2 Serious Adverse Event (SAE) Definition

An SAE is any untoward medical occurrence related to study participation that:

1. Results in death.
2. Is life-threatening (an event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death).
3. Requires in-patient hospitalization or prolongation of existing hospitalization.
4. Results in persistent or significant disability or incapacity.
5. Is a congenital abnormality or birth defect.
6. An important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient and may require intervention to prevent one of the other outcomes listed in the definition above (e.g. events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse).

4.3 Collecting and Reviewing Adverse Event and Outcome Information

During7 day and 28 day follow-up visits, whether in person or via telephone, the subjects will be asked about medically relevant changes in their health since the previous visit including hospitalization, and whether those hospitalizations were related to cardiovascular health.

The study team will record AE information for start dates/times occurring any time after informed consent is obtained until 28 days after the time of imaging agent infusion.

To assess relationship of an event to study procedure, the following guidelines are used:

- Unrelated
- Unlikely to be related
- Possibly related
- Probably related
- Definitely related

All adverse events must have their relationship to study participation, expectedness and severity of the event assessed by a qualified investigator, upon reporting of the event. These determinations will be made according to the professional opinion of the investigator using the package insert of

the imaging agent and the investigators assessment will be entered into the Redcap data collection software by research staff. All AEs will be followed by the investigator until the events are resolved, the subject is lost to follow-up, the condition is stabilized, or the AEs are otherwise explained.

4.4 Stopping Study Procedure Due to Adverse Event

In the use of this procedure there have been no reports of serious adverse reactions. Nevertheless, the injection and scan procedure will be stopped, and the results will be marked as incomplete if there are any adverse reactions during its administration as part of this study. The adverse reactions that will lead the investigators to stop the ^{124}I-evuzamitide PET scan include but are not limited to the following events: hypotension, fever, chills, nausea, vomiting and dizziness, as well as hypersensitivity reactions such as itching and various skin rashes.

The scan will also be stopped in cases of severe cardiac symptomology such as: chest pain, shortness of breath, palpitations, tachycardia. In such cases, other study procedures may be continued.

In addition to the planned 7 day and 28 day follow-up phone visits, subjects will be asked to contact the investigators if any additional adverse or allergic reactions are noted within the first 24 hours following the injection. If any such events are reported, the principal investigator will determine if they are potentially related to the administration of the imaging agent. Appropriate clinical care will be advised for any events determined by the investigator to be related to the scanning injection, and information about the reactions will be reported as described below.

5.ADVERSE EVENT REPORTING

5.1 Reporting to the Sponsor

The principal and participating investigator will report every serious adverse event (SAE) to the study sponsor, Attralus, as soon as possible, but no later than 48 hours after becoming aware of the event via phone, email or facsimile, and will complete the new SAE field on the Redcap database as soon as possible but no later than 48 hours after becoming aware of the event. The principal investigator will document the time of their awareness of the SAE in the study subject's binder

A participating investigator will also provide follow-up information on the SAE once new or updated information is available. The follow-up information will be added to the Redcap SAE field, as a follow-up to the initial entry.

Non-serious adverse events will be recorded on the Adverse Event Form. All non-serious entries related to study drug must be completed by the participating investigator within 5 business days.

5.2 Reporting to the IRB

Unanticipated Problem (UP):

An unanticipated problem is any incident, experience or outcome involving risks to subjects or others in any human subjects' research that meets all of the following criteria:

- Unexpected (in terms of nature, severity or frequency) given (a) the research procedures that are described in the IRB-approval protocol and informed consent document, and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in such research (e.g., there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in such research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic or social harm) than was previously known or recognized.

Reports of all events (including follow-up information) that meet the definition of an unanticipated problem posing risk to subjects or others must be submitted to the IRB within one week (5 business days) following the occurrence of the unanticipated problem or the investigator's acquiring knowledge of the unanticipated problem in accordance with IRB policy. Additionally, the investigator will submit a summary of all unanticipated problems that occurred since the beginning of the study at the time of continuing review.

5.3 Reporting to the FDA

Suspected Adverse Reaction:

In accordance with 21 CFR 312.32(a), a suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

The IND Sponsor will be responsible for all communication with the FDA and will report to the FDA, any adverse event that is serious, unexpected and for which there is reasonable possibility that the drug caused the adverse event. These must be reported to the FDA as soon as possible, but no later than 15 calendar days after the Sponsor determines that the information qualifies for reporting. Any fatal or life-threatening SARs will be reported to the FDA as soon as possible, but no later than 7 calendar days after the Sponsor determines that the information qualifies for such reporting.

All other serious adverse events will be included in the IND annual reports that are to be submitted to the FDA within 60 days of the anniversary date of the IND each year.

5.4 Reporting to Participants

The study team will notify all participants of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any incidental findings. In addition, the investigator will analyze the significance of any above findings to the ongoing clinical care of the subjects.

REGULATORY GUIDELINES

This study will be performed in accordance with the protocol, all applicable government laws, regulations, and guidance in whatever city and state it in conducted within, including policies with the Health Insurance Portability and Accountability Act of 1996 ("HIPAA"), and all other applicable medical privacy laws and regulations.

6.1 Institutional Review Board/Independent Ethics Committee

National regulations and ICH require that approval be obtained from an IRB or an IEC prior to participation of patients in research studies. Prior to the study onset, the protocol, any protocol amendments, ICFs, advertisements to be used for patient recruitment, and any other written information regarding this study to be provided to a patient or patient's legal guardian must be approved by the IRB or IEC.

All IRB and IEC approvals must be dated and contain IRB/IEC Chairman or designee authorization and must identify the IRB/IEC (e.g., name and address), the clinical protocol by title and/or protocol number, and the date of approval or favorable opinion was granted for the clinical research.

The Investigator is responsible for obtaining continuing review of the clinical research at least annually or more often if specified by the IRB or IEC. The Investigator must supply the Sponsor with written documentation of the approval of the continued clinical research.

The Investigator will make all attempts to ensure that the IRB or IEC is constituted and operates in accordance with Federal and ICH GCP and any local regulations.

6.2 Regulatory Authorities

Regulatory authorities will receive the protocol, amendments, reports on SAEs, and the Integrated Clinical Trial Report according to national and any local regulations.

6.3 Modification of the Protocol

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by the IRB that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IRB for approval prior to patients being enrolled under the amended protocol.

6.4 Informed Consent Form

Written informed consent in compliance with 21 Code of Federal Regulations (CFR) § 50 and ICH will be obtained from each patient prior to undergoing any protocol-specific tests or procedures that are not part of routine care.

The study purpose, methods and procedures will be explained to each potential participant, including all risks, benefits, compensation and alternatives. Subjects would in all cases be given ample time to consider participation and to consult family members or caregivers, if they wish. They would be given an opportunity to ask questions and have them answered. The potential subjects will have an opportunity to discuss the information provided directly (either in-person, on the telephone or on a video call) All forms of consent shall as a whole present information in sufficient detail relating to the research study and the circumstances of the consent process in a way that minimizes the possibility of coercion or undue influence.

6.5 Study Data Handling

All study results will be recorded upon paper CRFs, and such data will be recorded into a shared RedCap electronic database no later than five days following study visit.

Study data will be reviewed during the study in a systematic manner by a research worker who is not performing or administering the study procedures, in order to detect any deviations from intended protocol. Significant protocol exemptions will not be considered. Upon completion of the project, all study records will be audited internally once more to assure good clinical practice and FDA compliance.

Essential documents will be retained for the period required by applicable local law. The essential documents include the signed and dated final protocol, signed, and dated amendments(s), if applicable, signed and dated curriculum vitae of the Investigators, copies of the completed CRFs, signed ICFs, IRB approval and all related correspondence, financial agreements, regulatory approval, drug accountability, study correspondence, and patient identification codes.

6.6 Confidentiality

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs patients will not be identified by their names, but by the assigned patient number and initials.

Following the principles of the Good Clinical Practice, if local regulations specify a patient's number and initials will be used to identify the patient on their study records. Laboratory samples may be labeled with an independent numbering code, and the label will not contain any other personal identification information.

Please consider including the following sections:

- Describe how participants will be informed about AEs and SAEs, and study-related results on an individual or aggregate level. In addition, describe plans for detecting and managing incidental findings associated with study procedures.
- Describe the procedures for obtaining and documenting informed consent of study participants. In obtaining and documenting informed consent, the investigator must comply with applicable regulatory requirements (e.g., 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56) and should adhere to ICH GCP. Prior to the beginning of the trial, the investigator should have the IRB's written approval for the protocol and the written informed consent form(s) and any other written information to be provided to the participants.
- Describe the composition of the SMC or DSMB, frequency of interim data review, final data analysis and method of reviews. A separate DSMB Charter will provide further detail of DSMB membership, responsibilities and administration of the DSMB.
- Describe how monitoring of the conduct and progress of the clinical investigation will be conducted (i.e., who will conduct the monitoring, the type, frequency, and extent of monitoring, who will be provided reports of monitoring, if independent audits of the monitoring will be conducted). This section may refer to a separate detailed clinical monitoring plan.

- Plans for detecting, reviewing, and reporting deviations/violations from the protocol should be described. Provisions for approval of protocol exemptions can be described.

References:

1. [1] Griffin JM, Rosenblum H, Maurer MS. Pathophysiology and therapeutic approaches to cardiac amyloidosis. Circ Res 2021;128:1554–75.
2. [2] Gillmore JD, Maurer MS, Falk RH, Merlini G, Damy T, Dispenzieri A. Tcpyrophosphate scintigraphy for differentiating light-chain cardiac amyloidosis from the transthyretin- related familial and senile cardiac amyloidoses. 4. BS, Castano A. Circ Cardiovasc Imaging 2013;133:195–201.
3. [3] Bokhari S, Castano A, Pozniakoff T, Deslisle S, Latif F, Maurer MS. Tc- pyrophosphate scintigraphy for differentiating light-chain cardiac amyloidosis from the transthyretin- related familial and senile cardiac amyloidoses. Circ Cardiovasc Imaging 2013;6:195–201.
4. [4] Smiley DA, Einstein AJ, Mintz A, Shetty M, Chan N, Helmke ST, et al. Gene silencing therapy in hereditary (variant) transthyretin cardiac amyloidosis: A puzzling case of decreasing pyrophosphate uptake on scintigraphy. Circ Cardiovasc Imaging 2023:e015243.
5. [5] Maurer MS, Fontana M, Berk JL, Gustafsson F, Simoes M, Grogan M, et al. Primary Results from APOLLO-B, a Phase 3 Study of Patisiran in Patients with Transthyretin-Mediated Amyloidosis with Cardiomyopathy". Washington, DC; n.d.
6. [6] Morgenstern R, Yeh R, Castano A, Maurer MS, Bokhari S. Fluorine sodium fluoride positron emission tomography, a potential biomarker of transthyretin cardiac amyloidosis. J Nucl Cardiol 2018;25:1559–67.
7. [7] Pilebro B, Arvidsson S, Lindqvist P, Sundström T, Westermark P, Antoni G, et al. Positron emission tomography (PET) utilizing Pittsburgh compound B (PIB) for detection of amyloid heart deposits in hereditary transthyretin amyloidosis (ATTR). J Nucl Cardiol 2018;25:240– 8.
8. [8] Papathanasiou M, Kessler L, Carpinteiro A, Hagenacker T, Nensa F, Umutlu L. F- flutemetamol positron emission tomography in cardiac amyloidosis. J Nucl Cardiol 2020;18.
9. [9] Takasone K, Katoh N, Takahashi Y, Abe R, Ezawa N, Yoshinaga T. Non-invasive detection and differentiation of cardiac amyloidosis using (99m)Tc-pyrophosphate scintigraphy and (11)C-Pittsburgh compound B PET imaging. Amyloid 2020;27:266–74.
10. [10] Mockelind S, Axelsson J, Pilebro B, Lindqvist P, Suhr OB, Sundstrom T. Quantification of cardiac amyloid with [(18)F]Flutemetamol in patients with V30M hereditary transthyretin amyloidosis. Amyloid 2020;27:191–9.
11. [11] Genovesi D, Vergaro G, Giorgetti A, Marzullo P, Scipioni M, Santarelli MF. -Florbetaben PET/CT for Differential Diagnosis Among Cardiac Immunoglobulin Light Chain, Transthyretin Amyloidosis, and Mimicking Conditions. JACC Cardiovasc Imaging 2021;14:246–55.
12. [12] Cuddy SAM, Bravo PE, Falk RH, El-Sady S, Kijewski MF, Park M-A, et al. Improved quantification of cardiac amyloid burden in systemic light chain amyloidosis: Redefining early disease? JACC Cardiovasc Imaging 2020;13:1325–36.
13. [13] Ehman EC, El-Sady MS, Kijewski MF, Khor YM, Jacob S, Ruberg FL, et al. Early detection of multiorgan light-chain amyloidosis by whole-body ^{18}F-florbetapir PET/CT. J Nucl Med 2019;60:1234–9.
14. [14] Antoni G, Lubberink M, Estrada S, Axelsson J, Carlson K, Lindsjö L, et al. In vivo visualization of amyloid deposits in the heart with 11C-PIB and PET. J Nucl Med 2013;54:213–20.
15. [15] Lee SP, Lee ES, Choi H. C-Pittsburgh B PET imaging in cardiac amyloidosis. J Am Coll Cardiol Img 2015;8:50–9.
16. [16] Wall JS, Richey T, Macy S, Heidel E, Wooliver C, Kennel SJ. A novel method for quantifying peripheral tissue amyloid load by using the radiolabeled amyloidophilic peptide, p5. Amyloid 2013;20:21–6.
17. [17] Wall JS, Martin EB, Richey T, Stuckey AC, Macy S, Wooliver C. Preclinical Validation of the Heparin-Reactive Peptide evuzamitide as a Molecular Imaging Agent for Visceral Amyloidosis. Molecules 2015;20:7657–82.
18. [18] Kennel SJ, Stuckey A, McWilliams-Koeppen HP, Richey T, Wall JS. Tc-99m radiolabeled peptide p5 + 14 is an effective probe for SPECT imaging of systemic amyloidosis. Mol Imaging Biol 2016;18:483–9.
19. [19] Smiley D, Einstein AJ, Chan N, Mintz A, Santana DD, Shetty M, et al. In the thick of it: hereditary cardiac amyloidosis identified by 124I-AT-01 PET imaging but not cardiac MRI or SPECT 99mTc pyrophosphate scintigraphy. J Nucl Cardiol 2023;30:1258–62.