CLINICAL STUDY PROTOCOL

Screening for Cardiac Amyloidosis with Nuclear imaging in Minority Populations study (SCAN-MP)

Protocol Version:	4.2
Protocol Date:	June 24, 2022
ClinicalTrials.gov:	NCT03812172
Sponsor:	Columbia University Medical Center Clinical Cardiovascular Research Laboratory for the Elderly (CCRLE) 21 Audubon Ave. SB-0180 New York, NY 10032, USA
Sponsor-Investigator:	Mathew Maurer, MD Co-Principal Investigator 212-932-4537 Frederick Ruberg, MD Co-Principal Investigator 617-638-8733
Site Investigator:	Ed Miller Site Investigator 203-785-4127
Collaborating Investigators:	Gillian Murtagh, MD Abbott Diagnostics Agim Beshiri, MD Abbott Diagnostics

STUDY SYNOPSIS

Protocol Title	Screening for Cardiac Amyloidosis with Nuclear imaging in Minority Populations study (SCAN-MP)
Protocol Number	4.2
Design	This is a multicenter, diagnostic screening study to evaluate the prevalence of wild-type and Val122Ile ATTR cardiac amyloidosis (ATTR-CA) in a population of Black and Hispanic subjects with heart failure at participating sites. ATTR-CA is an age-related infiltrative cardiomyopathy that causes heart failure and death that is frequently unrecognized and underdiagnosed. Consented eligible patients will be evaluated for the presence and clinical manifestations of ATTR Cardiac Amyloidosis. Patients will undergo a Screening/Baseline visit, using nuclear medicine screening and TTR genetic test. Those who have myocardial retention of the imaging radiotracer with a Periguini score >=2 (uptake equal to bone) and/or H/CL ratio > 1.3 will be diagnosed with ATTR amyloidosis and followed up in 6 months and again at 12 months.
Study Sites	This study is to be conducted at five study sites: Recruitment and Screening at Harlem Hospital. All study procedures at NYP Allen Hospital, NYP Milstein Hospital, Boston University Medical Center and Yale New Haven Hospital.
Time on Study	The duration of patient participation in this study is approximately 12 months.
Primary Objective	To improve identification of minority patients with ATTR-CA and enhance the understanding of genotype-phenotype associations in Blacks/Hispanics with ATTR-CA due to the Val122Ile mutation or ATTR wild-type.
Sample Size	Up to 800 patients will be screened in this study.
Inclusion and Exclusion Criteria	Every participant must meet all of the following inclusion criteria to be eligible for enrollment in this study: 1. Black or Hispanic of Caribbean origin. 2. Age ≥60 years. 3. Diagnosis of heart failure, confirmed by one of two methods: a. Modified criteria utilized by Rich et al. which include a history of acute pulmonary edema or the occurrence of at least two of the following that improved with diuretic therapy without another identifiable cause: dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, bilateral lower extremity edema or exertional fatigue, and

	b. NHANES CHF criteria with a score ≥3 (see Appendix 1). 4. Left ventricular septal OR inferolateral wall thickness ≥12 mm. 5. Ejection fraction >30%. 6. Able to understand and sign the informed consent document after the nature of the study has been fully explained. The presence of any of the following excludes eligibility for enrollment in this study: 1. Primary amyloidosis (AL) or secondary amyloidosis (AA). 2. Prior liver or heart transplantation. 3. Active malignancy or non-amyloid disease with expected survival of less than 1 year. 4. Heart failure, in the opinion of the investigator, primarily caused by severe left-sided valve disease. *Note: if valve was repaired, subject may be considered as no longer with severe valve disease.* 5. Heart failure, in the opinion of the investigator, primarily caused by ischemic heart disease. 6. Ventricular assist device or anticipated within the next 6 months. 7. Impairment from stroke, injury or other medical disorder that precludes participation in the study. 8. Disabling dementia or other mental or behavioral disease. 9. Enrollment in a clinical trial not approved for co-enrollment. 10. Expected use of continuous intravenous inotropic therapy in the next 6 months. 11. High risk for non-adherence as determined by screening evaluation. 12. Inability or unwillingness to comply with the study requirements. 13. Chronic kidney disease with eGFR <15 mL/min/1.73 m2 or ESRD. 14. Weight >350 lb. 15. Nursing home resident. 16. Other reason that would make the subject inappropriate for entry into this study.
Aims	The specific aims of this epidemiologic investigation include: 1. To determine the prevalence of ATTR Val122Ile CA in Caribbean Hispanics and Blacks with HF. 2. To demonstrate that circulating RBP4 concentration is integral to TTR tetramer stability which in turn determines the clinical

	characteristics of ATTR CA. We will explore the following: 1. The prevalence of the Val122Ile genotype and ATTRm CA phenotype among Hispanics of Caribbean origin compared to Blacks. 2. Gender differences in ATTR-CA identified by myocardial uptake of Tc99-PYP or Tc99-HMDP. 3. The development of a risk based calculator based on clinical, electrocardiographic, echo and biochemical variables to identify ATTR-CA. 4. Disease progression and prognosis between Val122Ile vs. ATTRwt in this population so as to inform whether the genetic form of this condition results in a more malignant phenotype. 5. Differences in ATTR stability between ATTRm Val122Ile vs ATTRwt and non-amyloid controls which will associate with clinical features and outcomes. 6. Differences in RBP4 concentrations in plasma and urine in ATTRm Val122Ile vs ATTRwt and non-amyloid controls and their association with genetic penetrance in ATTRm V122I and prognosis.
Safety Assessments	The safety of study participants will be evaluated by: 1. Assessment of adverse events (AEs), including serious adverse events (SAEs). 2. Clinical laboratory safety tests (serum chemistry). 3. Vital sign measurements (blood pressure, pulse rate, and respiratory rate). 4. 12-Lead electrocardiogram (ECG). 5. 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	Baseline	6 Months* (±2 Weeks)	12 Months* (±4 Weeks)
Informed Consent	X			
Inclusion and Exclusion Criteria	X			
Baseline History		X		
Height and Weight†		X	X	X
Vitals Signs		X	X	X
Medications		X	X	X
Clinical Examination		X	X	X
Adverse Events Review		X	X	X
Electrocardiogram		X	X	X
Laboratory Analysis				
Basic Metabolic Panel		X	X	X
Hepatic Function Panel		X	X	X
Cardiac Biomarkers‡		X	X	X
Vitamin A/RBP4 in plasma/urine§		X	X	X
Prealbumin		X	X	X
TTR Kinetic Stability		X		X
TTR Gene Sequencing		X		
Ancestry Analysis		X		
Six-Minute Walk Test		X	X	X
Short Physical Performance Battery		X	X	X
QoL Questionnaires		X	X	X
Health Literacy Questionnaire		X		
Echocardiogram		X		X
^{99m}Tc-PYP Scintigraphy**		X		
Phone Call Follow-Up¶			X	X

* Follow-up visits are to be conducted on subjects who test positive to the Cardiac Amyloid Scintigraphy scan and/or are carrier of the V122I mutation. Window for the 6-month visit is ±2 weeks and for the 12-month visit is ±4 weeks.

† Height measured only at baseline visit. Subjects with weight >350 lb will not participate in the study.

‡ Includes BNP, NT-proBNP, galectin-3, troponin I and T.

§ Includes Vitamin A and RBP4 in on site spot urine.

¶ For participants with no TTR CA, a phone call follow-up will be conducted instead.

**In the event that PYP is unavailable due to supply shortage, the investigator has the option of using HDP until PYP becomes available again to ensure study continuation per FDA.

For every study visit, a checklist will be used to help ensure completion of all the study procedures. The checklist is also a guide that suggests the order in which the assessments should be performed (See Appendix 3).

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
6-MWD	Six-minute walk distance
6-MWT	Six-minute Walk Test
SPPB	Short Physical Performance Battery
99mTc-PYP	99mTechnetium pyrophosphate
99m Tc-HMDP	99m Hydroxymethylene Diphosphonate
AE	Adverse event
TTR	Transthyretin
ATTRm	Mutant Transthyretin amyloidosis
ATTRwt	Wild-type Transthyretin amyloidosis
ATTR-CA	Transthyretin cardiac amyloidosis
RBP4	Retinol-Binding Protein 4

1. INTRODUCTION

1.1 Background and Rationale

1.1.1 Transthyretin Biology

Transthyretin (TTR), also known as prealbumin, is a tetrameric protein produced predominantly by hepatocytes (>95% of TTR is liver-derived), with small fractions produced in the choroid plexus and retina.[1] The primary physiological role of TTR is to serve as a carrier of retinol (also known as vitamin A) through its interaction with retinol binding protein (RBP); it also plays a minor role as a carrier for thyroxine (T4). In humans, approximately 15% of T4 circulating in the plasma is bound to TTR; the remainder is predominantly bound to thyroxine-binding globulin.

Mutations in the TTR protein as well as idiopathic mechanisms lead to destabilization of the tetrameric form and dissociation into dimers and monomers. The misfolding of mutated monomers results in tissue deposition of oligomers and amyloid fibrils. Amyloid deposits typically contain both mutant (mt) and wild-type (wt) TTR in addition to serum amyloid P protein and glycosaminoglycans, and over time these deposits lead to significant tissue injury. The clinical manifestation of this process is called ATTR amyloidosis.

1.1.2 Transthyretin-mediated amyloidosis definition

Transthyretin-mediated amyloidosis (ATTR) is caused by deposition of TTR amyloid fibrils in various tissues. The hereditary form of ATTR is caused by an autosomal dominant mutation in the TTR gene that leads to destabilization of the TTR tetramer and aggregation of misfolded monomers; this, in turn, results in cardiac and neuronal extracellular deposition of TTR amyloid fibrils culminating in life-threatening cardiomyopathy and/or debilitating neuropathy, called ATTR mutant form or ATTRm. There are over 120 reported TTR genetic mutations[2] that phenotypically result in at least 2 distinct clinical syndromes of ATTRm: familial amyloidotic cardiomyopathy (FAC) and familial amyloidotic polyneuropathy (FAP), both of which are characterized by amyloid deposits of mutant and wild-type TTR.[3] ATTR wild-type (ATTRwt) is another form of ATTR, occurring more predominately in elderly males and characterized by deposition of wild-type TTR in the heart tissue.[4]

The Val122Ile mutation is the most common and estimated to be present in up to 4% of African Americans and in over 5% of West African populations.[5] Disease develops predominantly in males with the mean age at diagnosis of approximately 70 years, and symptom onset typically occurring after the age of 60. In the US there are estimated to be approximately 100,000- 150,000 African American males over age 65 who are heterozygous carriers of the V122I allele.[6] However, the extent of disease penetrance is currently unknown.

Also unknown is the prevalence of ATTRwt within the African-American population. While some cases have been documented it is unclear whether African-Americans develop wild-type disease with the same prevalence as non-African Americans. There are no data on the prevalence of ATTRwt or ATTRm amyloidosis in the Hispanic population in the US.

1.1.3 Pathogenesis and Outcome

Cardiomyopathy in ATTR (ATTR-CA) occurs when insoluble TTR amyloid fibrils deposit in cardiac tissue causing heart wall thickening with diastolic and systolic dysfunction, conduction defects, and arrhythmias, leading to congestive heart failure and death.

While there is a paucity of longitudinal clinical data on ATTR-CA, a few published reports have shown that ATTR patients have a significantly worse prognosis compared to other causes of heart failure, with rapid deterioration of functional capacity, including their ability to walk as measured by the 6-MWD, and quality of life (QOL) resulting in early mortality.

The median survival of Val122Ile patients from time of diagnosis in two retrospective studies was 27[7] to 41 months[7, 8] and in the prospective Transthyretin Amyloidosis Cardiac Study (TRACS) study median survival was 26 months.[9]

There is currently only one approved disease-modifying treatment option specifically for patients with ATTR-CA, Tafamidis Meglumine. Another therapy that has shown efficacy in TTR amyloid polyneuropathy is diflunisal (a non-steroidal anti-inflammatory agent) which when administered at a dose of 250 mg PO twice a day delayed progression of neuropathy when compared to placebo.[10] As ATTR-CA is progressive and many patients are anxious to receive a potentially disease modifying agent, there has been an increasing use of diflunisal in such patients, with a single report of safety over an intermediate time period in an uncontrolled study.[11]

1.1.4 ATTR-CM and Heart Failure

1.1.4.1 Heart failure (HF) is a growing epidemic among urban, multi-ethnic populations

The epidemic of HF disproportionately affects older and multi-ethnic populations. Blacks (incidence rate: 4.6 per 1000 person-years) have the highest incidence rate of HF, followed by Hispanic (3.5), white (2.4) and Chinese-American (1.0). The higher risk among Blacks has been related to differences in the prevalence of hypertension and diabetes mellitus, as well as socioeconomic status[12]. Additionally, Blacks are at high risk for the development of HF with preserved ejection fraction (HFpEF)[13]. Data from Medicare records revealed that hospitalization and readmission for HF was higher among Blacks and Hispanic patients than among non-Hispanic white patients[14]. Despite recent advances, HF remains a major public health problem with rising incidence/ prevalence and associated annual health care costs in excess of $32 billion[12, 13]. The incidence/prevalence of HF are strikingly age-dependent, with prevalence in adults > 80 years of age approaching 10% and mortality rates increasing exponentially with advancing age. Epidemiologic studies demonstrate that >50% of patients with HF have HFpEF. The hospitalization rate has continued to rise among subjects with HFpEF as compared to a flattening in those with systolic HF[13]. Large-scale clinical trials for the population with HFpEF have unfortunately not identified any specific therapies[15-17]. *Thus, identifying and treating patients with personalized and precise medical approaches from underserved minority communities at risk of HF are urgently needed*.

1.1.4.2 Amyloid infiltration is an under-recognized mechanism underlying HFpEF

With an increasing burden of disease and lack of efficacy in recently conducted clinical trials, a greater understanding of pathobiologic mechanism(s) that contribute to diastolic dysfunction and the genesis of HFpEF are warranted. Numerous biologic mechanisms that have been implicated in the genesis of myocardial stiffness[18-20] including intrinsic cardiomyocyte stiffness[19, 21] related to abnormal calcium homeostasis[22], the cytoskeleton (e.g. microtubules and intermediate filaments[23, 24] or titin[25, 26]) as well as, abnormalities in the extracellular matrix related to collagen and elastin[26-29]. Amyloid infiltration of the extracellular matrix can markedly alter myocardial stiffness resulting is upward and leftward shifts in the end diastolic pressure volume relation[30], and has been associated with most severe forms of diastolic dysfunction as assessed by Doppler imaging[31]. In-vitro length-tension experiments demonstrate increased diastolic force among subjects with amyloid in comparison to controls.[32] More recent data has demonstrated that transthyretin or TTR cardiac amyloidosis (TTR-CA) accounts for up to 15% of cases of HFpEF[33]. *Collectively, these data suggest that amyloid infiltration is not only a plausible pathobiologic mechanism in the genesis of HFPEF, but is under-appreciated and under-recognized.*

1.1.4.3 Pathobiology of ATTR-CA

TTR is a 127 amino acid protein, primarily synthesized in the liver, although other tissues including choroid plexus, the retinal pigment epithelium and kidney also synthesize TTR[34-37]. The known plasma functions of TTR are to carry thyroxine and retinol (vitamin A)-retinol binding protein complex. TTR binding to the smaller retinol-RBP complex prevents renal filtration of this retinoid-transport protein[38]. In its native state, TTR exists as a 55 kDa tetramer, i.e. four single chain TTR monomers form a tetrameric complex. The crystal structure and physical properties of TTR are well characterized[34-37]. Human TTR is encoded by a single copy gene on chromosome 18[39]. The TTR gene spans about 7 kB and contains 4 exons and 3 introns[39]. Although each monomer of TTR consists of only 127 amino acids, about 120 human TTR mutations have been identified[40]. More than 90 of these TTR variants are associated with human amyloidoses[40]. While the phenotypes are variable for the TTR-associated amyloidoses, polyneuropathy and cardiomyopathy represent the two most common clinical manifestations. These phenotypes result from extracellular deposition of amyloid fibrils composed of mutant and wild type (WT) TTR (the vast majority of the familial patients are heterozygotes), although the wild type protein alone in genetically normal aged individuals can form amyloid fibrils[37, 41, 42]. More than 20 TTR mutations have been specifically linked to cardiac involvement, but the Val122Ile mutation, accounts for the highest proportion of amyloid TTR cardiomyopathy cases, especially in Black Americans[5, 43]. The prevailing hypothesis (***Figure 1***) to explain amyloidogenic mutations is that the mutations destabilize the TTR tetramer, facilitating faster or more extensive dissociation, the rate-limiting step that precedes the formation of abnormally folded monomers that aggregate into amyloid

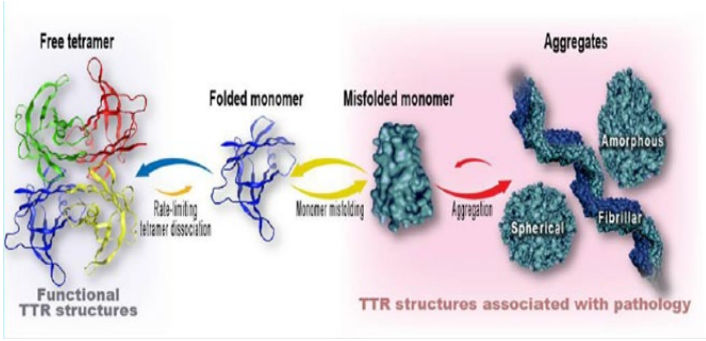


Figure 1. Native TTR is present in the circulation primarily as a tetramer but tetrameric TTR undergoes some dissociation into subunits. Mutations in TTR can result in an increased incidence in misfolded monomeric TTR, which is prone to aggregation, giving rise to spherical, amorphous and fibrillar TTR structures which are insoluble and result in amyloid deposition and ultimately amyloid-induced tissue damage.

fibrils[37, 42, 44]. These TTR amyloid fibrils are deposited extracellularly in tissues where they cause post-mitotic tissue damage and ultimately cell death. Collectively, the literature makes a compelling case that TTR amyloid infiltration is an under-appreciated cause of HFpEF.

1.2 ATTR-CM Population Burden

1.2.1 ATTR amyloid is an under-recognized cause of heart failure

Cardiac involvement in ATTR, also known as familial amyloid cardiomyopathy (FAC) when associated with variant and WT TTR aggregation, previously senile systemic amyloidosis (SSA) when associated solely with WT TTR aggregation, is caused by amyloid fibril infiltration of the myocardium. This results in diastolic dysfunction progressing to restrictive cardiomyopathy and HF. ATTR cardiomyopathy is a late onset disease with symptoms typically occurring in patients over 60 years old. Although there are more than 20 mutations which have been associated with specific cardiac involvement, one mutation, Val122Ile, has been reported with high frequency in Blacks (prevalence of 3.4% to 3.9%[5, 45, 46]). The prevalence of ATTR dominated by a cardiomyopathy phenotype is unknown, but is certainly under-diagnosed[43]. Both Black Val122Ile carriers and Black patients with ATTRwt, who present typically after age 60, are often mistakenly thought to have hypertensive heart disease[43]. The prevalence of the Val122Ile allele in older adults with manifest cardiovascular disease, such as HF, is much higher. Among 207 of 650 Blacks enrolled in the BEST Trial with NYHA Class III (92%) and IV (8%) HF, with a left ventricular ejection fraction <35%, the Val122Ile variant was found in 13 (6.3 %) of the subjects[47]. Of note, four of the 116 individuals in the under 60 years of age cohort (3.5%) were positive, whereas 9 of the 91 over 60 years of age (10%) had the amyloidogenic Val122Ile allele. Studies indicate that the Val122Ile mutation is 5 times more prevalent in Blacks with HF than in a community cohort of comparable age[47]. Notably, a mutation in TTR is not a prerequisite for developing ATTR cardiomyopathy. In the elderly, WT (normal) TTR may become structurally unstable for unknown reasons (this will be quantitatively explored herein), resulting in deposition of amyloid fibrils primarily in heart tissue and leading to HFpEF[48-51]. The frequency of TTR amyloid deposition in cardiac ventricles reported from autopsy studies, range from 1.8% to 25% [52, 53], with a rate of clinical cardiac disease pre-mortem of 34%[52]. More recent data demonstrates that among HFpEF subjects who were an average age of 74±14 at diagnosis, 21.2% had amyloid deposits on autopsy performed at an average age of 76±14 years. Of HFpEF patients ≥ age 75 years at HF diagnosis (n = 63), 20 (32%) had amyloid deposition vs. 4 of 50 (8%) patients < 75 years at HF diagnosis (p = 0.002). Of the 29 patients with amyloid deposition at autopsy, only 6 had a pre-morbid diagnosis of amyloid

at death[54]. The prevalence of ATTRwt is unknown, but is certainly under-diagnosed. Intriguingly, the vast majority (>90%) of ATTRwt patients in the reported series[9, 55-57] are White, while limited population based data suggests that the prevalence of ATTRwt does not differ among Whites and Blacks.[58] *Thus, TTR-CA is an under-recognized cause of HFpEF particularly among Blacks, among which ~4% of the population are allele carries of the Val122Ile TTR mutation that results in amyloid deposition in the heart. Additionally, despite similar prevalence of ATTRwt in Whites and Blacks,[58] reported series almost exclusively describes Whites as being affected*[9, 56, 59]. Collectively, these data suggest racial disparities in the identification of Black subjects with TTR cardiac amyloid, which is a focus of our proposed studies.

1.2.2 Are Hispanics affected by TTR-CA?

More than 53 million Hispanics currently live in the US, which constitutes 17% of the total US population, which make them the largest racial/ethnic minority. They represent the fastest-growing racial or ethnic population in the US, an ethnic group that is expected to constitute 30% of the total US population by 2050. While 64% of the US Hispanic population is of Mexican origin, 9% are of Puerto Rican origin, 3% each of Cuban, Salvadoran and Dominican origins, the remainder being of other Central American or South American origin. New York and in particular Washington Heights and Harlem have the highest percentages of Hispanics identifying as Black (up to 25%), compared to 2.5% of Hispanics nationwide. Overall, the Northeast region has the largest concentration of Black Hispanics, this is partly because of the large Puerto Rican, Dominican, and other mostly or partly African descended Hispanic populations in the region. Genetic data suggests that among Hispanics of Caribbean origin (e.g. Haiti, Dominican Republic, Cuba and Puerto Rico) that African ancestry ranges from 30-95%[60]. The Val122Ile mutation has been described almost exclusively in Blacks of African or Afro-Caribbean descent. Surprisingly, despite the common ancestry shared by many Hispanics in the Northeast of the US, published reports of TTR cardiac amyloid have not focused on this relevant population. Only one previous report in an urban mid-west city did not find that the Val122Ile mutation in any newborn of Hispanic descent[61], though whether these subjects were of Mexican or Caribbean origin was not specified. The proposed SCAN-MP (Screening for Cardiac Amyloidosis with Nuclear imaging in Minority Populations) study will provide critically important information on the impact of TTR-CA in the Hispanic population with significant African ancestry, which we hypothesize are affected, but currently unrecognized.

1.2.3 TTR-CA from mutant protein has more severe phenotype than wild type

We characterized the cardiac phenotype among subjects (n=88) diagnosed with biopsy proven TTR-CA at our institution. Subjects underwent DNA sequencing to confirm the presence of Val122Ile or ATTRwt. ATTR cardiac amyloid subjects were almost exclusively elderly males. Racial differences between Val122Ile and ATTRwt were distinct. Despite a younger age, subjects with Val122Ile mutation had a more advanced phenotype as evidenced by a higher troponin and reduced ejection fraction, as compared to ATTRwt. Additionally, survival in subjects with Val122Ile mutations was less (median survival of 2.53 years [95% CI; 1.7 to 3.2] compared to WT subjects with a median survival

of 4.3 years (95% CI of 2.5 to 5.4), p=0.0024 by log rank. Similar findings were confirmed in a multicenter. Transthyretin Cardiac Amyloid Study (TRACS).[30]

Table 1

Parameter	ATTRwt (N=57)	V122I (N=31)	P value
Demographics			
Age at Diagnosis (years)	77.2±5.8	69.5± 7.9	<0.001
Gender (% male)	94.7%	83.8%	0.124
Race (% Black)	1.7%	83.8.%	<0.001
Electrocardiography (%)			
Atrial Fibrillation/Flutter	26.80%	8.50%	0.119
Pacemaker	11.00%	1.20%	0.075
Laboratory			
BNP (pg/ml)	614±532.4	935±736.2	0.014
Troponin I (ng/ml)	0.1±0.3	0.2±0.5	0.335
eGFR (ml/min)	50.3±26.2	49.2±19.0	0.639
Echocardiogram			
LVIDd (mm)	39±15	41±13	0.963
IVS (mm)	16±7	16±6	0.567
EF (%)	43.8±19	27.5±16.1	<0.001
LV Mass (grams)	304.6±153.9	290.1±146.6	0.86
LV Mass Index (grams/m2)	11.6±6.3	12.1±5.0	0.911
LA size (mm)	37±22	35±21	0.149
Right Heart Catheterization			
RA Pressure (mmHg)	7.7±7.6	8.4±7.7	0.630
PA Pressure (mmHg)	33.6±23.3	32.7±22.5	0.398
PCWP (mmHg)	13.9±9.9	13.4± 10.4	0.654
PaSat (%)	41.2±30.0	40.2±27.0	0.013
Cardiac Output (L/min)	2.6±1.9	2.4±1.7	0.027
PVR (dynes)	87.9±131.4	135.5±235.7	0.005
PVR (woods)	1.1±1.6	1.9±2.9	0.007

Thus, despite the ability to identify subjects with the Val122Ile mutation by DNA sequencing, these patients present at a more advanced stage of disease than ATTRwt subjects for whom serologic testing is not available. Additionally, only 1 subject (1.7%) with ATTRwt was Black and only 1 subject with the Val122Ile mutation was Hispanic, despite our catchment area including Harlem, where 62% of the population is Black according to the 2010 census and Washington Heights, where 69% of the population is Dominican. Whether these differences are attributable to a different natural history or delayed diagnosis is unknown. With the advent of a non-invasive nuclear imaging approach to identify TTR-CA without the need for a biopsy, we have a unique ability to address the questions raised about the prevalence and outcomes of this condition in urban, minority populations.

1.3 Natural History of Transthyretin Cardiac Amyloidosis

The Transthyretin Amyloidosis Cardiac Study (TRACS)[9, 30] was a multi-center, prospective observational trial examining the natural history and disease progression in patients diagnosed with TTR-CA.

1.3.1 Clinical Characteristics and Outcome in ATTR-CA in TRACS

Mean patient age at TRACS enrollment was 74 years, with the majority male (92%). Of the twenty-nine patients (18 WT and 11 Val122Ile), twelve (41%) subjects died or underwent a cardiac transplant after a mean follow-up period of 478 days (range 31-807).

1.3.2 Predictors of Mortality

Age and gender did not differ between survivor and non-survivors. Subjects with Val122Ile mutations had a significantly higher mortality (72%, 8 of 11) compared with wild type patients (22%, 4 of 18). Survival analysis showed that in univariate analysis disease duration, a heart rate >70 bpm, lower stroke volume, a LVEF <50% and the presence of the Val122Ile mutation were all associated with shorter survival. However, in multivariate analysis only stroke volume and disease duration, but not the Val122Ile mutation, had independent associations with reduced survival. This suggests that subjects with a mutation may have a more malignant phenotype presenting at a later stage of the disease. To further explore the mechanisms underlying the reduction in stroke volume in subjects with TTR-CA, we employed non-invasive pressure volume analysis. TTR-CA is a disease characterized by significant abnormalities in passive diastolic function, progressive upward shifts in the end diastolic pressure volume relation (EDPVR), as well as decrements in the chamber contractility manifested by declines in the end systolic pressure volume ratio (ESPVR) and altered ventricular vascular coupling. Each of these could contribute to the inability of the ventricle to perform work. Using 2D echocardiographic measures of left ventricular volumes and mass with full Doppler studies, we estimated non-invasive pressure volume relations. As shown in the Figure 2, in TTR-CA, abnormalities in passive ventricular filling, reductions in chamber contractility and altered ventricular vascular coupling were each associated with a reduced ability of the ventricle to perform work, and ultimately associated with reduced survival in these subjects. By calculating the relationship between the isovolumetric pressure volume area and end diastolic pressure (PVAiso-EDP), we demonstrated that in TTR-CA, there are progressive declines in ventricular function over time (left panel), ventricular function at baseline was worse in subjects with the Val122Ile mutation than in ATTRwt subjects (middle panel) and that the decrement in PVAiso-EDP relation was independently associated with reduce survival (right panel).

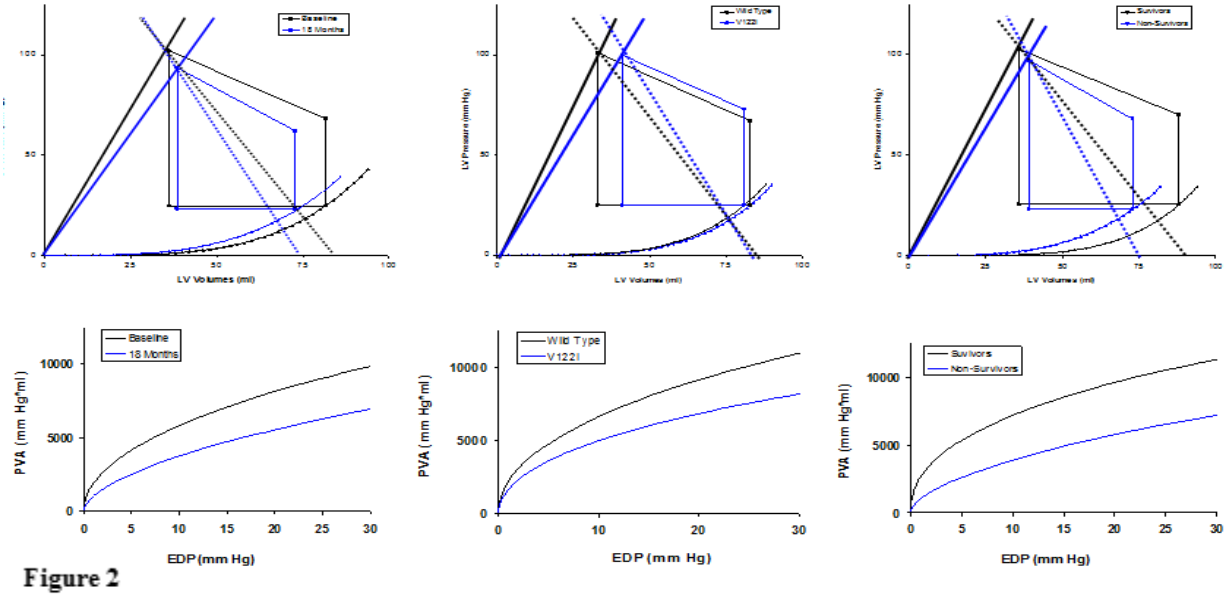


Figure 2

Pressure-Volume Relations:(upper panel) and iso-volumetric pressure-volume area (PVA-iso, lower panel): Baseline to 18-month follow-up (left panel), V122I versus Wild-Type at baseline (middle panel) and Survivors versus Non-survivors (right panel)). There were significant (e.g. $p<0.05$) increases in Ea, declines in Res and altered Ea/Res ratio from baseline to 18 months (right panel) as well as differences in baseline in Ea and Ea/Res ratio between wild-type and V122I (middle panel) as well as between survivors and non-survivors (right panel). Additionally, significant differences in PVA-iso curves were noted for all three comparisons.

These data show that subjects with Val122Ile had a more severe phenotype with a reduced ability of the ventricle to perform work. This could be due a more malignant course among subjects with the Val122Ile mutation compared to the ATTRwt genotype or are due to later presentation among the Blacks with Val122Ile than among ATTRwt subjects, as a result of racial disparities resulting in poor access to care. Accordingly, using such measures in a larger and unselected cohort of Blacks/Hispanics with both forms of TTR-CA, identified by cardiac amyloid scanning, we can evaluate the physiologic effect of the amyloid infiltration in those with and without a genetic cause. Thus, we can determine the true impact of the genotype on the phenotype and on outcomes in Blacks/Hispanics from the same geographic and socio-economic region.

1.4 Therapeutic Options

1.4.1 Novel Therapies Require Early Identification of Affected Individuals

A proven treatment based on lifespan extension for the familial TTR amyloidosis is liver transplantation; however, this strategy has numerous limitations, including a shortage of donors, a requirement for surgery, need for lifelong immunosuppression and the high cost. Furthermore, most patients, especially those with cardiac involvement, are not acceptable transplant candidates or require combined heart/liver transplantation, which is not feasible for the large population of affected older adults. Recent studies have provided insight into the pathogenesis of TTR amyloidosis and suggested new strategies for therapeutic intervention. The other approved therapeutic strategy is to kinetically stabilize the TTR tetramer (native state) through its binding to a small molecule[62, 63]. Approximately 70% of patients identified early in their course of disease and treated with tafamidis (a TTR stabilizer) remain progression-arrested, whereas in a demographically identical group, on placebo for 18 months before receiving the stabilizer, only ≈ 50% remain progression arrested[64]. We have demonstrated stabilization in a small open label trial the safety of diflunisal in subjects with ATTR cardiomyopathy[11]. Other therapeutic approaches approved for familial amyloid polyneuropathy include small interfering RNAs or antisense oligonucleotides to degrade TTR mRNA[65, 66]. These two strategies (antisense oligonucleotides and RNAi) have shown efficacy in recently reported trials of ATTR peripheral neuropathy[67, 68] and are expected to be effective for amyloid cardiomyopathy, as lowering the TTR concentration should decrease the TTR aggregation efficiency, a process thought to cause cardiomyopathy. All of the proposed therapies require early detection of subjects, because emerging therapies aim to prevent progression of disease by inhibiting newly synthesized TTR from converting into amyloid. These strategies do not directly reverse previous amyloid infiltration. *In summary, there are multiple emerging therapies for ATTR cardiomyopathy which have demonstrated therapeutic stabilization and silencing in clinical trials. However, it appears to be critical to recruit TTR-CA patients early in the course of their disease to maximize the efficacy of these therapies and if effective, to ameliorate the racial disparities in outcomes for this population.*

1.4.2 Technetium-based radiotracers can detect TTR-CA without the need for an amyloid biopsy

Guidelines have been established for formalizing the diagnosis of amyloidosis, requiring a biopsy of tissue delineating the presence of amyloid in either the heart or another organ.[69] Unfortunately, for a significant percentage of patients with TTR-CA, more easily performed biopsies, such as fat pad biopsies have a low yield.[56] The performance of endomyocardial biopsy has been restricted to large centers with significant expertise, not only in the performance of the biopsy, but also in the pathologically evaluation of the type of amyloid, which is critical to accurate diagnosis and appropriate treatment. Our own single center data has shown that ^{99m}Tc-PYP imaging has a sensitivity and specificity for detecting TTR cardiac amyloid from either ATTRwt or Val122Ile and differentiation form AL in of 97% and 100% of patients, respectively with an area under the curve of 0.997.[70] More recent multicenter data with ^{99m}Tc-PYP imaging from Columbia University, Boston University and the Mayo Clinic evaluated the utility of this non-invasive approach to diagnose TTR-CA without the need for endomyocardial biopsy[71]. Cardiac retention of ^{99m}Tc-PYP was visually scored using the ratio of myocardial uptake to the contralateral chest (the H/CL: heart contralateral ratio), which when >1.5 had a very high sensitivity (90%) and specificity (92%) for identifying the subjects with TTR-CA. Additionally, an even larger international collaboration employing ^{99m}Tc-DPD, ^{99m}Tc-PYP or ^{99m}Tc-HDP imaging; using the following grading system; Grade 0 – absent cardiac uptake; Grade 1 – mild uptake less than bone, Grade 2 - moderate uptake equal to bone; Grade 3 - uptake greater than bone, has demonstrated that the vast majority of subjects with biopsy proven WT TTR (89.5%) or Val122Ile TTR (97.6%) demonstrated grade 2/3 uptake indicative of TTR-CA. Among subjects who had grade 2/3 uptake using either of the three isotopes and no monoclonal protein (as evident by serum protein electrophoresis with immunofixation without a monoclonal band and free light chain ratio that was between 0.26 or 1.65), the specificity of nuclear scintigraphy for identifying TTR-CA (compared to AL, hypertrophic cardiomyopathy, HFpEF from hypertension or another infiltrative disease) was 100%![72] *These data demonstrate that TTR-CA can be reliably diagnosed in the absence of histology and these data serve as the basis for employing this non-invasive test to evaluate subjects at risk for TTR-CA in the SCAN-MP trial[73].*

1.4.3 Myocardial retention of Tc99-PYP is prognostic in TTR-CA

In a retrospective, cohort study[71] of 196 subjects with heart failure at 3 academic medical centers (Columbia University Medical Center, Boston University Medical Center and the Mayo Clinic) we evaluate cardiac retention of ^{99m}Tc-PYP using a quantitative heart-to-contralateral (H/CL) ratio. H/CL was calculated as total counts in a region of interest (ROI) over the heart divided by background counts in an identical size ROI over the contralateral chest. The outcome measured was time to death after ^{99m}Tc-PYP scan. Among the 144 subjects with TTR cardiac amyloid (the majority of who had ATTRwt [n=72] or the Val122Ile mutation ([n=24]), univariate analysis shown in Table 2 demonstrated that the H/CL >1.6 predicted worse survival (hazard ratio (HR) 4.273, 95% CI 1.27–14.37, P=0.02). In multivariate analysis encompassing age, gender, race, TTR mutation, NYHA class, troponin, BNP and eGFR, the HR for the H/CL ratio of >1.6 was still significant 6.2, 95% CI 1.5-30.4,

p=0.0043). In Kaplan-Meier analysis over a 4-year follow-up, survival was significantly worse if H/CL was >1.6 vs. <1.6 (P=0.0029 log-rank test). As shown in Figure 3, only 4 of 44 subjects (9.7%) with a H/CL < 1.6 died over the follow-up period while 32 deaths occurred in the cohort with a H/CL ratio of > 1.6 (35% mortality). Thus, not only can ^{99m}Tc-PYP imaging diagnose ATTR-CA without a biopsy (irrespective of genotype), but this approach has prognostic significance. These data collectively suggest that ^{99m}Tc-PYP scans can identify ATTR-CA early, enhance diagnostic accuracy and can provide prognostic information. *Accordingly, the use of such radiotracer imaging is a major advance in the field of cardiac amyloid which is being incorporated into clinical algorithms[73] and is being relied upon in the proposed SCAN-MP study.*

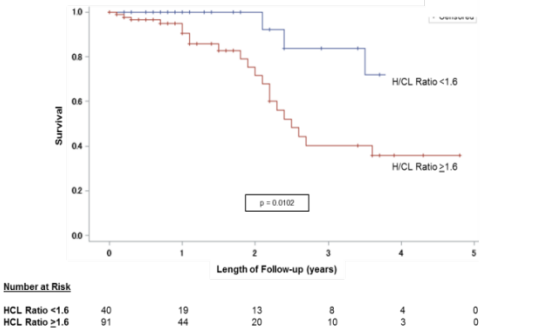
Table 2
Cox Proportional Hazards Univariable and Multivariable Predictors of Death Among Subjects with ATTR-CA[a]

	N	HR	95% CI	*P* Value
Univariable				
Age, y	132	1.073	(1.017-1.132)	0.0102
Modified BMI[b], kg·dl/m^2·g	129	0.974	(0.954-0.994)	0.0132
Male sex	132	1.111	(0.330-3.737)	0.8653
Black race	132	1.493	(0.634-3.513)	0.3589
ATTR mutation	132	0.827	(0.365-1.872)	0.6479
NYHA class, I-IV	110	5.232	(1.759-15.562)	0.0029
Troponin I ≥0.25, ng/ml	104	4.054	(1.812-9.072)	0.0007
BNP ≥500, pg/ml	108	2.896	(1.143-7.341)	0.0250
eGFR <60, ml/min	132	3.312	(1.445-7.592)	0.0047
LVEF <45, %	129	1.430	(0.640-3.193)	0.3835
H/CL Ratio ≥1.6	132	4.273	(1.271-14.370)	0.0189
Multivariable				
Model 1[c]	132	6.1939	(1.500-30.368)	0.0043
Model 2[d]	110	4.099	(1.045-16.083)	0.0431
Model 3[e]	101	4.383	(0.984-19.515)	0.0525
Model 4[f]	5	4.460	(1.167-17.042)	0.0288

Abbreviations: HR, hazards ratio; CI, confidence interval; BMI, body mass index; ATTR, transthyretin cardiac amyloid; NYHA, New York Heart Association; NT pro-BNP, amino-terminal pro-B-type natriuretic peptide; BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; LA, left atrium; IVSD, interventricular septal thickness at diastole; MCF, myocardial contraction fraction; H/CL, heart-to-contralateral.

Model 1: H/CL Ratio >1.6 adjusted by [age, male sex, black race, ATTR mutation].
Model 2: H/CL Ratio >1.6 adjusted by [age, male sex, black race, ATTR mutation, NYHA class].
Model 3: H/CL Ratio >1.6 adjusted by [age, male sex, black race, ATTR mutation, NYHA class, troponin I >0.25, BNP >500, eGFR<60].
Model 4: H/CL Ratio >1.6 adjusted by [age, male sex, black race, ATTR mutation, NYHA class, troponin T >0.0.02, NT pro-BNP >2000, eGFR<60].

Figure 3
Kaplan-Meier Survival Curves Among Subjects with ATTR-CA Comparing H/CL ratio <1.6 vs. ≥1.6 over the 5-year Study Duration.



1.4.4 Myocardial Contraction Fraction (MCF) to identification and prognosticate in TTR-CA

We previously described the MCF[74], defined as the ratio of LV stroke volume to myocardial volume and demonstrated that the MCF could successfully distinguish patients with HFpEF from athletes with physiologic hypertrophy and normal controls. The MCF provided a measure of ventricular function independent of chamber size that represents a volumetric measure of abnormal myocardial shortening, which was able to distinguish physiologic from pathologic hypertrophy. Measures of LV chamber performance are based on the premise that the myocardium is nearly incompressible and does not change volume significantly from end-diastole to end-systole.[75] Capitalizing on this fundamental principle of the incompressibility of the myocardium and by indexing the stroke volume to the myocardial volume, the MCF is an index of the volumetric shortening of the myocardium. During systole, the myocardium shortens and thickens, reducing its contained volume by the stroke volume. The MCF, though operationalized prior to the advent and widespread measurement of strain using echocardiography, is highly correlated with global longitudinal strain.[76] MCF is analogous to ejection fraction (EF) in that is a ratiometric measure that is easily obtainable, unit less and therefore does not need to be indexed to body size, age or gender. We have shown in a small cohort with cardiac amyloid in whom the average EF was preserved (51.2% +/- 13.1) while the MCF was significantly decreased (29.9% +/- 15.8) and was an independent predictor of survival.[77] Similar prognostic ability was

confirmed by colleagues at the Mayo Clinic in AL amyloid[76] and by colleagues in London.[78] More recently, the prognostic capacity of MCF was confirmed in TTR cardiac amyloidosis in the Transthyretin Amyloidosis Outcomes Survey (THAOS).[79] Classically, the voltage to mass ratio (V/M) has been employed to identify the phenotype of CA, but its use is confounded by the fact that many elderly patients with amyloid have a paced rhythm, making calculation of the V/M ratio impractical. Preliminary data demonstrates that the MCF with an area under the curve of 0.773 ($p<0.0002$, 95% CI 0.66–0.86) for identifying TTR-CA was similar to the V/M ratio with an AUC of 0.678 ($p=0.07$, 95% CI 0.54–0.79). *Accordingly, the MCF has potential as a simple measure to raise suspicion of TTR-CA as well as informing prognosis.*

1.4.5 A method to quantify TTR kinetic stability

In collaboration with Jeff Kelly and colleagues at Scripps, we have been studying the structure, energetics and dynamics of WT and mutant TTR in human plasma, towards development of mechanisms to identify individuals susceptible to TTR-CA and predict clinical benefit in treating TTR-CA. Using a published subunit exchange method for quantifying the kinetic stability of human TTR in plasma, we were able to demonstrate that tafamidis kinetically stabilizes TTR and that the extent of TTR kinetic stabilization correlated with the tafamidis plasma concentration.[80] Additionally, using this simple assay carried under physiological conditions, we were able to optimize the dose of tafamidis in a patient's plasma to maximize TTR kinetic stability.[81] We hypothesize that by studying the kinetic stability of WT TTR in plasma, that we can identify those at risk for developing TTR-CA (see below).

2. STUDY OBJECTIVES & STUDY DESIGN

2.1 Primary Objective

To enhance the understanding of genotype-phenotype associations in Blacks/ Hispanics with ATTR-CA due to the Val122Ile mutation or ATTRwt and to improve identification of minority patients with ATTR-CA.

2.1.1 Research Goal

The goal of our research is to change the approach to transthyretin cardiac amyloidosis, which is a devastating disease, by screening and early diagnosis, and by studying prospectively a cohort of older Blacks/Hispanics with heart failure in order to address the following aims and hypotheses:

Specific Aim 1: To determine the prevalence of ATTR CA in Caribbean Hispanics and Blacks with HF. The hypotheses to be tested include:

(1) the prevalence of ATTR CA will be 10-15%

(2) ATTRwt CA will be higher among Caribbean Hispanics compared to US Blacks, whereas the prevalence of ATTRm CA will be lower

(3) the gender distribution of ATTR CA identified by this active ascertainment approach will be similar

(4) disease progression will demonstrate that ATTRm is associated with a more rapid decline vs. ATTRwt

(5) the accuracy of our clinic-based risk calculator will be confirmed in this large prospectively recruited cohort

Specific Aim 2: To demonstrate that circulating RBP4 concentration is integral to TTR tetramer stability which in turn determines the clinical characteristics of ATTR CA. We will measure TTR tetramer stability and RBP4 concentration in serum and urine collected in Aim 1. The hypotheses to be tested include:

(1) TTR tetramer stability will be lower in ATTR CA and directly relate to RBP concentration

(2) TTR tetramer stability will associate with clinical features and

(3) Increased renal clearance of RBP4 in ATTR CA will be determined by measurement of RBP4 in plasma and urine.

2.1.2 Overview of Study Design

We will undertake a prospective cohort study among outpatient subjects with heart failure seen at one of five sites: (1) Columbia University Medical Center, (2) Allen Hospital of NY Presbyterian, (3) Harlem Hospital, (4) Boston University/Boston Medical Center and (5) Yale New Haven Hospital.

Black/Hispanic subjects over the age of 60 years with heart failure will undergo comprehensive cardiovascular examinations including: (1) complete medical history documenting the presence of HF and any symptoms that could be associated with TTR amyloidosis, as well as any additional co-morbid conditions or symptoms, (2) targeted physical exams including orthostatic blood pressure measurements, assessment for gallops and volume status (e.g. JVP, rales, edema) and determination of functional class, (3) standard 12 lead electrocardiogram, (4) measurement of plasma N-terminal B-type natriuretic peptide, troponin T and I RBP, Vitamin A and TTR kinetic stability, (5) RBP in urine, (6) six minute hall walks and (6) full two-dimensional and three-dimensional echocardiograms with Doppler flow assessment and tissue Doppler and speckle tracking (See procedures for details), (7) ^{99m}Tc-PYP or ^{99m}Tc-HMDP scintigraphy and (8) genetic analysis for the Val122Ile mutation in all subjects in a CLIA lab.

2.1.3 Patient Recruitment

The SCAN- MP study is designed to identify a sufficient number of cases to support the following analytical objectives:

1. Stratify prevalence of ATTR-CA in subjects with HF by relevant demographic characteristics (gender, race/ethnicity, genotype); and
2. Evaluate relationships among ATTR-CA and baseline risk factors (presence of Vall122Ile mutation and others) with disease outcomes.

To accomplish these objectives, a hybrid approach to cohort identification and selection is used that combines deliberate selection of subjects with heart failure who are at increased risk for ATTR-CA and selection of participants with relevant characteristics (age, gender, race/ethnicity, country of origin). Planned proportion of enrolled subjects by relevant characteristics is shown in the Table 3. Community involvement is essential for its success and recruitment will involve intensive community engagement through various means among the sites. In aggregate, we will recruit an equal number of men and women from the racial/ethnic groups. The expected marginal distributions of race/ethnicity are shown in Table 3. We will have overlapping ethnic groups among centers to minimize confounding of ethnicity by site. As recruitment of elderly members of minority groups is challenging, toward end of the recruitment period, participants will be asked to refer elderly persons to the study.

Table 3. Planned Enrollment by Age, Race, and Gender

Age (yr)	Black		Hispanic or Latino ethnicity		Total	
	M	F	M	F	M	F
≤75	150	150	50	50	200	200
>75	150	150	50	50	200	200

At CUMC we have seen 24,682 unique patients with HF over the last five years of which 2,969 (12%) self-identified as Black and 4,387 of 14,466 (29.9%) patients who declared their ethnicity were Hispanic and whose average age was 63 years. Harlem Hospital provides health care services to the primary service area of Harlem, Washington Heights/Inwood and the South Bronx with the vast

majority of residents being African American (44.5%) and Hispanic (40.8%). Thus, there are ~1500 potential subjects per year from which we aim to recruit at least 100 subjects per year at the CUMC site. For the Boston site, we propose to recruit an equal number of participants with similar demographic distribution. The overall patient population at Boston Medical Center is ethnically diverse with 39% Black and 15% Hispanic of all patients seen. A preliminary assessment of recruitment feasibility suggests that ~750 patients are seen annually with HF who are Black or Hispanic.

2.1.4 Selection Criteria

800 patients will be enrolled in this protocol. ***Inclusion Criteria*** will include: Black or Hispanic of Caribbean origin, Age $\geq$ 60 years with heart failure and able to understand and sign the informed consent document after the nature of the study has been fully explained, prior to any study procedures. ***Exclusion Criteria*** will include confirmed primary amyloidosis (AL) or secondary amyloidosis (AA), prior liver or heart transplantation and active malignancy or non-amyloid disease with expected survival of less than 1 year, or heart failure attributable to ischemic or valvular heart disease.

2.1.5 Study Procedures

The timing of all procedures in this study is outlined in Table 4. Eligible patients will be identified. Patients who are interested in the study will have a screening visit to evaluate for eligibility and obtain informed consent. If patients qualify for study and do consent they will have their study visit within 4 weeks of screening. At that visit they will have all of the following testing done.

Table 4. Study Procedures

Procedure	**Screening**	**Baseline**	**6 Months*** **(±2 weeks)**	**12 Months*** **(±4 weeks)**
Informed Consent	X			
Inclusion and Exclusion Criteria	X			
Baseline History		X		
Height and weight†		X	X	X
Vitals Signs		X	X	X
Medications		X	X	X
Clinical Examination		X	X	X
Adverse Events Review		X	X	X
Electrocardiogram		X	X	X
Laboratory Analysis				
Basic Metabolic Panel		X	X	X
Hepatic Function Panel		X	X	X
Cardiac Biomarkers‡		X	X	X
Vitamin A/RBP4 in plasma/urine§		X	X	X

Prealbumin		X	X	X
TTR Kinetic Stability		X		X
TTR Gene Sequencing		X		
Ancestry Analysis		X		
Six-Minute Walk Test		X	X	X
Short Physical Performance Battery		X	X	X
QoL Questionnaires		X	X	X
Health Literacy Questionnaire		X		
Echocardiogram		X		X
^{99m}Tc-PYP Scintigraphy**		X		
Phone call follow-up¶			X	X

* Follow-up visits are to be conducted on subjects who test positive to the cardiac amyloid nuclear scan and/or are carrier of the V122I mutation. Window for the 6-month visit is ±2 weeks and for the 12-month visit is ±4 weeks.

† Height measured only at baseline visit.

‡ Includes BNP, NT-proBNP, galectin-3, troponin I and T.

§ Includes Vitamin A and RBP4 in on site spot urine.

**In the event that PYP is unavailable due to supply shortage, the investigator has the option of using HDP until PYP becomes available again to ensure study continuation per FDA.

¶ For participants with no TTR CA, a phone call follow-up will be conducted instead.

2.1.5.1 Clinical Examination

A detailed[82] health investigation will be performed in all patients by a provider experienced in the care of patients with HF. This will include a medical history focused on the presence of HF as defined by standard clinical criteria (NHANES[83] and European Society of Cardiology[58] for HFpEF), etiology of HF, NYHA functional class assessed by standardized questionnaires[82] and co-morbid conditions; physical examination including vital signs both supine and upright, volume status, cardiopulmonary examinations and neurologic examination with focus on neuropathic findings. Exams will take ~45 minutes.

2.1.5.2 ^{99m}Tc-PYP Scintigraphy

A nuclear medicine technologist will perform planar cardiac imaging of the chest using dual-headed gamma cameras equipped with low energy, high resolution collimators. 10-25 mCi of ^{99m}Tc-PYP or will be administered intravenously and imaging will be performed after approximately 3 hours. The anterior and lateral planar views centered on the heart will be obtained simultaneously for a total of at least 750K counts/view (approx. 3-8 min of imaging). Cardiac retention will be assessed by both a semi-quantitative visual score (range: 0 [no uptake] to 3 [uptake greater than rib]) and a quantitative heart-to-contralateral (H/CL) ratio of total counts in a region of interest (ROI) over the heart divided by background counts in an identical size ROI over the contralateral chest including soft tissue, ribs, and blood pool. A visual score 2 or greater and/or calculated H/CL ratio 1.3 or greater, with myocardial retention of ^{99m}Tc-PYP confirmed in all cases by SPECT imaging with or without CT attenuation, will

be required to indicate TTR-CA according to prior published data.[70] The details of the imaging procedures for ^{99m}Tc-PYP or ^{99m}Tc-PYP imaging are shown in Tables 5a-5c below.

Table 5a

Acquisition Procedure - Planar Imaging	
System	Standard Gamma Camera
Participant Position	Supine on the imaging table - raise arms above the head
Collimator	LEHR
Matrix	256 x 256
Peak	140 KeV
Energy Window	15% to 20%
Zoom	1.46 (or closest)
Views	Anterior and Left Lateral
Acquisition Counts	Minimum 750,000 counts

Table 5b

Acquisition Procedure – SPECT Imaging	
System	Standard Gamma Camera
Participant Position	Supine on the imaging table - raise arms above the head
Orientation	Feet First
Organ	Cardiac
Collimator	LEHR
Matrix	64 x 64
Number of Angles	64
Peak	140 KeV
Energy Window	15% to 20%
Zoom	1.46 (or closest)
Acquisition Bit Depth	8 bit or 16 bit
Detector Configuration	90 degree (Cardiac)
Orbit Type	Non-circular or Circular
Orbit Acq	180 degrees
Start Position	0 degrees
Orbit Direction	Counter-Clockwise
Acquisition Type	Step and Shoot
Rotations	1
Time per Stop	30 sec
Total Time	~15 min

Table 5c

Imaging Protocol for CT Attenuation Correction (performed as available)	
Indication: CT attenuation correction	
Breathing Instructions:	Free breathing
Other Instructions:	raised arms above head

Duration:	3 mins
This imaging protocol utilizes a patient's radiation exposure that is as low as reasonable achievable while maintaining diagnostic image quality. Dose modulation software, when utilized, sets an appropriate exposure and CTDI proportional to the patient's size.	
PATIENT POSITION	Supine
SERIES DESCRIPTION	
SCOUT / TOPOGRAM	Standard
AXIAL, HELICAL OR VOLUME	Helical
Tube Potential	120 kV
Tube current (If NO mA modulation)	31 mA
SFOV (mm)	600/500
DFOV (mm)	600/500
DETECTOR COVERAGE (mm)	160x1.5
PITCH-SPEED	0.938
ROTATION TIME (s)	0.75
DOSE MODULATION	N/A
NOISE INDEX	N/A
Scan Length (mm)	216
DLP (mGy.cm)	46.299
CTDI PHANTOM (cm)	32
CTDIvol (mGy) (If NO mA modulation)	N/A
DOSE NOTIFICATION VALUE (mGy)	N/A
SCAN ACQUISITION DIRECTION	N/A
NUMBER OF PASSES	N/A
RECON 1	Filtered Back projection
SLICE THICKNESS (mm)	3
INTERVALS (mm)	3
ALGORITHM / KERNEL	B
ITERATIVE RECONSTRUCTION	N/A
SEND TO PACS	Yes

Each Tc-PYP or Tc-HMDP scan will be read by 3 independent readers blinded to clinical information. Previous unpublished data shows a high degree of agreement among four independent readers using a semi-quantitative score (0 to 3) with kappa ranging from 0.882 to 1.00 with an average of 0.95 for 50 scans read independently. When scores were dichotomized by positive (semi-quantitative score of 2 or 3) compared with negative (semi-quantitative score of 0 or 1), the degree of agreement among the readers was even higher (kappa of 0.891 to 1.00). (See Table 6 below) Using the quantitative score system of H/Cl ratio provided greater certainty in characterizing positive and negative scans.

Table 6. Degree of Agreement – DPD Scans Qualitative 0-1 vs 2-3

Group	Kappa	P value
Reader 1 vs. Reader 2	0.947	<0.0001
Reader 1 vs. Reader 3	0.947	<0.0001
Reader 1 vs. Reader 4	0.947	<0.0001

Reader 2 vs. Reader 3	0.891	<0.0001
Reader 2 vs. Reader 4	1.000.	<0.0001
Reader 3 vs. Reader 4	0.891	<0.0001

Based on an international multicenter collaboration, three 99m technetium-labeled radiotracers, 99mTc-99m-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), 99mTc-pyrophosphate (PYP), and 99mTc-hydroxymethylene diphosphonate (HDP), termed "bone avid" and traditionally used for bone scintigraphy, have been shown to have high diagnostic accuracy for the identification of patients with ATTR-CA when coupled with an assessment for monoclonal proteins to exclude the substrate for AL amyloidosis. In the event that PYP is unavailable due to supply shortage, the investigator has the option of using HDP until PYP becomes available again to ensure study continuation per FDA.

2.1.5.3 Two- Dimensional Echocardiography with Complete Doppler Analysis

A complete two-dimensional (2D) echo will be performed utilizing a commercially available GE Vivid-9 or Philips iU33 Echocardiography System. A standard imaging protocol will be performed. The following linear echocardiographic measurements will be obtained (by 2D or M-mode) in both end-diastole and end-systole: left ventricular (LV) internal dimension, septal wall thickness (SWT), and posterior wall thickness (PWT). Quantification of LV mass will be derived by the formula of Devereux RB et al[84] and by three dimensional-guided modified biplane Simpson's rule[85, 86]. LV and left atrial (LA) volumes (systolic and diastolic) will be measured by the biplane method of discs (modified Simpson's rule). LV ejection fraction and LA function will be calculated from these volume measurements. Doppler measures including peak early (E) and late (A) diastolic velocities from pulsed wave Doppler recordings of the mitral and the tricuspid flow velocities respectively, and E/A ratio will be calculated, along with deceleration time (DT) of early filling velocity and LV isovolumic relaxation time (IVRT). Pulsed-wave tissue Doppler imaging (TDI) will be obtained from the apical 4-chamber view for the lateral mitral annulus, medial mitral annulus and tricuspid annulus. Peak systolic (S), early (e') and late (a') diastolic velocities will be measured. Mitral-to-apical flow propagation will be measured using color M-mode technique.[87] Color-coded tissue Doppler will be obtained for derivation of global longitudinal strain[88] and speckle-tissue imaging (STI) on short-axis views to derive radial and circumferential strain and strain rate[89] according to ASE guidelines.[90] The myocardial contraction fraction, ratio of stroke volume to myocardial volume, will be calculated as previously described.[77] Pressure volume based measures including ventricular elastance, arterial elastance, the EDPVR and PVAiso-EDP relations will be estimated as previously described.[30]

2.1.5.3 Electrocardiogram

A standard 12 lead electrocardiogram will be performed with standard instrument sensitivity of 10 mm = 1 mV. A sum of precordial voltage (sum of S wave in lead V1 plus R wave in lead V_5 or V_6 [SVl + RV_5 or V_6]) will be calculated for all electrocardiograms. This sum will be used to compare data from patients with myocardial retention of radiotracer from controls. LVH will be defined when this sum is greater than 35 mm. When the sum was less than 15 mm, low voltage in the precordial leads is present.

Additional criteria to define low voltage only in the limb leads include no QRS deflection greater than 5 mm in any limb lead and low voltage in all leads is present when the average voltage in the three limb leads is <5 mm, and the average voltage in the chest leads is <10 mm.[91] Finally, the mass: voltage ratio will be calculated using the LV mass divided by the sum of S wave in lead VI plus R wave in lead V5 or V6 [SV1 + RV5 or V6]. The ECG tracings will be scanned and uploaded in RedCap.

2.1.5.4 Laboratory Analysis

Blood will be drawn and analyzed for standard chemistry (Chem-7), liver function, BNP, NT-proBNP, galectin-3, troponin I and T, vitamin A, retinol binding protein 4 (RBP4) and prealbumin. BNP, TnT, Gla-3, TnI and NT-proBNP will be measured through a research agreement with Abbott Laboratories, while the others are measured by local or affiliated research laboratories. Additionally, the kinetic stability of plasma TTR will be assessed at Scripps Laboratories using the published subunit exchange strategy, in which recombinant tagged TTR homotetramers are added to human TTR in plasma at equal concentrations to measure the rate at which the subunits exchange[80]. Tetramer dissociation is the rate-limiting step for subunit exchange and for amyloidogenesis. We hypothesize that those individuals at risk for developing WT ATTR-CA produce WT TTR exhibiting inferior kinetic stability. Quantification of TTR kinetic stabilization could serve as a surrogate biomarker for the prediction of clinical outcomes and could become part of a risk calculation or an early diagnosis strategy, after validation. Additional aliquots will be used for genetic analysis (see below) and stored at -80° for analysis in the future. Ancestry analysis will also be performed on all participants. Urine specimen will be analyzed for Vitamin A and RBP4 measurements, to learn whether its levels differ among subjects with TTR amyloidosis, and to learn more about the specific physiology of the disorder.

TTR Gene Sequencing: Genotyping will be performed on genomic DNA obtained from blood from participants for the Val122Ile mutation using a PCR-based assay in the CLIA certified laboratory at Boston University.

Ancestry Analysis: Ancestry information will be obtained after buffy coat samples are extracted from participants' blood. Analysis for ancestry will take place in a specialized laboratory at Columbia University.

CCRLE and NIH Repository: Several specimens collected during the study will be stored indefinitely in a -80°C freezer located at the CCRLE lab and used for future analysis. Some of these samples will ultimately be transferred to a NIH repository.

2.1.5.5 QOL Questionnaires

Two quality of life scales will be employed including two disease specific measures: the Kansas City Cardiomyopathy Questionnaire (KCCQ) and a more general instrument, the 12-Item Short Form Survey (SF-12v2).

2.1.5.6 Health Literacy Questionnaire

The participants' capacity to handle health -related information will be assessed with four instruments: Health Literacy, Trust, Perceived Discrimination, and English Proficiency.

2.1.5.7 Functional Capacity

Functional capacity will be determined by a hall walk of six minute duration[92] and a short physical performance battery.[93]

2.1.5.8 Phone Call Follow-Up

Participants who have no evidence of TTR CA and/or are not V122I mutation carriers will be followed-up by phone at 6 and 12 months to gather information about vital status and document reasons and dates of hospitalizations (if any occurred).

2.1.6 Hypotheses

Aim 1: To determine the prevalence of ATTR CA in Caribbean Hispanics and Blacks with HF

Hypothesis 1. Overall prevalence of ATTR CA: The prevalence of ATTR CA will be defined by the number of cases with both ATTRwt and ATTRm CA as a percentage of total enrollment. The background prevalence of Val122Ile in the US Black population is 3.4%, and we hypothesize a prevalence rate of 8% of ATTRm Val122Ile in our outpatient HF population. Since there are no data in Hispanics regarding ATTR CA prevalence, we further hypothesize that we will observe a prevalence of ATTRwt CA of 2-8% of the Hispanic population. Thus, we have conservatively defined the predicted prevalence of ATTR CA as 10-15%.

Hypothesis 2. Relative proportion of ATTR CA comprised by ATTRwt and ATTRm: As noted above, based upon the background allele frequency, we anticipate that Black patients will be much more likely to manifest ATTRm (Val122Ile) vs. ATTRwt CA, whereas Hispanic patients will be more likely to manifest ATTRwt CA. We will be able to explore the Val122Ile genotype frequency in the Hispanic population, and with the addition of cardiac amyloid scintigraphy scanning, the relative contribution of ATTRm in this population, which we postulate to be lower than Blacks. We will utilize genetic ancestry analysis data to further characterize race and ethnicity, and, as an exploratory analysis, evaluate the interaction of genetic variants identified with Val122Ile and ATTR CA as defined by radiotracer uptake.

Hypothesis 3. Proportion of gender: Published reports of ATTR Val122Ile are 75% male and reports of ATTRwt are 95% male. We hypothesize that highly sensitive cardiac amyloid scintigraphy testing will reveal a gender distribution of ATTR that is closer to 50%, based upon allele distribution. We will explore comorbidities, TTR stability, and RBP4 differences among men and women with ATTR to determine interactions with phenotypic expression.

Hypothesis 4. Progression of disease: Markers of disease progression are lacking in ATTR CA. We hypothesize that attainment of a hierarchical composite endpoint at 1-year of death, heart failure hospitalization, and 30% decline in 6-minute hall walk will be higher in ATTRm vs. ATTRwt CA. In addition, we will explore the capacity of specific markers to assess disease progression including: (1) established circulating biomarkers such as troponin I/T, NT-proBNP, BNP and Galectin-3, (2) novel biomarkers such as RBP4 and TTR concentration, (3) ECG, and

echocardiographic measures including longitudinal strain and MCF, (4) functional capacity by 6-minute hall walk and SPPB, and (5) QOL. Our preliminary data and published work[9] suggests reduced median survival for ATTRm Val122Ile CA (25 months) vs ATTRwt (43 months) in different race cohorts. We hypothesize that we will observe reduced survival and greater functional decline in ATTR Val122Ile vs ATTRwt within race cohorts.

Hypothesis 5. Performance of risk calculator: Our point-of-care risk calculation utilizing RBP4 and TTR concentration with echocardiographic and ECG determined parameters will be tested in this cohort. We will re-derive the prediction model based upon clinical variables noted above. We anticipate showing a similar degree of accuracy (ROC AUC > 0.95) for identification of cases with ATTR vs. controls

Specific Aim 2: To demonstrate that circulating RBP4 concentration is integral to TTR tetramer stability, which in turn determines the clinical characteristics of ATTR CA. We will measure TTR tetramer stability and RBP4 concentration in serum and urine collected in Aim 1. The overall hypotheses of this Aim are that measurements of TTR kinetic stability, RBP4 and TTR concentration will differentiate patients with ATTR CA vs. non-amyloid HF controls and associate with disease progression and outcomes. Specific hypotheses are as follows:

Hypothesis 1: TTR kinetic stability and RBP4 concentration: We hypothesize that at baseline, TTR kinetic stability will be lower in ATTR CA vs. non-amyloid HFpEF, and that stability will be inversely proportional to plasma RBP4 concentration. We further hypothesize that TTR kinetic stability will be lower for ATTRm vs. ATTRwt.

Hypothesis 2: TTR kinetic stability and disease progression: We hypothesize that TTR stability will associate with the specific outcomes of survival and disease progression detailed in Aim 1, sub-aim 4. We will explore further TTR stability among patients with indeterminate grade 1 cardiac amyloid scintigraphy scan results (early disease), and hypothesize that stability will be lower than non-amyloid HF controls.

Hypothesis 3: Urinary RBP4 concentrations: We hypothesize that RBP4 will be lower in plasma and higher in urine indexed for renal function by urinary creatinine, and vitamin A concentration, among patients with ATTR CA vs. non-amyloid controls. Reduced RBP4 binding, owing to TTR kinetic instability in blood, will result in increased clearance (and higher urine levels) of the protein. We will also explore RBP4 concentrations among patients with indeterminate grade 1 cardiac amyloid scintigraphy scan results, and hypothesize that serum RBP4 will be lower and urinary RBP4 higher than non-amyloid HF controls.

2.1.7 Statistical Considerations and Sample Size

Power and Sample Size: The total enrollment proposed for this study is of 800 subjects: 600 Blacks and 200 Hispanics. Amongst them, we expect approximately 80-160 ATTR CA subjects and 640-720 non-amyloid controls. The sample size planned for each race/ethnicity group achieves 80% power to

detect, as in Aim 1: (1) a prevalence of ATTR-CA of 10-15%, (2) a two-fold difference in the prevalence of ATTRm between Blacks and Hispanics (i.e., 0.08 vs 0.04, respectively), as well as, a similar difference in the prevalence of ATTRwt between Blacks and Hispanics, (3) a difference as small as 0.03 (if one truly exits) in the prevalence of ATTRwt with respect to gender (male) distribution between the two race/ethnic groups, and (4) in the ATTR CA group alone, we plan to measure the hierarchical composite endpoint of death, heart failure hospitalization, and 30% decline in 6-minute hall walk after 1 year A two-sided logrank test with an overall sample size of 80 subjects (40 in the ATTRwt and 40 in the ATTRm) achieves 82% power at a 0.05 significance level to detect a hazard ratio (HR) of 0.44 when the proportion of patients that did not progress in the mutant group is 0.60. This corresponds to a minimum difference of 15% in the probability of not having progressed past 12 months between the two groups.

Aim 2 focuses on comparing differences in TTR kinetic stability and RBP4 concentration between ATTR CA (N=80-120) and the non-amyloid group (N=680-720). These sample sizes generate >95% power to detect: Hypothesis 1) a minimum difference in RBP4 of 5, assuming SDs of 9 and 20 for ATTR CA and non-amyloid, respectively; Hypothesis 2) a mean change difference in TTR stability as small as 0.01, SD of 0.002.

2.1.8 Data Analysis Plan

Descriptive statistics will be used to summarize subjects' demographics and study outcomes. Sample proportions with exact 95% confidence intervals will be used to estimate the overall prevalence of ATTRm CA in Blacks and Hispanics and by sex. All analyses will be performed in SAS v.9.4 (Cary,NC), at a significance level of 0.05.

AIM 1: **Hypotheses 1-3:** The differences in proportions for prevalence (#1), genetic mutation status (#2), and sex (#3) will be assessed using Chi-squared/Fisher Exact tests. The genetic ancestry analysis will use 500K SNP data to perform principal components analysis (PCA) and determine the percentage of African admixture in all participants. To identify the associations of rare genetic variants in the TTR gene in ATTR CA cases vs controls, we will utilize burden testing.[94] **Hypothesis 4:** The main analysis will use the Kaplan-Meier method to estimate the probabilities of the composite endpoint, with medians (95% CI) being reported for each group. Cox regression analysis will be performed to generate the hazard ratio (95% CI), and to allow adjustment for other covariates such as sex or race/ethnicity, or explore interaction with other variables including cardiac biomarkers, echo parameters, or QOL. Alternatively, we will apply and compare Finkelstein-Schoenfeld's unmatched approach[95] to the matched win ratio,[96] both methods being used for reporting composite endpoints, with more appropriate priority given to the more clinically important event, e.g. mortality. **Hypothesis 5:** We will test the clinic-based risk calculator that was previously developed and validated in a smaller matched cohort study.[97] The proposed 4-variable model included: RBP4, LVEF, inter-ventricular septal thickness, mean limb lead QRS voltage, and generated an ROC AUC >90%. This model will be tested in our larger cohort and assessed with respect to prediction capability (AUC ROC) and goodness-of fit (Hosmer-Lemeshow test). We also plan to fit additional multivariable logistic regressions using the variables collected employing variable selection/model-building strategies and comparing the

resulting model(s) to the one proposed above. The variables in the final clinical risk model will be based on weights from the multivariate model.

AIM 2: **Hypothesis 1 and 3:** Means (95% CI) and/or medians (inter-quartile range) will be computed for all continuous measurements including RBP4/TTR concentrations. Pearson/Spearman correlation coefficients will be calculated to quantify the strength of the association between RBP4 plasma and urine concentrations in the overall ATTR CA cohort and separately by ATTRm vs. ATTRwt groups. Linear regression models will be used to assess differences in TTR kinetic stability between the two groups, adjusting for RBP4 concentration and vitamin A. **Hypothesis 2:** In addition to descriptive statistics, linear regression models will be employed to test the associations between RBP4 concentration, TTR stability, and cardiac amyloid scintigraphy heart to contralateral chest ratio between the two groups, ATTR CA vs non-amyloid HF controls, adjusting for clinical characteristics and retinol concentration.

2.2 Limitations and Assumptions

Limited subject recruitment: Our feasibility studies suggest completion of enrollment within the 5-year grant period, leveraging the resources available to us at the various sites. Furthermore, one of our sites (BMC) has already demonstrated recruitment of such a cohort in a currently funded NIH study. *Identification of other types of cardiac amyloidosis:* While unlikely, our target population may identify patients with light-chain (AL) amyloidosis. To account for other types of CA, the Co-PIs will scrutinize non-invasive testing data including echocardiography and electrocardiography for all recruited subjects. Any participant with normal TTR genotype but with testing or clinical features suggestive of CA will be referred for further characterization including serum immunofixation electrophoresis and free-light chain assay. Light-chain amyloidosis is unlikely to be identified in this small sample size given a reported incidence of 1:100,000. *Subject drop out or unwillingness to complete clinical evaluation for CA:* We do not anticipate dropout before the study blood sample is obtained (i.e. immediately after enrollment). Thus, we will be able to perform the TTR genotype analysis and RBP4/TTR measurements as described above. However, a subject might decline to proceed with all clinically recommended testing. Cardiac biopsy is needed to establish the diagnosis of ATTR which occurs in the setting of a monoclonal gammopathy and a diagnostic cardiac amyloid scintigraphy scan, hence some patients may require this as part of clinical care. We anticipate that the number of participants requiring cardiac biopsy will be quite small based upon clinical experience (< 10% of those with positive cardiac amyloid scintigraphy scan)[72]. *False negative rate of Cardiac Amyloid Scintigraphy Imaging*: In the large multicenter international study[72], with imaging performed with different isotopes (DPD, PYP and HDP) and at different times (1 and 3 hours) only 0.4% of biopsy proven ATTR CA patients had grade 0 scans, and 9% had grade 1 scans. Scan with Grades 0 and 1 result are more common with 3 hour imaging which is among the reasons we have chosen to image at 3 hours. We interpret grade 0 as being truly negative, thus the false negative rate is likely quite low. For those with grade 1 scans, while not adjudicated as positive and thus meeting diagnostic criteria for ATTR-CM, amyloid deposition may be present though quantitatively less, and below our diagnostic threshold. Our centers clinical experience with 1 hour imaging suggest that Grade 1 scans are not

common. Among the 110 patients with hypertensive, non-amyloid HFpEF, or HCM, 93% had a grade 0 scan, and 6% had a grade 1 scan. In this application, while we anticipate subjects with grade 1 uptake of CARDIAC AMYLOID SCINTIGRAPHY will be low (<10%), we will explore the significance of a grade 1 scan result in the following ways. First, we will perform SPECT/CT imaging in some subjects with grade 1 myocardial retention of radiotracer to distinguish true myocardial uptake versus blood pool in the LV cavity.. Those with the latter (grade 1 scans without myocardial retention of radiotracer but with blood pool in the LV cavity) will represent the false negative population, from which we can calculate the false negative rate. We can also determine if patients with grade 1 radiotracer uptake have a distinct clinical profile by biomarkers, echo, or functional measures and adverse outcomes as we are following patients prospectively in this study. The potential to have false positive with cardiac amyloid nuclear scintigraphy will be addressed by evaluation for AL cardiac amyloid in anyone who has myocardial retention of radiotracer greater or equal to grade 2, as this is the most common cause of a false positive scan. Every such case will be scrutinized and any subjects with normal TTR genotype but with testing or clinical features suggestive of CA will be referred for further characterization as part of routine clinical care for serum immunofixation electrophoresis and free-light chain assay to exclude light chain amyloidosis. While rare (incidence of 1:100,000), light-chain amyloidosis is the most common cause of a false positive scan. Other causes of a false positive nuclear scintigraphy that have been reported in the literature including hydroxychloroquine-mediated cardiotoxicity[98] and apolipoprotein A-1 amyloid cardiomyopathy[99] both of which are exceedingly rare and thus unlikely to be seen in our sample. *LVEF criterion:* ATTR CA is typically associated with HFpEF (LVEF>40%), but some patients with advanced disease can have moderate depressed systolic function. Notably, data is emerging that subjects with the Val122Ile variant more often present with a reduced EF. In order not to exclude this important cohort which is a focus of our efforts, we will enroll subjects with an EF >=30%. This may result in recruitment of controls with reduced EF, but we predict that exclusion of ischemic/valvular heart disease as the cause of HF will limit low EF control numbers. *Why is African ancestry not an inclusion criterion?* Given the association of the Val122Ile mutation with African ancestry, use as an inclusion criterion would enhance the identification of subjects. However, the strength of the association of Val122Ile with African ancestry is unknown. Additionally, requirement of African ancestry by genetic analysis would complicate enrollment. We will quantify ancestry[100] to determine its contribution to the observed phenotypes. *Will the Aim 2 on outcomes be confounded by participation in future clinical trials?* We will censor data on subjects enrolled in investigational trials in the longitudinal portion of the SCAN-MP study. If approved by FDA, we will not restrict access of eligible patients to any therapy that could be of clinical benefit. *Early vs. advanced disease:* We needed to choose between selecting a cohort with sufficient chance of identifying enough subjects with ATTR CA (those with HF) versus identification of subjects with early disease. Our active ascertainment of cases and use of Tc99-PYP or Tc99-HMDP, which identifies ATTR CA prior to echocardiographic changes[101, 102] coupled with inclusion of subjects with mild forms of HF, should ensure identification of subjects with earlier disease than current practice. *Reduced RBP4 expression/Vitamin A confounding:* We are not able, in this design, to specifically determine the expression of RBP4 as this would require liver biopsy and quantitative PCR. However, data suggest that RBP concentrations vary not by expression but rather by clearance[38], as we have postulated. Furthermore, we are measuring

serum vitamin A, and urinary creatinine, as a means to correct for confounding factors. *Unequal percentage of subjects with insulin resistance between groups*: As serum RBP4 concentration is increased in insulin resistance, to control for the potential confounder of unequal distribution of insulin resistance across our subject groups, we will adjust the analysis by history of diabetes and serum glucose. Our preliminary data has shown that glycated hemoglobin or lipids levels do not significantly interact with RBP4 concentrations in the target HFpEF population .

2.3 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.

3. ADVERSE AND SERIOUS ADVERSE EVENTS

3.1 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.

3.2 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).

3.3 Collecting and Reviewing Adverse Event and Outcome Information

During 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.

In addition to patient observations, relevant events and outcomes will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings found in the electronic medical record. The study team will record such information on Case Report Forms (CRFs) for each study visit and enter the information into the data collection system (RedCap) throughout the study. A checklist of potential adverse reactions to the imaging agent will be used to track all adverse events related to imaging agent for each subject. According to the package insert for Technetium Pyrophosphate, common side effects include flushing, hypotension, fever chills, nausea, vomiting and dizziness, as well as hypersensitivity reactions such as itching and various skin rashes

The study team will record AE information for start dates/times occurring any time after informed consent is obtained until 48 hours 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.

3.4 Stopping Study Procedure Due to Adverse Event

In the ordinary ongoing off-label 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 cardiac amyloid scintigraphy scanning portion of the study procedures include but are not limited to the events listed in the package insert: 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, 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.

3.5 Adverse Event Reporting

3.5.1 Reporting to the Sponsor

Each participating investigator must report every serious adverse event (SAE) to the IND sponsor at Columbia University Medical Center as soon as possible, but no later than 48 hours after becoming aware of the event via phone, email or facsimile, and must 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 site investigator must document the time of his or her awareness of the SAE in an email or facsimile to:

Name: Mathew S. Maurer, MD
Tel: (212) 932-4537
Fax: (212) 932-4538
Email: msm10@cumc.columbia.edu

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

Non-serious adverse events will be reported to the IND sponsor at Columbia University Medical Center on the Adverse Event Form through ordinary electronic data capture at RedCap. All non-serious entries related to adverse events must be completed by the participating investigator within 5 business days.

3.5.2 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.

3.5.3 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.

3.5.4 Reporting to Participating Sites

The IND Sponsor will notify all participating sites of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. In addition, the IND sponsor will review available reports concerning similar adverse events and analyze the significance of available information.

4. REGULATORY GUIDELINES

This study will be performed in accordance with the protocol, all applicable government laws, regulations, and guidances 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.

4.1 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.

4.2 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.

4.3 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.

4.4 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.

4.5 Study Data Recording and Retention

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

Essential documents should be retained for the period of time 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.

4.6 Confidentiality

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs patients should 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.

4.7 Lead Site monitoring of sub-site research activity

Columbia University Medical Center as lead site will conduct a pre-study visit, annual follow-up visits, weekly conference calls and a close-out visit, meeting with the sub-site investigators to review study goals and requirements, including but not limited to good clinical practice, regulatory documentation, informed consent process and documentation, eligibility criteria, study procedures, reportable events and source documentation. Qualifications of investigators and personnel, their training and delegation of authority will also be reviewed.

Within two weeks of each annual visit, the lead site will prepare a report of the monitoring visit findings including a list of records reviewed, any failure to report AEs, SAEs or UPs and general adherence to the protocol.

The lead site will report all SUSARs to the FDA as required by 21 CFR 312, document any non-compliance and, if necessary, remove the sub-site PI for major protocol or regulatory violations.

5. REFERENCES

1. Stangou AJ and Hawkins PN. Liver transplantation in transthyretin-related familial amyloid polyneuropathy. *Current opinion in neurology*. 2004;17:615-20.
2. Connors LH, Lim A, Prokaeva T, Roskens VA and Costello CE. Tabulation of human transthyretin (TTR) variants, 2003. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2003;10:160-84.
3. Yazaki M, Tokuda T, Nakamura A, Higashikata T, Koyama J, Higuchi K, Harihara Y, Baba S, Kametani F and Ikeda S. Cardiac amyloid in patients with familial amyloid polyneuropathy consists of abundant wild-type transthyretin. *Biochemical and biophysical research communications*. 2000;274:702-6.
4. Westermark P, Bergstrom J, Solomon A, Murphy C and Sletten K. Transthyretin-derived senile systemic amyloidosis: clinicopathologic and structural considerations. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2003;10 Suppl 1:48-54.
5. Jacobson DR, Pastore RD, Yaghoubian R, Kane I, Gallo G, Buck FS and Buxbaum JN. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. *N Engl J Med*. 1997;336:466-73.
6. Dharmarajan K and Maurer MS. Transthyretin cardiac amyloidoses in older North Americans. *Journal of the American Geriatrics Society*. 2012;60:765-74.
7. Connors LH, Prokaeva T, Lim A, Theberge R, Falk RH, Doros G, Berg A, Costello CE, O'Hara C, Seldin DC and Skinner M. Cardiac amyloidosis in African Americans: comparison of clinical and laboratory features of transthyretin V122I amyloidosis and immunoglobulin light chain amyloidosis. *Am Heart J*. 2009;158:607-14.
8. Connors LH, Doros G, Sam F, Badiee A, Seldin DC and Skinner M. Clinical features and survival in senile systemic amyloidosis: comparison to familial transthyretin cardiomyopathy. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2011;18 Suppl 1:157-9.
9. Ruberg FL, Maurer MS, Judge DP, Zeldenrust S, Skinner M, Kim AY, Falk RH, Cheung KN, Patel AR, Pano A, Packman J and Grogan DR. Prospective evaluation of the morbidity and mortality of wild-type and V122I mutant transthyretin amyloid cardiomyopathy: the Transthyretin Amyloidosis Cardiac Study (TRACS). *Am Heart J*. 2012;164:222-228 e1.
10. Berk JL, Suhr OB, Obici L, Sekijima Y, Zeldenrust SR, Yamashita T, Heneghan MA, Gorevic PD, Litchy WJ, Wiesman JF, Nordh E, Corato M, Lozza A, Cortese A, Robinson-Papp J, Colton T, Rybin DV, Bisbee AB, Ando Y, Ikeda S, Seldin DC, Merlini G, Skinner M, Kelly JW and Dyck PJ. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. *Jama*. 2013;310:2658-67.
11. Castaño A HS, Alvarez J and MS Maurer. Diflunisal for ATTR cardiac amyloidosis. *Congestive Heart Failure*. 2012.
12. Bahrami H, Kronmal R, Bluemke DA, Olson J, Shea S, Liu K, Burke GL and Lima JA. Differences in the incidence of congestive heart failure by ethnicity: the multi-ethnic study of atherosclerosis. *Arch Intern Med*. 2008;168:2138-45.
13. Bahrami H, Bluemke DA, Kronmal R, Bertoni AG, Lloyd-Jones DM, Shahar E, Szklo M and Lima JA. Novel metabolic risk factors for incident heart failure and their relationship with obesity: the MESA (Multi-Ethnic Study of Atherosclerosis) study. *J Am Coll Cardiol*. 2008;51:1775-83.

14. Mathew Maurer ILP, and Anne L. Taylor. *Chapter 49: Heart Failure in Special Populations: Racial and Ethnic Minorities, Elderly Persons, and Women*: Elsevier; 2010.
15. Massie BM, Carson PE, McMurray JJ, Komajda M, McKelvie R, Zile MR, Anderson S, Donovan M, Iverson E, Staiger C, Ptaszynska A and Investigators IP. Irbesartan in patients with heart failure and preserved ejection fraction. *N Engl J Med*. 2008;359:2456-67.
16. Cleland JG, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J and Investigators P-C. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. *Eur Heart J*. 2006;27:2338-45.
17. Yusuf S, Pfeffer MA, Swedberg K, Granger CB, Held P, McMurray JJ, Michelson EL, Olofsson B, Ostergren J, Investigators C and Committees. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. *Lancet*. 2003;362:777-81.
18. van Heerebeek L, Borbely A, Niessen HW, Bronzwaer JG, van der Velden J, Stienen GJ, Linke WA, Laarman GJ and Paulus WJ. Myocardial structure and function differ in systolic and diastolic heart failure. *Circulation*. 2006;113:1966-73.
19. Borbely A, van der Velden J, Papp Z, Bronzwaer JG, Edes I, Stienen GJ and Paulus WJ. Cardiomyocyte stiffness in diastolic heart failure. *Circulation*. 2005;111:774-81.
20. Bronzwaer JG and Paulus WJ. Matrix, cytoskeleton, or myofilaments: which one to blame for diastolic left ventricular dysfunction? *Prog Cardiovasc Dis*. 2005;47:276-84.
21. van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ, Kupreishvili K, Ijsselmuiden AJ, Schalkwijk CG, Bronzwaer JG, Diamant M, Borbely A, van der Velden J, Stienen GJ, Laarman GJ, Niessen HW and Paulus WJ. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. *Circulation*. 2008;117:43-51.
22. Lebeche D, Davidoff AJ and Hajjar RJ. Interplay between impaired calcium regulation and insulin signaling abnormalities in diabetic cardiomyopathy. *Nat Clin Pract Cardiovasc Med*. 2008;5:715-24.
23. Zile MR, Richardson K, Cowles MK, Buckley JM, Koide M, Cowles BA, Gharpuray V and Cooper Gt. Constitutive properties of adult mammalian cardiac muscle cells. *Circulation*. 1998;98:567-79.
24. Granzier HL and Irving TC. Passive tension in cardiac muscle: contribution of collagen, titin, microtubules, and intermediate filaments. *Biophys J*. 1995;68:1027-44.
25. LeWinter MM and Granzier H. Cardiac titin: a multifunctional giant. *Circulation*. 2010;121:2137-45.
26. Borbely A, Papp Z, Edes I and Paulus WJ. Molecular determinants of heart failure with normal left ventricular ejection fraction. *Pharmacol Rep*. 2009;61:139-45.
27. Gonzalez A, Lopez B, Querejeta R, Zubillaga E, Echeverria T and Diez J. Filling pressures and collagen metabolism in hypertensive patients with heart failure and normal ejection fraction. *Hypertension*. 2010;55:1418-24.
28. Bradshaw AD, Baicu CF, Rentz TJ, Van Laer AO, Boggs J, Lacy JM and Zile MR. Pressure overload-induced alterations in fibrillar collagen content and myocardial diastolic function: role of secreted protein acidic and rich in cysteine (SPARC) in post-synthetic procollagen processing. *Circulation*. 2009;119:269-80.
29. Ahmed SH, Clark LL, Pennington WR, Webb CS, Bonnema DD, Leonardi AH, McClure CD, Spinale FG and Zile MR. Matrix metalloproteinases/tissue inhibitors of metalloproteinases: relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease. *Circulation*. 2006;113:2089-96.

30. Bhuiyan T, Helmke S, Patel AR, Ruberg FL, Packman J, Cheung K, Grogan D and Maurer M. Pressure-Volume Relationships in Patients with Transthyretin (ATTR) Cardiac Amyloidosis Secondary to V122I Mutations and Wild Type TTR: TRACS (Transthyretin Cardiac Amyloid Study). *Circ Heart Fail*. 2010.
31. Lavine SJ. Genesis of the restrictive filling pattern: pericardial constraint or myocardial restraint. *J Am Soc Echocardiogr*. 2004;17:152-60.
32. Petre RE, Quaile MP, Wendt K, Houser SR, Wald J, Goldman BI and Margulies KB. Regionally heterogeneous tissue mechanics in cardiac amyloidosis. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2005;12:246-50.
33. Gonzalez-Lopez E, Gallego-Delgado M, Guzzo-Merello G, de Haro-Del Moral FJ, Cobo-Marcos M, Robles C, Bornstein B, Salas C, Lara-Pezzi E, Alonso-Pulpon L and Garcia-Pavia P. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. *Eur Heart J*. 2015;36:2585-94.
34. Goodman DS. *Plasma retinol-binding protein*. Orlando, FL.: Academic Press; 1984.
35. Hamilton JA and Benson MD. Transthyretin: a review from a structural perspective. *Cell Mol Life Sci*. 2001;58:1491-521.
36. Zanotti G and Berni R. Plasma retinol-binding protein: structure and interactions with retinol, retinoids, and transthyretin. *Vitam Horm*. 2004;69:271-95.
37. Buxbaum JN and Reixach N. Transthyretin: the servant of many masters. *Cell Mol Life Sci*. 2009;66:3095-101.
38. van Bennekum AM, Wei S, Gamble MV, Vogel S, Piantedosi R, Gottesman M, Episkopou V and Blaner WS. Biochemical basis for depressed serum retinol levels in transthyretin-deficient mice. *J Biol Chem*. 2001;276:1107-13.
39. Ingenbleek Y and Young V. Transthyretin (prealbumin) in health and disease: nutritional implications. *Annu Rev Nutr*. 1994;14:495-533.
40. Inoue S, Ohta M, Li Z, Zhao G, Takaoka Y, Sakashita N, Miyakawa K, Takada K, Tei H, Suzuki M, Masuoka M, Sakaki Y, Takahashi K and Yamamura K. Specific pathogen free conditions prevent transthyretin amyloidosis in mouse models. *Transgenic Res*. 2008;17:817-26.
41. Klabunde T, Petrassi HM, Oza VB, Raman P, Kelly JW and Sacchettini JC. Rational design of potent human transthyretin amyloid disease inhibitors. *Nat Struct Biol*. 2000;7:312-21.
42. Sekijima Y, Wiseman RL, Matteson J, Hammarstrom P, Miller SR, Sawkar AR, Balch WE and Kelly JW. The biological and chemical basis for tissue-selective amyloid disease. *Cell*. 2005;121:73-85.
43. Shah KB, Mankad AK, Castano A, Akinboboye OO, Duncan PB, Fergus IV and Maurer MS. Transthyretin Cardiac Amyloidosis in Black Americans. *Circ Heart Fail*. 2016;9:e002558.
44. Kelly JW. Mechanisms of amyloidogenesis. *Nat Struct Biol*. 2000;7:824-6.
45. Jacobson DR, Gorevic PD and Buxbaum JN. A homozygous transthyretin variant associated with senile systemic amyloidosis: evidence for a late-onset disease of genetic etiology. *Am J Hum Genet*. 1990;47:127-36.
46. Jacobson DR, Alexander AA, Tagoe C and Buxbaum JN. Prevalence of the amyloidogenic transthyretin (TTR) V122I allele in 14 333 African-Americans. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2015;22:171-4.
47. Buxbaum J, Jacobson DR, Tagoe C, Alexander A, Kitzman DW, Greenberg B, Thaneemit-Chen S and Lavori P. Transthyretin V122I in African Americans with congestive heart failure. *J Am Coll Cardiol*. 2006;47:1724-5.

48. Pages RA, Robbins J and Edelhoch H. Binding of thyroxine and thyroxine analogs to human serum prealbumin. *Biochemistry*. 1973;12:2773-9.
49. Saraiva MJ. Transthyretin mutations in hyperthyroxinemia and amyloid diseases. *Hum Mutat*. 2001;17:493-503.
50. Quintas A, Vaz DC, Cardoso I, Saraiva MJ and Brito RM. Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants. *J Biol Chem*. 2001;276:27207-13.
51. Hammarstrom P, Jiang X, Hurshman AR, Powers ET and Kelly JW. Sequence-dependent denaturation energetics: A major determinant in amyloid disease diversity. *Proc Natl Acad Sci U S A*. 2002;99 Suppl 4:16427-32.
52. Costa PP, Figueira AS and Bravo FR. Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. *Proc Natl Acad Sci U S A*. 1978;75:4499-503.
53. Tanskanen M, Peuralinna T, Polvikoski T, Notkola IL, Sulkava R, Hardy J, Singleton A, Kiuru-Enari S, Paetau A, Tienari PJ and Myllykangas L. Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. *Ann Med*. 2008;40:232-9.
54. Mirzoyev SA EW, Mohammed SF, Donovan SL, Roger VL, Grogan D, Redfield MM. Cardiac Amyloid Deposition is Common in Elderly Patients with Heart Failure and Preserved Ejection Fraction. *Circulation*. 2010;122.
55. Sikora JL, Logue MW, Chan GG, Spencer BH, Prokaeva TB, Baldwin CT, Seldin DC and Connors LH. Genetic variation of the transthyretin gene in wild-type transthyretin amyloidosis (ATTRwt). *Hum Genet*. 2015;134:111-21.
56. Ng B, Connors LH, Davidoff R, Skinner M and Falk RH. Senile systemic amyloidosis presenting with heart failure: a comparison with light chain-associated amyloidosis. *Arch Intern Med*. 2005;165:1425-9.
57. Givens RC, Russo C, Green P and Maurer MS. Comparison of cardiac amyloidosis due to wild-type and V122I transthyretin in older adults referred to an academic medical center. *Aging health*. 2013;9:229-235.
58. Gillmore JD and Hawkins PN. V122I transthyretin variant in elderly black Americans. *N Engl J Med*. 2015;372:1769.
59. Connors LH, Sam F, Skinner M, Salinaro F, Sun F, Ruberg FL, Berk JL and Seldin DC. Heart Failure Due to Age-Related Cardiac Amyloid Disease Associated With Wild-Type Transthyretin: A Prospective, Observational Cohort Study. *Circulation*. 2015.
60. Conomos MP, Laurie CA, Stilp AM, Gogarten SM, McHugh CP, Nelson SC, Sofer T, Fernandez-Rhodes L, Justice AE, Graff M, Young KL, Seyerle AA, Avery CL, Taylor KD, Rotter JI, Talavera GA, Daviglus ML, Wassertheil-Smoller S, Schneiderman N, Heiss G, Kaplan RC, Franceschini N, Reiner AP, Shaffer JR, Barr RG, Kerr KF, Browning SR, Browning BL, Weir BS, Aviles-Santa ML, Papanicolaou GJ, Lumley T, Szpiro AA, North KE, Rice K, Thornton TA and Laurie CC. Genetic Diversity and Association Studies in US Hispanic/Latino Populations: Applications in the Hispanic Community Health Study/Study of Latinos. *Am J Hum Genet*. 2016;98:165-84.
61. Yamashita T, Hamidi Asl K, Yazaki M and Benson MD. A prospective evaluation of the transthyretin Ile122 allele frequency in an African-American population. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2005;12:127-30.
62. Johnson SM, Connelly S, Fearns C, Powers ET and Kelly JW. The Transthyretin Amyloidoses: From Delineating the Molecular Mechanism of Aggregation Linked to Pathology to a Regulatory-Agency-Approved Drug. *J Mol Biol*. 2012.

63. Coelho T, Merlini G, Bulawa CE, Fleming JA, Judge DP, Kelly JW, Maurer MS, Plante-Bordeneuve V, Labaudiniere R, Mundayat R, Riley S, Lombardo I and Huertas P. Mechanism of Action and Clinical Application of Tafamidis in Hereditary Transthyretin Amyloidosis. *Neurol Ther*. 2016.
64. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Plante-Bordeneuve V, Lozeron P, Suhr OB, Campistol JM, Conceicao IM, Schmidt HH, Trigo P, Kelly JW, Labaudiniere R, Chan J, Packman J, Wilson A and Grogan DR. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology*. 2012;79:785-92.
65. Benson MD, Kluve-Beckerman B, Zeldenrust SR, Siesky AM, Bodenmiller DM, Showalter AD and Sloop KW. Targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides. *Muscle Nerve*. 2006;33:609-18.
66. Kurosawa T, Igarashi S, Nishizawa M and Onodera O. Selective silencing of a mutant transthyretin allele by small interfering RNAs. *Biochemical and biophysical research communications*. 2005;337:1012-8.
67. Suhr OB, Coelho T, Buades J, Pouget J, Conceicao I, Berk J, Schmidt H, Waddington-Cruz M, Campistol JM, Bettencourt BR, Vaishnaw A, Gollob J and Adams D. Efficacy and safety of patisiran for familial amyloidotic polyneuropathy: a phase II multi-dose study. *Orphanet journal of rare diseases*. 2015;10:109.
68. Wang AK, Coehlo T, Waddington-Cruz M, Polydefkis MJ, Dyck PJ, Scheinberg M, Plante-Bordeneuve V, Berk J, Barroso F, Adams D, Brannagan T, Whelan C, Merlini G, Drachman B, Heitner S, Conceicao I, Schmidt H, Vita G, Campistol JM, Gamez J, Gane E, Gorevic P, Oliveria ASB, Monia B, Hughes SG, Kwoh J, McEvoy BW, Baker BF, Shenker A, Millns H, Bergemann R, Ackermann EJ, Gertz M and Benson MD. Safety and Efficacy of Inotersen in Patients with Hereditary Transthyretin Amyloidosis with Polyneuropathy (NEURO-TTR). *Ann Neurol*. 2017;82:S108-S109.
69. Gertz MA, Comenzo R, Falk RH, Fermand JP, Hazenberg BP, Hawkins PN, Merlini G, Moreau P, Ronco P, Sanchorawala V, Sezer O, Solomon A and Grateau G. Definition of organ involvement and treatment response in immunoglobulin light chain amyloidosis (AL): a consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis, Tours, France, 18-22 April 2004. *Am J Hematol*. 2005;79:319-28.
70. Bokhari S, Castano A, Pozniakoff T, Deslisle S, Latif F and Maurer MS. (99m)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.
71. Castano A, Haq M, Narotsky DL, Goldsmith J, Weinberg RL, Morgenstern R, Pozniakoff T, Ruberg FL, Miller EJ, Berk JL, Dispenzieri A, Grogan M, Johnson G, Bokhari S and Maurer MS. Multicenter Study of Planar Technetium 99m Pyrophosphate Cardiac Imaging: Predicting Survival for Patients With ATTR Cardiac Amyloidosis. *JAMA Cardiol*. 2016;1:880-889.
72. Gillmore JD, Maurer MS, Falk RH, Merlini G, Damy T, Dispenzieri A, Wechalekar AD, Berk JL, Quarta CC, Grogan M, Lachmann HJ, Bokhari S, Castano A, Dorbala S, Johnson GB, Glaudemans AW, Rezk T, Fontana M, Palladini G, Milani P, Guidalotti PL, Flatman K, Lane T, Vonberg FW, Whelan CJ, Moon JC, Ruberg FL, Miller EJ, Hutt DF, Hazenberg BP, Rapezzi C and Hawkins PN. Non-Biopsy Diagnosis of Cardiac Transthyretin Amyloidosis. *Circulation*. 2016.
73. Maurer MS. Noninvasive Identification of ATTRwt Cardiac Amyloid: The Re-emergence of Nuclear Cardiology. *Am J Med*. 2015.
74. King DL, El-Khoury Coffin L and Maurer MS. Myocardial contraction fraction: a volumetric index of myocardial shortening by freehand three-dimensional echocardiography. *J Am Coll Cardiol*. 2002;40:325-9.

75. King DL, Coffin Lel K and Maurer MS. Noncompressibility of myocardium during systole with freehand three-dimensional echocardiography. *J Am Soc Echocardiogr*. 2002;15:1503-6.
76. Milani P DA, Gertz MA, Lacy MQ, Baudi FK. Kumar SK, Maurer MS, Klarich K, Hayman SR, Leung N, Dingli D, Lust LA, Lin Y, Kapoor P, Go RS, Hwa YL, Zeldenrust SR, Kyle RA, Rajkumar SV and M Grogan. In Patients with Light-Chain (AL) Amyloidosis Myocardial Contraction Fraction (MCF) Is a Simple, but Powerful Prognostic Measure That Can be Calculated from a Standard Echocardiogram (ECHO). *Blood*.
77. Tendler A, Helmke S, Teruya S, Alvarez J and Maurer MS. The myocardial contraction fraction is superior to ejection fraction in predicting survival in patients with AL cardiac amyloidosis. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2015;22:61-6.
78. Fontana M, Pica S, Reant P, Abdel-Gadir A, Treibel TA, Banypersad SM, Maestrini V, Barcella W, Rosmini S, Bulluck H, Sayed RH, Patel K, Mamhood S, Bucciarelli-Ducci C, Whelan CJ, Herrey AS, Lachmann HJ, Wechalekar AD, Manisty CH, Schelbert EB, Kellman P, Gillmore JD, Hawkins PN and Moon JC. Response to Letters Regarding Article, "Prognostic Value of Late Gadolinium Enhancement Cardiovascular Magnetic Resonance in Cardiac Amyloidosis". *Circulation*. 2016;133:e450-1.
79. Steidley DE, Schumacher J, Carlsson M, Ong ML and Maurer M. Myocardial Contraction Fraction: A Volumetric Index for Predicting Mortality in Transthyretin Cardiomyopathy. *Journal of Cardiac Failure*. 22:S48.
80. Rappley I, Monteiro C, Novais M, Baranczak A, Solis G, Wiseman RL, Helmke S, Maurer MS, Coelho T, Powers ET and Kelly JW. Quantification of transthyretin kinetic stability in human plasma using subunit exchange. *Biochemistry*. 2014;53:1993-2006.
81. Cho Y, Baranczak A, Helmke S, Teruya S, Horn EM, Maurer MS and Kelly JW. Personalized medicine approach for optimizing the dose of tafamidis to potentially ameliorate wild-type transthyretin amyloidosis (cardiomyopathy). *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2015;22:175-80.
82. Kubo SH, Schulman S, Starling RC, Jessup M, Wentworth D and Burkhoff D. Development and validation of a patient questionnaire to determine New York Heart Association classification. *J Card Fail*. 2004;10:228-35.
83. Schocken DD, Arrieta MI, Leaverton PE and Ross EA. Prevalence and mortality rate of congestive heart failure in the United States. *J Am Coll Cardiol*. 1992;20:301-6.
84. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I and Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol*. 1986;57:450-8.
85. Mor-Avi V, Sugeng L, Weinert L, MacEneaney P, Caiani EG, Koch R, Salgo IS and Lang RM. Fast measurement of left ventricular mass with real-time three-dimensional echocardiography: comparison with magnetic resonance imaging. *Circulation*. 2004;110:1814-8.
86. Takeuchi M, Nishikage T, Mor-Avi V, Sugeng L, Weinert L, Nakai H, Salgo IS, Gerard O and Lang RM. Measurement of left ventricular mass by real-time three-dimensional echocardiography: validation against magnetic resonance and comparison with two-dimensional and m-mode measurements. *J Am Soc Echocardiogr*. 2008;21:1001-5.
87. Garcia MJ, Ares MA, Asher C, Rodriguez L, Vandervoort P and Thomas JD. An index of early left ventricular filling that combined with pulsed Doppler peak E velocity may estimate capillary wedge pressure. *J Am Coll Cardiol*. 1997;29:448-54.

88. Hoffmann R, Altiok E, Nowak B, Heussen N, Kuhl H, Kaiser HJ, Bull U and Hanrath P. Strain rate measurement by doppler echocardiography allows improved assessment of myocardial viability inpatients with depressed left ventricular function. *J Am Coll Cardiol*. 2002;39:443-9.
89. Amundsen BH, Helle-Valle T, Edvardsen T, Torp H, Crosby J, Lyseggen E, Stoylen A, Ihlen H, Lima JA, Smiseth OA and Slordahl SA. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. *J Am Coll Cardiol*. 2006;47:789-93.
90. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W and Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2015;16:233-70.
91. Carroll JD, Gaasch WH and McAdam KP. Amyloid cardiomyopathy: characterization by a distinctive voltage/mass relation. *Am J Cardiol*. 1982;49:9-13.
92. Laboratories ATSCoPSfCPF. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166:111-7.
93. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA and Wallace RB. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *Journal of gerontology*. 1994;49:M85-94.
94. Lee S, Abecasis GR, Boehnke M and Lin X. Rare-variant association analysis: study designs and statistical tests. *Am J Hum Genet*. 2014;95:5-23.
95. Finkelstein DM and Schoenfeld DA. Combining mortality and longitudinal measures in clinical trials. *Stat Med*. 1999;18:1341-54.
96. Pocock SJ, Ariti CA, Collier TJ and Wang D. The win ratio: a new approach to the analysis of composite endpoints in clinical trials based on clinical priorities. *Eur Heart J*. 2012;33:176-82.
97. Dungu JN, Papadopoulou SA, Wykes K, Mahmood I, Marshall J, Valencia O, Fontana M, Whelan CJ, Gillmore JD, Hawkins PN and Anderson LJ. Afro-Caribbean Heart Failure in the United Kingdom: Cause, Outcomes, and ATTR V122I Cardiac Amyloidosis. *Circ Heart Fail*. 2016;9.
98. Chang ICY, Bois JP, Bois MC, Maleszewski JJ, Johnson GB and Grogan M. Hydroxychloroquine-Mediated Cardiotoxicity With a False-Positive (99m)Technetium-Labeled Pyrophosphate Scan for Transthyretin-Related Cardiac Amyloidosis. *Circ Cardiovasc Imaging*. 2018;11.
99. Quarta CC, Obici L, Guidalotti PL, Pieroni M, Longhi S, Perlini S, Verga L, Merlini G and Rapezzi C. High 99mTc-DPD myocardial uptake in a patient with apolipoprotein AI-related amyloidotic cardiomyopathy. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2013;20:48-51.
100. Alexander DH, Novembre J and Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res*. 2009;19:1655-64.
101. Glaudemans AW, van Rheenen RW, van den Berg MP, Noordzij W, Koole M, Blokzijl H, Dierckx RA, Slart RH and Hazenberg BP. Bone scintigraphy with (99m)technetium-hydroxymethylene diphosphonate allows early diagnosis of cardiac involvement in patients with transthyretin-derived systemic amyloidosis. *Amyloid : the international journal of experimental and clinical investigation : the official journal of the International Society of Amyloidosis*. 2014;21:35-44.
102. Bokhari S, Shahzad R, Castano A and Maurer MS. Nuclear imaging modalities for cardiac amyloidosis. *J Nucl Cardiol*. 2014;21:175-84.

6. APPENDICES

APPENDIX 1 HEART FAILURE CLINICAL SCORE

Clinical Variables	Score
Dyspnea/difficulty breathing	
Trouble with breathing (shortness of breath)	
Hurrying on the level or up slight hill	1
At ordinary pace on the level?	1
Do you stop for breath when walking at own pace?	2
Do you stop for breath after 100 yards on the level?	2
Physical examination	
Heart rate (beats/min)	
91 to 110	1
111 +	2
Rales/crackles	
Either lower lung field	1
Either lower and either upper lung field	2
Jugulovenous distension	
Alone	1
Plus edema	2
Plus hepatomegaly	2
Chest X-ray film	
Cephalization of pulmonary vessels	1
Interstitial edema	2
Alveolar fluid plus pleural fluid	3
Interstitial edema plus pleural fluid	3

APPENDIX 2 THE BORG SCALE

PLEASE, GRADE YOUR LEVEL OF SHORTNESS OF BREATH AND FATIGUE ACCORDING TO THIS SCALE:

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight (light)
3	Moderate
4	Somewhat severe
5	Severe (heavy)
6	
7	Very severe
8	
9	
10	Very, very severe (maximal)

APPENDIX 3 NUTRITION FACTS OF A CONTAINER OF A PINT OF ICE CREAM

Nutrition Facts	
Serving Size	1/2 cup
Servings per container	4
Amount per serving	
Calories 250	Fat Cal 120
	%DV
Total Fat 13g	20%
Sat Fat 9g	40%
Cholesterol 28mg	12%
Sodium 55mg	2%
Total Carbohydrate 30g	12%
Dietary Fiber 2g	
Sugars 23g	
Protein 4g	8%

* Percent Daily Values (DV) are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

Ingredients: Cream, Skim Milk, Liquid Sugar, Water, Egg Yolks, Brown Sugar, Milkfat, Peanut Oil, Sugar, Butter, Salt, Carrageenan, Vanilla Extract.

APPENDIX 4 CASE REPORT FORMS

SCHEDULE OF ACTIVITIES – SCREENING

Order	Activity	Done	Not Done	Notes
1	Informed consent	☐	☐	
2	Inclusion and Exclusion Criteria	☐	☐	
3	Demographics and Contact Information	☐	☐	

Form Completed by: _____

Visit: **BL** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __

SCHEDULE OF ACTIVITIES – BASELINE

Order	Activity	Done	Not Done	Notes
1	Baseline history	☐	☐	
2	Medications	☐	☐	
3	Height and Weight	☐	☐	
4	Vital signs	☐	☐	
5	Clinical examination	☐	☐	
6	Electrocardiogram	☐	☐	
7	Short physical performance battery	☐	☐	
8	Six-minute walk test	☐	☐	
9	Echocardiogram	☐	☐	
10	IV catheter placement	☐	☐	
11	Blood/urine samples collection	☐	☐	
	a. Basic metabolic panel	☐	☐	
	b. Hepatic function panel	☐	☐	
	c. NT-proBNP, BNP and Galectin-3	☐	☐	
	d. Troponin I and T	☐	☐	
	e. Vitamin A / RBP4 in plasma	☐	☐	
	f. Vitamin A / RBP4 in urine	☐	☐	
	g. Prealbumin	☐	☐	
	h. TTR kinetic stability	☐	☐	
	i. TTR gene sequencing	☐	☐	
	j. Ancestry analysis	☐	☐	
12	Injection of radioisotope (^{99m}Tc-PYP or ^{99m}Tc-HMDP)	☐	☐	
13	QoL questionnaires	☐	☐	
14	Health Literacy Questionnaire	☐	☐	
15	Cardiac Amyloid Scintigraphy scan	☐	☐	

Form Completed by: _____

Visit: **M6** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __

SCHEDULE OF ACTIVITIES – MONTH 6

Order	Activity	Done	Not Done	Notes
1	Weight	☐	☐	
2	Vital signs	☐	☐	
3	Medications	☐	☐	
4	Clinical examination	☐	☐	
5	Adverse events review	☐	☐	
6	Electrocardiogram	☐	☐	
7	Blood/urine samples collection			
	a. Basic metabolic panel	☐	☐	
	b. Hepatic function panel	☐	☐	
	c. NT-proBNP, BNP and Galectin-3	☐	☐	
	d. Troponin I and T	☐	☐	
	e. Vitamin A / RBP4 in plasma	☐	☐	
	f. Vitamin A / RBP4 in urine	☐	☐	
	g. Prealbumin	☐	☐	
8	Short physical performance battery	☐	☐	
9	Six-minute walk test	☐	☐	
10	QoL questionnaires	☐	☐	

Form Completed by: _____

SCHEDULE OF ACTIVITIES – MONTH 12

Order	Activity	Done	Not Done	Notes
1	Weight	☐	☐	
2	Vital signs	☐	☐	
3	Medications	☐	☐	
4	Clinical examination	☐	☐	
5	Adverse events review	☐	☐	
6	Electrocardiogram	☐	☐	
7	Blood/urine samples collection			
	a. Basic metabolic panel	☐	☐	
	b. Hepatic function panel	☐	☐	
	c. NT-proBNP, BNP and Galectin-3	☐	☐	
	d. Troponin I and T	☐	☐	
	e. Vitamin A / RBP4 in plasma	☐	☐	
	f. Vitamin A / RBP4 in urine	☐	☐	
	g. Prealbumin	☐	☐	
	h. TTR kinetic stability	☐	☐	
8	Short physical performance battery	☐	☐	
9	Six-minute walk test	☐	☐	
10	QoL questionnaires	☐	☐	
11	Echocardiogram	☐	☐	

Form Completed by: _____

HEART FAILURE CLINICAL SCORE – NHANES CRITERIA

Clinical Variables	Check if present
Dyspnea/difficulty breathing	
Trouble with breathing (shortness of breath)	
Hurrying on the level or up slight hill	☐ 1
At ordinary pace on the level?	☐ 1
Do you stop for breath when walking at own pace?	☐ 2
Do you stop for breath after 100 yards on the level?	☐ 2
Physical examination	
Heart rate (beats/min)	
91 to 110	☐ 1
111 +	☐ 2
Rales/crackles	
Either lower lung field	☐ 1
Either lower and either upper lung field	☐ 2
Jugulovenous distension	
Alone	☐ 1
Plus edema	☐ 2
Plus hepatomegaly	☐ 2
Chest X-ray film	
Cephalization of pulmonary vessels	☐ 1
Interstitial edema	☐ 2
Alveolar fluid plus pleural fluid	☐ 3
Interstitial edema plus pleural fluid	☐ 3
Score:	__ __

Form Completed by: _____

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria	Yes	No
1. Black or Hispanic of Caribbean origin.	☐	☐
2. Age ≥60 years.	☐	☐
3. Diagnosis of heart failure, confirmed by two methods:	☐	☐
a. Modified criteria utilized by Rich et al. which include a history of acute pulmonary edema or the occurrence of at least two of the following that improved with diuretic therapy without another identifiable cause: dyspnea on exertion, paroxysmal nocturnal dyspnea, orthopnea, bilateral lower extremity edema or exertional fatigue, and	☐	☐
b. NHANES CHF criteria with a score ≥3.	☐	☐
4. Left ventricular septal OR inferolateral wall thickness ≥12 mm.	☐	☐
5. Ejection fraction >30%	☐	☐
6. Able to understand and sign the informed consent document after the nature of the study has been fully explained.	☐	☐

Exclusion Criteria	Yes	No
1. Primary amyloidosis (AL) or secondary amyloidosis (AA).	☐	☐
2. Prior liver or heart transplantation.	☐	☐
3. Active malignancy or non-amyloid disease with expected survival of less than 1 year.	☐	☐
4. Heart failure, in the opinion of the investigator, primarily caused by severe left-sided valve disease. *Note: if valve was repaired, subject no longer with severe valve disease.*	☐	☐
5. Heart failure, in the opinion of the investigator, primarily caused by ischemic heart disease.	☐	☐
6. Ventricular assist device or anticipated within the next 6 months.	☐	☐
7. Impairment from stroke, injury or other medical disorder that precludes participation in the study.	☐	☐
8. Disabling dementia or other mental or behavioral disease.	☐	☐
9. Enrollment in a clinical trial not approved for co-enrollment.	☐	☐
10. Expected use of continuous intravenous inotropic therapy in the next 6 months.	☐	☐
11. High risk for non-adherence as determined by screening evaluation.	☐	☐
12. Inability or unwillingness to comply with the study requirements.	☐	☐
13. Chronic kidney disease with eGFR <15 mL/min/1.73 m2 or ESRD.	☐	☐
14. Weight >350 lb.	☐	☐
15. Nursing home resident.	☐	☐
16. Other reason that would make the subject inappropriate for entry into this study.	☐	☐
Specify: _____		

Form Completed by: _____

DEMOGRAPHICS AND CONTACT INFORMATION

Demographics

1. Name

   Last Name: _____

   First Name: _____

2. Gender

   ☐ Male ☐ Female

3. Child Bearing Potential

   If the subject is female is she of childbearing potential?

   ☐ Yes ☐ No

   If No, why? (check one reason only)

   ☐ ≥2 yrs postmenopausal ☐ Bilateral oopherectomy ☐ Hysterectomy

   ☐ Other (specify): _____

4. Date of Birth: __ __ / __ __ / __ __ __ __ (mm/dd/yyyy)

5. Date of Enrollment: __ __ / __ __ / __ __ __ __ (mm/dd/yyyy)

6. Age at Enrollment: __ __ years

7. Racial Identity (check all that apply)

   ☐ American Indian/Alaska Native

   ☐ Asian

   ☐ Black or African American

   ☐ Native Hawaiian or Other Pacific Islander

   ☐ White

   ☐ Unknown or Not Reported

8. Hispanic Origin

   ☐ Yes

   ☐ No

   ☐ Unknown or Not Reported

9. Country of Origin

☐ Bahamas	☐ El Salvador	☐ Honduras	☐ Senegal
☐ Belize	☐ Gambia	☐ Ivory Coast	☐ St Croix
☐ Costa Rica	☐ Ghana	☐ Jamaica	☐ St Lucia
☐ Cuba	☐ Guinea	☐ Nigeria	☐ St Maarten
☐ Dominican Republic	☐ Guyana	☐ Panama	☐ Trinidad and Tobago
☐ Ecuador	☐ Haiti	☐ Puerto Rico	☐ Venezuela

☐ Other (specify): _____

10. Marital Status

- ☐ Married
- ☐ Divorced/separated
- ☐ Widowed
- ☐ Living as married/living with partner
- ☐ Single (never married)
- ☐ Did not answer

11. Highest level of education

- ☐ Less than high school
- ☐ High school/GED
- ☐ 2-year or 4-year college degree
- ☐ Graduate or post-graduate degree
- ☐ Did not answer

12. Insurance

- ☐ Medicare
- ☐ Medicaid
- ☐ Military/VA
- ☐ Private/Other
- ☐ Self/None

Contact Information

1. Primary Address

   Address: _____

   City, State, Zip Code: _____

   Home phone number: (__ __ __) __ __ __ - __ __ __ __

   Mobile phone number: (__ __ __) __ __ __ - __ __ __ __

2. Secondary Address (if applicable)

   Address: _____

   City, State, Zip Code: _____

   Home phone number: (__ __ __) __ __ __ - __ __ __ __

3. Social Security Number (SSN)

   __ __ __ - __ __ - __ __ __ __

4. Contacts

   Primary Contact

   Full Name: _____

   Address: _____

   City, State, Zip Code: _____

   Home phone number: (__ __ __) __ __ __ - __ __ __ __

   Mobile phone number: (__ __ __) __ __ __ - __ __ __ __

   Secondary Contact

   Full Name: _____

   Address: _____

   City, State, Zip Code: _____

   Home phone number: (__ __ __) __ __ __ - __ __ __ __

   Mobile phone number: (__ __ __) __ __ __ - __ __ __ __

5. Primary Care Provider

   Full Name: _____

   Address: _____

   City, State, Zip Code: _____

   Home phone number: (__ __ __) __ __ __ - __ __ __ __

   Mobile phone number: (__ __ __) __ __ __ - __ __ __ __

6. Cardiologist

   Full Name: _____

   Address: _____

   City, State, Zip Code: _____

   Home phone number: (__ __ __) __ __ __ - __ __ __ __

   Mobile phone number: (__ __ __) __ __ __ - __ __ __ __

Form Completed by: _____

BASELINE HISTORY

Medical History

Have you ever been told by a doctor, nurse or any other health professional that you had any of the following conditions?	Yes	No
1. Congestive heart failure	☐	☐
2. Atrial fibrillation or atrial flutter	☐	☐
3. Other arrhythmias or irregular heart beat	☐	☐
4. Coronary artery disease	☐	☐
5. Myocardial infarction or heart attack	☐	☐
6. Hypertension	☐	☐
7. Hyperlipidemia or hypercholesterolemia	☐	☐
8. Peripheral vascular disease (blockages in the blood vessels or arteries of the legs	☐	☐
9. Stroke	☐	☐
10. Chronic kidney disease	☐	☐
11. Hyperthyroidism	☐	☐
12. Hypothyroidism	☐	☐
13. Chronic hepatitis	☐	☐
14. Hepatic cirrhosis	☐	☐
15. Anemia	☐	☐
16. Chronic obstructive pulmonary disease (COPD), chronic bronchitis or emphysema	☐	☐
17. Asthma	☐	☐
18. Osteoarthritis	☐	☐
19. Carpal tunnel syndrome	☐	☐
20. Spinal stenosis	☐	☐
21. Polyneuropathy	☐	☐
22. Benign prostatic hypertrophy (BPH) or enlarged prostate	☐	☐
23. Gastric or duodenal ulcer	☐	☐
24. Cancer (not skin cancer but melanoma)	☐	☐
25. Skin cancer (not melanoma)	☐	☐
26. Diabetes	☐	☐
27. Does subject have an implantable cardioverter defibrillator?	☐	☐
28. Does subject have a pacemaker?	☐	☐
If ‘Yes’, is this a biventricular pacemaker?	☐	☐

Form Completed by: _____

Risk Factors

1. Do you ever drink beer, wine liquor or any drink containing alcohol?

Yes	No	Did not answer
☐	☐	☐

(If 'No', or 'Did not answer', skip to Question 2)

a. If 'Yes', on average how many days per week do you drink alcohol?

<1 per week								Did not answer
☐	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐

b. If 'Yes', on a typical day when you drink, how many drinks do you have? (Consider a 'drink' to be a 12 oz. beer, 5 oz. glass of wine, 12 oz. wine cooler, 1 ½ oz. shot of liquor or a mixed drink made with 1 shot.) __ __

c. If 'Yes', how many times in the past month have had more than 4 or more drinks? __ __

2. Have you smoked more than 100 cigarettes in your entire life?

Yes	No	Don't know	Did not answer
☐	☐	☐	☐

(If 'No', skip to Question 4)

3. Do you now smoke cigarettes every day, some days, or not at all?

Every day	Some days	Not at all	Did not answer
☐	☐	☐	☐

4. Have any of your blood relative died from early heart disease?

(Early heart disease means <65 years in women and <55 years in men. Blood relatives refer to parents, siblings, and children.)

Yes	No	Did not answer
☐	☐	☐

Hospitalizations

Were you hospitalized in the last 6 months? ☐ Yes ☐ No

If 'yes', how many times? __ __

If 'yes', how many of these hospitalizations were due to heart failure? __ __

Form Completed by: _____

Visit: **BL** ☐ **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

BODY WEIGHT AND HEIGHT

1. Body Weight: __ __ __ Kg
2. Height: __ __ . __ m

VITAL SIGNS

	Supine	Standing
3. Blood Pressure (mmHg)	__ __ __ / __ __ __	__ __ __ / __ __ __
4. Pulse Rate (bpm)	__ __ __	__ __ __
5. Respiratory Rate (rpm)	__ __	__ __

PHYSICAL EXAM

1. JVP (cm H_20) ☐ **≤ 5** ☐ **6 - 8** ☐ **≥ 9**

	Present	Absent
2. Rales	☐	☐
3. Gallop S3	☐	☐
4. Gallop S4	☐	☐
5. Hepatomegaly	☐	☐
6. Edema	☐	☐

7. NYHA Class as per PI ☐ **I** ☐ **II** ☐ **III** ☐ **IV**
8. Incidental Findings

9. Adverse Events

LABORATORY

Incidental Findings

Form Completed by: _____

Visit: **ALL** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __

MEDICATIONS

List both prescription and over-the-counter medications the patient is taking.

Medication	Dose	Units	Route	Frequency			Start Date	Stop Date
				# Times	Time Interval	PRN		
1					D W M	☐		
2					D W M	☐		
3					D W M	☐		
4					D W M	☐		
5					D W M	☐		
6					D W M	☐		
7					D W M	☐		
8					D W M	☐		
9					D W M	☐		
10					D W M	☐		
11					D W M	☐		
12					D W M	☐		
13					D W M	☐		
14					D W M	☐		
15					D W M	☐		
16					D W M	☐		
17					D W M	☐		
18					D W M	☐		
19					D W M	☐		
20					D W M	☐		
21					D W M	☐		
22					D W M	☐		
23					D W M	☐		
24					D W M	☐		
25					D W M	☐		

Visit: **BL** ☐ **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

NYHA FUNCTIONAL CLASS QUESTIONNAIRE

Please Read to Patient:

"I will be asking you a few questions about your health status. Please, respond to the best of your ability with the answer that you feel best describes your health. All answers need to be yours and based on your own experience." *(Please ask clinical staff, relatives and other visitors to leave the room if they are with you).*

Questions can be answered as Yes/No or with one of 4 responses:

Never or none of the time
Rarely, a little of the time, or on occasion
Some of the time, a moderate amount of the time
Frequently, most of the time

Answer all applicable questions.

1. a. How often do you walk up and down stairs? (8-12 steps)

$_1$☐ Never → b. Do you avoid stairs because it makes you tired or short of breath?
$_0$☐ No $_1$☐ Yes **Go to #2**

$_2$☐ Rarely
$_3$☐ Sometimes
$_4$☐ Frequently
→ c. How often do you get short of breath or tired when you walk up or down a flight of stairs at a normal pace under normal conditions?
$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently
d. How often do you get short of breath or tired when you walk up or down a flight of stairs quickly?
$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently **Go to #2**

2. a. How often do you engage in strenuous work or prolonged exertion at work or play?

$_1$☐ Never → b. Do you think you would get short of breath or tired if you engaged in these activities?
$_0$☐ No $_1$☐ Yes **Go to #3**

$_2$☐ Rarely
$_3$☐ Sometimes
$_4$☐ Frequently
→ c. How often do you get short of breath or tired doing strenuous work or prolonged exertion?
$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently **Go to #3**

3. a. How often do you go for walks, either outside or inside, on level ground at a normal pace under normal conditions?

$_1$☐ Never → b. Do you avoid walks because it will make you short of breath?

$_0$☐ No $_1$☐ Yes **Go to #4**

$_2$☐ Rarely
$_3$☐ Sometimes
$_4$☐ Frequently

c. How often would you get short of breath or tired if you walked less than 1 block?

$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently

d. How often would you get short of breath or tired if you walked more than 2 blocks?

$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently

e. How often would you find yourself walking more slowly than usual?

$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently **Go to #4**

4. a. How often do you get short of breath or tired when you are sitting doing nothing or when you are sleeping?

$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently **Go to #5**

5. a. How often do you walk up hills?

$_1$☐ Never → b. Do you avoid walking up hills because it makes you short of breath or tired?

$_0$☐ No $_1$☐ Yes **Go to #6**

$_2$☐ Rarely
$_3$☐ Sometimes
$_4$☐ Frequently

c. How often do you get short of breath or tired walking up hills in normal weather?

$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently **Go to #6**

6. a. How often do you go out in the cold/windy or hot/humid weather?

$_1$☐ Never → b. Do you avoid cold/windy or hot/humid weather because it makes you short of breath or tired?
$_0$☐ No $_1$☐ Yes **Go to #7**

$_2$☐ Rarely
$_3$☐ Sometimes
$_4$☐ Frequently
→ c. How often do you get short of breath or tired when you go outside in the cold, windy or hot, humid weather?
$_1$☐ Never $_2$☐ Rarely $_3$☐ Sometimes $_4$☐ Frequently **Go to #7**

7. a. On a scale of 1-10, with 10 being perfectly normal and 1 being near death, how would rate yourself?

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10

Form Completed by: _____

Visit: **BL** ☐ **6M** ☐ **12M** ☐ Date: __ __ / __ __ / __ __ __ __ Time: __ __ : __ __ AM ☐ PM ☐ Study ID#: __ __ __ __

ELECTROCARDIOGRAM

Quantitative

Ventricular Rate(bpm) _____

PR Interval (msec) _____

QRS Axis (degrees) _____

QRS Duration (msec) _____

SV_1 + RV_5 or V_6 (mm) _____

Total QRS Voltage (mm) _____

Low QRS Voltage ☐ Yes ☐ No

QT Interval (msec) _____

QTc Interval (msec) _____

Overall Assessment

Rhythm

☐ Sinus Rhythm ☐ Atrial Fibrillation

☐ Other, specify: _____

Electrical Pacing on ECG ☐ Yes ☐ No

If yes, ☐ Atrial Pacing

☐ Ventricular Pacing

☐ Atrial Ventricular Pacing

ST or T-wave changes ☐ Yes ☐ No

If yes, specify reason:

A-V Block ☐ Yes ☐ No

If yes, ☐ 1st degree

☐ 2nd degree, Type I

☐ 2nd degree, Type II

☐ 3rd degree heart block

☐ Other, specify: _____

Ventricular Conduction Defect ☐ Yes ☐ No

If yes, ☐ Left Bundle Branch Block

☐ Incomplete Left Bundle Branch Block

☐ Left Anterior Hemiblock

☐ Left Posterior Hemiblock

☐ Right Bundle Branch Block

☐ Incomplete Right Bundle Branch Block

☐ Intraventricular Conduction Delay

Infarct pattern/Poor R-wave progression ☐ Yes ☐ No

If yes, infarct pattern is observed in this locations:

☐ Anteroseptal

☐ Anterior

☐ Anterolateral

☐ Lateral

☐ Inferior

☐ Other, specify: _____

Form Completed by: _____ Investigator's Signature: _____

Visit: **BL** ☐ **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

SHORT PHYSICAL PERFORMANCE BATTERY (SPPB)

1. Balance Tests

a. Side-by-Side Stand

- ☐ Held for 10 s → 1 point
- ☐ Not held for 10 s Time: __ . __ __ s
- ☐ Not attempted

→ 0 points, **go to #2**

b. Semi-Tandem Stand

- ☐ Held for 10 s → 1 point
- ☐ Not held for 10 s Time: __ . __ __ s
- ☐ Not attempted

→ 0 points, **go to #2**

c. Tandem Stand

- ☐ Held for 10 s → 2 point
- ☐ Held for 3 to 9.99 s → 1 point
- ☐ Held for < than 3 s
- ☐ Not attempted

→ 0 points

d. Balance Ordinal Score: ___ (sum points)

If not attempted, select reason:

1 ☐ Tried but unable
2 ☐ Participant could not hold position unassisted
3 ☐ Not attempted, you felt unsafe
4 ☐ Not attempted, participant felt unsafe
5 ☐ Participant unable to understand instructions
6 ☐ Other (specify): _____
7 ☐ Participant refused

2. Gait Speed Test

a. Test #1

Time for 4-meter: __ __ . __ __ s

b. Test #2

Time for 4-meter: __ __ . __ __ s

c. Walking aid

☐ None ☐ Cane ☐ Walker

d. Record best of the two times

Best time: __ __ . __ __ s

If not attempted, select reason:

1 ☐ Tried but unable
2 ☐ Participant could not walk unassisted
3 ☐ Not attempted, you felt unsafe
4 ☐ Not attempted, participant felt unsafe
5 ☐ Participant unable to understand instructions
6 ☐ Other (specify): _____
7 ☐ Participant refused

e. Gait ordinal score: ___

0 ☐ Could not do

1 ☐ >8.70 s

2 ☐ 6.21-8.70 s

3 ☐ 4.82-6.20 s

4 ☐ <4.82 s

3. Repeated Chair Stand Test

a. Safe to stand five times?

☐ Yes ☐ No

b. If five stands done, record time:

Time to complete five stands: __ __ . __ __ s

c. Chair stand ordinal score: ___

0 ☐ Unable to complete 5 chair stands or completes stands in >60 s

1 ☐ If chair stand time is ≥16.70 s and ≤60 s

2 ☐ If chair stand time is ≥13.70 s and <16.70 s

3 ☐ If chair stand time is ≥11.20 s and <13.70 s

4 ☐ if chair stand time is <11.20 s

If not attempted, select reason:

1 ☐ Tried but unable

2 ☐ Participant could not stand unassisted

3 ☐ Not attempted, you felt unsafe

4 ☐ Not attempted, participant felt unsafe

5 ☐ Participant unable to understand instructions

6 ☐ Other (specify): _____

7 ☐ Participant refused

4. Total Score

Points: ____ (sum of scores above)

Form Completed by: _____

SIX-MINUTE WALK TEST

PROMPT: "The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation."

Answer any questions the participant may have.

PROMPT: "Are you ready to do that? Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. Start now, or whenever you are ready."

1. Is participant using an assistive device? ☐ No ☐ Yes
   If "Yes"… ☐ Cane ☐ Walker
2. Supplemental oxygen during the test? ☐ No ☐ Yes
3. Measurements

	Baseline	End of Test
Time	__ __:__ __	__ __:__ __
Heart Rate (bpm)	__ __ __	__ __ __
SpO_2%	__ __ __	__ __ __
Borg Scale		
Dyspnea	__ __	__ __
Fatigue	__ __	__ __

4. Stopped or paused before 6 minutes? ☐ No ☐ Yes
   Reason: _____
5. Symptoms at end of exercise (select all that apply):
   - ☐ Angina
   - ☐ Dizziness
   - ☐ Hip pain
   - ☐ Leg pain
   - ☐ Calf pain
   - ☐ Other (specify): _____
6. Total distance walked in 6 minutes: __ __ __ meters

Form Completed by: _____

Visit: **BL** ☐ **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

THE KANSAS CITY CARDIOMYOPATHY QUESTIONNAIRE (KCCQ)

The following questions refer to your **heart failure** and how it may affect your life. Please read and complete the following questions. There are no right or wrong answers. Please mark the answer that best applies to you.

1. **Heart failure** affects different people in different ways. Some feel shortness of breath while others feel fatigue. Please indicate how much you are limited by **heart failure** (shortness of breath or fatigue) in your ability to do the following activities over the past 2 weeks.

Please place an **X** in one box on each line

Activity	**Extremely Limited**	**Quite a bit Limited**	**Moderately Limited**	**Slightly Limited**	**Not at all Limited**	**Limited for other reasons or did not do the activity**
Dressing yourself	☐	☐	☐	☐	☐	☐
Showering/Bathing	☐	☐	☐	☐	☐	☐
Walking 1 block on level ground	☐	☐	☐	☐	☐	☐
Doing yardwork, housework or carrying groceries	☐	☐	☐	☐	☐	☐
Climbing a flight of stairs without stopping	☐	☐	☐	☐	☐	☐
Hurrying or jogging (as if to catch a bus)	☐	☐	☐	☐	☐	☐

2. Compared with 2 weeks ago, have your symptoms of **heart failure** (shortness of breath, fatigue or ankle swelling) changed?
My symptoms of **heart failure** have become…

Much worse	Slightly worse	Not changed	Slightly better	Much better	I've had no symptoms over the last 2 weeks
☐	☐	☐	☐	☐	☐

3. Over the past 2 weeks, how many times did you have **swelling** in your feet, ankles or legs when you woke up in the morning?

Every morning	3 or more times a week, but not every day	1–2 times a week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐

4. Over the <u>past 2 weeks</u>, how much has **swelling** in your feet, ankles or legs bothered you?
   It has been…

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no swelling**
☐	☐	☐	☐	☐	☐

5. Over the <u>past 2 weeks</u>, on average, how many times has **fatigue** limited your ability to do what you want?

All of the time	Several times per day	At least once a day	3 or more times per week but not every day	1–2 times per week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐	☐	☐

6. Over the <u>past 2 weeks</u>, how much has your **fatigue** bothered you?
   It has been…

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no fatigue**
☐	☐	☐	☐	☐	☐

7. Over the <u>past 2 weeks</u>, on average, how many times has **shortness of breath** limited your ability to do what you wanted?

All of the time	Several times per day	At least once a day	3 or more times per week but not every day	1–2 times per week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐	☐	☐

8. Over the <u>past 2 weeks</u>, how much has your **shortness of breath** bothered you?
   It has been…

Extremely bothersome	**Quite a bit** bothersome	**Moderately** bothersome	**Slightly** bothersome	**Not at all** bothersome	I've had **no shortness of breath**
☐	☐	☐	☐	☐	☐

9. Over the <u>past 2 weeks</u>, on average, how many times have you been forced to sleep sitting up in a chair or with at least 3 pillows to prop you up because of **shortness of breath**?

Every night	3 or more times a week, but not every day	1–2 times a week	Less than once a week	Never over the past 2 weeks
☐	☐	☐	☐	☐

10. **Heart failure** symptoms can worsen for a number of reasons. How sure are you that you know what to do, or whom to call, if your **heart failure** gets worse?

Not at all sure	**Not very** sure	**Somewhat** sure	**Mostly** sure	**Completely** sure
☐	☐	☐	☐	☐

11. How well do you understand what things you are able to do to keep your **heart failure** symptoms from getting worse? (for example, weighing yourself, eating a low salt diet, etc.)

Do not understand at all	Do not understand very well	Somewhat understand	Mostly understand	Completely understand
☐	☐	☐	☐	☐

12. Over the <u>past 2 weeks</u>, how much has your **heart failure** limited your enjoyment of life?

It has **extremely** limited my enjoyment of life	It has limited my enjoyment of life **quite a bit**	It has **moderately** limited my enjoyment of life	It has **slightly** limited my enjoyment of life	It has **not limited** my enjoyment of life at all
☐	☐	☐	☐	☐

13. If you had to spend the rest of your life with your **heart failure** the way it is right now, how would you feel about this?

Not at all satisfied	Mostly dissatisfied	Somewhat satisfied	Mostly satisfied	Completely satisfied
☐	☐	☐	☐	☐

14. Over the <u>past 2 weeks</u>, how often have you felt discouraged or down in the dumps because of your **heart failure**?

I felt that way **all of the time**	I felt that way **most of the time**	I **occasionally** felt that way	I **rarely** felt that way	I **never** felt that way
☐	☐	☐	☐	☐

15. How much does your **heart failure** affect your lifestyle? Please indicate how your **heart failure** may have limited your participation in the following activities over the <u>past 2 weeks</u>.

Please place an **X** in one box on each line

Activity	**Severely** limited	Limited **quite a bit**	**Moderately** limited	**Slightly** limited	**Did not** limit at all	Does not apply or did not do for other reasons
Hobbies, recreational activities	☐	☐	☐	☐	☐	☐
Working or doing household chores	☐	☐	☐	☐	☐	☐
Visiting family or friends out of your home	☐	☐	☐	☐	☐	☐
Intimate relationships with loved ones	☐	☐	☐	☐	☐	☐

Form Completed by: _____

Visit: **BL** ☐ **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

12-ITEM SHORT FORM SURVEY (SF-12v2™)

This survey asks for your views about your health. This information will help you keep track of how you feel and how well you are able to do your usual activities. Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
1☐	2☐	3☐	4☐	5☐

2. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1☐	2☐	3☐
b. Climbing several flights of stairs	1☐	2☐	3☐

3. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Accomplished less than you would like	1☐	2☐	3☐	4☐	5☐
b. Were limited in the kind of work or other activities	1☐	2☐	3☐	4☐	5☐

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Accomplished less than you would like	1☐	2☐	3☐	4☐	5☐
b. Did work or other activities less carefully than usual	1☐	2☐	3☐	4☐	5☐

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
1☐	2☐	3☐	4☐	5☐

6. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Have you felt calm and peaceful?	1☐	2☐	3☐	4☐	5☐
b. Did you have a lot of energy?	1☐	2☐	3☐	4☐	5☐
c. Have you felt downhearted and depressed?	1☐	2☐	3☐	4☐	5☐

7. During the past 4 weeks, how much of the time has our physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
1☐	2☐	3☐	4☐	5☐

Form Completed by: _____

HEALTH LITERACY

Read to subject:

"This information is on the back of a container of a pint of ice cream."

Questions

	Answer Correct? Yes	No
1. If you eat the entire container, how many calories will you eat? **Answer** ☐ 1,000 is the correct answer	☐	☐
2. If you are allowed to eat 60 g of carbohydrates as a snack, how much ice cream could you have? **Answer** Any of the following is correct: ☐ 1 cup (or any amount up to 1 cup) ☐ Half the container Note: If patient answers "2 servings," ask "How much ice cream would that be if you were to measure it into a bowl?"	☐	☐
3. Your doctor advises you to reduce the amount of saturated fat in your diet. You usually have 42 g of saturated fat each day, which includes 1 serving of ice cream. If you stop eating ice cream, how many grams of saturated fat would you be consuming each day? **Answer** 33 is the only correct answer	☐	☐
4. If you usually eat 2500 calories in a day, what percentage of your daily value of calories will you be eating if you eat one serving? **Answer** 10% is the only correct answer	☐	☐
Pretend that you are allergic to the following substances: Penicillin, peanuts, latex gloves, and bee stings.		
5. Is it safe for you to eat this ice cream? **Answer** ☐ No	☐	☐
6. (Ask only if patient responds "no" to question 5): Why not? **Answer** Because it has peanut oil.	☐	☐

Total Correct _____

Form Completed by: _____

Visit: **BL** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __

TRUST

	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
1. I doubt that my doctor really cares about me as a person.	☐	☐	☐	☐	☐
2. My doctor is usually considerate of my needs and puts them first.	☐	☐	☐	☐	☐
3. I trust my doctor so much I always try to follow his/her advice.	☐	☐	☐	☐	☐
4. If my doctor tells me something is so, then it must be true.	☐	☐	☐	☐	☐
5. I sometimes distrust my doctor's opinion and would like a second one.	☐	☐	☐	☐	☐
6. I trust my doctor's judgments about my medical care.	☐	☐	☐	☐	☐
7. I feel my doctor does not do everything he/she should for my medical care.	☐	☐	☐	☐	☐
8. I trust my doctor to put my medical needs above all other considerations when treating my medical problems.	☐	☐	☐	☐	☐
9. My doctor is a real expert in taking care of medical problems like mine.	☐	☐	☐	☐	☐
10. I trust my doctor to tell me if a mistake was made about my treatment.	☐	☐	☐	☐	☐
11. I sometimes worry that my doctor may not keep the information we discuss totally private.	☐	☐	☐	☐	☐

Form Completed by: _____

PERCEIVED DISCRIMINATION

Everyday Discrimination Scale (Short Version)

In your day-to-day life how often have any of the following things happened to you?

	Almost everyday	At least once a week	A few times a month	A few times a year	Less than once a year	Never
1. You are treated with less courtesy or respect than other people.	☐	☐	☐	☐	☐	☐
2. You receive poorer service than other people at restaurants or stores.	☐	☐	☐	☐	☐	☐
3. People act as if they think you are not smart.	☐	☐	☐	☐	☐	☐
4. People act as if they are afraid of you.	☐	☐	☐	☐	☐	☐
5. You are threatened or harassed.	☐	☐	☐	☐	☐	☐

Follow-up Question (Asked only of those answering "A few times a year" or more frequently to at least one question.): What do you think is the main reason for these experiences? (check more than one if volunteered).

Recommended options:

1. ☐ Your Ancestry or National Origins
2. ☐ Your Gender
3. ☐ Your Race
4. ☐ Your Age
5. ☐ Your Religion
6. ☐ Your Height
7. ☐ Your Weight
8. ☐ Some other Aspect of Your Physical Appearance
9. ☐ Your Sexual Orientation
10. ☐ Your Education or Income Level

Other possible categories to consider:

1. ☐ A physical disability
2. ☐ Your shade of skin color
3. ☐ Your tribe
4. ☐ Other (specify):_____

Form Completed by: _____

Visit: **BL** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __

ENGLISH PROFICIENCY

	Poor	Fair	Good	Excellent
1. How well do you speak English	☐	☐	☐	☐
2. How well do you write English	☐	☐	☐	☐
3. How well do you read English	☐	☐	☐	☐

Form Completed by: _____

Visit: **BL** ☐ **12M** ☐ Date: __ __ / __ __ / __ __ __ __ Time: __ __ : __ __ AM ☐ PM ☐ Study ID#: __ __ __ __ __

ECHOCARDIOGRAM

Dimensions	***Value***
LV End Dias. Internal Dimension (cm)	
LV End Sys. Internal Dimension (cm)	
LVOT Dimension (cm)	
Left Atrial Diameter (cm)	
Inferior Vena Cava (cm)	
IVC Collapse >50% ☐ Yes ☐ No	

Volume / Area	***Value***
LV End-Diastolic Volume (ml) *(via Simpson's, Devereux and Bi-Plane)*	
LV End-Systolic Volume (ml) *(via Simpson's, Devereux and Bi-Plane)*	
LV Stroke Volume (ml)	
LV Ejection Fraction (%)	
Cardiac Output (l/min)	
LA Volume (ml) *(via Bi-Plane)*	
RA Volume (ml) *(via Bi-Plane)*	
RV EDA (cm^2)	
RV ESA (cm^2)	
RV FAC (%)	

Mass / Thickness	***Value***
LV Myocardial Mass End Diastole *(via Simpson's, Devereux and Bi-Plane)*	
LV Myocardial Mass End Systole *(via Simpson's, Devereux and Bi-Plane)*	
Intraventricular Septal Thickness *(via 2D)*	
Posterior Wall Thickness *(via 2D)*	
RV Wall Thickness *(via 2D)*	
LV Wall Thickness at Mid-Cavity *(via 2D)*	

Cardiac Valves

	Regurgitation			
	Normal	Mild	Moderate	Severe
Aortic	☐	☐	☐	☐
Mitral	☐	☐	☐	☐
Pulmonic	☐	☐	☐	☐
Tricuspid	☐	☐	☐	☐

Wall motion assessment
☐ Normal
☐ Global hypokinesis
☐ Regional wall motion abnormalities

Abnormal segments:

Hemodynamic Variables	***Value***
AV Velocity Time Integral (cm) *(area under curve traced in CW)*	
Isovolumetric Relaxation Time *(sec - measured from LVOT in PW)*	
Isovolumetric Contraction Time *(sec - measured from LVOT in PW)*	
Aortic Ejection Time (sec)	
LVPEP (sec)	
MV Peak E Wave Velocity (cm/sec)	
MV Peak A Wave Velocity (cm/sec)	
Mitral E/A Ratio	
Mitral Deceleration Time (sec)	
Mitral Inflow Deceleration Slope	
Mitral Wave A Duration (sec)	
TV Regurgitant Jet Velocity (cm/sec)	
TV Max Pressure Gradient (mmHg)	
TV Excursion – TAPSE (cm)	

Tissue Doppler	***Value***
MV Lateral Velocity (cm/sec)	
MV Septal Velocity (cm/sec)	
TV Annular Velocity (cm/sec)	

Strain	***Value***
Global Longitudinal Strain	
Average Apical Segment Strain	
Average Mid-Segment Strain	
Average Basal Segment Strain	

Incidental Findings

Form Completed by: _____ Investigator's Signature: _____

Site: **BU** ☐ **Milstein** ☐ Visit: **BL** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __

^{99m}Tc-PYP CARDIAC AMYLOID SCINTIGRAPHY Imaging Sheet

Vital signs

Time collected: __ __ : __ __ (24-hour clock)
(To be collected before dosing)

Position: ☐ Sitting ☐ Supine

Systolic blood pressure: __ __ __ mmHg

Diastolic blood pressure: __ __ __ mmHg

Heart rate: __ __ __ bpm

Injection Details

☐ ^{99m}Tc-PYP ☐ ^{99m}Tc-HMDP

Injectate lot number: _____

Assay date: __ __ / __ __ / __ __ __ __ (mm/dd/yyyy)

Assay time: __ __ : __ __ (24-hour clock)

Assay activity: _____ mCi

Injection time: __ __ : __ __ (24-hour clock)

Injection site:

☐ Antecubital

☐ Hand

☐ Other, specify: _____

Residual time: __ __ : __ __ (24-hour clock)

Residual activity: _____ mCi

Total injected activity: _____ mCi

Planar Imaging Details

Imaging start time: __ __ : __ __ (24-hour clock)

Imaging stop time: __ __ : __ __ (24-hour clock)

Time between injection and acquisition: (ideally 3 hrs)
__ __ : __ __ (hh:mm)

Image duration: _____ counts
(750,000 minimum counts)

SPECT/CT Imaging Details *(if applicable)*

Was SPECT/CT imaging performed? ☐ Yes ☐ No

Imaging start time: __ __ : __ __ (24-hour clock)

Imaging stop time: __ __ : __ __ (24-hour clock)

Time between injection and acquisition:
__ __ : __ __ (hh:mm)

Imaging protocol:

☐ SPECT

☐ Gated SPECT

☐ Gated SPECT/CT
Type of scan: ***CTAC scan***
Applied to: ***Rest***
Tube voltage (kV): ***120***
Tube current (mAs): _____
Radiation dose (DLP): _____

Adverse Reactions (AR)

Any AR to radiotracer injection? ☐ Yes ☐ No

If 'Yes', please check all that apply:

☐ Flushing ☐ Itching

☐ Hypotension ☐ Skin rash

☐ Fever ☐ Chest pain

☐ Chills ☐ Palpitations

☐ Nausea ☐ Tachycardia

☐ Vomiting ☐ Shortness of breath

☐ Dizziness ☐ Other *(specify)*: _____

Difficulties with Scan

Please note any difficulties in obtaining the scan. For example, difficulty positioning the participant, participant motion, etc. and any action taken to correct it.

Form Completed by: _____

Site: **BU** ☐ **CUMC** ☐ Visit: **BL** Date of Visit: __ __ / __ __ / __ __ __ __ Subject ID: __ __ __ __
First Reader ☐ **Second Reader** ☐ **Third Reader** ☐

CARDIAC AMYLOID SCINTIGRAPHY Findings Sheet

Nuclear Findings

Planar myocardial radiotracer uptake pattern:

☐ Absent ☐ Diffuse
☐ Focal ☐ Focal on diffuse

Semi-quantitative visual grading of radiotracer uptake – based on "Planar" images:

☐ Grade 0: no uptake and normal bone uptake
☐ Grade 1: uptake less than rib uptake
☐ Grade 2: uptake equal to rib uptake
☐ Grade 3: uptake greater than rib uptake with mild/absent rib uptake

Was SPECT imaging performed? ☐ Yes ☐ No

If 'Yes':

Semi-quantitative visual grading of radiotracer uptake – based on "Planar" and "SPECT" images:

☐ Grade 0: no uptake and normal bone uptake
☐ Grade 1: uptake less than rib uptake
☐ Grade 2: uptake equal to rib uptake
☐ Grade 3: uptake greater than rib uptake with mild/absent rib uptake

Quantitative interpretation of radiotracer uptake:

H/CL ratio: __ . __ __

If SPECT or SPECT/CT done, blood pool activity:

☐ Absent ☐ Minimal ☐ Significant

Technical Findings

Study difficulties:

☐ None
☐ Participant motion
☐ High background
☐ Inadequate count density
☐ Dose infiltration
☐ Delay in imaging
☐ Poor labeling
☐ Other, specify: _____

Artifact Findings

Incidental Findings

(Bone findings, hot spots, pleural or pericardial effusion, etc.)

Comments

Conclusion

Overall interpretation:

☐ Not suggestive of ATTR
☐ Strongly suggestive of ATTR
☐ Equivocal for ATTR / Possible early ATTR

Study quality:

☐ Uninterpretable
☐ Poor
☐ Fair
☐ Good
☐ Excellent

Form Completed by: _____ Investigator's Signature: _____

Visit: **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

ON-SITE FOLLOW-UP

1. Was the study visit conducted? ☐ Yes ☐ No

   a. If no, did the patient drop out of the study? ☐ Yes ☐ No

      If yes, date of drop out: __ __ / __ __ / __ __ __ __

      If yes, provide reason subject dropped out, and ***go to #2***:

   b. If no, did the subject die? ☐ Yes ☐ No

      If yes, date of death: __ __ / __ __ / __ __ __ __

      Is death related to study participation? ☐ Definitely ☐ Possibly ☐ Not related

      If yes, provide description/narrative, and ***go to #2***:

   c. If no, provide reason subject missed visit:

2. Has the patient been hospitalized? ☐ Yes ☐ No

   If yes, number of hospitalizations: __ __

   If yes,

	Reason	Causality			Hospital	Date of admission	Date of discharge
		HF	CVA	Other CVD			
1		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
2		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
3		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
4		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
5		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __

3. Was the patient seen in the ER? ☐ Yes ☐ No

   If yes, number of ER visits: __ __

   If yes,

	Reason	Causality			Hospital	Date of admission	Date of discharge
		HF	CVA	Other CVD			
1		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
2		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
3		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
4		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
5		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __

4. Has the patient had any ambulatory procedures/interventions (no overnight stay) ☐ Yes ☐ No

   If yes, number of procedures __ __

   If yes,

	Reason	Causality			Hospital	Date of procedure
		HF	CVA	Other CVD		
1		☐	☐	☐		__ __ / __ __ / __ __
2		☐	☐	☐		__ __ / __ __ / __ __
3		☐	☐	☐		__ __ / __ __ / __ __
4		☐	☐	☐		__ __ / __ __ / __ __
5		☐	☐	☐		__ __ / __ __ / __ __

5. Has the patient been infected with COVID-19? ☐ Yes ☐ No

   If yes, date of COVID-19 diagnosis: __ __ / __ __ / __ __ __ __

Visit: **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

Form Completed by: _____ Investigator's Signature: _____

Visit: **6M** ☐ **12M** ☐ Date of Visit: __ __ / __ __ / __ __ __ __ Study ID#: __ __ __ __

PHONE CALL FOLLOW-UP

1. Was any data collected? ☐ Yes ☐ No

   If yes, ☐ Phone ☐ Chart review ☐ Phone and chart review

   If no, reason: _____

   If no, deceased? ☐ Yes ☐ No

   If yes, date of death: __ __ / __ __ / __ __ __ __

   Is death related to study participation? ☐ Definitely ☐ Possibly ☐ Not related

   Description / Narrative:

2. Has the patient been hospitalized? ☐ Yes ☐ No

   If yes, number of hospitalizations: __ __

   If yes,

Reason	Causality			Hospital	Date of admission	Date of discharge
	HF	CVA	Other CVD			
1	☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
2	☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
3	☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
4	☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
5	☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __

3. Was the patient seen in the ER? ☐ Yes ☐ No

   If yes, number of ER visits: __ __

   If yes,

Form Completed by: _____ Investigator's Signature: _____

	Reason	Causality			Hospital	Date of admission	Date of discharge
		HF	CVA	Other CVD			
1		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
2		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
3		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
4		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __
5		☐	☐	☐		__ __ / __ __ / __ __	__ __ / __ __ / __ __

4. Has the patient had any ambulatory procedures/interventions (no overnight stay) ☐ Yes ☐ No

   If yes, number of procedures __ __

   If yes,

	Reason	Causality			Hospital	Date of procedure
		HF	CVA	Other CVD		
1		☐	☐	☐		__ __ / __ __ / __ __
2		☐	☐	☐		__ __ / __ __ / __ __
3		☐	☐	☐		__ __ / __ __ / __ __
4		☐	☐	☐		__ __ / __ __ / __ __
5		☐	☐	☐		__ __ / __ __ / __ __

5. Has the patient been infected with COVID-19? ☐ Yes ☐ No

   If yes, date of COVID-19 diagnosis: __ __ / __ __ / __ __ __ __

Form Completed by: _____ Investigator's Signature: _____

SERIOUS ADVERSE EVENT

Part A. Event Type

1. Type of adverse experience (check all that apply)

☐ Hospitalization or prolongation of hospitalization
(specify reason: _____)

☐ Persistent or significant disability. Date: __ __ / __ __ / __ __ __ __

☐ Life threatening. Date: __ __ / __ __ / __ __ __ __

☐ ER / Urgent Care Visit (specify reason: _____)

☐ Important medical event that represents significant risk or harm to the participant and may require medical or surgical intervention to prevent one of the other outcomes (e.g. hospitalization, persistent disability, death). Date: __ __ / __ __ / __ __ __ __

(Specify event: _____)

☐ Death: **SKIP TO PART C**

Part B. Nonfatal Events

2. Was the participant hospitalized overnight for this experience?

☐ No **(Skip to Question 3)**

☐ Yes, complete the following:

Hospital Name: _____

Street Address: _____

City: _____

State: _____

Zip Code: _____

Date participant **entered** the hospital: __ __ / __ __ / __ __ __ __ Check here if unknown: ☐

Date participant **left** the hospital: __ __ / __ __ / __ __ __ __ Check here if unknown/ongoing: ☐

3. Did the participant receive treatment for the experience in an ER or Urgent Care without being hospitalized overnight?

☐ No **(Skip to Question 4)**

☐ Yes, Complete the following:

Institution: _____

Street Address: _____

City: _____

State: _____

Zip Code: _____

Date of visit __ __ / __ __ / __ __ __ __ Check here if unknown: ☐

Form Completed by: _____ Investigator's Signature: _____

4. Describe this adverse experience. What was the condition or symptoms the participant was having? What tests or procedures were performed?

5. Describe any relevant history, including pre-existing conditions, medications, etc. Skip to Part D when finished.

Form Completed by: _____ Investigator's Signature: _____

Part C. Fatal Events

6. Date of death: __ __ / __ __ / __ __ __ __ Check here if unknown: ☐
7. Where did the death occur?
   - ☐ In the hospital
   - ☐ In the ER
   - ☐ Out of hospital (includes DOA, Rehab. Center, Hospice or Nursing Home; **Skip to 10**)
   - ☐ Unknown (**Skip to 10**)
8. Hospital or ER Name: _____
   Street Address: _____
   City: _____
   State: _____
   Zip Code: _____
   Date participant **entered** the hospital or ER: __ __ / __ __ / __ __ __ __ Check here if unknown: ☐
9. **For out of hospital death cases, including deaths that occur in an ER, skip to 10.**
   For hospital deaths only: describe the reason for the hospitalization, the symptoms the participant was having, and conditions treated. Also, any events, procedures, or tests that occurred before the participant died. (Skip to Part D when finished). Hospital records may be requested by the Central Study Coordinator.

Form Completed by: _____ Investigator's Signature: _____

10. For out-of hospital death (including those that occur in the ER): list the location (including city and state), social setting, and type and duration of symptoms. Specific symptoms of interest include: angina; pain or discomfort in the chest, left arm, shoulder or jaw; increased fatigue or tiredness; shortness of breath; dizziness; palpitations; paralysis; loss of speech.
    Be as specific as possible when reporting information such as the precise location of death, when the patient was last seen alive, who last saw the patient alive, and location of those final sightings in relation to the site where the participant died. The Central Study Coordinator may request any information about the patient's treatment during the last six months of life. If you need more space, write on the back of this form.

Part D. SAE Summary

11. Outcomes: Indicate any of the specific following conditions that occurred as part of this experience (mark all that apply).
    - ☐ Arrhythmia, atrial fibrillation or flutter
    - ☐ Heart Attack
    - ☐ Stroke or TIA
    - ☐ New or worsening Heart Failure/Heart Failure Exacerbation
    - ☐ Chest pain (hospitalized only)
    - ☐ Kidney Failure / Dialysis
    - ☐ GI Bleeding
    - ☐ Infection
    - ☐ Arrhythmia, Ventricular
    - ☐ Amputation of part of leg, including toes, because of poor circulation/gangrene
    - ☐ Other problems with heart, brain or circulation (specify: _____)

Form Completed by: _____ Investigator's Signature: _____

12. Was this event caused or worsened by enrollment in the study or any of its procedures?

- ☐ No
- ☐ Possibly
- ☐ Definitely

If possible or definitely, why?

_____

_____

13. Investigator Signature (Indicate when reviewed and approved by Clinic PI)

Signature: _____ Date: __ __ / __ __ / __ __ __ __

Adverse Events Log

Protocol: ______________________________ IRB #: ______________________

Principal Investigator: ______________________________ Site: ______________________

Subject ID #: ______________________________

Adverse Event (Please use medical terminology)	Serious Adverse Event	Date Site Became Aware	Start Date	End Date - OR - Continuing	Severity (Grade)	Relatedness to Study Participation	Outcome	Report to External Entities (If applicable)	PI Initial & Date
	☐ No ☐ Yes * * Complete SAE Form	____/_____/20____ (mm/dd/yyyy) ☐ PI notified	____/_____/20____ (mm/dd/yyyy)	____/_____/20____ (mm/dd/yyyy) ☐ Continuing	☐ 1 - Mild ☐ 2 - Moderate ☐ 3 - Severe ☐ 4 - Life-threatening ☐ 5 - Death	☐ Definitely ☐ Probably ☐ Possibly ☐ Unlikely ☐ Not Related ☐ Unknown	☐ Recovered/Resolved ☐ Recovered/Resolved with Sequelae ☐ Not Recovered/Not Resolved ☐ Fatal ☐ Other (specify): __________	IRB ☐ Yes ☐ No FDA ☐ Yes ☐ No Sponsor ☐ Yes ☐ No	
	☐ No ☐ Yes * * Complete SAE Form	____/_____/20____ (mm/dd/yyyy) ☐ PI notified	____/_____/20____ (mm/dd/yyyy)	____/_____/20____ (mm/dd/yyyy) ☐ Continuing	☐ 1 - Mild ☐ 2 - Moderate ☐ 3 - Severe ☐ 4 - Life-threatening ☐ 5 - Death	☐ Definitely ☐ Probably ☐ Possibly ☐ Unlikely ☐ Not Related ☐ Unknown	☐ Recovered/Resolved ☐ Recovered/Resolved with Sequelae ☐ Not Recovered/Not Resolved ☐ Fatal ☐ Other (specify): __________	IRB ☐ Yes ☐ No FDA ☐ Yes ☐ No Sponsor ☐ Yes ☐ No	
	☐ No ☐ Yes * * Complete SAE Form	____/_____/20____ (mm/dd/yyyy) ☐ PI notified	____/_____/20____ (mm/dd/yyyy)	____/_____/20____ (mm/dd/yyyy) ☐ Continuing	☐ 1 - Mild ☐ 2 - Moderate ☐ 3 - Severe ☐ 4 - Life-threatening ☐ 5 - Death	☐ Definitely ☐ Probably ☐ Possibly ☐ Unlikely ☐ Not Related ☐ Unknown	☐ Recovered/Resolved ☐ Recovered/Resolved with Sequelae ☐ Not Recovered/Not Resolved ☐ Fatal ☐ Other (specify): __________	IRB ☐ Yes ☐ No FDA ☐ Yes ☐ No Sponsor ☐ Yes ☐ No	

Version 2.1 June 2017