Title:	Novel Cardiac Magnetic Resonance Imaging to Define a Unique Restrictive Cardiomyopathy in Sickle Cell Disease
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CLINICAL RESEARCH PROTOCOL

Protocol Title:	Novel Cardiac Magnetic Resonance Imaging to Define a Unique Restrictive Cardiomyopathy in Sickle Cell Disease
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Protocol Signature Page

I, the Site Principal Investigator, agree to conduct this study in full accordance with the provisions of this protocol.

I have read and understand the information in this protocol and will ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about the obligations incurred by their contribution to the study.

_Charles T. Quinn, M.D., M.S.____
Name of Site Principal Investigator

_____ _____
Signature of Site Principal Investigator Date of Signature

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1. Abbreviations

AE ..... Adverse event
AT1R..... Angiotensin-1 receptor
ALT ..... Alanine transaminase
AST..... Aspartate transaminase
CBC ..... Complete blood count
CMR..... Cardiac magnetic resonance imaging
ECV..... Extracellular volume fraction
EF ..... Ejection fraction
GCP ..... Good clinical practices
Hb ..... Hemoglobin
HbF ..... Fetal hemoglobin
HbS..... Sickle hemoglobin
HbSβ^0..... Sickle-β^0-thalassemia
HbSS..... Sickle cell anemia (homozygous)
HCT..... Hematocrit
ICH..... International conference on harmonisation
IRB..... Institutional review board
IVRT..... Isovolumic relaxation time
LDH..... Lactate dehydrogenase
LGE..... Late gadolinium enhancement
LV..... Left ventricle (or left ventricular)
MDA..... Malonaldehyde
mPAP..... Mean pulmonary artery pressure
MR ..... Magnetic resonance
MRI ..... Magnetic resonance imaging
MR-pro-ANP ..... Mid-regional pro-atrial natriuretic peptide
NOX ..... NADPH oxidase
NT-pro-BNP ..... N-terminal pro-hormone of brain natriuretic peptide
PAH..... Pulmonary arterial hypertension
PAI-1 ..... Plasminogen activator inhibitor-1
PVR..... Pulmonary vascular resistance
Rac..... Ras-related C3 botulinum toxin substrate (Rho-family GTPase)
RBC ..... Red blood cell
RCM..... Restrictive cardiomyopathy
RAS..... Renin-angiotensin system
ROS ..... Reactive oxygen species
RV ..... Right ventricle (or right ventricular)
RVEDV..... Right ventricular end diastolic volume
RVESV..... Right ventricular end systolic volume
SAE..... Severe adverse event
SCD ..... Sickle cell disease
TDI ..... Tissue Doppler imaging
TGFβ1..... Transforming growth factor β-1
TRJV..... Tricuspid regurgitant jet velocity
VCG ..... Vectorcardiographic algorithm
VSD..... Ventricular septal defect
WB ..... Whole blood
3-d..... Three-dimensional

2. Synopsis

Sickle cell disease (SCD) causes progressive cardiopulmonary morbidity, beginning in childhood, which can ultimately be fatal. As a group, cardiopulmonary complications, such as acute chest syndrome and sudden death, are now the most common causes of death in SCD, especially in adolescents and adults.

Patients with SCD have features of both an anemia-related, high cardiac output state and a **restrictive cardiomyopathy (RCM)**. *We propose that this **unique RCM** is an overlooked and understudied complication of SCD.* RCM could explain the modest increases in pulmonary artery pressure in patients with SCD, as measured by cardiac catheterization or estimated by tricuspid regurgitant jet velocity (TRJV), which has often been attributed to a primary pulmonary arterial hypertension (PAH). RCM could also be the cause of unexplained sudden cardiac death in SCD, which is a feature of other forms of RCM.

***Our overarching hypothesis** is that increased **ROS-mediated AT1R-TGFβ1 signaling is pro-fibrotic** and, in combination with vaso-occlusive ischemia-reperfusion injury, results in an age-dependent, **progressive RCM** that can be detected by non-invasive cardiac imaging.*

This pilot, longitudinal, observational study uses a novel, comprehensive, multimodal cardiac imaging strategy, combining cutting-edge cardiac magnetic resonance imaging (CMR) and echocardiographic tissue Doppler imaging (TDI), to demonstrate **the unique RCM of SCD,** characterizing its frequency and the temporal evolution over a 2-year period. We will also correlate the RCM phenotype with biomarkers of ROS and RAS-TGFβ1 signaling.

This research could change our understanding of how SCD affects the heart and lungs. We propose studies that will change the current concept of primary pulmonary vasculopathy to a *cardiomyopathy-centered* model with secondary pulmonary vascular changes leading to sudden death. **This translational pilot study will *deliver* a novel, clear, quantifiable CMR phenotype with established diagnostic performance that will be used in phase II/III clinical trials to test anti-fibrotic therapy to prevent or reverse SCD-related RCM.**

3. Background and Rationale

SCD is the name for a group of recessive genetic disorders caused by mutant sickle hemoglobin (HbS). The most common and severe form of SCD is sickle cell anemia (HbSS), the homozygous state for the HbS mutation ($\beta^{6\ glu\rightarrow val}$). Other forms of SCD result from compound heterozygous states with HbS and other β-hemoglobinopathies, such as β^0-thalassemia (sickle-β^0-thalassemia). HbS is insoluble in the deoxygenated state. So, as HbS-containing red blood cells traverse the circulation undergoing cycles of oxygenation and deoxygenation, HbS repeatedly forms rigid polymers that damage the red blood cell (RBC) membrane and drastically shorten RBC lifespan. These HbS-containing RBCs can also obstruct the microcirculation before their premature demise. Consequently, individuals with SCD have chronic hemolytic anemia and episodic vaso-occlusive crises. Approximately 1 in 700 African-Americans has SCD at birth, and as many as 100,000 individuals are affected in the United States. [1,2].

Elevated Pulmonary Artery Pressure in SCD

Sickle cell disease (SCD) causes progressive cardiopulmonary morbidity, beginning in childhood, which can ultimately be fatal. [3] As a group, cardiopulmonary complications, such as acute chest syndrome and sudden death, are now the most common causes of death in SCD, especially in young adults. [4,5] Pulmonary arterial hypertension (PAH) has been the focus of much recent research and debate. [6,7] Echocardiographic measurements of tricuspid regurgitant jet velocity (TRJV) are commonly used to estimate the prevalence of PAH (TRJV > 2.5 m/s), which has consistently been approximately 30% in both children and adults. Indeed, an elevated TRJV is associated with early mortality in adults, [8] although not children. [9]

Multiple epidemiologic studies associate elevated right ventricular (RV) pressures with adverse events in patients with SCD. [8,10,11] These data have been interpreted to support the idea that hemolysis-related endothelial dysfunction causes PAH, which is responsible for the observed increase in RV pressure.

However, in a series of recent studies, patients were selected to undergo right heart catheterization based on echocardiographic screening. [12-14] These studies showed the prevalence of PAH (defined simply as mPAP ≥ 25 mmHg) is low, ranging from 6-10%, and that the prevalence of moderate to severe PAH is significantly less. The pulmonary vascular resistance (PVR) of these patients is markedly lower than that reported in other pulmonary hypertension populations. [12] In one study, the PVR in all the patients with SCD was within the normal range. [15] Consequently, the number of adverse events in these patients with very mild elevation in pulmonary pressures exceeds that expected in patients with idiopathic pulmonary hypertension. [16] These observations have led us to propose that the primary mechanism for cardiac morbidity and mortality including sudden death is not primary PAH, but rather **a unique restrictive cardiomyopathy (RCM)**.

Restrictive Cardiomyopathy

Several lines of data suggest that **increased right ventricular (RV) and pulmonary arterial (PA) pressure in SCD is secondary to the restrictive left ventricular (LV) physiology found in restrictive cardiomyopathy (RCM)** and not a primary form of pulmonary arterial hypertension (PAH). These lines of evidence include: **(1) It is well known that impaired diastolic filling is common in all SCD patients**, including young patients, before they develop increased RV pressures.[17] In a study by Hankins et al., all children between the ages of 6-18y had an abnormal E/e' ratio on echocardiography, a marker of increased left atrial (LA) pressure.[18] This finding was also seen in a group SCD patients undergoing sleep studies.[19] Although it has been postulated that impaired relaxation and restrictive filling is secondary to iron deposition, Hankins et al. showed no significant iron deposition by MRI T2* measurements. It has also been postulated that the diastolic dysfunction seen in these patients is secondary to hypertrophy; however, Hankins et al. also showed that no patients had abnormal age-corrected indexed LV mass. **(2) The diastolic dysfunction seen in SCD appears to precede the PA pressure elevation.** A study by Eddine et al. showed abnormalities in nearly all diastolic filling parameters in a group of 54 children with SCD, but none had significant elevations of tricuspid regurgitant jet velocity (TRJV).[20] Dham et al. reported a group of SCD children with minimal TRJV increase but with a high prevalence diastolic dysfunction.[21] **(3) There is a high rate of sudden death**, in excess of what is seen in the idiopathic PAH population, in the SCD population, with relatively low PA pressures. While not consistent with what is seen in the pediatric and adult PAH population, this high rate of sudden death is consistent with that seen in RCM, in which there is an excessive rate of sudden death (28% in a pediatric cohort; 2-year survival 50% and 5-year survival 25% in some series).[22,23] As in SCD, the risk of sudden death is particularly high in young patients with idiopathic RCM associated with PAH.

The standard definition of RCM includes (1) dilated atria, (2) normal or nearly normal left ventricular systolic function without significant ventricular dilatation; (3) no echocardiographic evidence of hypertrophic cardiomyopathy; and (4) no evidence of constrictive pericarditis. Supportive evidence for RCM includes a restrictive mitral inflow pattern by pulsed-wave Doppler. The cardiomyopathy in sickle cell disease is characterized by dilated atria, normal or supra-normal systolic function, impaired relaxation, and restrictive filling patterns. However, the ventricles are typically mildly dilated, likely due to the anemia-related high output state. We propose that the cardiomyopathy of SCD is **a unique RCM phenotype** with normal to mildly dilated left ventricle with abnormal diastolic relaxation, increasingly elevated left atrial pressures, and normal systolic function as a result of myocardial fibrosis (**Figure 1**). It is this cardiomyopathy that results in elevated post-capillary PVR, secondary PAH, and elevated TRJV.

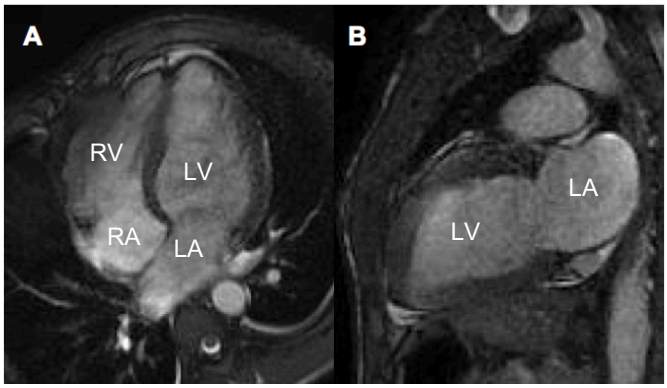

Figure 1. The restrictive cardiomyopathy of SCD. Four chamber (A) and two chamber (B) SSFP images from a 20 y.o. HbSS patient showing moderate bi-atrial dilatation, left ventricular dilatation, and normal systolic function. The left atrial volume was 79 ml/m^2 (normal ~ 45 ± 9 ml/m^2). The left ventricular end-diastolic volume was 121 ml/m^2 (normal ~ 92 ±10 ml/m^2). The ejection fraction was 66%.

Cardiac Magnetic Resonance Imaging (CMR)

CMR will be used to characterize cardiac structure and function in this study of children and adults with SCD. CMR provides functional data (systolic and diastolic) and myocardial tissue characterization. CMR is ideal for measuring ventricular volumes and masses, because it is not restricted by geometric assumptions, provides high contrast images, and is inherently three-dimensional (3-d). CMR has been validated against *ex vivo* samples for measurements in adult and pediatric ventricles. [24-27] The intra- and inter-observer variability for standard cardiac MRI measures is 3-8%. [28,29] Compared to echocardiography, CMR is also less variable and more reproducible, allowing smaller sample sizes for clinical studies (**Figure 2**).

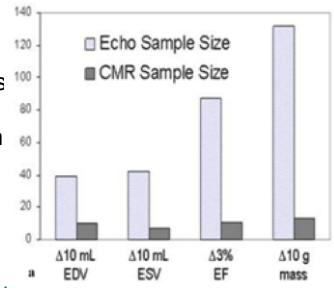

Figure 2. CMR is less variable and more reproducible than echocardiography in patients undergoing repeated LV measurements using both modalities. Standard deviations of CMR measurements are significantly lower than echocardiography. Decreased variability and greater reproducibility affords 80-90% smaller sample sizes to detect differences in cardiac structure and function.

Diastolic assessment with CMR is validated for assessment of myocardial relaxation and filling dynamics. [30,31] Using velocity sensitive phase contrast imaging of the mitral valve inflow and the left ventricular myocardium, diastolic parameters including isovolumic relaxation time (IVRT), early to late inflow ratio (E/A ratio), deceleration time (DT), and mitral valve inflow to septal velocity ratio (E/e') are measured with both accuracy and precision compared with echocardiography (**Figure 3)**. [32,33] Using standard planimetry, the left atrial volume provides an indirect measure of the filling pressure. There are also novel deformational diastolic parameters that can be measured including diastolic strain rate, untwisting, and torsion (**Figures 4, 5**). [34]

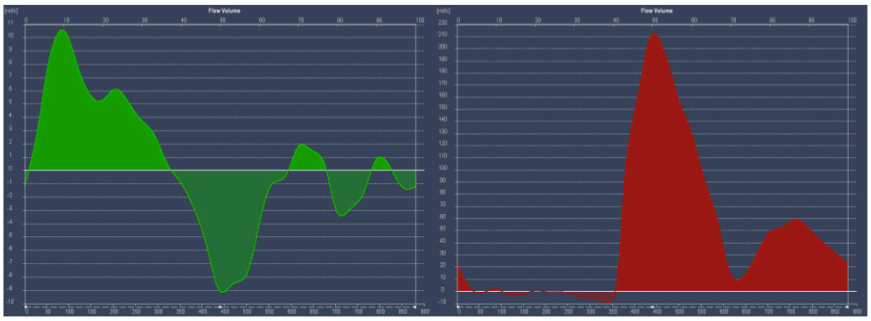

Figure 3. Restrictive LV filling in SCD. Myocardial velocity (green) and mitral valve (red) inflow phase contrast curves from a SCD patient at CCHMC. The mitral valve inflow shows a markedly increased E/A ratio and the E/e' is elevated consistent with restrictive filling.

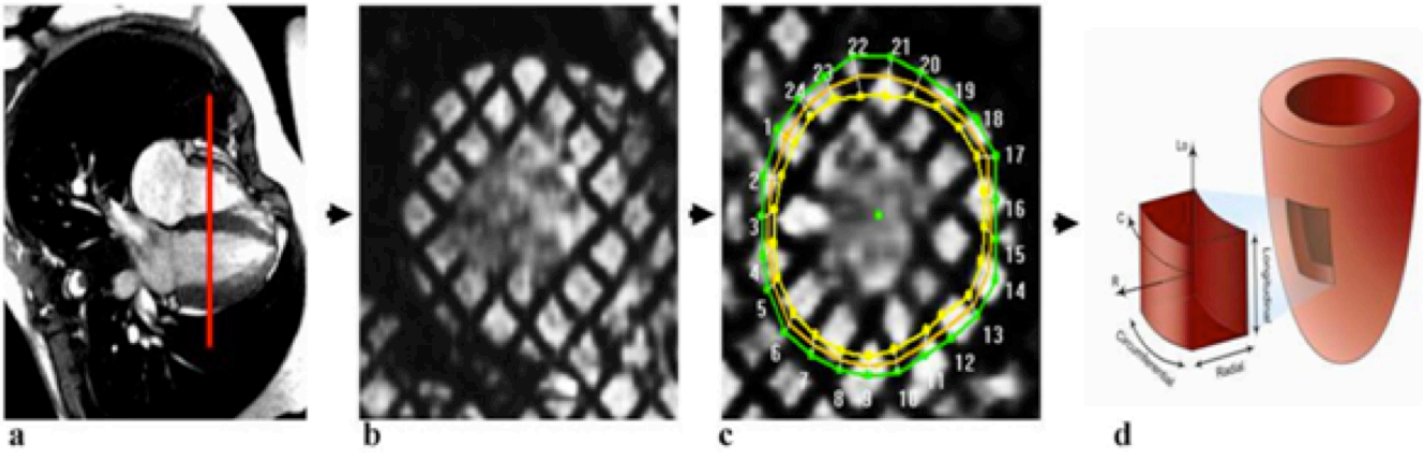

Figure 4. CMR and regional myocardial strain. The short axis of the mid-ventricle is obtained from the four-chamber view (a) at the level of the papillary muscles with a tag sequence (b). Mesh overlaying of the tag image using HARP (c). Regional Myocardial Strain (d): In this idealized LV diagram, normal strains are represented: wall thickening (radial strain, ε_{rr}), circumferential shortening (circumferential strain, ε_{cc}), and longitudinal compression (longitudinal strain, ε_{ll}). Peak composite (shown) or regional ε_{cc} can be shown graphically or exported to a spreadsheet for data analysis.

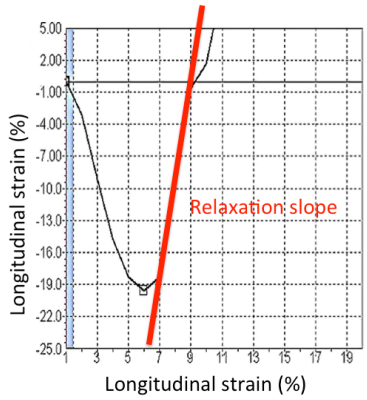

Figure 5. CMR-based deformational diastolic parameters. Diastolic slope derived from longitudinal deformation curve allows quantitative measurement of the relaxation rate.

Myocardial tissue characterization by CMR broadly involves determining signal characteristics before and after administration of IV contrast and comparing quantitative parameters with data from normal myocardium. [35] Myocardium can be differentiated from fat, fluid, inflammation, and fibrosis. [36] Regions of myocardial inflammation can be identified using T2-weighted imaging, secondary to higher fluid content in these areas of myocardial tissue. Similarly, regions of edema can be seen in active myocarditis and infiltrative cardiomyopathies. Gadolinium–diethylene-triamine pentaacetic acid (Gd-DTPA) is a partially extracted extracellular contrast agent that resides in the interstitial space of territories that receive myocardial blood flow. Territories with scar have replacement fibrosis, more interstitial space, and as a result, they retain gadolinium. **Late gadolinium enhancement (LGE)** is the most widely used and investigated CMR technique for myocardial characterization (**Figure 6**). [37,38] LGE sequences obtained approximately 10 minutes after gadolinium injection result in distinct image contrast. Gadolinium is paramagnetic resulting in significant signal differentiation. In T1-weighted pulse sequences normal myocardium is set to appear black with regions of gadolinium accumulation appearing bright. Enhanced (white) regions indicate areas of gadolinium accumulation and suggest the presence of inflammation, fibrosis, or scar.

The specific pattern of tissue characteristics can identify a given cardiomyopathy. For example, cardiac amyloidosis frequently demonstrates diffuse global subendocardial LGE. In contrast, cardiac sarcoidosis demonstrates patchy myocardial LGE of either the LV or right ventricle. Unlike other common non-ischemic cardiomyopathies, hypertrophic cardiomyopathy (HCM) is known to have a unique structural phenotype that classically involves asymmetric LV wall thickening of the basal interventricular septum. In hypertrophic cardiomyopathy, LGE is often present in a patchy midwall distribution, particularly at the insertion point of the left and right ventricles and regions of greatest hypertrophy. Non-ischemic dilated cardiomyopathies are generally recognized to have a typical midwall linear LGE pattern that is commonly located in the ventricular septum. In contrast to chronic cardiomyopathies, active myocarditis typically demonstrates focal increases in midwall and subepicardial signal on T2-weighted images, signaling the presence of edema in these areas. Farther out from the initial onset of myocarditis, epicardial LGE may be seen in areas of residual fibrosis. Using these tissue characteristics, the etiology of cardiomyopathy can be derived in many cases and may **obviate invasive myocardial biopsy**.

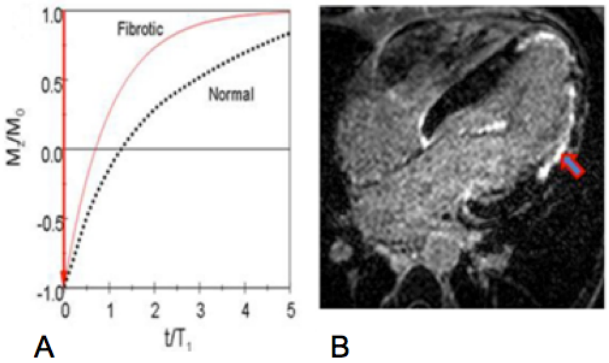

Figure 6. Late gadolinium enhancement (LGE). Panel A: CMR of fibrotic myocardium highlights differences hydrogen nuclei (spins) present throughout tissues. After applying radiofrequency energy in a magnetic field, spins in fibrotic tissue relax faster (i.e. have a shorter T1) to equilibrium compared to those in normal muscle, a difference that can be detected using the LGE imaging technique. Panel B: Sample LGE image in the 4 chamber from patient with myocardial infarction (white area) as indicated by the arrow.

The majority of studies of SCD patients showed no significant areas of LGE. However, pathology (autopsy) data show significant areas of fibrosis. Small microscopic areas of fibrosis are not detected using standard post-Gd LGE techniques. Consequently, new CMR techniques have recently been developed to **quantify the extent of diffuse myocardial fibrosis** present at a given time point. Microscopic fibrosis has been shown to be quantified by the partition coefficient of gadolinium, which can be measured with T1 mapping. With this technique, decreases in the T1 time of the myocardium reflect increased interstitial space (microfibrosis) and increased gadolinium concentration throughout the myocardium. Thus, the relative distribution of gadolinium between the blood pool and myocardium can be used to **estimate the amount of fibrosis within the myocardium**. When quantified as the extracellular volume fraction (ECV) metric (**Figure 7)**, [39] this relative distribution of gadolinium in the myocardium has been shown to correlate with the presence of fibrosis on surgical biopsies in patients with HCM. [40]

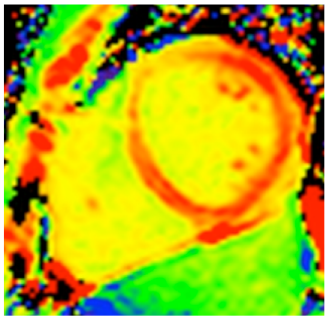

Figure 7. CMR evidence of microscopic fibrosis. ECV map of the LV at 3T in an 8 y.o. Duchenne Muscular Dystrophy patient at CCHMC without evidence of significant LGE. The mid-ventricular ECV was 35±4 % suggesting diffuse microscopic fibrosis. Normal value from the literature is 26±3%. [37]

Preliminary Data

In ***our preliminary study*** of 7 SCD patients (**Table 1**), all 7 had dilated atria and LVs, 6/7 had abnormal relaxation, and 5/7 had restrictive filling patterns with evidence of increased filling pressures. Consequently, 5/7 fit the criteria for the RCM phenotype. None had evidence of LGE by standard techniques. However, upon implementing the fibrosis imaging scheme using ECV parametric maps (**Figure 7**), our data show similar findings to those from SCD mice, where relaxation is impaired and ECV is increased. The image data were confirmed by histopathology showing increased perivascular fibrosis and myocardial fibrosis.

Table 1. Selected Cardiac MRI parameters in 7 SCD patients referenced to normal values as mean z-scores.

Parameter	Value ± SD	Mean z-score
LV end-diastolic volume index(ml/m^2)	124 ± 14	**2.96**
LV ejection fraction (%)	67 ± 6	0.92
LV mass (g/m^2)	82 ± 7	**2.56**
LV Diastolic Function		
LA volume index (ml/m^2)	65 ± 14	**3.85**
Early diastolic strain rate [Inf/Lat] (s^{-1})	59 ± 11	**4.17**
Mitral Inflow E /A	2.8 ± 0.4	1.26

Overview of Research Plan and Deliverables

We will now apply this **novel and comprehensive imaging strategy**, CMR with LGE and quantification of the extent of diffuse myocardial fibrosis using the ECV metric (with supplementation by echocardiographic TDI), **for the first time** to a representative group of children and adults with SCD to demonstrate **the unique RCM of SCD**.

This research could change our understanding of how SCD affects the heart and lungs. We propose studies that will change the current concept of primary pulmonary vasculopathy to a *cardiomyopathy-centered* model with secondary pulmonary vascular changes leading to sudden death.

This **translational pilot study** will generate the prerequisite **for phase II/III clinical trials** to test anti-fibrotic therapy **to prevent SCD-related RCM**; specifically, this study will provide ***a novel, clear, quantifiable CMR phenotype with established diagnostic performance characteristics***.

4. Specific Aims

This pilot study will provide the critical data needed to design phase II/III clinical trials to test anti-fibrotic therapy, such as losartan, as a means to prevent or reverse SCD-related RCM.

4.1. Aim 1: Frequency of RCM phenotype

Estimate the proportion of the RCM phenotype in SCD in a representative sample of children and adults with SCD and how the proportion varies by age groups in a cross-sectional sample. We will also quantify the extent of diffuse myocardial fibrosis using the extracellular volume fraction (ECV) metric.

Hypothesis: *The proportion of the RCM phenotype will be 30% in the overall study population, and the proportion increases in older age strata.*

4.2. Aim 2: Stability of RCM phenotype

Estimate the stability (resolution, non-progression, or progression) of the RCM phenotype over a 2-year period to determine its suitability as an outcome measure for a future clinical trial in children and adults with SCD who are imaged yearly.

Hypothesis: *The RCM phenotype, once detected by imaging, will not spontaneously resolve.*

4.3. Aim 3: Association of RCM with TRJV

Explore the association between tricuspid regurgitant jet velocity (TRJV) and RCM phenotype.

Hypothesis: *Individuals with a high TRJV (>2.5 m/s) will be more likely to have RCM features and more extensive fibrosis than those with a normal TRJV (≤2.5 m/s).*

4.4. Aim 4: Biomarkers

Estimate the within-patient and between-patient variability of a comprehensive panel of biomarkers for heart failure, ROS production, and RAS-TGFβ1 signaling, as well as a panel of clinical laboratory tests. Explore the correlations between the RCM phenotype and the panel of biomarkers.

Hypothesis: *These biomarkers will have performance characteristics to serve as discriminative surrogate markers in future clinical trial*s.

5. Primary Endpoint

The primary endpoint is **the RCM phenotype**, which is defined for this study as the following:

- Normal LV ejection fraction (%);
- Impaired diastolic relaxation: ($d\varepsilon_{cc}/dt$) 2 z-scores below normal;
- Restrictive left ventricular filling: deceleration time 2 z-scores below normal for age;
- LA indexed volume 2 z-scores above normal; and
- No evidence of infiltrative disease, hypertrophic cardiomyopathy, or constrictive pericarditis.

All normal values will be age-appropriate when available.

6. Study Population

6.1. Overview and Age Strata

We will enroll a maximum of 30 participants in the entire study across **three age strata**: A, 6 – 13.9 years; B, 14 – 20.9 years; and C, 21 years and older (**Figure 8**). 25 participants are required to fill all strata, but up to 5 additional participants will be enrolled, if needed, to replace any drop-outs to maintain complete, active enrollment of 25 in the study. Enrollment in all strata should proceed simultaneously (until each is filled). Stratum A will have 5 participants, while strata B and C will each have 10 participants. Participants in stratum A can have any TRJV. Participants will be selected for

strata B and C such that half will have a normal TRJV (≤2.5 m/s) and the other half an elevated TRJV (>2.5 m/s). Once filled, each stratum will be closed to enrollment.

Figure 8. Definitions of Strata by Age, Group Size, and TRJV

Stratum A 6-13.9 years **Total N=5** Any TRJV	**Stratum B** 14-20.9 years **Total N=10** TRJV ≤2.5 m/s, N=5 TRJV >2.5 m/s, N=5	**Stratum C** ≥21years **Total N=10** TRJV ≤2.5 m/s, N=5 TRJV >2.5 m/s, N=5

6.2. Inclusion Criteria

*The following inclusion criteria apply to **all study participants**:*

1. Sickle cell anemia (HbSS) or sickle-β^0-thalassemia (HbSβ^0) confirmed by hemoglobin separation and identification techniques.
2. Ability to cooperate with and undergo CMR without sedation or anesthesia.
3. Ability to cooperate with and undergo echocardiogram.
4. Written informed consent in accordance with the institutional policies and federal guidelines must be provided by the participant (if ≥18 years of age) or parent or legally authorized guardian (if the participant is <18 years of age). Minor participants ≥11 years of age will be requested to provide assent.

*The following additional inclusion criterion applies to **Age Stratum A**:*

1. Age 6 to 13.99 years.

*The following additional inclusion criteria apply to **Age Stratum B**:*

1. Age 14 to 20.99 years.
2. Detectible and quantifiable TRJV with reported value.

*The following additional inclusion criteria apply to **Age Stratum C**:*

1. Age ≥21 years.
2. Detectible and quantifiable TRJV with reported value.

Note: If the tricuspid valve insufficiency peak gradient (TV gradient) is provided on an echocardiogram report rather than the TRJV directly, the TRJV can be back-calculated as follows:

$$TRJV = \sqrt{(TV\ gradient)/4}$$

6.3. Exclusion Criteria

1. Current chronic transfusion therapy (defined as regular, approximately monthly, transfusions of packed red blood cells given for at least 6 consecutive months for the treatment of prevention of SCD-related complications with the plan to continue this therapy at the time of potential enrollment).
2. Any contraindication to MRI or physical or behavioral factor that could degrade the quality of MRI data or interfere with a participant's tolerance of the MRI, such as permanent or semi-permanent metallic implants, including pacemakers and defibrillators, or severe claustrophobia.
3. Known ventricular septal defect (VSD) documented in medical record.
4. Estimated GFR <60 mL/min/1.73 m^2 (estimated by serum creatinine or cystatin-C).
5. Pregnancy (documented by serum or urine pregnancy test).

Note: Participants are allowed to participate in the SCD Stable Isotope Protocol (2011-2977) concurrently with this study.

6.4. Withdrawal and Replacement of Subjects

Participants may withdraw from the study at any time. Study personnel may withdraw participants who are deemed to be non-compliant with study procedures. Another eligible participant may replace the withdrawn participant. The study data of such withdrawn participants will not be discarded and may be analyzed in post hoc analyses.

Although chronic transfusion therapy is an exclusion criterion, it is not a reason for withdrawal of subjects if begun after enrollment.

7. Study Design and Monitoring

7.1. Study Procedures

Participants will have minimum of 4 study visits. Participants will undergo CMR and echocardiography at study entry (visit 1) and then yearly for two years (visits 2 and 3). Study procedures are detailed in **Table 2.**

Visit windows for each of the 4 visits will be ± 3 days. Some of the procedures for a visit can be performed on different days, if necessary, within this window. Visits 0 and 1 may also be combined for participants' convenience, if desired. If done separately, the maximum time between visits 0 and 1 is 3 months.

Table 2. Study Procedures by Study Visit

Study Procedure	Visit 0 (consent/pre-baseline)	Visit 1 (baseline)	Visit 2 (12 months)	Visit 3 (24 months)
Confirm eligibility	X			
Informed consent	X			
Review of medical records	X	X	X	X
History and physical[1]	X[1]	X	X	X
Clinical laboratories[1]	X[1]	X	X	X
Cardiac biomarkers[1]	X[1]	X	X	X
ROS biomarkers[1]	X[1]	X	X	X
RAS-TGFβ-1 biomarkers (blood and urine)[1]	X[1]	X	X	X
Genetic testing		X		
Pre-CMR hydration status[2]		X	X	X
Pre-CMR pregnancy status[2]		X	X	X
CMR		X	X	X
Echocardiography		X	X	X
Electrocardiogram		X	X	X
AE monitoring		X	X	X
Interim history		X	X	X
Reimbursement		X	X	X

Notes:

[1]Blood and urine tests may be obtained on Visit 0 or Visit 1 after consent obtained.

[2] Prior to CMR there must be assessment and documentation of (1) hydration status—must not be dehydrated; (2) renal function—must have eGFR ≥60 mL/min/1.73 m^2; and (3) pregnancy status—must have negative serum or urine pregnancy test. These results must be known before the CMR and Echo.

7.2. Laboratory Studies and Biomarkers

1. Clinical laboratories:

   Complete blood count, reticulocyte count, HbF quantitation, LDH, AST, bilirubin fractions, plasma free Hb, serum creatinine, ektacytometry, cystatin-C, urinalysis, and first-morning and 24-hour urine collection for protein, creatinine, microalbumin, and osmolality. *Rationale:* these tests are markers of SCD-related severity, renal function and/or indirect markers of hemolysis that will be correlated with clinical and imaging outcomes. In addition, a pregnancy test will determine if females of childbearing potential may proceed with the study.

2. Cardiac biomarkers:

   Plasma concentrations of the natriuretic peptides, NT-pro-BNP and MR-pro-ANP, and cardiac troponins. *Rationale*: these tests are markers of heart failure (including RCM) that will be correlated with clinical and imaging outcomes.

3. Biomarkers of ROS:

   RBC ROS measured by flow cytometry; plasma MDA by HPLC; RBC glutathione level; relative NOX1, NOX2, NOX4 and NOX5 expression in RBCs (WB); activity of Rac GTPases by pull-down. *Rationale:* these tests are indicators of oxidative stress that we hypothesize lead to the RCM phenotype that will be correlated with clinical and imaging outcomes.

4. Biomarkers of RAS-TGFβ1:

   Plasma for renin activity, aldosterone level, active TGFβ1, and PAI-1 activity; urine and serum for angiotensin-II and serum forangiotensinogen (oxidized and reduced forms for both); and AT1R expression on platelets and RBCs. *Rationale:* these assays are for markers of signaling through the RAS-TGFβ1 pathways, that we hypothesize lead to the RCM phenotype that will be correlated with clinical and imaging outcomes.

5. Genetic testing:

   Screen for single nucleotide polymorphisms in ~100 genes that encode components of the RAS-TGFβ1 pathways. *Rationale:* these are polymorphisms of components in the RAS-TGFβ1 pathways that could modulate the development of the RCM phenotype that we hypothesize results from excessive TGFβ1 signaling.

7.3. MRI Protocol

CMR will be performed on a Philips Achieva 1.5T 70 cm bore scanner with a 32 channel dedicated cardiac coil. Parallel imaging with SENSE reconstruction (SENSE factor=2) will be used for acceleration. Acquisitions will be gated using a standard vectorcardiographic algorithm (VCG). Subjects will be asked to breath hold as tolerated for the scanning sequences; subjects unable to breath hold will be scanned using “breathe-through” techniques that have become standard at most pediatric CMR centers, and has been shown to be accurate. Standard sequences will be performed including localizers and steady-state free precession cine CMR in the ventricular long and short axis planes for chamber sizes, EF, and ventricular mass. Once “standard” imaging is completed, cine myocardial tag imaging will be performed in short axis and 2 long axis planes of the mid-papillary slice of the left ventricle via the SPAMM (spatial modulation of magnetization) technique. Certified CMR technologists will perform all cardiac CMR scans with continuous guidance from pediatric CMR physicians. The following sequences and procedures will be performed on all subjects:

1. Three plane SSFP localizer.
2. Axial Black Blood stack using a single shot technique
3. 2 Chamber, 4 Chamber, 3 chamber, and short axis cine SSFP
4. Single short axis T2* image

5. Phase Contrast imaging of the aortic valve, pulmonary valve, branch pulmonary arteries, atrioventricular valves.
6. Tissue PC imaging of the myocardium in the short axis, 4 chamber, and 2 chamber
7. T1 mapping using a MOLLI sequence in the short axis and long axis planes
8. Intravenous injection of 0.2 mmol/kg Gd - DTPA
9. Seven minutes post Gd injection, Look-Locker based TI scout
10. Eight minutes post Gd injection LGE Short Axis and long axis images
11. Ten minutes post Gd injection T1 mapping using a MOLLI sequence in the short axis and long axis planes

CMR Image Processing:

The CMR images will be stored in standard DICOM format in a password protect computer in the PI's office for analysis using cardiac software analysis packages [Mass – Medis, Leiden, Netherlands and HARP/SENC – Diagnasoft, Cary, NC].

CMR Image Analysis:

Global Ventricular Function: Ventricular and atrial chamber sizes, myocardial mass, and ejection fraction will be measured using standard planimetric software. Endocardial and epicardial contours will be drawn manually including segmenting large trabeculations (MEDIS, Leiden, The Netherlands). The location of the atrioventricular valve planes will be confirmed by referencing the images to the 2- and 4-chamber images. An experienced analyst and CMR physician will draw all contours. The ventricular mass will be calculated as the difference between the ventricular epicardial and endocardial volumes multiplied by the estimated myocardial density (1.06 g/ml). The volume and mass will be indexed to the body surface area (BSA), calculated by the Mosteller algorithm. As an internal data verification, the ventricular stroke volume (RVEDV – RVESV) will be compared with the phase contrast derived cardiac output after excluding shunting.

The left ventricular mid-ventricular systolic circumferential, longitudinal, and radial strains and the diastolic relaxation slope will be calculated using HARP algorithm with the 4 chamber and short axis CSPAMM images. (HARP/SENC, Diagnasoft, Cary, NC). A dedicated research expert will perform all strain analyses.

The ECV will be measured using a combination of MRmap 1.3 (Danial Messroghli, Berlin) and MATLAB. MRmap allows creation of the T1 maps from MOLLI image sets. Using Matlab (Mathworks, Natck, MA), a parametric image is created according to the following equations where R is the relaxation rate. ECV and HCT are expressed as percentages.

$$\Delta R1_{myo} = 1/T1_{myo-postGd} - 1/T1_{myo-preGd}$$
$$\Delta R1_{blood} = 1/T1_{blood-postGd} - 1/T1_{blood-preGd}$$
$$ECV = \Delta R1_{myo} / \Delta R1_{blood} * (100 - HCT)$$

7.4. Echocardiography (TDI) Protocol

All patients will undergo a limited echocardiogram just prior to the MRI. The echocardiogram will be focused to look at the Doppler tissue velocities of the myocardium, the atrioventricular inflows, and the TRJV. All echocardiograms will be performed and all measurements will be made centrally in the core echocardiography laboratory at CCHMC. As noted in our previous losartan in SCD grant, agitated saline will be used to increase the contrast of the TRJV.

Pre-procedure:

- Assess for contraindications to agitated saline: pregnancy and known VSD

- All patients will have peripheral IV (20 gauge or larger) in upper extremity for both the echocardiogram and the MRI. A topical numbing spray or anesthetic ointment may be used to minimize the discomfort of IV placement.

Left Ventricular Diastolic Function

- Mitral valve inflow
- Mitral valve Doppler tissue imaging (DTI) (both lateral wall annulus and septal annulus)
- Pulmonary venous Doppler velocity profile (AP4C, sample volume in pulmonary vein upstream from ostium, ensure A wave is clearly seen)
- Describe atrial septal bowing if any, use PFO Doppler velocity for interatrial gradient

Right Atrial Pressure estimation

- IVC collapse (sniff test) from longitudinal abdominal view -Hepatic vein flow reversal – color Doppler from longitudinal abdominal view

Atrial septum

- 2d and color Doppler of the atrial septum interrogating for ASD/PFO

RV/PA Pressure

- TR Doppler peak velocity -PR peak and end-diastolic velocity -Repeat TR Doppler peak velocity during the administration of 10 ml of agitated saline IV

Pulmonary artery imaging

- MPA Pulmonary Doppler flow profile

8. Sample Size and Analysis Plan

This **pilot study** will provide the critical data **needed to design phase II/III clinical trials** to test anti-fibrotic therapy, such as losartan, as a means **to prevent or reverse SCD-related RCM**. Total sample size is based upon the degree of precision of the estimate of the true proportion of the RCM phenotype (see Aim 1) in combination with pragmatic issues.

8.1. Aim 1: Frequency of RCM phenotype

The primary endpoint of this aim is diagnosis of RCM as defined by CMR. Sample proportion and confidence interval will be used to make inference on the true proportion of the RCM phenotype in a population of children and adults with SCD. We will enroll a total of n=25 subjects across three different age strata (n=5 for age 6-13, n=10 for age 14-20 and n=10 for age ≥21). With a 95% level of confidence, the proposed sample of 25 would estimate the true proportion with an error margin≤0.2. If the RCM prevalence in the SCD population is ≥21% and the prevalence in a normal population does not exceed 5%, the proposed sample would also provide a ≥80% power to detect a higher RCM frequency in the SCD population. The expected (hypothesized) prevalence is 30%. Given that the expected proportion of RCM in a normal population of children and adults would be close to zero, this is an excellent power for this pilot study. We will also explore whether the proportion of RCM changes across the three age strata using the Freeman-Halton extension of the Fisher exact probability test for a 2x3 contingency table. We will also quantify the extent of diffuse myocardial fibrosis using the partition coefficient of gadolinium for each participant with RCM, and perform secondary exploratory correlations with clinical, laboratory, and imaging variables.

25 participants are required to fill all strata, but up to 5 additional participants will be enrolled, if needed, to replace any drop-outs to maintain complete, active enrollment of 25 in the study.

8.2. Aim 2: Stability of RCM phenotype

In this aim, we will estimate the temporal stability (e.g., resolution, non-progression, or progression) of the RCM phenotype over a 2-year period to determine its suitability as an outcome measure for a future clinical trial. The primary endpoint is RCM classification repeatedly diagnosed at enrollment and every year post enrollment. The proportion of changes in classification from baseline (RCM or no RCM) at the time of the 2 yearly follow-up CMR studies will be used to make inference on the temporal stability. We hypothesize that the RCM phenotype, once detected by imaging, will not spontaneously resolve. Therefore, poor CMR test performance would be the change from RCM to no RCM phenotype compared to any preceding study. Acceptable CMR test performance would be the persistence of the RCM phenotype from one CMR study to the next (baseline to year 1; year 1 to year 2). Whether the *degree* of the RCM phenotype changes with time (e.g., progresses, or remains stable) is not a factor in this analysis, as both outcomes are biologically expected. We will conclude that the RCM phenotype by CMR is acceptable for use in clinical trials as an imaging outcome if no more than 1 subject (<5% of the total sample size) with the CMR-defined RCM phenotype has resolution of the RCM phenotype on any follow-up CMR study.

8.3. Aim 3: Association of RCM with TRJV

In this exploratory aim, we will test for association between the RCM phenotype, as the binary dependent variable, and the TRJV both as a continuous regressor and as a dichotomous (≤2.5 vs >2.5 m/s) independent variable. This dichotomy has already been established as clinically and prognostically meaningful in adults with SCD. For this secondary analysis, we will combine all the age strata into a single study population, and we will not test for this association across age strata because of small sample size in each. We also hypothesize that RCM will be associated with a higher TRJV independent of age. Analysis will be by GLM and binary logistic regression.

8.4. Aim 4: Biomarkers

In this exploratory aim, we will estimate the within-patient and between-patient variability of a comprehensive panel of biomarkers for heart failure, ROS production, and RAS-TGFβ1 signaling, as well as a panel of clinical laboratory tests. The goal of this aim is to describe the performance characteristics of the biomarkers to determine their suitability for future clinical trials.

9. Study Sites and Personnel

Cincinnati Children's Hospital Medical Center (CCHMC). Drs. Charles Quinn, Michael Taylor, and Robert Fleck will be responsible for the overall conduct of the study. Dr. Charles Quinn will oversee the identification and enrollment of participants at CCHMC and coordinate all multi-center activities. CMR will be overseen and interpreted by Drs. Michael Taylor, Robert Fleck, and Jeffrey Towbin. Echocardiography with be overseen and interpreted by Drs. Michael Taylor and Jeffrey Towbin. Dr. Lin Fei will be responsible for statistical design and analysis. All study investigators (see page 1) will responsible for design, analysis, and publication of the study. Children and young adult participants (age ≤21 years) will be recruited at this site.

University of Cincinnati College of Medicine. Adult participants (age ≥18 years) will be recruited at this site, overseen by Dr. George Atweh. All other study procedures will take place at CCHMC.

10. Recruitment

Study personnel may approach potential participants during clinically indicated inpatient or outpatient encounters. IRB-approved brochures will be mailed to potentially available participants and provided to them in person during medical and/or research encounters. We will post IRB-approved flyers in approved locations in the hospital and clinics of study sites to solicit direct contact by interested participants. The study *may* also be advertised to our patient population by phone calls, the research website, and at outreach activities. Recruitment and participation in this study will not interfere with or delay standard medical care.

11. Potential Risk

This study involves few risks to subjects, including: (1) discomfort from venipuncture and IV catheter placement, (2) anxiety related to echocardiography, (3) anxiety or claustrophobia during the MRI, and (4) adverse effects of the MR contrast agent, gadolinium. Echocardiography and cardiac MRI are safe, noninvasive procedures that are often performed as standard of care for children and adults with SCD to screen for or diagnose cardiopulmonary complications of SCD. The side effects of gadolinium include headache, nausea and local discomfort (at the IV site). Rarely (less than 1%) low blood pressure and light-headedness occurs. This can be treated immediately with intravenous fluids. Very rarely (less than one in one thousand), patients may be allergic to gadolinium. Allergic reactions are most commonly hives and itchy eyes, but more severe reactions have been reported.

Gadolinium contrast is safe for patients with SCD who are not dehydrated. There is some evidence to suggest that gadolinium-based contrast agents may be associated with the development of nephrogenic systemic fibrosis (NSF) in patients with severe renal insufficiency, i.e. in patients with acute or chronic severe renal impairment [estimated GFR (eGFR) <30 mL/min/1.73 m^2 for children >2 years of age, adolescents, and adults], or acute renal failure of any cause. Adequate hydration status will be ensured before gadolinium is administered (by patient questionnaire and examination). Subjects with renal impairment, i.e. an eGFR <60 mL/min/1.73 m^2, will be excluded. The eGFR result **must** be available before gadolinium-based contrast injection.

Recent research studies have found that in people who have had 4 or more MRI scans with gadolinium-based contrast agents, small amounts of gadolinium can be found in the brain after the MRI scan has been completed. The Food and Drug Administration has stated that no adverse health effects have been identified from this residual gadolinium. The FDA has recommended that gadolinium-based contrast agents should be used when it is necessary to provide the required information. We have made the determination that the use of a gadolinium-based contrast agent is necessary. Information about this potential risk will be included in the informed consent document and process.

There is also the possible risk of the loss of confidentiality by inadvertent release of protected health information (Section 10 details the procedures to minimize this possibility).

12. Procedures to Maintain Confidentiality

Subject confidentiality will be maintained by the investigators, the investigators' associates and co-workers, and by all administrators who are part of the project. Confidentiality will be maintained according to ICH E6; 4.8.10, part O: "Records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential."

The investigators, their staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the trial and the safety of subjects. The subject/legal guardian may obtain the results of clinical labs, tests and procedures obtained for this research study.

Subjects will be identified by a unique identifier. Any publications will reflect only unique identifiers. All information related to this study will be kept in secure and locked offices.

Any clinical data on paper forms that needs to be stored separately from a participant's official medical record will be kept in individual study binders that are secured in locked cabinets. Any data on computer will be accessible only by password access. Only members of the research team will have access to these physical and electronic files.

Study data will be shared between Study Site investigators in accordance with the HIPAA waivers signed by each participant/parent/guardian.

13. Potential Benefits

This research will establish improved screening and diagnostic techniques for patients with SCD. The potential benefits of this study will mainly accrue to the class (individuals with SCD) rather than the participants. It is possible that this research will uncover a medically important issue that would benefit the participant if treated as a result of the discovery, but this is not the focus of the study. All potentially medically significant findings will be reported to the patient and his or her primary hematologist for counseling and decisions about further testing, treatment, or both.

14. Risk/Benefit Analysis

The potential benefits (section 11) of participation in the study outweigh the few, generally minor risks (section 10). The formal IRB risk assessment is: slightly more than minimal risk but with potential for direct benefit to participants.

15. Protection of Human Subjects

The following will be done to ensure safety of human subjects:

- IRB approval will be obtained at each institution implementing this study.
- All Informed Consent Forms (ICFs), including parental permission forms and assent forms where applicable must also be approved by the sponsor or designee.
- Patients and parents will be provided with the risks and benefits and give informed consent for the study.
- Assent will be sought for patients based on local/institutional IRB requirements
- All Institutional (IRB and HIPPA) and NIH requirements for human studies will be met.
- Exclusion criteria are in place that ensure safety of the drug and exclude vulnerable groups prone to adverse events (e.g., pregnant or lactating women are not enrolled and pregnancy tests will be performed at each visit).
- Adults with diminished capacity will not be enrolled
- Children will be enrolled only with proper parental permission (age <18 years) and assent (age 11 years and older)

Compliance with Good Clinical Practice (GCP) guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. The study (protocol, informed consent, advertisements, subject information sheets, and Investigator CV and credentials) should be reviewed and approved by the Institutional Review Board (IRB) or ethics committee. Changes to the protocol will be approved by the IRB prior to being implemented. Subjects must sign written informed consent prior to undergoing any study procedures.

The investigators and institutions affiliated with this study will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source documents.

16. Data and Safety Monitoring Plan

There is no Data Safety and Monitoring Board for this study because this in an non-interventional study with only yearly visits for cardiac imaging with few risks to participants. Adverse event (AE) reporting will be **limited to the 30 days following each yearly study visit** to capture any possible complications, whether SCD-related or not, associated with MRI, gadolinium, echocardiography, and phlebotomy/IV placement.

An adverse event (AE) is defined is defined as any untoward medical occurrence in a study subject that does not necessarily have a causal relationship with the study. An AE can be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the study.

The Common Toxicity Criteria (CTC v4.0) criteria will be used, with SCD-specific modifications noted below (**Table 3**). Rationale for the modifications: SCD causes multisystem chronic, progressive organ injury and intermittent and unpredictable "crises" or vaso-occlusive complications and infections. Baseline and expected laboratory values are also different in this patient population as compared to normal individuals. If the event is not found in the CTC v4.0 criteria, the event will be captured and rated mild, moderate, severe, life-threatening, or death per the investigator (**Table 4**).

Table 3. CTC v4.0 modifications for SCD

Parameter	Grade 2	Grade 3	Grade 4
Hemoglobin (g/dL)	5.5-6.9	4.0-5.4	<4.0
Total WBC (x10^9/L)	1.0-1.000	0.5-0.999	<0.5
ANC (x10^9/L)	0.5-0.999	0.2-0.499	<0.2
Platelets (x10^9/L)	50-79	20-49	<20
Total bilirubin (mg/dL)	5.0-10.0	10.1-20.0	20.0
AST (IU/L)	150-300	301-1000	>1000
ALT (IU/L)	150-300	301-1000	>1000
Creatinine	Doubling of the baseline serum creatinine level OR a value of >1.0 mg/dL	1.6-2.0	>2.0

Table 4. Severity descriptors for events not found in CTC v4.0

Severity	Numerical Value	Description
Mild	1	Aware of sign, symptom, or event, but easily tolerated; does not interfere with daily routine
Moderate	2	Discomfort enough to interfere with daily routine and may require some therapeutic intervention
Severe	3	Incapacitating, significantly affects clinical status; requires therapeutic intervention
Life Threatening	4	Life-Threatening; immediate intervention required
Death	5	Adverse event causes death.

The investigator is also responsible for determination adverse event attribution to determine if the event is related the protocol (**Table 5**). The following table outlines these definitions:

Table 5. AE attribution definitions

Unrelated	The event is unrelated to the protocol.
Possibly Related	The event or severity of event is not usually associated by the clinical condition or standard of care for the condition, but there is no strong evidence to link the event to the protocol.
Probably Related	The event or severity of event is such that it can likely be correlated to a protocol activity.
Definitely Related	There is a strong correlation between the event and a protocol related activity.

Expected AEs are a known sign, symptom, finding, or condition related to SCD, MRI, gadolinium, echocardiography, and phlebotomy/IV placement (**Tables 6 and 7**). Expected events (Grade 2-5) will be

recorded and will include the following information only: (a) nature of event; (b) start date/stop date; and (c) highest grade.

Reporting of Expected AEs: grades 2-5 are to be reported to the IRB according to local IRB requirements.

Table 6. Expected events, conditions or findings associated with SCD

Acute chest syndrome	Hematuria	Pyelonephritis
Albuminuria	Hemiplegia	Renal failure
Amenorrhea	Hemolysis	Renal insufficiency
Anemia	Hepatic sequestration	Renal papillary necrosis
Aplastic crisis	Hepatomegaly	Reticulocytosis
Arthralgia	Hospitalization	Retinopathy
Avascular necrosis of bone	Hyperbilirubinemia	Retinal hemorrhage
Bacteremia	Hypersplenism	Rhabdomyolysis
Bone infarction	Hypertension	Seizure
Cardiac arrhythmia	Hypocalcemia	Septicemia/sepsis
Cardiomegaly	Hyposthenuria	Silent infarct (stroke)
Cerebrovascular accident	Hypertension	Skin ulcer
Cholecystitis	Hypoxemia	Splenic sequestration
Cholelithiasis	Ileus	Splenomegaly
Cognitive dysfunction	Infection: bacterial or viral	TIA
Constipation	Jaundice	Transfusion
Cranial nerve palsy	Leukocytosis	Vaso-occlusive crisis
Decreased renal function	Meningitis	
Decreased lung function	Nephropathy	
Delayed growth/puberty	Osteomyelitis	
Depression	Pain: any body site	
Dizziness	Priapism	
Electrolyte imbalance	Proteinuria	
Elevated urobilinogen	Pneumonia	
Elevated serum transaminases	Pulmonary embolism	
Fever	Pulmonary hypertension	
Hand-foot syndrome (dactylitis)	Pulmonary radiographic infiltrate	

Table 7. Expected events, conditions or findings associated with MRI, IV placement/phlebotomy, gadolinium, and/or echocardiography

Allergic reactions	Erythema	Nausea
Anaphylaxis	Headache	Pain/discomfort
Anxiety	Hives	Rash
Claustrophobia	Hypotension	Syncope
Dizziness	Itching	Thrombophlebitis
Edema	Light-headedness	Vomiting

Unexpected AEs are those that have not been previously associated with SCD, MRI, gadolinium, echocardiography, and phlebotomy/IV placement. Information on unexpected AEs will include a description of the event, specific tests and/or treatments and the results thereof, and outcome parameters.

Unexpected AEs will be followed until resolution (or study completion) from initial start date at which time the event will be defined as: (a) ongoing; (b) resolved or stabilized without sequelae; or (c) resolved or stabilized with sequelae.

Reporting of Unexpected AEs: grades 2-5 are to be reported to the IRB according to local IRB requirements unless meeting the definition of a serious adverse event (SAE), described below.

Serious adverse Events: For the purpose of this study, an SAE is considered one that:

- Results in death;
- Is life-threatening (an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe);
- Requires inpatient hospitalization or a prolongation of an existing hospitalization which, in the opinion of the investigator, is *not* attributable to the subject's SCD (hospitalization is not uncommon for the subjects in this study based on the nature of their illness) but is at least *possibly* related to any study-related activity;
- Results in a persistent or significant disability/incapacity greater than that which existed at baseline;
- Results in a congenital anomaly/birth defect; or
- Is, in the opinion of the investigator, an important medical event.

Reporting of SAEs: SAEs will be reported to the IRB according to IRB policy. SAEs that require expedited reporting (within 7 calendar days of the investigator's initial receipt of the information) are:

- Unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the protocol-related documents and given the characteristics of the participant population being studied;
- Related or possibly related to a participant's participation in the research; and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

17. Quality Assurance Monitoring Plan

Monitoring will be performed to ensure the study is conducted, documented, and reported in accordance with the IRB approved protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements.

For this observational study (no intervention or therapy involved), the Cancer and Blood Diseases Institute at CCHMC will monitor eligibility, consent documentation, and CRF to source data verification for the first patient enrolled and for at least 5% of the patients enrolled since the last monitor visit on an annual basis. This schedule will be subject to change based on enrollment, the degree of risk or severity of monitoring findings, and other study management issues. At the end of each monitoring visit, findings and follow-up actions will be summarized in a monitoring report and will be provided to the study PIs.

18. Plan for Informed Consent

Informed consent is an ongoing process that includes the signing of an informed consent document. Subjects are required to sign an informed consent prior to undergoing any study procedures or assessments, in accordance with International Conference on Harmonisation (ICH) E6; 4.8, "Informed Consent of Trial Subjects." When substantial modifications are made to the informed consent, the IRB may require that all subjects currently enrolled in the study will be re-consented; ICH E6; 4.8 guidelines would still apply.

Subjects will be provided with a copy of the informed consent that explains the purpose of the study, the study procedures and assessments, risks and benefits and alternative choices in clear, understandable language. Subjects will also be provided with the telephone numbers of the investigator and qualified personnel who can assist with their questions and concerns.

The following is the consent process that will be used for this project.

- Subjects are recruited from patient populations or from eligibility criteria obtained preparatory to research.
- A copy of the informed consent is given to the patient or parent before signing the consent. The content of these documents, and the nature of the study, is discussed with the subject, patient and parents or guardians before obtaining their signature. If there are no questions regarding the studies or the content of the consent forms, the subject, patient and/or guardian are requested to sign the consent in the presence of a member of the investigation team.
- An investigator/designee involved in the study conducts the consent process.
- The consent is obtained by a study investigator/designee in person and the investigator signs the consent.
- Subjects are given copies of the consent with signatures to keep.
- Assent to participate in the study will be obtained from children as per each site IRB policy
- Prior to use, advertisements will be reviewed and approved by the IRB for participating institutions.
- Subjects who entered the study as minors with the permission of the parents/legal guardians will be asked to provide informed consent upon reaching the age of majority.

To safeguard the participation of children in this study, we will do the following (hereafter “parent” means “parent or legal guardian”):

- Obtain parent’s written consent
- Assent will be obtained from children of appropriate age (11 years of age and older).
- Inform parents of alternative options, including non-participation and standard of care
- Inform parents that participation is voluntary and that they are free to withdraw from the study at any time.
- Inform parents that choosing not to participate in the study will not result in any penalty or loss of benefits to which the patient is otherwise entitled
- Whenever possible and appropriate, purposefully solicit that the parent and child are still willing to participate (e.g., when new information regarding the risks/benefits is available)

19. Cost of Participation

The patient and/or his insurance will be billed for all standard of care treatments. Participants will not be charged for any procedures associated with the research.

20. Compensation

Participants will be reimbursed for participation in this study to compensate them for their time, inconvenience, and meals related to the study procedures and visits.

If additional study visits are required for Visit 1, 2 or 3, (e.g., if a study procedure has to be repeated for quality), an additional $180 will be provided per extra visit.

Visit	Visit 0	Visit 1	Visit 2	Visit 3
Compensation	$60	$180	$180	$180

If needed, families can be provided with a travel voucher for transportation to and from the hospital for study visits.

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