PROBENECID IMPROVES CARDIAC FUNCTION IN SUBJECTS WITH A FONTAN CIRCULATION AND AUGMENTS CARDIOMYOCYTE CALCIUM HOMEOSTASIS

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TRPV2 AGONISTS IN FONTAN CIRCULATION PATIENTS

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1. ABSTRACT

Patients with single ventricle physiology (either a single morphologic left or right ventricle) have a form of very complex congenital heart disease that is fatal without intervention. The development of the Fontan procedure to separate the systemic from the pulmonary circulation has resulted in improved survival well into adulthood; however, this procedure is palliative and results in a circulation that is ultimately fraught with multisystem complications related to high central venous pressure, low cardiac output, valvular and ventricular dysfunction (both systolic and diastolic). At least 30% have occult diastolic disease.

There are currently very few proven pharmacologic options available for these patients. The recent discovery of transient receptor potential vanilloid (TRPV) channels, particularly TRPV2 channels, in the cardiovascular system is promising as a potential pathway for pharmacologic intervention for Fontan patients. Probenecid, a drug best known as a treatment for gout or as a penicillin adjunct, acts as a TRPV2 agonist and has recently become the subject of study as a model therapy for the treatment of cardiomyopathy due to its positive inotropic and lusitropic effects. The purpose of this pilot study is to determine if probenecid will improve magnetic resonance (MRI) parameters of systolic and/or diastolic dysfunction as well as associated symptoms in patients with a Fontan circulation. We will quantitatively assess functional improvement with pre- and post-treatment cardiopulmonary exercise testing.

2. INTRODUCTION

2.1. Background

Thirty-five of every 100 000 live births are complicated by the presence of single ventricle physiology. Some malformations consist of a single left ventricle (i.e. tricuspid atresia) while others result in a single right ventricle (i.e. hypoplastic left heart syndrome). Although there are inherent differences in lifelong functionality based on the morphology of a single right versus a single left ventricle, the prognosis of single ventricle patients as a whole was transformed by the introduction of the Fontan operation. This palliative procedure has certainly improved survival, but patients with a Fontan circulation experience a vast array of multisystem physiologic consequences related to high central venous pressure and valvular and ventricular dysfunction

(both systolic and diastolic). Despite early palliation, the ventricle in Fontan circulations remains particularly vulnerable to failure. Up to 40% of adult Fontan patients exhibit heart failure during late follow-up.

Although management of heart failure in adults with structurally normal hearts has been well-studied, the same does not apply to heart failure in congenital heart disease. Many of the same drugs are used by congenital cardiologists, although somewhat empirically, since the body of evidence to support the long-term efficacy of such drugs is lacking. Transient receptor potential vanilloid (TRPV) channels are Ca^{2+} channels that have been fairly recently discovered in the cardiovascular system, including in cardiac myocytes, where TRPV2 channels are more abundant than TRPV1 channels. Probenecid, an FDA-approved drug that has been in use for other purposes (i.e. gout) for more than 40 years, activates TRPV2 channels in cardiomyocytes and increases intracellular Ca^{2+}. This results in improved myocardial contractility and relaxation. Importantly, it does not induce apoptosis, malignant arrhythmias, or adverse changes in peripheral vascular resistance. These findings suggest that TRPV2 activation, via probenecid, could represent a novel and clinically relevant approach for treating cardiac dysfunction in adolescents and adults with Fontan palliation for single ventricle congenital heart defects.

2.2. Preliminary Studies

Probenecid has been shown to induce a TRPV2 dependent cytosolic influx of calcium resulting in increased cell contractility in isolated cardiomyocytes. This effect has also been shown ex vivo in hearts and in vivo in mice. There are ongoing studies in vivo in mice and pigs, and investigators have proposed trials in humans; however, to date, we are not aware of any published data using Probenecid for the treatment of heart failure in humans. There are also no studies looking at the application of Probenecid as a heart failure management drug in patients with a Fontan. These endeavors are a practical next step given that Probenecid is already an FDA-approved drug and has a long history of use for other medical purposes with a low side-effect profile. Probenecid has also been safely used in children as an adjunct to treatment with penicillin and other antibiotics. It has also been used as an adjunct in the treatment of malaria in children, with a defined dosing regimen, and no evidence of drug intolerance.

3. PURPOSE OF THE STUDY

The primary objective of this study is to determine if Fontan patients treated with probenecid for four weeks will experience increased systolic and diastolic function (as measured via standard and advanced MRI parameters) compared with four weeks of placebo.

Secondary objectives of this study include the following:

- Fontan patients treated with probenecid for four weeks will experience improved exercise performance compared with four weeks of placebo.
- Determine if there is a difference in the effect probenecid has on patients with a single right versus a single left ventricle after a Fontan procedure.

4. STUDY DESIGN

4.1. Study Description

This will be a prospective randomized, placebo-controlled cross-over pilot study of up to 22 patients who have had a prior Fontan operation for single ventricle physiology who are ≥ 10 years old. Including teenagers younger than 18 years old is important for the following reasons: 1) The adult Fontan patient population is small, 2) Most adult Fontan patients > 18 years old have single left ventricle physiology; therefore, single right ventricle patients (i.e. with Hypoplastic Left Heart Syndrome (HLHS)) would be under-represented, as surgical palliation allowing survival beyond infancy was reported to be successful in 1981, with many initial problems with early connections that were created; and 3) Patients in the 10-18 year old age group could potentially also benefit from probenecid with little risk associated risk.

4.2. Study Duration

The entire study duration is estimated to be 3 years total to allow sufficient time to recruit patients and conduct the study (estimated 2 years), and analyze the data (estimated 1 year). From the time of study consent to the time of study completion, we expect study participation to be 12 weeks for each participant.

5. SELECTION AND RECRUITMENT OF PARTICIPANTS

We will select up to 22 patients meeting the criteria below. Potentially eligible patients will be identified from existing patients of the Fontan Management Clinic or the Adult Congenital Heart Disease clinic, and will be evaluated for eligibility criteria prior to enrollment; eligibility screen failures will be noted in the enrollment log. Patients will be recruited at the time of a clinic visit, hospital stay or over the phone following a recruitment letter. Letters will be sent 2 weeks prior to calling potential participants so they are informed about the study and can opt-out if desired.

Investigators may reach out to colleagues at nearby institutions to present an overview of the study. IRB approved promotional material will be provided to these cardiologists to disperse to their potentially eligible/interested Fontan patients. The cardiologists from outside our institution will refer these potential subjects to our study team. Study staff will engage the potential subjects and begin the screening/recruitment process.

5.1. Inclusion Criteria

Subjects who meet all of the following criteria will be eligible for the study:

1. ≥ 10 years old
2. Single ventricle congenital heart disease status post Fontan procedure.
3. Impaired ventricular function as assessed by preexisting echocardiographic studies and any available MRI studies.
    a. LV inclusion criteria: Ejection fraction by cMRI or echo assessment of <50% or moderate to severe dysfunction.

b. RV inclusion criteria: Ejection fraction by cMRI of <45% or moderate to severe dysfunction. Or, given the ASE recommendation to avoid use of 2-D imaging quantification for assessment of right ventricular systolic function, a peak global longitudinal strain value as assessed by a single reviewer with a value greater than -17% will also be included. Peak global longitudinal strain analysis will be performed for all eligible single right ventricles noted by subjective echo reports to have abnormal systolic function if no qualifying cardiac MRI assessment of ejection fraction is available.

5.2. Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study:

1. Clinically unstable or ongoing illness.
2. Evidence of untreated Fontan pathway obstruction.
3. Presence of uncontrolled arrhythmias.
4. Evidence of moderate or greater atrioventricular valve regurgitation.
5. Pregnancy.
6. History of sulfonamide allergy
7. Known G6PD deficiency
8. Patients on certain drugs that have potentially dangerous interactions with probenecid other than for prophylactic reasons: doripenem, zalcitabine, deferiperone, citalopram, methotrexate, ciprofloxacin, amoxicillin, cefprozil, cefpodoxime, cefotaxime, meropenem, ertapenem, valganciclovir, ganciclovir, ziovudine ketorolac, cefdinir, cephalexin, dapsone, indomethacin, and piperacillin.Each subject's medication list will be reviewed prior to study participation.
9. Impaired renal function as defined by a GFR < 60mL/min/1.73 m^2 within the last year.
10. Patients at a higher risk for arrhythmia including those with a prior history of arrhythmia including atrial and ventricular dysrhythmia or those on established anti-arrhythmic therapy.
11. Admission to the hospital due to a clinically significant arrhythmia within the previous month.
12. Greater than moderate atrioventricular regurgitation as denoted on most recent echo report.
13. Patients with atrio-pulmonary Fontan
14. Currently enrolled in an interventional drug trial or completed an interventional drug trial within the past 30 days.
15. Not appropriate for MRI screening due to having an implanted device.

6. STUDY PROCEDURES

Once patients are enrolled, they will be randomized to receive either probenecid or placebo as the first treatment.. Randomization procedures are described below under "Statistical Analysis".

All patients will have a baseline assessment immediately preceding initiation of probenecid or placebo. This will include a physical exam to assess any clinical symptoms, review of cardiac symptoms, blood work, pregnancy test for those girls/women of child bearing potential, PEDS QL questionnaire completion, exercise testing and MRI, as described in detail below. Patients will then receive either probenecid or placebo for 28(±7 days) of treatment according to their randomization group, after which the same testing will be repeated. Following 28(±7 days) of washout, patients will undergo the same testing procedures as during the first period of study but with the alternate treatment. To assist in achieving congruence between clinical testing performed and desired pre-intervention testing, we will coordinate with each patient and his/her cardiologist prior to the clinical visit.

Patients will receive a one month supply of oral probenecid therapy with the following dosing regimen:

- For patients (adults and pediatric) ≥ 40 kg: 500 mg by mouth twice daily.
- For children 10-17 years old and < 40 kg: 250 mg by mouth twice daily

Note: This dosing regimen is based upon available literature of those used in both adults and children and with the guidance of a clinical pharmacist. A dose of 20-25 mg/kg/day divided in 2 daily doses has been well-tolerated in children for 3 days in 1 study (Sowunmi, A. et al. May 2004. Tropical Medicine and International Health; Craft JC, et al. Antimicrobial Agents and Chemotherapy. 1979). The standard maximum adult dose is 2000 mg/day. Probenecid is available as a 500 mg tablet, and there is no commercially available compound.

During the placebo treatment arm, patients will receive 28(±7 days) of placebo therapy. Oral placebo therapy will be twice daily, with an identical dosing regimen as the placebo.

The investigators will review all clinical data to assess for adverse experiences during the period from enrollment through the end of study. The investigators will evaluate any changes in clinical symptomatology, and determine if the change is clinically important and different from expected in the course of treatment of Fontan patients. This will be assessed by a clinical research coordinator with a direct telephone call to the study participant at 4 time points during the 28(±7 days) of therapy with the first call taking place within 2 days of commencement of each treatment (probenecid or placebo) and weekly thereafter. Patients who manifest new symptoms will be asked to return to the CCHMC Heart Institute for further assessment. All subjects will also be clinically evaluated before and after each arm of study by a clinician.

6.1 Quality of Life Assessment

All subjects will complete the Pediatric Quality of Life Assessment 4.0 Generic Core Module (PedsQLCore) at each visit. The PedsQL Core questionnaire is a modular instrument for

measuring health-related quality of life in adolescents through adults ages 10 and up. Participants will complete the designated Peds QL Core for their respective age group.

Schedule of Procedures

	Treatment Period A			Wash Out	Treatment Period B		
Procedures	**Before treatment**	**28 (±7days)**	**Immediately After Treatment**	**28 (±7days)**	**Before Treatment**	**28 (±7days)**	**Immediately After treatment**
Informed consent	X						
Demographics, medical history, concomitant medication	X				X		
Physical Exam	X		X		X		X
Review of symptoms	X		X		X		X
Blood sample - NT-proBNP, CBC, CMP, LDH and, GGT	X		X		X		X
Pregnancy Test (Urine)	X		X		X		X
Cardiopulmonary Exercise Tests (CPET)	X		X		X		X
MRI	X		X		X		X
Drug dispensing/administration	X				X		
Adverse events	X	X	X	X	X	X	X
Drug adherence check			X				X
PEDS QL	X		X		X		X

6.2 Process of Obtaining Informed Consent

The Fontan database at CCHMC (IRB 2016-2425) will be used to screen for this study. Patients meeting eligibility criteria will be approached for participation during a regularly scheduled clinic visit at CCHMC. Alternatively, a letter of introduction approved by the IRB will be mailed to potential participants along with a consent document. In the letter, patients will be given the opportunity to opt-out of the study by calling the study line. After ten days, study personnel will call patients to further explain the study. If there is interest in the study, study personnel will schedule a research visit for the patient.

When the subject arrives to the visit, study personnel (research coordinator or MD) will again provide a verbal explanation about the study and discuss the risks and benefits that may come with participation. Adequate time will be given for the participant or legal guardian to read the informed consent and all questions will be answered. If the participant and, if applicable, a legally authorized representative, agree to participate in the study, informed consent will be obtained. Assent will be obtained from all participants between the ages of 10-17, inclusive.

We will stress that non-participation in the study will not affect the subject's ability to obtain care at Cincinnati Children's Hospital.

No research procedures will be done prior to obtaining assent/consent.

6.3 Laboratory Procedures

Each participant will have blood drawn for assessing NT-proBNP, CBC, CMP, GGT, and LDH both before and immediately after each 4 week drug-treatment period. The blood will be processed through the CCHMC clinical laboratory.

6.4 Imaging Procedures: MRI

Image acquisition: Cardiac magnetic resonance imaging will be performed on all eligible study patients at baseline and repeated at the intervals specified above. Studies will be performed on non-sedated patients utilizing a Philips Ingenial 1.5T clinical magnet. Patients will have weight and height measured to calculate body surface area. Studies will be terminated in case of patient instability or development of claustrophobia. A resuscitation cart is available. Patients are monitored by ECG and respiratory gating within the presence of a physician and an MRI technician.

CMR Scanning: Studies will be ECG gated with leads placed prior to scanning. The multichannel body array and spine coils will be used. The patient will be placed head first into the MRI scanner. Parallel imaging will be used on all scans. The breath holding approach will be used for sections 3 and greater; if the patient is unable to cooperate, multiple signal averages will be used instead.

A standard protocol for image acquisition will include localizer images for planning purposes followed by single sequence free-precession (SSFP) cine imaging performed in the vertical long axis as well as horizontal long axis and short axis stacks at a slice thickness of 6-8 mm with sufficient coverage to quantify atrial and ventricular volumes in both systole and diastole. Phase-encoded velocity mapping will be performed at the level of the AV valve leaflet tips at end-diastole as well as at the ventricular outlet for verification of volumetric analysis. Velocity encoding will be optimized to avoid flow aliasing while maximizing flow velocity fidelity. Additional phase contrast imaging through the branch pulmonary arteries and/or pulmonary veins will be performed to quantify relative pulmonary and systemic blood flow. During the initial scan, a SSFP respiratory-navigated 3D whole-heart sequence will also be performed during late diastole to characterize any additional extracardiac abnormalities which might influence image interpretation.

The CMR techniques and scans to be used in this study are as follows:

1. Static steady state free precession (SSFP) imaging: Contiguous axial images forming a full volume data set of the thorax will be obtained. These images will be used to assess general cardiovascular anatomy to ensure that no unexpected lesions are present.
2. Multi-planar reformatting: The axial images in #1 will be reformatted to calculate the exact slice position and orientation to obtain the 4-chamber and short axis ventricular views for cine images and perpendicular to flow in the main (MPA) and branch pulmonary arteries (BPA) and across the aortic valve for PC-MRI.
3. Cine imaging: Off-axis, retrospectively gated cine images are obtained in the 4-chamber view and as a stack of ventricular short axis images from base to apex for ventricular performance parameters (20-30 phases, depending upon the heart rate).
4. Phase-encoded velocity mapping (VMAP): "Through-plane" retrospective, phase-encoded velocity maps will be obtained at the aortic annulus and in the MPA and branch pulmonary arteries. Initially, a velocity encoding (VENC) of 150 cm/s will be utilized. After each acquisition, images will be checked and if velocities exceeded the VENC, the images will be repeated with an increased VENC.
5. Native T1: The modified Look-Locker inversion recovery (MOLLI) sequence will be used to obtain a non-contrast native T1 value at 1 short axis level (mid ventricle).

Imaging Data Analysis: All study images will be reviewed and interpreted by a qualified investigator with expertise in congenital cardiac MRI. Institutional post-processing software (CMR42, Circle Cardiovascular Imaging, Calgary, Canada) will be utilized for off-line image analysis. Contouring of the single ventricle at end-systole and end-diastole will be performed to quantify ventricular volumes and calculation of ejection fraction as a measure of global systolic function. Phase contrast quantification of flow including blood volume and diastolic parameters such as deceleration time, E and A wave velocities, and pulmonary vein flow patterns will be assessed. Flow volumes will be utilized to verify accuracy of volumetric analysis and to generate flow patterns for temporal analysis to quantify diastolic function as previously described[33,34]. Tissue velocities obtained by phase contrast imaging will also be analyzed at the level of the AV valve annulus for measurement of E' velocity and additional diastolic parameters. Additional off-line processing of horizontal long-axis tissue strain and strain rate will be performed via feature-tracking software (CMR42, Circle Cardiovascular Imaging, Calgary, Canada)) for further characterization of myocardial mechanics.

Images will be analyzed for global ventricular function, blood flow, ventricular strain and diffuse fibrosis (native T1 value).

Global ventricular function: From the cine images of the ventricular short axis, the endocardial and epicardial borders will be traced (semi-automatically) and ventricular performance parameters computed using standard analysis packages (e.g. CMR42, Circle Cardiovascular Imaging, Calgary, Canada). Ventricular volumes will be determined by multiplying the slice thickness with the segmented area. The volumes of each slice will be integrated along the ventricular longitudinal axis using Simpson's rule to extract the total volume of the ventricle in a cardiac phase. This will be applied at end diastole (for end-diastolic volume - EDV) and at end-systole (for end systolic volume - ESV). Stroke Volume=EDV–ESV. Ejection Fraction=Stroke Volume/EDV. Cardiac output=stroke volume multiplied by heart rate. Ventricular mass=1.05 * (epicardial volume minus the endocardial volume).

PC-MRI: Integration of the velocities of each voxel of the vessel cross-section represents the flow at a given phase of the cardiac cycle. Further integration of all PC-MRI data over the cardiac cycle represents flow in one heartbeat and multiplying by heart rate yields cardiac output. Regurgitant fraction (%) = ([reverse flow]/[forward flow])X100.

Ventricular Strain: From the cine images of the ventricular short axis and long axis, endocardial and epicardial borders will be traced (semi-automatically)_ and circumferential, radial, and longitudinal strain measures will be obtained. In addition, strain rate, rotational dispersion, time to maximum strain rate will also be recorded.

Native T1: Standard analysis packages (e.g. CMR42, Circle Cardiovascular Imaging, Calgary, Canada) will be utilized to analyze both regional and global diffuse fibrosis as a signal intensity map at the mid-ventricular level from the native MOLLI sequence. The raw signal intensity on these specialized sequences are the T1 relaxation times of the myocardium.

6.5. Other Non-invasive procedures

6.5.1 Cardiopulmonary Exercise Tests (CPET)

Each participant will undergo both a pre- and post-intervention cardiopulmonary exercise test at CCHMC. Participants will perform the clinical maximal ramp-incremental exercise test. The CCHMC Exercise cart will be used for cardiopulmonary exercise testing in all cases.

6.5.2 Pregnancy Testing:

Females of childbearing potential include any female who has experienced menarche. Urine pregnancy test (for all females of child-bearing potential) will be conducted at all visits at CCHMC. A positive pregnancy test will exclude the subject from further participation in the study. Pregnant subjects who are discontinued from the study will be transitioned into an alternate therapy at the discretion of the Investigator.

6.5.3. Clinical Data

The following data will be collected via either chart review with participant verification, or direct patient questioning.

- Age
- Gender
- Anthropometric data
- Cardiac diagnoses; type of congenital heart disease
- Previous cardiac surgeries; date and type
- Previous cardiac catheterizations; date and type
- Other major medical problems
- Current medications
- Clinical status (New York Heart Association (NYHA)

7. INVESTIGATIONAL AGENT

7.1 Description

Probenecid is a uricosuric and renal tubular transport blocking agent that inhibits the tubular reabsorption of urate and increasing the urinary excretion of uric acid. It also inhibits the tubular secretion of penicillin and increases plasma penicillin levels. Probenecid is FDA approved in adults and children >2 years of age as adjuvant to therapy with penicillin, ampicillin, methicillin, oxacillin, cloxacillin, or nafcillin. Additionally, it is FDA approved for the treatment of hyperuricemia associated with gout and gouty arthritis in adults. Studies have shown that probenecid also activates the TRPV2 channel. Activation of the TRPV2 channel results in increased intracellular calcium levels and increased cell contractility in isolated cardiomyocytes.

Probenecid reaches peak serum concentrations in 1-5 hours after oral administration in adults and in 3-9 hours in children. Probenecid is highly protein bound and is extensively metabolized in the liver (oxidation and glucuronide conjunction) to active and inactive metabolites. Approximately 75-88% is excreted in the kidney and the remainder is excreted unchanged in the urine. The half-life of probenecid is 3 to 17 hours.

Probenecid is contraindicated in patients with a history of allergic reaction to probenecid, children <2 years of age, it is not recommended in patients with known blood dyscrasias or uric acid kidney stone, and in patients with an acute gout attack.

There are two main types of drug interactions with probenecid. The first type of interaction involves probenecid and decreased clearance of acidic medications and resulting plasma concentrations of the following medications: doripenem, zalcitabine, deferiperone, citalopram, methotrexate, ciprofloxacin, amoxicillin, cefprozil, cefpodoxime, cefotaxime, meropenem, ertapenem, valganciclovir, ganciclovir, ziovudine and ketorolac. We will exclude patients on these drugs from the study unless they are on these drugs short-term for prophylaxis .

The second type of interaction involves the use of probenecid and the reversal of the uricosuic effects of the following medications: magnesium salicylate, sodium thiosulfate, aspirin, choline magnesium trisalicylate and bismuth subsalicylate. As probenecid is not being utilized for its properties of urinary excretion of uric acid, it is unlikely that these drug interactions of this type will be of clinical significance in the patient population being studied. Probenecid's inhibition of secretion of acidic medications and clinical impact on its potential use as an inotropic agent is unknown.

Adverse effects reported with probenecid include alopecia, puritis, urticaria, and Stevens Johnson-syndrome. Gastrointestinal effects including nausea, vomiting, and anorexia have been reported. Isolated cases of aplastic anemia, leukopenia, neutropenia, thrombocytopenia and hepatic necrosis have been reported. Probenecid is categorized as a sulfonamide without an aromatic amine and therefore the incidence of cross-allergenicity between classes of sulfonamides is low; however, anaphylaxis and hypersensitivity reaction have been reported. Precipitation of gout attacks and uric acid stones with or without hematuria have been reported. Nephrotic syndrome is rare. Headache, dizziness and fever have been reported.

7.1.1 How Supplied

Probenecid is available as a 500 mg tablet. It will be supplied by Cincinnati Children's Hospital Medical Investigational Pharmacy. Each participant will be given a one month supply in a bottle with an attached MEMS® cap. The MEMS® cap is an adherence monitoring device that will be used to determine participant compliance with the medication. Each time the cap is removed from the bottle, it will record the date and time. If the cap is removed from the bottle more than once within fifteen minutes, the cap will only record the first dispensing action. Along with the utilization of the MEMS® caps, a tablet count will be completed by a designated study staff member prior to dispensing the one month supply as well as after the 28 (±7days) period to ensure consistency in the data obtained. Participants will also be provided with a pocket diary, in the event that they go on vacation and are unable to take their bottle with them. In this case, they will be instructed to record how many tablets they removed from the bottle at once so as to have a record of why the cap data is discrepant.

7.1.2 Storage

Probenecid is stored at room temperature, 20° to 25°C (68° to 77°F) and should be protected from light.

7.1.3 Proposed Dose

- For patients (adults and pediatric)≥ 40 kg: 500 mg by mouth twice daily
- For children 10-17 years old and < 40 kg: 250 mg by mouth twice daily

8. DATA COLLECTION AND MANAGEMENT

All data will be recorded on study-specific case report forms (CRFs). Participants will be assigned a study identification number that will be reported on all CRFs and source documents to protect patients' confidentiality.

Data will be collected by designated research coordinator(s) and the physicians participating in the study, with paper forms stored securely in locked file cabinets. A study database will be designed in the Research Electronic Data Capture (REDCap) system, a secure, HIPAA-compatible online platform housed on secure research servers behind the CCHMC institutional firewall, and available free to all UC and CCHMC investigators. REDCap includes user-specific access rights to prevent unauthorized access beyond study personnel.

All data will be recorded on study specific case report forms (CRFs). Participants will be given a study identification number that should be reported on all CRFs and source documents. The key which links this study number to participant's direct identifying information will be stored in a separate, secure location with access to only authorized individuals.

The following information will be collected for this study:

Demographics:

a. Date of birth

b. Gender
c. Race / Ethnicity

Clinical Data/Assessments:

a. Height / Weight, Heart rate, Blood pressure, O2 Saturation
b. Cardiac diagnosis; type of congenital heart disease
c. Major medical history
d. Previous surgeries
e. Concomitant Medications
f. NYHA classification

a. Physical Exam/Review of Systems Pertinent to Congenital Heart Disease and Fontan PopulationPresence of cough
b. Presence of wheeze
c. Chest pain presence
d. Shortness of breath presence
e. Heart palpitations, heart murmur, S3/S4 gallop presence
f. Swelling presence in legs
g. Varicose vein presence
h. Current illnesses
i. Dizziness or syncope presence
j. Ascites presence
k. Presence of jugular vein distention
l. Palpability liver and spleen
m. Presence of weight loss, change in appetite, loose stools, blood in stool
n. Recent stroke symptoms

Laboratory Assessment:

a. NT-proBNP
b. CBC
c. CMP

d. LDH
e. GGT

Cardiopulmonary Exercise Tests (CPET):

a. Spirometry
b. Blood pressure
c. Resting EKG
d. Power output
e. Gas exchange
f. Ventilation
g. Pulse oximetry

MRI:

a. Ventricular volumes
b. Ejection fraction
c. Ventricular mass
    a. Cardiac output
    b. Cardiac index
    c. SVC flow
    d. IVC flow
    e. Aortic flow
    f. Mitral inflow

d. Strain

a. Global circumferential
b. Global longitudinal

e. T1 Mapping

Patient Disposition

Data will be collected by designated research coordinator(s) and the physicians participating in the study, with paper forms stored securely in locked file cabinets. Data will be managed by an experienced team of clinical data specialists and data coordinators using Data Management Center (DMC) standard operating procedures and guidelines. Appropriate data cleaning methods will

be applied and final analysis dataset(s) with any necessary calculations/derivations will be prepared by a SAS programmer before sending the data to the project statistician.

A study database will be designed in the Research Electronic Data Capture (REDCap) system, a secure, HIPAA-compatible online platform housed on secure research servers behind the CCHMC institutional firewall, and available free to all UC and CCHMC investigators. REDCap includes user-specific access rights to prevent unauthorized access beyond study personnel.

We will take the following precautionary measures to protect the privacy and confidentiality of the participants' research and/or medical record:

1. Computerized study data will be stored on a secure network that prohibits access by unauthorized users.
2. A password will be required to access the computerized study data.
3. Only approved individuals associated with the research project will have access to the study records.

9. STATISTICAL ANALYSIS

9.1 Study Endpoints

The primary endpoints are change in systolic and diastolic function on probenecid vs. placebo (measured via standard and advanced MRI parameters).

The secondary endpoint will be exercise performance on probenecid vs. placebo.

9.2 Statistical Analyses

Randomization and blinding: The randomization plan will be conducted according to Standard Operating Procedure (SOP) ST-43.01.01. Briefly, the study statistician will prepare a randomization plan to stratify randomization and sequence of treatments, based on the study protocol, and will test the randomization protocol to ensure study parameters are met. An independent (non-study) statistician will produce the final randomization list and will provide it to the investigational pharmacy who will be responsible for implementing the randomization and maintaining this randomization list until the study is unblinded.

All primary analyses will be conducted as intention to treat (ITT), with all outcomes tested between probenecid and placebo for all randomized patients. Safety analyses will, however, include a modified ITT, including any participant taking at least one study dose. The main outcomes will be pre- to post-treatment changes in EF and E/E' ratio; pre- to post-treatment changes in exercise performance between probenecid and placebo groups will also be evaluated. Transformations of measurements (e.g., log transformation or nonparametric rank transformation) will be considered as necessary to meet the assumptions of the models. The analysis will consider the sequence of treatments, the patient effect (within sequence), the period of study (first or second arm), and the treatment effect (probenecid or placebo) as potential factors impacting the pre-to-post change in the outcomes of interest. Mixed modeling will be used to test these effects, with consideration of repeated measures within patient represented by the cross-over design.

An exploratory analysis will test for differences in drug response between left and right single ventricle patients, recognizing this analysis will not be well-powered. Because the two physiologic groups will not be randomized relative to one another, demographic and clinical covariates that may differ between these strata will also be considered in mixed modeling. An interaction term will be introduced into the model and tested to evaluate whether treatment effects statistically differ by physiologic group (e.g., treatment*group). For all analyses, two-sided $p<0.05$ will be considered significant.

9.3 Sample Size Calculation

Power estimates for this study were based on preliminary data for EF, from the randomized cross-over designed study of adult patients with HFrEF. In the full analysis sample of 15 patients, the difference in the EF response between probenecid and placebo treatment in the 1-week cross-over design was normally distributed with mean difference of 7.7% and standard deviation (SD) of 7.6.

Assuming power of 80%, $\alpha=0.05$, and a 2-sided test, a sample size of 17 patients would be required to complete both arms of the study to detect this magnitude of effect. This sample size would also provide 73% power to detect the observed differences in E/E' ratio noted in the pilot study after 1 week of treatment.

To conservatively account for potential differences in drug effect between adults and children and other stochastic variability, as well as the potential for participant dropout during the crossover design, this study will enroll up to 22 patients, which would allow for up to 30% dropout.

10. RISKS AND BENEFITS

10.1 Potential Benefits

Patients who participate in this study may experience improvement in their ventricular function, exercise performance and tolerance, and symptoms of heart failure (if present) as a result of treatment with probenecid. If this occurs, they will be considered for possible continuation of therapy.

10.2 Potential Risks

There is minimal risk associated with this study. Risks are only related to cardiopulmonary exercise testing and to the potential side effects of probenecid therapy.

Probenecid has a long and reassuring safety record; however the following are potential adverse reactions:

> Serious reactions: Hemolytic anemia, aplastic anemia, leukopenia, thrombocytopenia, hepatic necrosis, anaphylaxis, nephrotic syndrome
>
> Common but mild/reversible reactions: Headache, dizziness, anorexia, nausea, vomiting, gingival soreness, urinary frequency, renal colic, anemia, dermatitis, pruritus, flushing, fever, gout exacerbation.

Care will be taken to avoid potential drug interactions by reviewing each subject's existing medication list prior to enrolling in the study. Probenecid has numerous known and well documented drug interactions, most of which will not be relevant to our study population. Patients

receiving any of the following medications will be excluded from the study: doripenem, zalcitabine, deferiperone, citalopram, methotrexate, ciprofloxacin, amoxicillin, cefprozil, cefpodoxime, cefotaxime, meropenem, ertapenem, valganciclovir, ganciclovir, ziovudine ketorolac, cefdinir, cephalexin, dapsone, indomethacin, and piperacillin.

Exercise testing also carries some risks, including mechanical trauma (i.e. from a fall), arrhythmias, syncope, and cardiac arrest. To minimize these risks, a trained exercise physiologist will perform this procedure. If the patient is considered moderate to high risk, a physician will also be present In addition, heart rhythm, vital signs, and perceived exertion will be monitored continuously throughout the test. Commonly reported symptoms include fatigue, shortness of breath, and muscle soreness; however, these symptoms do not typically result in any real risk to the patient. With the cardiopulmonary testing, patients frequently complain of discomfort or claustrophobia wearing the face mask, but again, this does not carry a true clinical risk. Chest pain is occasionally a complaint, but the risk of myocardial infarction is extremely rare in our patient population. The test will be discontinued if there is any concern for a pathologic arrhythmia, coronary ischemia, other evidence that the patient cannot safely proceed with the test, or if the patient expresses discomfort with proceeding due to symptoms or overwhelming anxiety.

Pregnancy:

Female participants
Pregnancy is an exclusion criteria for the study. Participants will have pregnancy testing at all study visits to CCHMC prior to any study procedures. Participants will also be instructed to use birth control for the duration of the study. Acceptable methods of birth control will be discussed with the patient by the PI or designee.

Loss of Confidentiality: There is a minimal risk for loss of confidentiality associated with this study. Precautions will be taken to minimize the possibility of disclosure of personal information. This risk will be minimized by utilizing traditional precautions for the storage of paper records (lock and key) and electronic records (password protection and use of secure servers). These precautions are outlined in the Privacy and Confidentiality section of this protocol. Patient identifiers will not be used in reports or publications from this study.

10.3 Risk/Benefit Analysis

The proposed study has minimal risks associated with it, which are reasonable in relation to the knowledge that will be gained and used to create interventional programs in this high-risk population. This study is being listed as “more than minimal risk” due to the very rare possibility of serious adverse reactions to probenecid.

☐ Minimal risk but with direct benefit to participants

☐ Minimal risk but without direct benefit to participants

☒ More than minimal risk but with direct benefit to participants

☐ More than minimal risk but without direct benefit to participant

11 ASSESSMENT OF SAFETY

11.1 Adverse Events

An adverse effect (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose. Adverse events will be recorded from the time of the first dose of study drug until resolution (or study completion).

Events that are unexpected and considered to be related or possibly related to the study procedures as well as breaches of confidentiality, and protocol violations must be reported to the IRB as soon as possible after discovery of the event. All adverse events will be classified by the PI for relationship (Related, Possibly Related, Unlikely Related, Not Related); severity (Mild, Moderate, or Severe); and expectedness (Expected or Unexpected). Events not meeting the criteria for prompt reporting will be reported to the IRB at the time of continuing review.

11.2 Serious Adverse Events

A Serious Adverse Event (SAE) is any undesirable occurrence associated with the use of a drug in a patient occurring at any dose, whether or not considered drug related, when the outcome is:

- Death,
- Life-threatening (i.e., participant was at substantial risk of dying at the time of the AE),
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions or resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life,
- Congenital anomaly/birth defect in the offspring of a subject who received study medication, or
- Other serious (important) medical events that may jeopardize the participant and may require medical or surgical intervention to prevent one of the other outcomes are also considered to be SAEs.

11.2.1 Relationship

All adverse events will be classified by the PI for relationship to the study drug as: Related, Possibly Related, Unlikely Related, Not Related.

11.2.2 Severity

All adverse events will be classified for severity by the PI as: Mild, Moderate, or Severe.

11.2.3 Expectedness

All adverse events will be classified for expectedness by the PI as: Expected or Unexpected. Very rare but potentially expected events associated with the use of probenecid include:

- Nausea
- Vomiting
- Dizziness
- Headache
- Anorexia
- Gingival soreness
- Urinary frequency
- Renal colic
- Anemia
- Dermatitis
- Pruritus
- Flushing
- Fever

11.3 Adverse Event Reporting

Expedited Reporting

SAEs require expedited reporting when meeting the following criteria:

- Serious
- Unexpected
- At least possibly related to the study agent or other protocol specific activity

SAEs meeting the above criteria are required to be reported to the FDA as follows:

- If characterized as fatal or life-threatening, within 7 calendar days of the sponsor's initial receipt of the information
- If non-fatal or non-life threatening, within 15 calendar days.

Events that are considered unexpected and possibly related to the conduct of the study will be reported to the IRB within seven days of discovery.

The DSMB will review SAEs within 48 hours after initial receipt of the information by the investigator(s) to review the PIs assignment of SAE as related or unrelated to treatment; to confirm the grading of toxicity, and assure that the study may continue.

Significant unplanned deviations from the protocol will also be reported as stipulated above. All other serious adverse events and non-serious adverse events will be reported at the time of submission of annual reports. Likewise, minor deviations will be reported in annual reporting.

12 DATA SAFETY AND MONITORING PLAN

Data will be collected electronically or on data collection forms that will not be labeled with the patient's name, only the study number. The list of matching codes to the patient's name will be handled and retained by the study coordinator and investigator. To ensure proper use and continued protection of these data, the data collection sheets will not be given to any individuals except those co-investigators and study coordinators performing data-entry.

Electronic participant binders will be maintained with names of the subject, pertinent clinical data, and files with signed, scanned and certified consent forms and copies of pertinent clinical and research related forms. All participant binders and study records will be stored on a password protected network accessible to only research personnel. A separate enrollment database linking subject identification number to identifying information (first name, last name, medical record number, and date of birth) will also be maintained on a password-protected computer network. Access to this information will be restricted to the PI, research staff and the IRB.

13 DATA SAFETY AND MONITORING BOARD (DSMB)

A Data Safety and Monitoring Board (DSMB) will be assembled to monitor for adverse outcomes. It will be comprised of 2 certified cardiologists with expertise in clinical trials and one biostatistician and will periodically review and evaluate the accumulated data for enrollment, eligibility criteria, and safety data, and will make recommendations for any alterations to the protocol or any safety concerns. The DSMB will meet after 2, 12, and 22 patients have completed the study protocol, or at least annually, and will also meet if a serious adverse event occurs.

Recruitment into the study will be temporarily halted under the following conditions:

1) If two subjects have the same Grade 3 or above AE that is thought to be related to study participation
2) Three or more subjects have a Grade 3 or 4 Adverse Event
3) One SAE occurs that is thought to be related to study participation

The DSMB charter provides additional details regarding the DSMB.

14 PRIVACY & CONFIDENTIALITY

The privacy and confidentiality of patient information will be maintained in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations. All research personnel who work on this study must complete HIPPA and the Collaborative Institutional Training Initiative module on human research with direct subject interaction. No identifying data will be used in any publications that were a result from this work.

15 PARTICIPATION COST AND PAYMENTS

There is no cost to participate in this study. Participants will not be charged for the tests that are done for research purposes however, participants will still be responsible for the usual costs of medical care. To compensate for time and travel, each participant will receive up to $240 total for full study participation. For attending each study visit, the participant will receive $50. However, if the participant also returns their MEMS bottle and cap with any remaining medication at visit 2 and visit 4, they will receive an additional $20 at each of those visits. Participants will also be reimbursed for travel at the federal mileage rate when traveling ≥ 50 miles one way and lodging at the discretion of the study team.

BIBLIOGRAPHY

FONTAN'S CIRCULATION

- Piran S, Veldtman G, Siu S, Webb GD, Liu PP. Heart failure and ventricular dysfunction in patients with single or systemic right ventricles. Circulation. 2002;105(10):1189–94.
- Szabó G, Buhmann V, Graf A, Melnitschuk S, Bährle S, Vahl CF, Hagl S. Ventricular energetics after the Fontan operation: contractility-afterload mismatch. The Journal of thoracic and cardiovascular surgery. 2003;125(5):1061–9.
- Gewillig MH, Lundström UR, Deanfield JE, Bull C, Franklin RC, Graham TP, Wyse RK. Impact of Fontan operation on left ventricular size and contractility in tricuspid atresia. Circulation. 1990;81(1):118–27.
- Rychik J, Veldtman G, Rand E, Russo P, Rome JJ, Krok K, Goldberg DJ, Cahill AM, Wells RG. The precarious state of the liver after a Fontan operation: summary of a multidisciplinary symposium. Pediatric cardiology. 2012;33(7):1001–12.
- Kouatli AA, Garcia JA, Zellers TM, Weinstein EM, Mahony L. Enalapril Does Not Enhance Exercise Capacity in Patients After Fontan Procedure. Circulation. 1997;96(5):1507–1512.
- Mahle WT, Wang A, Quyyumi AA, McConnell ME, Book WM. Impact of spironolactone on endothelial function in patients with single ventricle heart. Congenital heart disease. 4(1):12–6.
- Ishibashi N, Park I-S, Waragai T, Yoshikawa T, Murakami Y, Mori K, Mimori S, Ando M, Takahashi Y, Doi S, Mizutani S, Nakanishi T. Effect of carvedilol on heart failure in patients with a functionally univentricular heart. Circulation journal : official journal of the Japanese Circulation Society. 2011;75(6):1394–9.
- Coats L, O'Connor S, Wren C, O'Sullivan J. The single-ventricle patient population: a current and future concern a population-based study in the North of England. Heart (British Cardiac Society). 2014.
- Fogel MA, Weinberg PM, Fellows KE, Hoffman EA. A Study in Ventricular Ventricular Interaction : Single Right Ventricles Compared With Systemic Right Ventricles in a Dual-Chamber Circulation. Circulation. 1995;92(2):219–230.
- Notomi Y. Maturational and Adaptive Modulation of Left Ventricular Torsional Biomechanics: Doppler Tissue Imaging Observation From Infancy to Adulthood. Circulation. 2006;113(21):2534–2541.
- d'Udekem Y, Iyengar AJ, Galati JC, Forsdick V, Weintraub RG, Wheaton GR, Bullock A, Justo RN, Grigg LE, Sholler GF, Hope S, Radford DJ, Gentles TL, Celermajer DS, Winlaw DS. Redefining expectations of long-term survival after the Fontan procedure: twenty-five years of follow-up from the entire population of Australia and New Zealand. Circulation. 2014;130(11 Suppl 1):S32–8.
- Kiesewetter CH, Sheron N, Vettukattill JJ, Hacking N, Stedman B, Millward-Sadler H, Haw M, Cope R, Salmon AP, Sivaprakasam MC, Kendall T, Keeton BR, Iredale JP, Veldtman GR. Hepatic changes in the failing Fontan circulation. Heart (British Cardiac Society). 2007;93(5):579–84.

- Guha IN, Bokhandi S, Ahmad Z, Sheron N, Cope R, Marshall C, Veldtman G. Structural and functional uncoupling of liver performance in the Fontan circulation. International journal of cardiology. 2013;164(1):77–81.
- Kendall TJ, Stedman B, Hacking N, Haw M, Vettukattill JJ, Salmon AP, Cope R, Sheron N, Millward-Sadler H, Veldtman GR, Iredale JP. Hepatic fibrosis and cirrhosis in the Fontan circulation: a detailed morphological study. Journal of clinical pathology. 2008;61(4):504–8.

TRPV2 CHANNELS

- Rubinstein J, Lasko VM, Koch SE, Singh VP, Carreira V, Robbins N, Patel AR, Jiang M, Bidwell P, Kranias EG, Jones WK, Lorenz JN. Novel role of transient receptor potential vanilloid 2 in the regulation of cardiac performance. American journal of physiology. Heart and circulatory physiology. 2014;306(4):H574–84.
- Robbins N, Koch SE, Rubinstein J. Targeting TRPV1 and TRPV2 for potential therapeutic interventions in cardiovascular disease. Translational Research. 2013;161(6):469–476.
- Koch SE, Tranter M, Robbins N, Luther K, Singh U, Jiang M, Ren X, Tee T, Smith L, Varma P, Jones WK, Rubinstein J. Probenecid as a noninjurious positive inotrope in an ischemic heart disease murine model. Journal of cardiovascular pharmacology and therapeutics. 2013;18(3):280–9.
- Katanosaka Y, Iwasaki K, Ujihara Y, Takatsu S, Nishitsuji K, Kanagawa M, Sudo A, Toda T, Katanosaka K, Mohri S, Naruse K. TRPV2 is critical for the maintenance of cardiac structure and function in mice. Nature communications. 2014;5:3932.

PROBENECID SAFETY

- Craft JC, Feldman WE, Nelson JD. Clinicopharmacological evaluation of amoxicillin and probenecid against bacterial meningitis. Antimicrobial Agents and Chemotherapy. 1979;16(3):346–352.
- Sowunmi A, Fehintola FA, Adedeji AA, Gbotosho GO, Falade CO, Tambo E, Fateye BA, Happi TC, Oduola AMJ. Open randomized study of pyrimethamine-sulphadoxine vs. pyrimethamine-sulphadoxine plus probenecid for the treatment of uncomplicated Plasmodium falciparum malaria in children. Tropical medicine & international health : TM & IH. 2004;9(5):606–14.
- Sowunmi A, Adedeji AA, Fateye BA, Fehintola FA. Comparative effects of pyrimethamine-sulfadoxine, with and without probenecid, on Plasmodium falciparum gametocytes in children with acute, uncomplicated malaria. Annals of tropical medicine and parasitology. 2004;98(8):873–8.

- Vree TB, Van Ewijk-Beneken Kolmer EWJ, Wuis EW, Hekster YA. Capacity-limited renal glucuronidation of probenecid by humans. Pharmaceutisch Weekblad. 1992;14(5):325–331.
- Selen A, Amidon GL, Welling PG. Pharmacokinetics of probenecid following oral doses to human volunteers. Journal of Pharmaceutical Sciences. 1982;71(11):1238–1242.

CARDIAC MRI

- Buss SJ, Krautz B, Schnackenburg B, Abdel-Aty H, Santos MFB, Andre F, Maertens MJ, Mereles D, Korosoglou G, Giannitsis E, Katus HA, Steen H. Classification of diastolic function with phase-contrast cardiac magnetic resonance imaging: validation with echocardiography and age-related reference values. Clinical research in cardiology : official journal of the German Cardiac Society. 2014;103(6):441–50.
- Rathi VK, Doyle M, Yamrozik J, Williams RB, Caruppannan K, Truman C, Vido D, Biederman RW. Routine evaluation of left ventricular diastolic function by cardiovascular magnetic resonance: a practical approach. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance. 2008;10:36.
- Moody WE, Taylor RJ, Edwards NC, Chue CD, Umar F, Taylor TJ, Ferro CJ, Young AA, Townend JN, Leyva F, Steeds RP. Comparison of magnetic resonance feature tracking for systolic and diastolic strain and strain rate calculation with spatial modulation of magnetization imaging analysis. Journal of Magnetic Resonance Imaging. 2015;41(4):1000–1012.
- Thattaliyath BD, Forsha DE, Stewart C, Barker PCA, Campbell MJ. Evaluation of Right Ventricular Myocardial Mechanics Using Velocity Vector Imaging of Cardiac MRI Cine Images in Transposition of the Great Arteries Following Atrial and Arterial Switch Operations. Congenital heart disease. 2015.