Colchicine in Postoperative Fontan Patients (CPFP)

Protocol Number: 1

National Clinical Trial (NCT) Identified Number: NCT03575572

Principal Investigators: Albert Rocchini, MD, Stephanie Goldstein, MD

Funded by: University of Michigan

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Summary of Changes from Version 1.4 to 1.5:

Affected Section(s)	Summary of Revisions Made	Rationale
4.1, 9.4.6	Addition of interim analysis	Due to slower than anticipated recruitment, the study team has decided to perform an interim analysis.
8.3.3.3, 8.3.5	Spacing out the frequency of Safety Monitoring Committee meetings if there are less than 5 new patients enrolled	Since Fontan operations are often seasonal, there can be stretches of 6-8 months where there are 0-2 new patients enrolled. This modification allows for the SMC to postpone their next review to the next scheduled time if there are less than 5 new patients enrolled.

Summary of Changes from Version 1.3 to 1.4:

Affected Section(s)	Summary of Revisions Made	Rationale
4.2, 5.1, 9.3	Lowering the lower age limit of included patients from 24 months to 20 months	Fontan operations are occurring slightly earlier than 24 months. There is no difference in risk profile or mechanism of action with the administration of colchicine to a slightly younger age, as it has been studied to be safe and effective in children as young as less than one year of age[1,2]. This

		age change, however, significantly increases the patient population who will qualify for screening in for this study.
5.1, 6.1.2, 7.1	Alteration of the definition of "blood dyscrasia" or "myelosuppression"	After review of literature and discussion with Dr. Rajen Mody a clinical trialist in Pediatric Oncology, the definition of myelosuppresion and blood dyscrasia was altered to be: total WBC < 4.0, ANC < 0.1, ALC < 0.1, or platelets < 10. Many Fontan patients have lower WBC and ALC numbers at baseline[3] without an increase in infection risk[4]. Altering this criteria allows for increased eligible patients without putting patients at any increased risk of adverse events.
5.5	Update to compensation for patients and families	The compensation of a small toy for patients and a $25 gift card was already in process and part of the informed consent. This mistake was recognized upon this amendment and therefore updated.

Summary of Changes from Version 1.2 to 1.3:

Affected Section(s)	**Summary of Revisions Made**	**Rationale**
4.3, 6.1.2, 7.1, 8.2	Altering the wording regarding discontinuation of the study intervention secondary to GI distress, nausea and vomiting.	Most postoperative Fontan patients have some degree of nausea and vomiting. Upon review of the historical cohort that was not treated with the study intervention, 8 out of 12 had at 1-3 days of nausea and vomiting 1-4 times per day. Due to this, we are requesting an alteration of the study intervention discontinuation wording to include "above expected degree."
7.1, 8.2	Adding a clause that states we can continue or restart the study intervention if a noted adverse event is not related or unlikely to be related to the intervention.	Patients who have an event that is not related or unlikely to be related to the study intervention are being withdrawn from the study, despite the adverse event being

		unrelated to the study intervention based on the wording of the protocol.
8.2	Addition of "within" to the sentence: follow up labs "will be obtained within 1 week following discontinuation to ensure resolution"	If the clinical team obtains repeat labs earlier than 1 week that prove resolution of the adverse event, repeated studies are not necessary.
1.3, 8.3.4	Added a range of days for phone call follow up	It is sometimes not possible to reach families on particular days. This allows a range of time to reach the family in follow up.

Summary of Changes from Version 1.1 to 1.2:

Affected Section(s)	**Summary of Revisions Made**	**Rationale**
5.2	**Addition of exclusion criteria.**	**Based on recommendations from IRBMED**

Summary of Changes from Version 1.0 to 1.1:

Affected Section(s)	**Summary of Revisions Made**	**Rationale**
1.2, 1.3, 5.5, 8.2	**Addition of call to family prior to informed consent as screening for potential interest.**	**This was recommended as a first step to gauge interest in families, rather than approach them without time to think about their potential involvement.**
6.1.2, 7.1	**Alter the definition of concerning dyscrasia related to the study intervention.**	**Patients may have altered CBCs after surgery that is not related to the study intervention. Therefore, we altered the definition to account for this.**
6.2.2, 6.3.3	**Update the information about the colchicine information currently used at Michigan medicine**	**This was a recommendation from the IDS subcommittee based on the current hospital formulary. It was taken from the new colchicine package insert.**

1.1, 1.2, 1.3, 3, 8.1, 9.1, 9.2, 9.4.2,	**Delete the blood cytokine portion of the protocol.**	**Upon scientific review during grant proposal, this was thought to be unnecessary for the purposes of our hypothesis. It was not funded, and we have removed it from our protocol.**
10.1.5	**Updated contact information**	**The email that was listed was the non-institutional email for the Co-PI**

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.]

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: The effect of empiric Colchicine on inflammatory cytokines in postoperative Fontan pleural drainage

Study Description: Prolonged pleural drainage is a common complication of postoperative Fontan patients. There is elevation of inflammatory cytokines in the Fontan population out of proportion to age-matched controls, with increase over time. Colchicine is an anti-inflammatory agent with a low side-effect profile that may target the pro-inflammatory cytokines seen elevated in post-operative Fontan patients. The hypothesis of this study is that empiric Colchicine will decrease pro-inflammatory cytokines and therefore may decrease the duration of pleural drainage in post-operative Fontan patients.

Objectives:

Primary Objective: Determine if Colchicine is associated with decreased inflammatory cytokines (TNF-α) within the pleural space in post-operative Fontan patients compared to a historical cohort of Fontan patients

Secondary Objective:

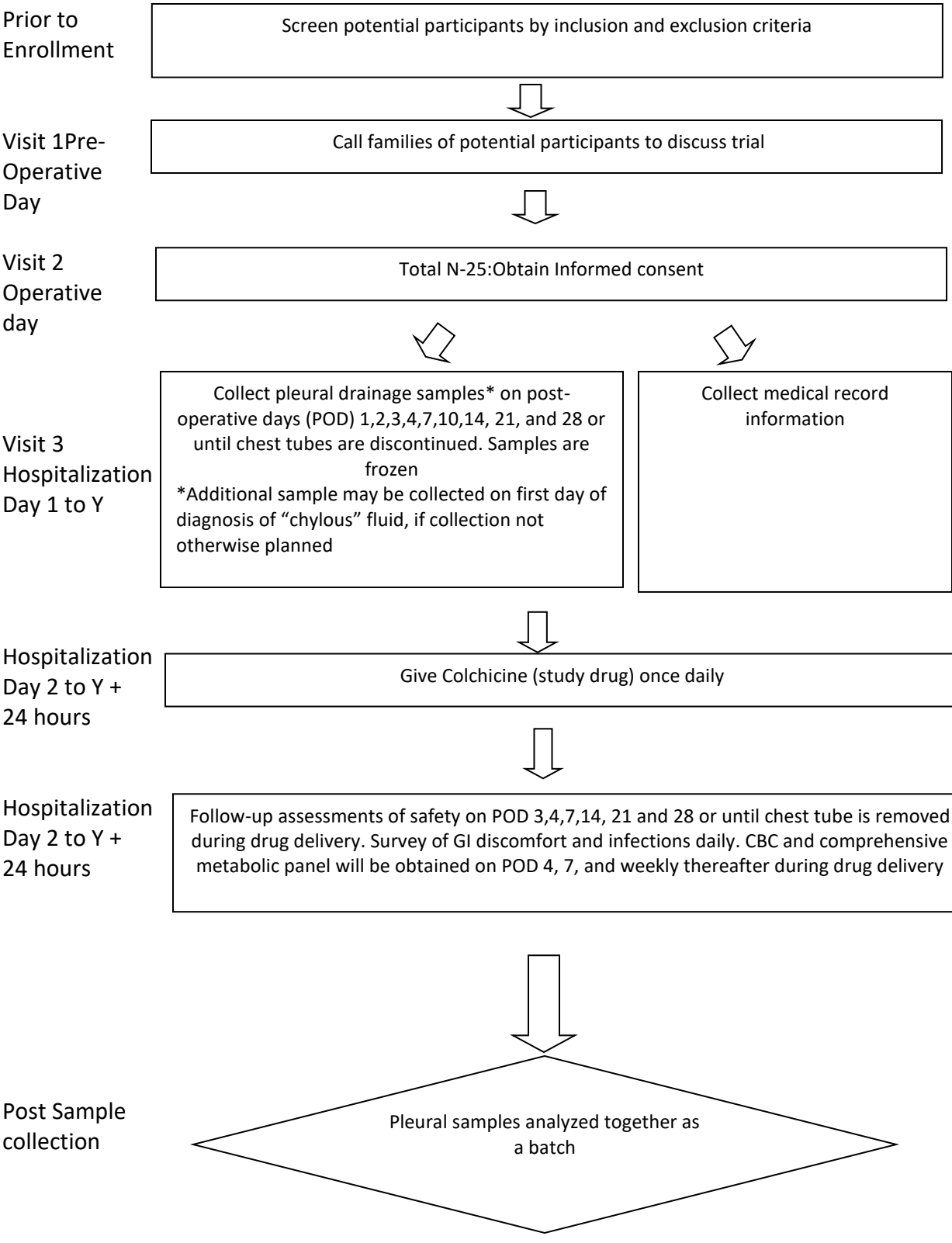
1. Examine the cytokine concentrations in Fontan patients treated empirically with Colchicine in comparison to a cohort of Fontan patients at a given time-point postoperatively.

2. Determine if Colchicine is associated with decreased duration of pleural drainage in post-operative Fontan patients compared to a cohort of Fontan patients
3. Determine if Colchicine reduces hospital length of stay following Fontan procedure

Endpoints:	Primary Endpoint: Change in Cytokine concentrations (TNF-α) from postoperative day 1 (POD1) to the day most distal from surgery (DMD) in pleural fluid in patients s/p Fontan palliation treated with Colchicine Secondary Endpoints: 1. Cytokine concentrations (particularly TNF-α, MIP-1βm, IL-17A, IL-8, and INF-γ) in Fontan patients treated with Colchicine at a given time-point postoperatively 2. Duration of pleural drainage in Fontan patients treated with Colchicine. 3. Hospital length of stay in Fontan patients treated with Colchicine
Study Population:	25 patients undergoing Fontan operation ages 24 months to 5 years and 364 days at the University of Michigan Congenital Heart Center.
Phase:	2
Description of Sites/Facilities Enrolling Participants:	The study will be performed at one site: The University of Michigan
Description of Study Intervention:	Colchicine, an anti-inflammatory drug will be given on postoperative day 2. Colchicine will be given at 0.6 mg once daily for the duration of chest tube output plus 24 hours after chest tube removal with a maximum of 4 weeks duration.
Study Duration:	24 months
Participant Duration:	10 days, on average based on median hospitalization in control cohort.

1.2 SCHEMA

Example #1 Flow diagram *(e.g., randomized controlled trial)*



Y= chest tube removal date or a second chest intervention

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening Day -30 to -1	Enrollment/Baseline Visit 1: Hospitalization, POD 1[a]	Hospitalization, POD 2	Hospitalization, POD 3	Hospitalization, POD 4	Hospitalization, POD 5	Hospitalization, POD [b]	Hospitalization, POD 7	Hospitalization, POD 10	Hospitalization, POD 14	Hospitalization, POD 21	Hospitalization, POD 28	Hospitalization, POD Z	Phone call, [c]+5-14 days
Informed consent	x													
Demographics	x													
Operation*	x													
Pleural fluid collection		x	x	x	x		x	x	x	x	x	x		
Review and record relevant medical history and medication history	x	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	x		
Drug delivery once daily[c]			x	----------	----------	----------	----------	----------	----------	----------	----------	x		
CBC, Renal function panel	X													
CBC, comprehensive metabolic panel					X			x		x	x	x		
Complete Case Report Forms (CRFs) with AE recording	X	X	X	X	X	X	X	X	X	X	X	X	X	

a: POD is defined as midnight to midnight (calendar day).
b: This collection will only be obtained on the first day of the diagnosis of "chylous" fluid
c: Drug delivery continues for duration of chest tube drainage or re-intervention in the chest + 24 hours for a maximum duration of 4 weeks
Z: Hospital discharge
*: operation is per clinical care; not part of the study

2 INTRODUCTION

2.1 STUDY RATIONALE

Prolonged pleural drainage and chylothorax are common complications post Fontan procedure and are associated with short and long term morbidities such as prolonged hospital stay, protein losing enteropathy, and plastic bronchitis. Our previous study showed a statistically significant increase in pro-inflammatory cytokines (specifically IL 8, IL-17A, INF-ɣ, TNF-α, MIP-1β) localized to the pleural space in comparison to age-matched controls. Our study also showed a longer duration of pleural drainage and thus longer hospitalization in the Fontan study group. While prolonged pleural drainage in this population is likely multifactorial, with suspected etiologies including thoracic duct injury, and increased lymphatic pressures, our hypothesis is that localized inflammation contributes to this prolonged pleural drainage. Colchicine is an anti-inflammatory medication that has been used in pericarditis, persistent pericardial effusions, Familial Mediterranean Fever, and Gout and many other inflammatory diseases, and has mechanisms of action that target TNF-α and INF-ɣ, among other inflammatory cytokines. We hypothesize that addition of colchicine to empiric treatment of post-operative Fontan patients will

decrease inflammatory cytokines within the pleural space and therefore may decrease the duration of pleural drainage, thus shortening hospitalization and potential hospital-associated complications.

2.2 BACKGROUND

The Fontan procedure, introduced in 1971, is the final of three stages of palliation for patients born with single ventricle heart disease and involves creation of a total cavo-pulmonary connection typically performed at 2-3 years of age.[5] This procedure has enabled many children born with previously fatal defects to live into adulthood. With advancements in surgical techniques and perioperative management, morbidity and mortality from Fontan palliation has improved but remain significant.[6] One challenging problem in the immediate postoperative care of the Fontan patient is prolonged pleural drainage. The rate of pleural effusions in patients with functional single ventricles ranges from 12% to 45%[7], and remains a dominant morbidity associated with increased postoperative length-of-stay. It has also been associated with an increased risk of protein losing enteropathy and plastic bronchitis and diminished short- and long-term survival.[8–10] Currently, treatment methods include Fontan fenestration, aggressive diuresis, low fat diet, parenteral nutrition, and time.[11] Octreotide administration, thoracic duct ligation, and pleurodesis are reserved for refractory drainage with variable results.[12] Rare alternative treatment methods have been attempted to no avail including diaphragmatic fenestration[13] and angiotensin-converting enzyme inhibitor use,[14] however, no treatment methods have ideal results and patients requiring treatment often continue to drain.

The etiology for prolonged pleural drainage in a Fontan patient is likely multifactorial. Fontan patients have hemodynamic characteristics that favor pleural fluid accumulation including elevated central venous pressures, elevated right ventricle (RV) end-diastolic pressure from poor ventricular compliance or neo-aortic valve regurgitation, elevated pulmonary vascular resistance, or significant aortopulmonary collaterals. The inadvertent surgical disruption of lymphatic channels during the Fontan surgery may cause extravasation of chylous fluid and compromise local lymphatic function. Activation of the inflammatory and complement cascades during the course of cardiopulmonary bypass (CPB) can cause vascular capillary leak to promote the development of effusions.[7] Patients undergoing the Fontan procedure have been shown to have significantly increased postoperative plasma levels of inflammatory cytokine and complement production compared to preoperative levels.[15] Also, cytokine and complement levels were significantly higher in patients who had the Fontan procedure with CPB compared to patients who had the Fontan procedure off pump.[16]

A recent study from our institution showed a significant increase in pro-inflammatory cytokines (IL-17A, IFN-γ, MIP-1β, and TNF-α) localized to the pleural space in the postoperative Fontan population in comparison to age-matched controls undergoing CPB. Inflammatory cytokines ((IL-8, MIP-1β, and TNF-α) increased over time in the Fontan population, while an anti-inflammatory cytokine, IL-10, decreased over time. The control population had no such changes. TNF-α in particular was significantly elevated in Fontan patients as compared to controls on DMD from surgery, it had a significant increase over time in the Fontan group, whereas the control group had no such change. The change over time in comparison to the control group was also significantly different. [17] This suggests an exaggerated ongoing inflammatory response or process in the postoperative Fontan population that is not a result of cardiopulmonary bypass alone, particularly in the well described pro-inflammatory cytokine TNF-α.

Colchicine is a widely used and widely recognized drug and has been used to treat acute and recurrent pericarditis[18–21], to reduce postoperative pericardial and pleural effusions, shortening postoperative hospital stays after cardiac surgeries in adults[22,23], and even reduce post-pericardiotomy

syndrome[24,25]. It was also shown to acutely suppress local cardiac production of inflammatory cytokines in patients with acute coronary syndrome [26]. Colchicine modulates multiple pro-inflammatory pathways. It prevents microtubule assembly, thereby disrupting inflammasome activation, microtubule-based inflammatory cell chemotaxis, generation of leukotrienes, and cytokines, and phagocytosis. [27] It also impairs neutrophil function by impacting inflammatory pathways and mediators of neutrophil activation. It is used in multiple inflammatory conditions, including gout, Behçet's syndrome, primary biliary cirrhosis, alcohol-induced liver cirrhosis, psoriasis, Sweet's syndrome, scleroderma, sarcoidosis, and Familial Mediterranean Fever. [1,28–30] Colchicine blunts TNF-α induced activation of macrophages and reduces the number of TNF-α receptors on the surface of macrophages and endothelial cells. [27] It also decreases levels of the proinflammatory cytokines IL-1β, INF-ɣ, IL-18, and IL-6, among others. Our previous study demonstrates that, TNF-α and INF-ɣ were shown to be significantly elevated in the pleural fluid of postoperative Fontan patients, and increased over time [17]. We propose the novel hypothesis that Colchicine, given empirically to postoperative Fontan patients, decreases the pro-inflammatory cytokines within the pleural space and decreases the duration of pleural drainage in post-operative Fontan patients, thus contributing to shortened hospitalizations.

While the above studies were all done in adults, Colchicine has been shown to be safe and efficacious in children, even as young as less than 1 year of age[1,31,32]. It is most commonly given in children with Familial Mediterranean Fever (FMF), and has minor side-effects [1,2]. Typical side effects seen are GI distress (diarrhea, cramping) and mildly increased transaminases, but these can be controlled by a decrease in the dose.[2] Dosage typically given to children for Familial Mediterranean Fever ranges from 0.3-2 mg /day in 1-2 divided doses. [1,2,31–38] The FDA approved label dose for children ages 4-6 years is 0.3-1.8 mg daily.

Significance

The etiology of pleural effusions in patients undergoing the Fontan operation is likely multifactorial, and many studies indicate that an inflammatory response contributes to this process. Our prior study showed a significant elevation in pro-inflammatory cytokines localized to the pleural space in post-operative Fontan patients as compared to age-matched controls[17].

Our study will investigate the novel hypothesis that Colchicine, given empirically, to post-operative Fontan patients decreases the pro-inflammatory cytokines within the pleural space and therefore may decrease the duration of pleural drainage, thus contributing to shortened hospitalizations. If this is true, further studies can be done to extend to multiple centers and to evaluate for potentially decreased long-term morbidity in post-operative Fontan patients.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The most commonly reported side effect of Colchicine is GI discomfort, nausea, vomiting, and diarrhea. This is a short term risk and has been shown to be mitigated by dose decrease [37]. As stated in the FDA

approved drug label, these effects are usually mild, transient and reversible upon lowering the dose. They typically present within 24 hours of initiation of therapy. Survey of symptoms will be performed on POD 3 and 4, 24 and 48 hours after initiation of therapy, and POD 7 and weekly thereafter for as long as the patient remains on the therapy drug for monitoring. Blood dyscrasias including myelosuppression, leukopenia, granulocytopenia, thrombocytopenia, pancytopenia and aplastic anemia have been reported with colchicine used in therapeutic doses. A CBC will be collected during blood draws on POD 4 (2 days after initiation of drug), and POD 7 and weekly thereafter for as long as the patient remains on the therapy drug for monitoring. Colchicine-induced neuromuscular toxicity and rhabdomyolysis have been reported with chronic treatment. This study period consists of short term treatment, and should not be applicable. Colchicine is a P-gp and CYP3A4 substrate, therefore life-threatening and fatal drug interactions have been reported in patients treated with colchicine given with P-gp and strong CYP34A inhibitors. If a patient is on these classes of drugs, colchicine will not be given.

There is a slight increased risk of infection as part of the collection protocol. The samples are collected in a sterile manner from a self-sealing chest tube. This is an immediate risk, and there is no long range risk as part of this. As an alternative to needle puncture into the tubing, collections could be obtained via the collection box, however this is a collection of multiple days of pleural drainage and is therefore inaccurate for the purposes of this study. There is a slightly increased risk of infection secondary to blood draws as well, however this is no higher than would be for a clinically indicated blood draw. There is a risk of loss of private or confidential information.

2.3.2 KNOWN POTENTIAL BENEFITS

Colchicine acts as an anti-inflammatory medication. Its primary indication is for gout, an inflammatory process. It has also been used in pericarditis, recurrent pericarditis, pericardial and pleural effusions, and post-pericardiotomy syndrome in adults with notable improvements in duration of effusions [18–24]. It is FDA approved for use in young children, primarily for Familial Mediterranean Fever, another inflammatory disease. There may be a benefit of decreasing hospital length of stay, as Fontan patient's length of stay is proportional to their duration of chest tube drainage [17]. If chest tube duration is decreased, this may decrease the risk of long term complications such as protein losing enteropathy and plastic bronchitis, as these were associated with chest tube drainage longer than 2 weeks [8,10]

There is no specific benefit to the sample collection itself.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Colchicine is a medication that has been widely used and very well tolerated in pediatric patients. The potential for decreased duration of chest tube drainage and decreased hospital length of stay with a possible decrease for long term complications associated with Fontan physiology as well, outweighs the risk of mild GI discomfort and diarrhea, which is the most commonly reported side effect associated with the medication. Additionally, in the studies cited above, if there were complaints of GI discomfort or diarrhea, they were ameliorated by a reduction in dose. The warnings of dyscrasias, neuromuscular toxicity and rhabdomyolysis are rare and have been reported in cases of overdose or excessive accumulation.

Obtaining the samples is necessary to determine if the drug is affecting the proposed mechanism of action of the Fontan pleural drainage (inflammation within the pleural space). The risk of infection is

low, and among the baseline cohort of patient who underwent a similar collection schedule, there were no infectious complications.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Determine if Colchicine is associated with decreased inflammatory cytokines (TNF-α) within the pleural space in post-operative Fontan patients compared to a historical cohort of Fontan patients	Change in Cytokine concentrations (TNF-α) from postoperative day 1 (POD1) to day most distal from surgery (DMD) in pleural fluid in patients s/p Fontan palliation treated with Colchicine	Cytokines correlate with the findings from the original study on the Fontan cohort. Certain cytokines in the Fontan patients were elevated (TNF-α, MIP-1β, IL-8, IL-17) as compared to age-matched controls, and increased over time, whereas controls did not. This endpoint allows us to evaluate if colchicine effects these changes as the mechanism of action if there is decreased duration of pleural drainage.
Secondary		
1. Examine the cytokine concentrations in postoperative Fontan patients treated empirically with Colchicine in comparison to a cohort of postoperative Fontan patients at a given time-point postoperatively 2. Determine if empiric treatment with Colchicine is associated with decreased duration of pleural drainage in post-operative Fontan patients compared to a cohort of Fontan patients not treated with Colchicine 3. Determine if empiric treatment with Colchicine is associated with decreased length of stay in postoperative Fontan patients as compared to a	1. Cytokine concentrations (particularly TNF-α, MIP-1β, IL-17A, IL-8, and INF-γ) in comparison to postoperative Fontan patients at a given time-point postoperatively 2. Duration of pleural drainage in Fontan patients treated with Colchicine 3. Hospital length of stay in Fontan patients treated with Colchicine	1. Cytokines correlate with the findings from the original study on the Fontan cohort. Certain cytokines were higher (TNF-α, MIP-1β, IL-8, IL-17) as compared to age-matched controls, and increased over time, whereas controls did not. This endpoint allows us to evaluate if colchicine effects these changes as the mechanism of action if there is decreased duration of pleural drainage 2. The duration of chest tube drainage in postoperative Fontan patients correlates to short and long term morbidity. Our

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
cohort of Fontan patients not treated with Colchicine		hypothesis is that elevated cytokines contribute to longer drainage, therefore decreasing duration of chest tube drainage with colchicine treatment is a clinically meaningful result. 3. Hospital length of stay was proportional to chest tube drainage, therefore if empiric Colchicine is associated with decreased chest tube drainage, hospital length of stay may be another clinically meaningful result.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This study's hypothesis is that certain pro-inflammatory cytokines (TNF-α, MIP-1β, IL-17A, IL-8 and INF-γ) are significantly reduced in Fontan patients treated with colchicine as compared to a historical cohort of Fontan patients. The secondary hypotheses is that Colchicine, when given empirically to post-operative Fontan patients, decreases the median duration of chest tube drainage and hospital length of stay in comparison to a cohort of Fontan patients.

This is a phase II trial for efficacy of Colchicine (study intervention) in this population.

The study is a single-arm, single site clinical trial in which all enrolled Fontan patients will be given the empiric drug. Selection bias will be minimized as all consenting, eligible Fontan patients will be given the empiric drug and this will be in comparison to a historical cohort of Fontan patients who underwent surgery all at the same institution where surgical technique is similar. See section 6.1.2 for specific dosing and administration of the study drug.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study intervention group will be compared with a historical control group. This control group of patients is from the previous study in children ages 20 months to 5 years and 364 days, who underwent Fontan palliation at the University of Michigan between April 2016 – October 2016. The study was similar, without the study intervention of colchicine administration.

4.3 JUSTIFICATION FOR DOSE

In children, Colchicine is most commonly given in children with Familial Mediterranean Fever (FMF), and has minor side-effects [1,2]. Dosage in clinical use ranges from 0.3-2 mg /day in 1-2 divided doses [1,2,31–38]. We chose 0.6 mg once daily as the dose based on the largest number of studies with this dosing range to maximize effect and minimize side effects. In addition, the drug is formulated in 0.6 mg tablets. There will be no titration. If there are complaints of GI distress out of proportion to the expected degree, the dose will be decreased to 0.3 mg daily (1/2 tablet). The drug is available in 0.6mg tablets, however children within the age-range in this study will not be able to take the medication in this form. The tablet will be dissolved in 2-3 mL of either water, juice (non-grapefruit containing) or milk to make a solution that is able to be given to children of this age.[39,40] This process will be performed per standard hospital procedures. The drug may NOT be crushed. The drug may be given via mouth, G-tube, NG tube, or NJ tube. The drug will be prepared beside for immediate use (given within one hour).

4.4 END OF STUDY DEFINITION

The end of the study participation is defined as completion of the last visit shown in the Schedule of Activities (SoA) Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Parent stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 20 months to 5 years and 365 days
4. Diagnosed with single ventricle heart disease requiring Fontan palliation
5. Undergoing Fontan palliation at the University of Michigan Congenital Heart Center
6. Ability to take oral or enteral medication

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current or recent (within 14 days) use of medication with P-gp and strong or moderate CYP34A inhibitors or protease inhibitors
2. Pre-existing myelosuppression as defined as a total white blood cell count less than 4.0 K/uL, absolute neutrophil count less than 0.1 K/uL, absolute lymphocyte count less than 0.1 K/uL, or platelets less than 10 L/uL on routine pre-operative labs.
3. Current use of clarithromycin, cyclosporine, atorvastatin, simvastatin, pravastatin, fluvastatin, gemfibrozil, fenofibrate, finofibric acid, and benzafibrate.
4. Renal impairment as defined by serum creatinine greater than 3 times baseline or as deemed by the clinical inpatient team.
5. Hepatic impairment as defined by serum transaminases greater than 3 times the upper limit of normal or as deemed by the clinical inpatient team
6. Less than 20 months or greater than 5 years of age (5 years and 364 days).
7. Children who weigh under 10 kilograms.
8. Known allergic reactions to Colchicine
9. Treatment with another investigational drug within 3 months
10. Other conditions or postoperative complications that would increase the risk to the patient as determined by the study team, including but not limited to need for mechanical support (ECMO), or need for re-intervention within their chest more than 4 hours after arrival to the ICU.

5.3 LIFESTYLE CONSIDERATIONS

Not applicable

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) will not be rescreened, as it would be a change in their postoperative clinical course such that they would be ineligible to receive the study intervention.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

- *Target study sample size:* 25 patients undergoing Fontan palliation. Anticipated number to be screened is 45 patients (assuming approximately 70% consent to the study).
- *Anticipated accrual rate:* The Fontan operation typically occurs between the calendar months of March through October. The study will likely reach its target sample size after 2 years of recruitment seasons based on the number of Fontan operations the University of Michigan Congenital heart center performs each year.
- *Anticipated number of sites:* Subjects will be enrolled at the University of Michigan only, and will be having their operation at our institution.

- Source of participants*:* Eligible subjects will be determined by the surgical schedule. Patients will then be screened for inclusion criteria by the medical record as well as parental interview. Subjects parents will be called pre-operatively to discuss the trial. Informed consent documents will be sent to their home for review. Signatures will be obtained either at their pre-operative clinic visit or in the waiting room while their child is undergoing surgery. This will be completed within 3 weeks prior to enrollment.
- *Recruitment venues:* as above.
- *How potential participants will be identified and approached:* Potential candidates will be identified based on the surgical schedule. Any patient who is scheduled to have a Fontan operation will be eligible to be approached for screening.
- *Specific strategies that will be used to recruit and retain historically under-represented populations in order to meet target sample size and conform with the NIH Policy on Inclusion of Women and Minorities as Participants In Research Involving Human Subjects:* Any patient who is scheduled to undergo the Fontan operation will be recruited/screened for the study. This includes males, females, and any ethnic group.
- This study includes a vulnerable population: Children as part of the definition of the study inclusion criteria, the subject must be 20 months to 5 years and 364 days of age.

Patients enrolled will receive a small toy and their family will receive a $25 gift card to Amazon.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Colchicine is an alkaloid approved in 1961 for the use in Familial Mediterranean Fever in adults and children 4 years of age or older. As described in section 2.2 and 2.3, it has been widely used for decades in other indications, such as Gout, recurrent pericarditis, pericardial effusions and other inflammatory diseases. This drug is commercially available and is approved in children 4 years and older.

There is no control intervention.

6.1.2 DOSING AND ADMINISTRATION

Colchicine will be given on postoperative day 2. Colchicine will be given at 0.6 mg once daily for the duration of chest tube output plus an additional 24 hours after chest tube removal. It will be given in the morning with the 0900 medication administration or within 12 hours of that time. It does not need to be timed relative to a meal. There will be no dose escalation. If the patient has complaints or evidence of GI distress (as some patients are too young to voice complaints) or nausea out of proportion to the expected degree, or diarrhea for more than 24 hours, the dose will be reduced to 0.3 mg (1/2 tab) given once daily. If the GI distress out of the expected degree continues for more than 24 hours following that change, the medication will be stopped. Expected degree is defined as more than 2 x per day for more than 3 days, based on the historical cohort of postoperative Fontan patients.

If the patient has chylous fluid, there will be no change in continuation of colchicine. If the patient is deemed to have clinically significant renal or hepatic dysfunction (3x baseline creatinine or 2x upper limit of normal for AST/ALT), the drug will be discontinued. If there is evidence of blood dyscrasias on screening CBCs, as defined as 20% lower than the a total white blood cell count less than 4.0 K/uL, absolute neutrophil count less than 0.1 K/uL, absolute lymphocyte count less than 0.1 K/uL, or platelets less than 10 L/uL , the drug will be discontinued. Other signs of toxicity that may warrant dose reduction or discontinuation (per the label) if severe: neuropathy, purpura, rash, rhabdomyolysis, muscle pain or weakness.

After initial chest tube removal, if a patient has re-accumulation such that a chest tube is replaced within 48 hours, Colchicine will be continued for the duration of the new chest tube plus an additional 24 hours.

The maximum duration of drug delivery will be 4 weeks, at which point it will be deemed treatment failure and the medication will be terminated. The minimum duration of drug delivery will be 2 days.

The drug is formulated in 0.6mg tablets. The tablet will be dissolved in 2-3 mL of either water, juice (non-grapefruit containing) or milk to make a solution that is able to be given to children of this age.[39,40] This process will be performed per standard hospital procedures. The drug may NOT be crushed. The drug may be given via mouth, G-tube, NG tube, or NJ tube. The drug will be prepared beside for immediate use (given within one hour).

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The University of Michigan hospital inpatient pharmacy will distribute the medication from their inpatient supply in pill format with instructions on creating the appropriate solution. The bedside nurse will dissolve the tablet in solution, and administer the medication.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Distributed by:
Prasco Laboratories
Mason, OH 45040
Colchicine tablets, USP, **0.6 mg,** are purple, capsule-shaped, film-coated tablets, scored on one side and debossed with “AR;374” on the other side. There is no control product.

6.2.3 PRODUCT STORAGE AND STABILITY

Store Colchicine Tablets, USP at room temperature between 68°F and 77°F (20°C and 25°C). Keep Colchicine Tablets, USP in a tightly closed container. Keep Colchicine Tablets, USP out of the light.

6.2.4 PREPARATION

The drug is formulated in 0.6mg tablets. The tablet will be dissolved in 2-3 mL of either water, juice (non-grapefruit containing) or milk to make a solution that is able to be given to children of this age.[39,40] This process will be performed per standard hospital procedures. The drug may NOT be crushed. The drug may be given via mouth, G-tube, NG tube, or NJ tube. The drug will be prepared beside for immediate use (given within one hour).

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This study is neither blinded nor randomized. It is a single arm trial.

6.4 STUDY INTERVENTION COMPLIANCE

The study intervention will be completed entirely during a patient's hospitalization, and therefore will be documented and monitored by the study team. If a dose is missed, i.e. due to vomiting, refusal, missed administration, etc., the dose will be logged as missed and dosing will resume the following day.

6.5 CONCOMITANT THERAPY

Patients will be screened at this study's initiation. If the patient has been on a study drug in the 3 months prior to the start of this trial, they will be excluded. As mentioned in section 5.2, concomitant use of atorvastatin, simvastatin, pravastatin, fluvastatin, lovastatin, gemfibrozil, fenofibrate, fenofibric acid or benzafibrate (themselves associated with myotoxicity) or cyclosporine with colchicine may potentiate the development of myopathy and therefore patients taking these medications will be excluded.

If it is imperative that a subject begin taking the following excluded medications such as drugs known to inhibit CYP34A and P-glycoprotein during the study, they will then be withdrawn from the study.

Grapefruit and grapefruit juice may also interact and should not be consumed during colchicine treatment.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from the drug administration and sample collection does not mean discontinuation from the study. The subject's medical record information will continue to be collected and they will be included in the analysis. As stated in section 6.1.2, the medication dose will be cut in half in the event of GI complaints or symptoms above the expected degree and these will be reported as adverse events (AEs). If the patient continues to have GI complaints or symptoms (nausea, abdominal pain, diarrhea) above the expected degree, for more than 24 hours after dose decrease, the study drug will be discontinued and these will also be reported as AEs.

Also as stated in section 6.1.2, If the patient is deemed to have renal or hepatic dysfunction on screening comprehensive metabolic panels, as defined by the clinical team or if the serum creatinine is greater than 3 times the baseline, or if the transaminases are 2 time higher than the upper limit of normal, the study drug will be discontinued. If there is evidence of blood dyscrasias on screening CBCs, as defined as a total white blood cell count less than 4.0 K/uL, absolute neutrophil count less than 0.1 K/uL, absolute lymphocyte count less than 0.1 K/uL, or platelets less than 10 L/uL , the drug will be discontinued and the finding will be reported as an adverse event (AE). If these laboratory abnormalities are not related or unlikely to be related to the study intervention as defined in section 8.3.3.2 and the abnormalities normalize within 48 hours, the study intervention may be restarted upon discussion with the clinical team and/or the safety monitoring board as necessary.

Pleural samples will be discontinued and the drug will be discontinued in the event that a patient has a re-intervention in their chest (i.e. re-operation, chest exploration more than 4 hours after arrival in ICU). Re-placement of chest tubes is not included in the definition of re-intervention in a patient's chest. Blood samples for screening purposes (see SOA in section 1.3) will be obtained as long as the patient is on the study drug.

There is no specific additional data that will need to be recorded at the time of study intervention discontinuation, aside from the medical record/history data already recorded as part of the study design.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive the drug for 2 days despite continued chest tube drainage

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form but do not receive the study intervention may be replaced. Subjects who sign the informed consent form and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study will not be replaced.

7.3 LOST TO FOLLOW-UP

Not applicable. The study is during their inpatient postoperative hospital stay.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The study intervention, Colchicine, will be given once for the duration of chest tube drainage plus 24 hours after chest tube discontinuation, as described in Section 6.1.2. The medication will be put into solution (as described in Section 6.1.2) by the bedside registered nurse.

Pleural fluid studies
3 mL of pleural fluid will be collected from chest tubes in sterile fashion on postoperative days 1, 2, 3, 4, 7, 10, 14, 21 and 28. The self-sealing chest tube will be cleaned with Betadine and a sterile needle no larger than 23G with a sterile syringe will be inserted into the tubing with negative pressure to collected the fluid. Samples will be centrifuged at 500 RPM x 5 minutes, the supernatant will be collected and placed into a storage tube, and then frozen at -80 degrees Celsius until all samples are collected. At that time, all samples will be analyzed concurrently to decrease variability in laboratory technique. Pleural fluid will be tested using the Bio-rad Bio-Plex ProHuman Cytokine 17-plex Assay (#M5000031YV) on the

Bio-Rad MAGPIX Multiplex Reader[41] for the following inflammatory cytokines[42]: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17A, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), interferon-gamma (IFN-γ), monocyte chemoattractant protein (MCP-1), and macrophage inflammatory protein (MIP-1β).

Samples will also be obtained if the clinical team deems the pleural fluid "chylous" prior to initiation of standard of care therapy[a]. If a patient is started on standard of care treatment for high-output effusions, one additional sample will be obtained on the day of therapy initiation if not on the prior collection schedule, and then the protocol will revert to the prior collection schedule.

If a patient has chest tubes replaced for re-accumulation of pleural fluid, samples will be collected on the day of placement, for the first 3 days, and then weekly thereafter.

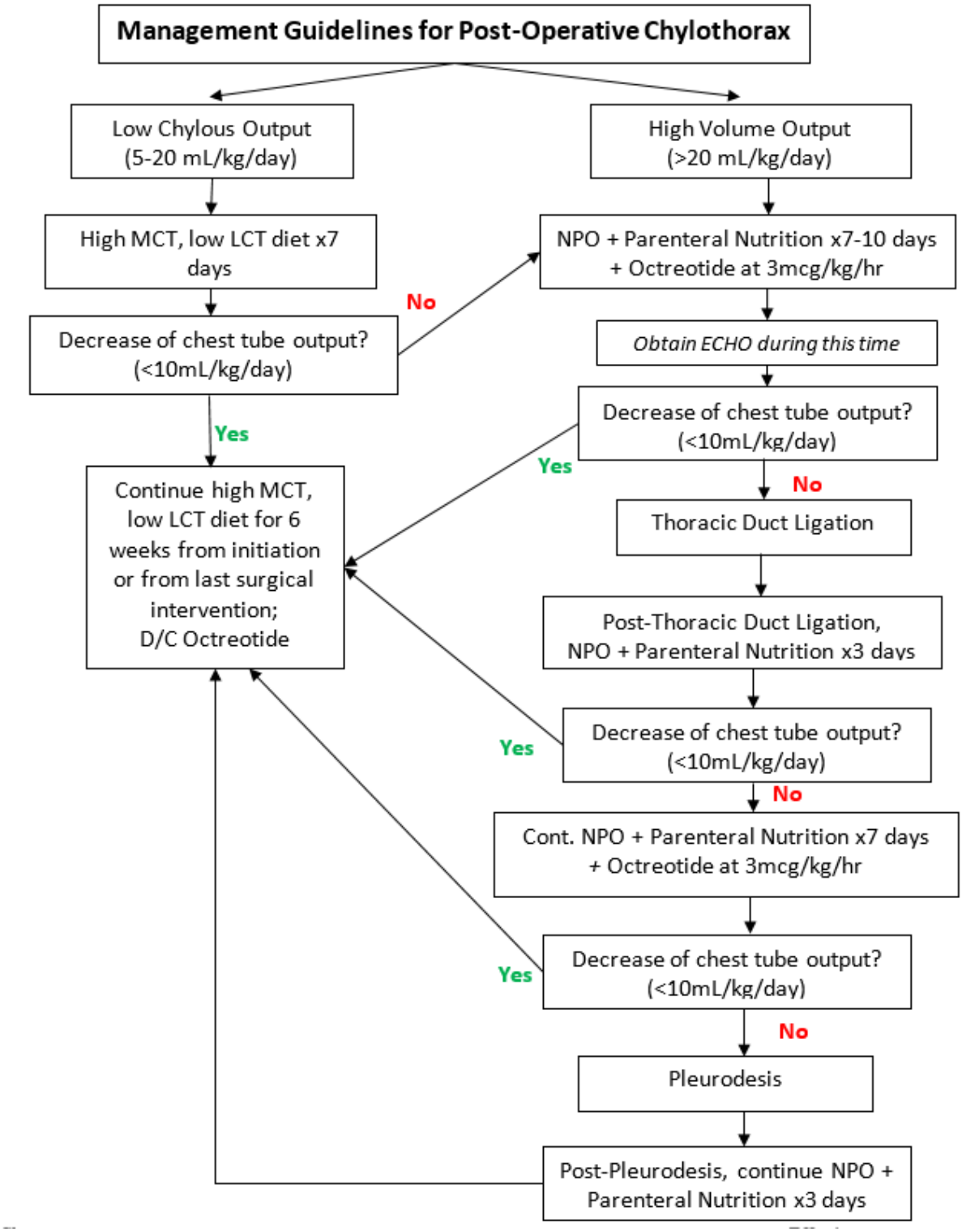
Blood studies

A complete blood count and comprehensive metabolic panel will be obtained on post-operative days as described in Section 1.3 (SoA). The CBCs will be run at the CLIA certified hospital laboratory to decrease variability.

All blood samples and pleural fluid samples will be collected by a registered nurse, physician, or phlebotomist.

[a]Standard of Care therapy

If a patient has chylous output at any time upon initiation of enteral nutrition as defined by the clinical team (testing fluid for triglycerides is variable by clinician and there is no clear standard definition), standard of care protocol will be followed and colchicine will be continued. If a patient has high-output effusions > 20 mL/kg/day continuing on POD 5 that are not deemed clinically "chylous," the high-output protocol will be followed (see protocol for institutional standard of care flowsheet below). One additional sample will be obtained on the day of therapy initiation if not on the prior collection schedule, and then the protocol will revert to the prior collection schedule.



Patient data

Patient demographics will be collected including age, ethnicity, and admission weight and height. Any chromosomal abnormality or other organ anomaly will be noted. The cardiac diagnosis will be recorded with special attention to ventricular morphology. Preoperative echocardiogram findings will be

recorded, especially ventricular function and atrioventricular valve regurgitation. Preoperative heart catheterization data will be recorded when available including ventricular end-diastolic pressure, pulmonary artery pressure, and transpulmonary gradient pressure.

Intraoperative data to be collected include the surgical procedure and bypass, cross clamp, and deep hypothermic arrest times. Any need for intraoperative steroids will be noted.

In the postoperative course, total daily pleural fluid output, total chest tube days, and presence of chyle will be recorded. The total ventilator time, hospital time, and daily maximum vaso-inotropic score will be recorded.[43] Post-operative steroids and duration of therapy will be recorded. Administration of NSAIDs or spironolactone will be noted. Any complications will be recorded including need for extracorporeal membrane oxygenation (ECMO), renal replacement therapy (RRT), infection, unplanned surgical procedure or catheterization, cardiopulmonary resuscitation, and death. Finally, treatment for pleural drainage will be documented including low fat diet, need for periods of NPO with total parenteral nutrition, octreotide infusion, thoracic duct ligation, and pleurodesis.

The bedside nurse or study staff will complete an AE survey for the patient regarding GI complaints at 24 and 48 hours post initiation of study intervention and weekly thereafter.

The patient will have standard post-operative clinical care.

8.2 SAFETY AND OTHER ASSESSMENTS

Serum creatinine will be obtained within 30 days preoperatively via a renal function panel as part of the standard of care pre-operative screening. If, after surgery, the patient is unstable; i.e. needs mechanical support (ECMO) or re-exploration greater than 4 hours after surgery, the patient will not be a candidate for the study and will not have samples collected or receive the study intervention. Two milliliters of blood will be obtained for CBC/comprehensive metabolic panel as described in Section 1.3 and 8.1. CBCs and comprehensive metabolic panels will be obtained as indicated in Section 1.3 and if blood dyscrasias, renal impairment or liver impairment are noted as described in section 6.1.2, the study drug will be discontinued. If these abnormalities are not or unlikely to be related to the study intervention and resolve within 48 hours, the study drug may be restarted as described in section 7.1. GI complaints will be monitored as described in Section 1.3 and if present out of proportion to the expected degree, study drug dose will be decreased or stopped as described in section 6.1.2. The CLIA certified hospital laboratory will perform these laboratory analyses. If the patient develops hives, rash, or anaphylaxis, the study intervention will be discontinued.

Pleural Samples will be obtained via semi-sterile technique as described in section 8.1. Diagnosis of infection will be monitored as described in Section 1.3. If a patient has diagnosed infection by the clinical team, either surgical site, blood, or urine, this will be noted as an adverse event.

All surveys and laboratory samples will be collected by a registered nurse or physician.

If a patient is deemed to have a blood dyscrasia, elevation in creatinine or transaminases requiring discontinuation of the study intervention, a repeat CBC or comprehensive metabolic panel, whichever is pertinent, will be obtained within 1 week following discontinuation to ensure resolution. If the patient

has GI complaints requiring discontinuation, symptoms will be monitored for 2 days following the last dose to ensure resolution.

Medical chart review will be performed and specific data recorded as described in Section 8.1.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 Classification of an Adverse Event

8.3.3.1 SEVERITY OF EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.3 EXPECTEDNESS

The safety monitoring committee (SMC), made up of a physician, Adult-Congenital Cardiologist, who has had experience managing postoperative Fontan patients as well as experience using colchicine in a clinical environment, and a nurse practitioner in Pediatric Cardiology who works extensively with the Fontan population and is actively involved in multiple ongoing research studies will be responsible for determining whether an adverse event (AE) is expected or unexpected based on the approved drug label and the informed consent document. Neither the physician nor the NP are part of the study team. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. The SMC will meet once every 4 months to review all AEs and SAEs, unless there have been fewer than 5 new patients enrolled, at which time that meeting can be postponed to the next scheduled meeting time.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during hospitalization and interviews of a study participant parents or guardians presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Principal investigator or nurse coordinator will record all reportable events with start dates occurring any time the first sample is obtained until 7 days after the last day of dosing of the study drug. This will be determined by a follow up phone call 5-14 days after the last day of dosing of the study drug. Events will be followed for outcome information until resolution or stabilization.

8.3.5 ADVERSE EVENT REPORTING

We will report all AEs to our SMC and our institution's IRB as per the IRBMED reporting guidelines. The principal investigator is responsible for completing a CRF and signing off on all serious AEs. The PI or the nurse coordinator will be responsible for completing a CRF and signing off on all moderate or mild AEs. Serious AEs will be reported to the PI within 24 hours of occurrence. Moderate AEs will be reported to the PI within 3 days of occurrence to the PI, and mild AEs will be reported within 1 week to the PI. The SMC will review all AEs every 4 months unless there have been fewer than 5 new patients enrolled, at which time that meeting can be postponed to the next scheduled meeting time.

Any surgically-related complications and post-operative complications that occur prior to initiation of the study intervention will not be repor
ted per the standard process for reporting. These complications will be recorded in our study database as post-operative complications.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

AEs and SAEs will be reported to the IRB as per their SAE reporting guidelines. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event

(e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the IRB.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the SMC and should be provided as soon as possible.

This study will comply with all applicable regulations found in 21 CFR 312.32.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Parents or guardians of participants will be notified regarding any serious AE felt by the SMC to be definitely related to the study intervention. In the event that routine safety monitoring laboratory results have values out of range, this will be discussed with the inpatient clinical team.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); 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.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported per the IRB requirements.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint: Change in Cytokine concentrations (TNF-α) from POD 1 to DMD in pleural fluid

  Change in TNF-α (ChangeTNFα) in pleural fluid will be calculated as:

  $$\text{ChangeTNF}\alpha = \text{TNF}\alpha_{POD1} - \text{TNF}\alpha_{DMD}$$

  The calculated change in TNF-α in Fontan patients treated with Colchicine ($\text{ChangeTNF}\alpha_{Colchicine}$) will be compared with a historical cohort of Fontan patients ($\text{ChangeTNF}\alpha_{Control}$)

  The null and alternative hypotheses for the primary endpoint will be as following:

  H_0: ChangeTNFαColchicine = $\text{ChangeTNF}\alpha_{Control}$ vs. H_a: $\text{ChangeTNF}\alpha_{Colchicine} \neq \text{ChangeTNF}\alpha_{Control}$

- Secondary Efficacy Endpoint(s):
  - Cytokine concentrations (particularly TNF-α, MIP-1βm, IL-17A, IL-8, and INF-γ) at a given time-point postoperatively

    Each cytokine concentration in Fontan patients treated with Colchicine will be measured at a given postoperative time point (ex. $\text{TNF}\alpha_{Colchicine\_t_i}$) and will be compared with a historical cohort of Fontan patients not treated with Colchicine (ex. $\text{TNF}\alpha_{Control\ t_i}$)

    The null and alternative hypotheses for the secondary endpoint (1) will be as following:

  H_0: $\text{TNF}\alpha_{Colchicine\_t_i} = \text{TNF}\alpha_{Control\ t_i\_}$ vs. H_a: $\text{TNF}\alpha_{Colchicine\_t_i} \neq \text{TNF}\alpha_{Control\ t_i}$, t_i = POD 1, POD 2, ⋯ , DMD_

  - Duration of pleural drainage in days, from date of Fontan surgery to chest tube drainage discontinuation

    Duration of pleural drainage (DaysPleuralDrain) will be calculated as:

  DaysPleuralDrain = Date of chest tube drainage discontinuation – Date of Fontan surgery

    The calculated duration of pleural drainage in Fontan patients treated with Colchicine ($\text{DaysPleuralDrain}_{Colchicine}$) will be compared with a historical cohort of Fontan patients ($\text{DaysPleuralDrain}_{Control}$)

The null and alternative hypotheses for the secondary endpoint (2) will be as following:

H_0: $DaysPleuralDrain_{Colchicine} = DaysPleuralDrain_{Control}$ vs. H_a: $DaysPleuralDrain_{Colchicine} \neq DaysPleuralDrain_{Control}$

- Hospital length of stay in days, from date of Fontan to hospital discharge date

  Postoperative hospital length of stay (HospitalLOS) will be calculated as:

  HospitalLOS = Date of hospital discharge - Date of Fontan surgery

  The calculated hospital length of stay in Fontan patients treated with Colchicine ($HospitalLOS_{Colchicine}$) will be compared with a historical cohort of Fontan patients ($HospitalLOS_{Control}$)

  The null and alternative hypotheses for the secondary endpoint (3) will be as following:

  H_0: $HospitalLOS_{Colchicine} = HospitalLOS_{Control}$ vs. H_a: $HospitalLOS_{Colchicine} \neq HospitalLOS_{Control}$

9.2 SAMPLE SIZE DETERMINATION

To determine if Colchicine is associated with decreased inflammatory cytokines (TNF-α) within the pleural space in postoperative Fontan patients compared to a historical cohort of Fontan patients, change in TNF-α from POD 1 to DMD in pleural fluid in the Fontan patients treated with Colchicine will be compared to a historical cohort of Fontan patients, using two-sample t-test or Wilcoxon rank sum test. Our original Fontan study demonstrated that pleural TNF-α in Fontan patients were significantly increased from POD 1 to DMD (n=25; median 30.29 to 66.97, p<.0001)[17]. Assuming its median TNF-α change from POD 1 to DMD (32.02) and range/4 of TNF-α at POD 1 (16.91) as mean and estimated standard deviation of change in TNF-α from a historical cohort of Fontan patients, sample size estimate was calculated using PASS 11 (Kaysville, UT) as follows;[44]

A sample size of 25 Fontan patients treated with Colchicine is required to achieve at least 80% power to detect a 45% or greater decrease in TNF-α at DMD from POD 1 compared to a historical cohort of Fontan patients, based on two-sided 0.05 significance level using two-sample t-test. As described in Section 5.5, anticipated number to be screened is 45 patients during the study period. Assuming approximately 70% consent to the study, enrolling 25 Fontan patients will be feasible.

9.3 POPULATIONS FOR ANALYSES

Patients undergoing Fontan operation ages of 20 months -5 years at the University of Michigan Congenital Heart Center will be included in the analysis. The analysis will be based on an Intention-to-Treat (ITT) approach.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

All analyses will be performed using SAS version 9.4 (SAS Institute, Cary, NC, USA), with statistical significance level of 0.05 using two-sided tests. Descriptive statistics of demographic and clinical characteristics will be presented as frequency and percentage (%) for categorical variables, and mean and standard deviation (SD) or median and interquartile range (IQR), depending on distributional assumption, for continuous variables. A detailed statistical analysis plan for each specific aim will be described in the sections below.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary endpoint of the study is change in Cytokine concentrations (TNF-α) from POD 1 to DMD in pleural fluid. As described in section 9.1, change in TNF-α (ChangeTNFα) in pleural fluid will be calculated as:

$$\text{ChangeTNF}\alpha = \text{TNF}\alpha_{POD1} - \text{TNF}\alpha_{DMD}$$

The calculated change of TNF-α in patients s/p Fontan palliation treated with Colchicine will be reported as mean and SD or median and IQR, as appropriate, and will be compared with a historical cohort of Fontan patients using two-sample t-test or Wilcoxon rank sum test.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

The secondary endpoints are:

1. Cytokine concentrations (particularly TNF-α, MIP-1βm, IL-17A, IL-8, and INF-γ) at a given time-point postoperatively

   Each cytokine concentration measured at a given postoperative time point in Fontan patients treated with Colchicine will be reported as mean and SD or median and IQR, as appropriate, and will be compared with a historical cohort of Fontan patients not treated with Colchicine at the corresponding postoperative time-point, using two-sample t-test or Wilcoxon rank sum test.

2. Duration of pleural drainage in Fontan patients treated with Colchicine

   Duration of pleural drainage in days, from date of Fontan surgery to chest tube drainage discontinuation in Fontan patients treated with Colchicine will be calculated and reported as median and IQR and compared with a historical cohort of Fontan patients using Wilcoxon rank sum test.

3. Hospital length of stay in Fontan patients treated with Colchicine

   Hospital length of stay in days will be calculated from date of Fontan surgery to hospital discharge date and reported as median and IQR. Similar to duration of pleural drainage, the

calculated hospital length of stay in Fontan patients treated with Colchicine will be compared to a historical cohort of Fontan patients, using Wilcoxon rank sum test.

9.4.4 SAFETY ANALYSES

Each AE described in Section 7 and 8 will be reported including start date/time, stop date/time, severity, and duration. Frequency and severity of AEs will be presented using summary statistics. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be listed in a table.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Baseline characteristics, including demographics and pre- and intra-operative characteristics, will be compared between Fontan patients with Colchicine and a historical cohort of Fontan patients, using Chi-square test or Fisher's exact test for categorical variables and two-sample t-test or Wilcoxon rank sum test for continuous variables, as appropriate.

9.4.6 PLANNED INTERIM ANALYSES

There will be an interim analysis after 9 patients have been enrolled (5 patients completing the full course of treatment). This will entail simple descriptive statistics and will not alter the remainder of the study design or power calculations.

9.4.7 SUB-GROUP ANALYSES

No sub-group analyses are planned due to small number of sample size anticipated.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

No tabulation of individual participant data is planned. Instead, each cytokine data for individual participant will be graphically presented over time point.

9.4.9 EXPLORATORY ANALYSES

In addition to the individual participant data, summary statistics (such as mean or median, as appropriate) of each cytokine data will be also graphically presented over time point.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to administering study

intervention. The following consent materials are submitted with this protocol : Informed Consent document.

10.1.1.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the parent/guardian of the participant will be asked to read and review the document. The investigator will explain the research study to the parent/guardian of the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the parent/guardian's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. The parent/guardian of the participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The parent/guardian of the participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The parent/guardian of the participant will sign the informed consent document prior to any procedures being done specifically for the study. Parent/guardians of the participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the parent/guardian of the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the investigator.

All research activities will be conducted in as private a setting as possible.

Authorized representatives of the investigator, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored in the University of Michigan secure network drive. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the University of Michigan research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the University of Michigan.

A Certificate of Confidentiality is not applicable.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the University of Michigan server and will be monitored and maintained by the Michigan Congenital Heart Outcomes Research and Discovery (MCHORD) program.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the University of Michigan laboratory. These samples could be used to research the causes of prolonged pleural drainage in postoperative Fontan patients, its complications and other conditions for which individuals with Fontan physiology are at increased risk, and to improve treatment. will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the MCHORD program.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Co-Principal Investigator**
Albert Rocchini, MD, Professor	*Stephanie Goldstein, MD, Fellow*
University of Michigan	*University of Michigan*
1540 E. Hospital Dr. *Ann Arbor, MI 48109*	*1540 E. Hospital Dr* *Ann Arbor, MI 48105*
734-763-6242	*734-936-8765*
rocchini@med.umich.edu	*stablowz@med.umich.edu*

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of a Safety Monitoring Committee (SMC) composed of individuals with the appropriate expertise, including a nurse practitioner and a physician who are each independent investigators and experts in the field of pediatric cardiology with an emphasis on Fontan physiology and long term outcomes. The SMC will meet at least quarterly, unless there have been fewer than 5 new patients enrolled, at which time that meeting can be postponed to the next scheduled meeting time, to assess safety and efficacy data. Prior to the initiation of the study, each data element that the SMC needs to assess will be clearly defined. The SMC will provide its input to IRB as indicated.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- The Co-Principal Investigator will conduct the monitoring of the data collection or will collect the data herself. Monitoring will occur bi-weekly in the format of either on-site meeting or via teleconference with the study team data collectors. There will be 100% data verification.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the AE surveys will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into RedCAP, a 21 CFR Part 11-compliant data capture system provided by the The University of Michigan. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harminosation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers x years after the completion of the primary endpoint by contacting Stephanie Goldstein.

In addition, this study will comply with the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study

leadership has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

Not applicable.

10.3 ABBREVIATIONS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
ANCOVA	Analysis of Covariance
CBC	Complete blood count
CLIA	Clinical Laboratory Improvement Amendments
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DMD	Day most distal
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FMF	Familial Mediterranean Fever
G-CSF	Granulocyte-Colony Stimulating Factor
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP	Good Laboratory Practices
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IL	Interleukin
IND	Investigational New Drug Application
INF	Interferon
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ITT	Intention-To-Treat
MCP	Monocyte Chemoattactant Protein
MIP	Macrophage Inflammatory Protein
MOP	Manual of Procedures
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
NPO	Nil per os
OHRP	Office for Human Research Protections
PI	Principal Investigator
POD	Postoperative day
QA	Quality Assurance
QC	Quality Control
RV	Right Ventricle
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TNF	Tumor Necrosis Factor

UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale

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