Therapeutic Potential for Aldosterone Inhibition in Duchenne Muscular Dystrophy

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Study Sponsor: National Institutes of Health

PRINCIPAL INVESTIGATOR: SUBHA RAMAN, MD
Email: raman.1@osu.edu

DSMB CHAIR: CHRISTOPHER SPURNEY, MD
E-mail: cspurney@childrensnational.org

BIOSTATISTICIAN: XIN HE, PHD
E-mail: xinhe@umd.edu

REGULATORY OFFICER: JONATHAN KALTMAN, MD
Email: kaltmanj@nhlbi.nih.gov

PROJECT MANAGER: SUZANNE SMART, BS
Email: suzanne.smart@osumc.edu

Data Coordinating Center (DCC): Ohio State University

INVESTIGATOR SIGNATURE PAGE
Version/Date: Version 9; 12/07/2017
Principal Investigator: Subha Raman, MD
Title: Therapeutic Potential for Aldosterone Inhibition in Duchenne Muscular Dystrophy
Study Sponsor: National Heart, Lung, and Blood Institute
INSTRUCTIONS: Please have the Principal Investigator print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page by surface mail to: 473 W. 12th Ave, Suite 200 Attention: Raman Lab Columbus, OH 43210
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 45, 50, 56, and 312, and the International Conference on Harmonization (ICH) document "Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance". Further, I will conduct the study in keeping with local, legal, and regulatory requirements. As the Site Principal Investigator, I agree to conduct ***Therapeutic Potential for Aldosterone Inhibition in Duchenne Muscular Dystrophy*** by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of Study Principal Investigator. **PI name** **Site Principal Investigator (Print)** **Site Principal Investigator (Signature)** **Date**

Protocol Synopsis

Title	Therapeutic Potential for Aldosterone Inhibition in Duchenne Muscular Dystrophy
Short Title	Aldosterone Inhibition in DMD
Clinical Phase	3
Study Objectives	Demonstrate non-inferiority of spironolactone vs. eplerenone in preserving cardiac and pulmonary function in DMD patients with preserved LV ejection fraction.
Study Design	Randomized trial
Primary Mechanistic Endpoints	Measure impact on 12-month cardiac MRI measures of LV strain and pulmonary function parameters.
Secondary Mechanistic Endpoints	Evaluate biomarkers of muscle injury that predict treatment response.
Cohort Size	52 (26 in each arm)
Study Duration	12 months
Treatment Description	Once daily oral tablet – spironolactone 50 mg or eplerenone 50 mg
Inclusion Criteria	i) Boys age $\geq$7 years with DMD confirmed clinically and by mutation analysis able to undergo cardiac magnetic resonance (CMR) without sedation, and ii) LV EF ≥45%±5% by clinically-acquired echocardiography, cardiac nuclear scan or CMR done within 2 months of enrollment.
Exclusion Criteria	a) Non-MR compatible implants, b) severe claustrophobia, c) gadolinium contrast allergy, d) kidney disease, e) prior use of or allergy to aldosterone antagonist, or f) use of other investigational therapy.

Study Contacts and Participating Centers

CLINICAL CORE PRINCIPAL INVESTIGATOR	Subha Raman, MD, Ohio State University (Study and Site PI) Other participating centers and Site PIs: Nationwide Children's Hospital – Linda Cripe, MD UCLA – Nancy Halnon, MD University of Utah – Michael Puchalski, MD University of Colorado – Scott Auerbach, MD University of Kansas – Jeffrey Statland, MD Vanderbilt University – Jonathan Soslow, MD

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Glossary of Abbreviations

ACEI - Angiotensin converting enzyme inhibitors

AE – Adverse event

ARB - Angiotensin receptor blocker

CMR – Cardiac magnetic resonance

CRF – Case report form

DCC – Data coordinating center

DMD – Duchenne muscular dystrophy

DSMB – Data safety monitoring board

Ecc – Myocardial circumferential strain

EF – Ejection fraction

FDA – Food and Drug Administration

FVC – Forced vital capacity

GFR – Glomerular filtration rate

IND – Investigational new drug

HIPAA - Health Insurance Portability and Accountability Act

LGE – Late gadolinium enhancement

LV – Left ventricle

MRA – Mineralocorticoid receptor antagonist

MRI – Magnetic resonance imaging

NIH – National Institutes of Health

OSHA – Occupational Safety and Health Administration

OSU – Ohio State University

PFT – Pulmonary function testing

PHI – Personal health information

PI – Principal investigator

RCT – Randomized clinical trial

SAE – Serious adverse event

SAP – Statistical analysis plan

SAR – Suspected adverse reaction

SUSAR – Serious and unexpected suspected adverse reaction

USEPA – United States Environmental Protection Agency

WHO – World Health Organization

Study Definitions Page

Mineralocorticoid receptor antagonist	A class of medicines also known as aldosterone antagonists
Magnetic resonance imaging	A noninvasive way to make pictures of structures inside the body such as the heart
Pulmonary function tests	Tests that indicate how well the breathing structures are working
Osteopontin	A protein measured in the bloodstream that indicates muscle damage

1. ESSENTIAL STUDY COMPONENTS

1.1 Hypothesis/Research Question

Hypothesis 1.1*: Spironolactone 50 mg qd is noninferior to eplerenone 50 mg qd when assessing 12-month change in myocardial strain (Ecc) in DMD patients with preserved ejection fraction.*
Hypothesis 1.2a*: Less myocardial injury by baseline LGE score predicts greater preservation of Ecc in DMD boys treated with mineralocorticoid receptor antagonist (MRA).*
Hypothesis 1.2b*: Higher baseline osteopontin, a muscle injury marker, predicts greater Ecc improvement among DMD patients treated with MRA.*
Hypothesis 1.3*: There is similar decline of forced vital capacity (FVC%) before vs. after treatment with MRA.*

1.2 Primary Objective

Demonstrate noninferiority of spironolactone vs. eplerenone in preserving cardiac function in DMD patients with preserved LV ejection fraction.

1.3 Secondary Objectives

Demonstrate noninferiority of spironolactone vs. eplerenone in preserving pulmonary function in DMD patients with preserved LV ejection fraction.

Evaluate biomarkers of muscle injury that predict treatment response.

1.4 Primary Endpoint

12-month change in left ventricular strain (Ecc) by cardiac magnetic resonance (CMR)

2 BACKGROUND AND RATIONALE

2.1 Background

Duchenne muscular dystrophy (DMD), the most common severe form of muscular dystrophy, is an X-linked disorder in which the sarcolemmal protein dystrophin is effectively absent. Males with DMD typically die in the third and fourth decades of life of cardiopulmonary disease[1]. Mouse models of DMD, autopsy data, and *in vivo* human studies using magnetic resonance-based late gadolinium enhancement imaging (LGE) have all shown that progressive myocardial damage is well underway before left ventricular ejection fraction (LV EF) becomes abnormal[2].

Exertional symptoms and signs of myocardial disease are typically absent as skeletal muscle disease progressively limits functional capacity in affected boys. Thus, cardiac involvement can go undetected until LV dysfunction and myocardial fibrosis are advanced without the use of more sensitive biomarkers. While echocardiography remains a useful tool to evaluate LV dysfunction, cardiac magnetic resonance (CMR) with LGE is advantageous for DMD patients since it identifies myocardial injury before decline in EF is apparent by echocardiography[3]. Further, greater reproducibility affords efficient sample sizes for cardiomyopathy clinical trials in patients with rare diseases[4]. CMR's increasing availability at major DMD clinical centers has afforded earlier cardiomyopathy detection, and has helped refine current management

to typically include agents such as angiotensin converting enzyme inhibitors (ACEI) once damage is evident. This strategy, however, may not be sufficient, with prior studies showing inexorable decline in systolic function with or without ACEI or angiotensin receptor blocker (ARB) therapy[5].

We previously tested mineralocorticoid receptor antagonism (MRA) added to ACEI while EF was still normal in a mouse model that mimics the myocardial damage seen in DMD patients[6]. This combination significantly reduced myocardial injury and improved (made more negative) LV circumferential strain (Ecc), a sensitive and early marker of LV systolic dysfunction. Earlier intervention is also motivated by the recognition that myocardial injury, irrespective of underlying cause, engages matricellular proteins such as osteopontin that promote ventricular remodeling prior to overt heart failure[7]. Recognizing the urgent need for effective cardioprotective regimens in this high-risk and understudied population, we sought to translate these preclinical findings with clinical evaluation of currently-available drugs.

2.2 Preclinical and Clinical Experience

As detailed in our originally-approved proposal and reiterated in our Year 1 progress report, the approach for the clinical work to be done in this project is to be based on findings from i) mouse experiments comparing eplerenone and spironolactone and ii) results of a pilot RCT comparing eplerenone 25 mg qd to placebo in boys with DMD and preserved systolic function (LVEF ≥45%) but evident myocardial injury in at least one LV segment by LGE-CMR (NCT01521546). Completed murine experiments suggest comparable effect of both aldosterone antagonists spironolactone and eplerenone that is blunted by concomitant corticosteroid use. Major findings of the pilot RCT (confidential; in review) include: 1) no hyperkalemia or other adverse events with eplerenone 25 mg qd, 2) continued decline in LV strain that was less with eplerenone 25 mg qd vs. placebo ($p<0.05$), 3) less response to therapy with greater baseline extent of myocardial injury, and 4) 12-month change in the tissue injury biomarker osteopontin paralleled 12-month change in extent of myocardial damage by LGE. While boys not on concomitant corticosteroid therapy comprised only 6/40 (15%) of those completing baseline and follow-up exams precluding analyses achieving statistical significance, qualitative assessment of their results suggest a much greater benefit as compared to the majority of subjects who were also on corticosteroids in addition to study drug. These findings suggest that comparison of spironolactone to eplerenone in boys not on steroid therapy warrants further investigation. Additionally, given the modest benefit of eplerenone 25 mg qd seen in the pilot RCT without adverse effects, we anticipate improved efficacy with 50 mg qd, a dose that has established safety in both pediatric and adult clinical trials. Similarly, we feel that it is appropriate to start with a dose of 50 mg qd for the spironolactone arm as this has been shown to be effective in prior heart failure[8-10] and pediatric trials[11, 12].

3 STUDY POPULATION RATIONALE

Boys with DMD confirmed clinically and by mutation analysis age ≥7 years able to undergo CMR without sedation will be enrolled from 4 collaboration centers with clinical programs dedicated to cardiomyopathy in neuromuscular disease: Ohio State University/Nationwide Children's Hospital, University of California Los Angeles, University of Colorado, University of Utah, University of Kansas/Saint Luke's Hospital, and Vanderbilt University. All sites are well versed in all aspects of DMD clinical research, and have met key requirements for participation including ongoing quality assurance procedures. Inclusion criteria are: i) LV EF ≥45%±5% by clinically acquired echocardiography, cardiac nuclear scan or CMR done within 2 months of enrollment. Exclusion criteria are: a) non-MR compatible implants, b) severe claustrophobia, c) gadolinium contrast allergy, d) kidney disease, e) prior use of or allergy to aldosterone antagonist, or f) use of other investigational therapy. Scoliosis rods and other orthopedic hardware that are often present in DMD patients are typically MR-compatible and do not interfere significantly with imaging. Baseline and 12-

month cystatin C, a reliable measure of GFR in patients with muscle breakdown[13], was normal in all subjects in the pilot RCT and will be measured in this trial at the same time points to ensure normal renal function

4 INVESTIGATIONAL THERAPEUTIC REGIMEN

4.1 Investigational Therapeutic Regimen

The investigational therapeutic regimen is eplerenone 50 mg PO qd or spironolactone 50 mg PO qd in addition to background therapy that may include ACEI or ARB.

4.2 Rationale for Protocol Mandated Procedures

Protocol-mandated procedures include CMR with contrast, which provides highly reproducible measurements of LV structure and function as well as extent of myocardial injury by the LGE imaging technique. Also, PFTs measure pulmonary function that can decline over time in boys with DMD. Preclinical studies showed benefit in both cardiac muscle as well as diaphragm, supporting the inclusion of procedures that measure cardiac and pulmonary function in the clinical trial. Lastly, blood draws are mandated for monitoring of potassium levels throughout the study period, given the possible risk of hyperkalemia with MRA therapy. Blood is also needed at baseline and 12-month follow-up to help identify biomarkers of treatment response.

5 KNOWN AND POTENTIAL RISKS AND BENEFITS

5.1 Investigational Therapeutic Regimen Risks

5.1.1 Medications

Eplerenone may cause the following side effects: headache, dizziness, diarrhea, stomach pain, cough, excessive tiredness, flu-like symptoms, breast enlargement or tenderness. The following symptoms are uncommon, but may occur: chest pain, tingling in arms and legs, loss of muscle tone, weakness or heaviness in legs, confusion, lack of energy, cold, gray skin, irregular heartbeat.

Spironolactone may cause the following minor side effects: vomiting, diarrhea, stomach pain or cramps, dry mouth, thirst, dizziness, unsteadiness, headache, enlarged or painful breasts, difficulty maintaining or achieving an erection, deepening of voice, increased hair growth on parts of the body, drowsiness, tiredness, restlessness. The following symptoms are uncommon, but may occur: muscle weakness, pain or cramps; pain, burning, numbness or tingling in the hands or feet; inability to move arms or legs, changes in heartbeat, confusion, nausea, extreme tiredness, unusual bleeding or bruising, lack of energy, loss of appetite, pain in upper right part of stomach, yellowing of skin or eyes, flu-like symptoms, rash, hives, itching, difficulty breathing or swallowing, vomiting blood, blood in stools, decreased urination, fainting.

5.1.2 Procedures

Possible discomforts associated with being in the MRI scanner include noise from the scanner, which is minimized with earplugs or headphones. Some people may also feel claustrophobic.

Patients with pacemakers, aneurysm clips, neurostimulators or any other metal implant not compatible with MRI will not be enrolled. If a subject has metal in his body that may have been missed during the screening process, this metal could move. In addition, an increase in the temperature of the object may occur. The

screening process will reveal whether or not one may be at risk. If metal is discovered during the scan, the scan will be stopped and the subject will be taken out of the room, and then evaluated by a physician; he/she will decide if any treatment is needed.

When the contrast is injected, one may feel warmth, coolness or a sensation of local pressure or pain at the injection site. Less frequently reported are dizziness, nausea, headache and a metallic taste in the mouth. There is a slight risk (less than 1%) of allergic reaction to the contrast. Rare reactions are vomiting, sleepiness that lasts longer than normal, changes in vision, diarrhea, anxiety, difficulty breathing, chest pain, increased heart rate, trembling, joint-pain or allergy-like symptoms such as hives, itching or an irritation in the throat. A severe reaction (anaphylactic shock) is extremely rare, and if not treated, death can occur. Each subject is continuously monitored by appropriate personnel during the exam.

A rare but serious skin disease has been linked to MRI contrast agents. This disease affects patients with very serious kidney problems. Patients with these serious kidney problems will not be enrolled in the study.

Some individuals undergoing pulmonary function tests may feel some slight soreness of the chest muscles due to the effort involved in the testing. During the test, some people cough. Rarely, pulmonary function testing may result in a collapsed lung. If someone's lung collapses, a temporary tube may need to be placed inside the chest to help re-inflate the lung.

5.1.3 Tests

The risks associated with having an IV placed and/or blood drawn are mild discomfort, bruising, bleeding, blood clot and a very slight risk of infection at the needle puncture site. Some persons who have needle punctures become lightheaded, nauseous or faint.

5.1.4 Confidentiality

All study-related information will remain confidential and secured in a coded fashion in a password-protected system for clinical research data management.

Records may be reviewed by the following groups:

- Office for Human Research Protections or other federal, state, or international regulatory agencies;
- U.S. Food and Drug Administration;
- The enrolling center's Institutional Review Board;
- The sponsor supporting the study, their agents or study monitors; and
- The subject's insurance company (if charges are billed to insurance).

If this study is related to a subject's medical care, study-related information may be placed in his permanent hospital, clinic, or physician's office records. Authorized staff not involved in the study may be aware that a subject is participating in a research study and have access to such information.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This website will not include information that can identify any subject. At most, the website will include a summary of the results.

Subjects will also be asked to sign a separate Health Insurance Portability and Accountability Act (HIPAA) research authorization form as the study involves the use of some protected health information.

5.2 Benefits

There may be no direct benefits to the individual participant in this study. Cardiac MRI images will be read by a cardiac imaging specialist. If any incidental findings are identified as a result of participation in this research study, participants will be notified of the results. With their permission, test results will also be shared (verbally and/or written) with their primary physicians or an appropriate health care professional. It is emphasized at enrollment and throughout the follow-up period that participation in this research study is not meant to replace one's usual health care.

6 SELECTION OF PARTICIPANTS

6.1 Participant Selection

Potential participants will be selected from the Duchenne muscular dystrophy and cardiology clinics at participating centers. Additionally, participants will be solicited via Parent Project for Muscular Dystrophy emails and other advertisements.

6.2 Inclusion Criteria

Boys with DMD confirmed clinically and by mutation analysis age ≥7 years able to undergo CMR without sedation will be included if LV EF is ≥45% ±5%by clinically acquired echocardiography, cardiac nuclear scan or CMR done within 2 months of enrollment.

6.3 Exclusion Criteria

Excluded from enrollment are boys with a) non-MR compatible implants, b) severe claustrophobia, c) gadolinium contrast allergy, d) kidney disease, e) prior use of (within 1 month of enrollment) or allergy to aldosterone antagonist, or f) use of other investigational therapy.

7 STUDY DESIGN

This is a randomized, double-blind clinical trial of once-daily oral eplerenone vs. spironolactone on top of background therapy. 52 boys with DMD will be enrolled to undergo baseline and 12-month follow-up cardiac MRI, PFTs and blood tests plus safety labs drawn at 1, 2, 3, 6 and 9 months. Subjects will also fill out a quality of life questionnaire, an upper extremity function questionnaire and have goniometry of their elbow joint at baseline and 12 months.

8 STUDY THERAPY

8.1 Study Therapy

The study therapy is one of two once-daily oral mineralocorticoid receptor antagonist drugs (also known as aldosterone antagonists) – eplerenone 50 mg or spironolactone 50 mg.

8.2 Concomitant Medications

Concomitant medications may include an angiotensin converting enzyme inhibitor or angiotensin receptor blocker (ACEI or ARB) and a beta-blocker.

8.3 Prophylactic Medications

It is anticipated that some patients may be on prophylactic medications for osteoporosis, gastrointestinal reflux disease, and other conditions associated with prior corticosteroid use.

8.4 Prohibited Medications

These medications may not be taken during the study: the antibiotics clarithromycin (Biaxin) and telithromycin (Ketek); the anti-fungal medications itraconazole (Sporanox) and ketoconazole (Feoris,Nizoral); the anti-retroviral medications indinavir (Crixivan), nefinavir (Viracept), saquinavir (Fortovase, Invirase), ritonavir (Norvir), amprenavir (Agenerase), lopinavir (Kaletra), atazanavir (Reyataz), fosamprenavir (Lexiva,Telzir), tipranavir (Aptivus), darunavir (Prezista); potassium supplements or other potassium sparing diuretics such as Aldactazide, amiloride (Midamor, Moduretic), or triamterene (Dyrenium, Dyazide, Maxzide).

8.5 Modification or Discontinuation of Study Therapy

Study therapy will be stopped if the blood potassium level exceeds 5.5 mmol/L at any time point. Only in instances of a hemolyzed blood sample resulting in such a value can resuming study therapy be considered. In such instances, a repeat blood test showing a potassium level <5.0 mmol/L is required before resuming study therapy can be considered by the site PI.

Study therapy will also be stopped if a blood sample cannot be obtained within a reasonable number of attempts.

The addition of new medications to a subject's therapy will not modify any study procedures unless the medication is one of those prohibited above. If the new medication is on the prohibited list, the subject will immediately be taken off the study drug and be given the option to continue follow-ups without being on the study drug or to withdraw from the study.

9 PREMATURE TERMINATION OR WITHDRAWAL

9.1 Study Stopping Rules

Any potassium level exceeding 5.5 mmol/L in a non-hemolyzed blood sample mandates premature termination of study drug for the remainder of the trial period. Such participants may still undergo 12-month follow-up procedures.

9.2 Participant Withdrawal Criteria

Any participant wishing to withdraw can do so at any time during the study.

9.3 Termination for Noncompliance

Noncompliance with study medication for more than 7 days during any 3-month follow-up period will prompt termination for noncompliance. Failure to complete any follow-up within the allotted visit window may result in termination for noncompliance.

10 STUDY PROCEDURES

10.1 Enrollment/Consenting

Only research staff certified in human subjects research will be involved in enrollment and consenting.

10.2 Screening

Screening will occur in the clinics of participating sites.

10.3 Clinical Study Assessments

Prior to enrollment, subjects will be assessed in the clinics of the participating sites. Assessment will include basic demographics, medical history, physical exam and medication review. Prior exposure to corticosteroids (type, dose, duration) will also be recorded at enrollment. The study may be discussed at this time and informed consent may be obtained.

10.4 Visit Activities

Prior to enrollment ('Visit 1'), assessment will include: age, race, ethnicity; past medical history; height and weight; concomitant medications (names, start date, end date); review of inclusion/exclusion criteria; informed consent. PFT results from 1 year ± 3 months prior to the baseline visit will also be collected at this time.

Baseline visit ('Visit 2', which may be concurrent with Visit 1) will include: randomization for study drug; medication training, patient/family education, dispensing of medication (3 month supply), drug diary review; blood tests; cardiac MRI scan (may be acquired ± 7 days); pulmonary function test (may be clinically acquired if ± 14 days) and 2 questionnaires (quality of life and upper extremity function).

Visit 3 (2 weeks post-enrollment) will consist of the following: medication adherence/drug diary review, AE/SAE, hospitalizations, cardiovascular events or death, confirmation of continued participation.

Visits 4 through 8 (1, 2, 3, 6, 9 months post-enrollment) will consist of the following: medication adherence/drug diary review, AE/SAE, hospitalizations, cardiovascular events or death, confirmation of continued participation; follow up of AE/SAE to subject's primary physician; blood test for potassium level.

In addition, study medication will be dispensed for Visits 6, 7 and 8 (months 3, 6, and 9 post-enrollment).

Visit 9 (12 months post-enrollment) will consist of the following: medication adherence/drug diary review, return of any remaining study drug, AE/SAE, hospitalizations, cardiovascular events or death; follow up of AE/SAE to subject's primary physician; blood tests; cardiac MRI scan; pulmonary function test (may be clinically acquired if ± 14 days) and 2 questionnaires (quality of life and upper extremity function).

Visits 2 and 9 (and 1 if done concurrently with 2) will occur at the study site. The rest of the visits will be done by phone with blood draws performed local to the patient.

10.5 Visit Windows

Visit 1 – Pre-enrollment

Visit 2 – Baseline procedures (note: Pre and Baseline visits may be concurrent)

Visit 3 – 2 weeks post-enrollment

Visit 4 – 1 month post-enrollment ± 7 days

Visit 5 – 2 months post-enrollment ± 7 days

Visit 6 – 3 months post-enrollment ± 14 days

Visit 7 – 6 months post-enrollment ± 14 days

Visit 8 – 9 months post-enrollment ± 14 days

(note: blood tests for visits 4-8 may be done locally to the subject)

Visit 9 – 12 months post-enrollment ± 14 days

10.6 Study Treatment Assignment Procedures

10.6.1 Clinical testing

Subjects will undergo a cardiac MRI scan at baseline and 12 months. Pulmonary function testing will also be performed at baseline and 12 months. Clinically ordered pulmonary function testing is acceptable if performed at the enrolling center ± 14 days of baseline and/or 12 month visits.

10.6.2 Blood testing

Blood will be drawn at baseline and 12 months for potassium, hematocrit, and cystatin C/GFR for immediate processing. If the subject is under 18 years old, blood will also be processed for BUN and creatinine in order to more accurately estimate GFR in pediatric subjects. Additional blood will be drawn for future analysis, including but not limited to osteopontin and genetic modifiers.

Blood will be drawn at 1, 2, 3, 6, and 9 months for potassium.

10.6.3 Research sample collection

Research blood samples will be collected at baseline and 12 months (Visits 2 and 9).

10.7 Blinding and Randomization

10.7.1 Randomization Process

Assignment to either eplerenone or spironolactone once daily is performed by a randomization scheme that ensures equal distribution of subjects into both arms. The study statistician will perform a block randomization scheme with each block containing 4 assignments. Each block of 4 will contain two assignments of eplerenone and two assignments of spironolactone in a random order. The total number of blocks needed depends on the preset allocation for each individual site.

10.7.2 Blinding of Site

Study treatments will be administered in a double-blind fashion (i.e., subjects,parents/legal guardians of the subjects and site research staff will not know the treatment assigned, with the exception of the dispensing site pharmacist and study biostatistician).

The following study procedures will be in place to ensure double-blind administration of study treatments:

1. Access to the randomization list will be strictly limited and controlled
2. Eplerenone and Spironolactone capsules will be compounded to be identical in appearance.
3. Packaging and labeling of treatments will be identical.
4. Site pharmacist will label each bottle in a blinded fashion prior to dispensing (eg: Spironolactone/Eplerenone 50 mg capsule)

The site pharmacist will be responsible for dispensing appropriate treatment assigned to the subject by referring to the randomization list sent to each site pharmacy by the study biostatistician

10.7.3 Unblinding Requirements and Procedures

Unblinding will not occur until all visits and collection of all data for all visits and all subjects has been completed. The statistician will then perform primary data analysis, followed by results review and further analysis by members of the investigative team. Once the results have been published, the rest of the sites and the subjects may be unblinded.

If an event occurs that would require unblinding to a subject's study drug assignment, the PI at the site where the subject enrolled would contact the site pharmacy who would directly inform the site PI of the study drug assignment within 24 hours. The site research team would then contact the DCC who would work with the site to ensure appropriate and timely IRB and DSMB reporting. Should the event occur at a time when the site PI or pharmacy cannot be reached, subjects will be instructed to immediately stop the study drug until the site PI or pharmacy can be reached.

10.8 Medication Management

10.8.1 Storage

All study drugs will be stored in a secure pharmacy area. Storage areas will either be staffed by pharmacists and technicians 24 hours per day or locked during off-hours. Storage areas will remain at or around room temperature (25°C) in accordance with FDA labels for each drug.

10.8.2 Administration

Subjects will be given study drug in 3 month supplies from each site's pharmacy.

10.8.3 Tracking

Drug inventory tracking will be maintained by each site's pharmacy.

10.8.4 Returns

Any drug remaining at each visit will be collected and sent to the site's pharmacy for disposal. The pharmacy will count the excess pills and keep a record of accountability documentation before disposing of the drugs.

10.8.5 Compliance

Subjects and their families will be asked to keep a drug diary where they record the day and time that each pill is taken. These will be reviewed at each visit to ensure compliance. Bottles and any remaining drug will also be checked and counted before returning to the site pharmacy for disposal.

10.8.6 Destruction or Final Storage

Any remaining drugs will be disposed of by each site's pharmacy in accordance with OSHA and USEPA regulations.

10.9 Compliance Monitoring

At least quarterly teleconferences with study coordinators will be held to review compliance, quality and all other aspects of the study. More frequent conference calls or in-person site visits will be conducted as needed, particularly when concerns arise during the course of compliance monitoring.

11 SAFETY MONITORING

11.1 Overview

Event reporting will be in accordance with the policies of the participating sites and all federal, state, and local policies.

11.2 Definitions

11.2.1 Adverse Event (AE)

An adverse event (AE) is defined as any untoward or unfavorable medical occurrence associated with the use of a drug in humans, whether or not considered drug related (21 CFR 312.32(a)). An adverse event may include any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product (ICH E6, 1.2).

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with:

- **Study mandated procedures:** cardiac MRI, pulmonary function tests, blood tests, study drug intake

11.2.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the investigational drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

Management of any SAR will be consistent with each site's standard of care.

11.2.3 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, package insert, or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the IND.

"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigational brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a)).

An unexpected adverse event will be considered unanticipated if there is a reasonable possibility that it is related to the research and if it suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

11.2.4 Serious Adverse Event

An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of the investigator, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious if, based on appropriate medical judgment, they may jeopardize the

participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

11.3 Adverse Events Grading and Attribution

11.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects.

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

11.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE CRF. Final determination of attribution for safety reporting will be determined by the PI/DSMB. The relationship of an adverse event to study procedures will be determined using the descriptors and definitions provided in Table 5.

Table 5. Attribution of adverse events

Code	Descriptor	Relationship to the primary investigational product and/or other concurrent mandated study therapy or study procedure
1	Unrelated	The adverse event is clearly not related.
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

11.4 Collection and Recording of Adverse Events

11.4.1 Collection Period

Adverse events (including SAEs) will be collected throughout the time during which a subject is taking study drug, which may be up to and including 12 months.

11.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Questioning the subject in an objective manner.
- Receiving an unsolicited complaint from the subject.

In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 11.3, *Grading and Attribution of Adverse Events.*

11.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 11.2, *Definitions*) on the appropriate AE/SAE CRF regardless of the relationship to study procedure.

All adverse events must be recorded by the site on the appropriate AE/SAE CRF within 5 business days of the site learning of the adverse event(s). Please refer to Section 11.5 for reporting of meeting serious criteria.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

11.5 Reporting of Serious Adverse Events

11.5.1 Reporting of Serious Adverse Events to Sponsor

This section describes the responsibilities of the site investigator to report serious adverse events to the sponsor via the DCC CRF. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Site investigators must report all serious adverse events (see Section 11.2.3, Serious Adverse Event), regardless of relationship or expectedness to study procedures within 24 hours of discovering the event.

11.5.2 Sponsor Reporting to Health Authority

The PI reports to the health authority.

11.5.3 Expedited Reporting within 15 Calendar Days

The sponsor must notify the appropriate authorities and all participating investigators as soon as possible, or within 15 calendar days if the adverse event is classified as one of the following (21CFR312.32 (c)(1)):

- Serious and unexpected suspected adverse reaction **(**SUSAR**)** (see Section 11.2.1.1, *Suspected Adverse Reaction* and Section 11.2.2, *Unexpected Adverse Event*). Expedited reporting of SUSARs are to include, in an aggregate analysis, specific events that occur more frequently in this patient population as a result of study procedures than previously reported.
- Any findings from other studies, whether or not conducted under an IND that suggest a significant risk in humans. This includes findings from animal or *in vitro* testing that suggest a significant risk in humans. Ordinarily, such a finding would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the trial, will be reported.
- Increased rate of occurrence of serious suspected adverse reactions. The PI must report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure or package insert.

11.5.4 Expedited Reporting within 7 Calendar Days

Study personnel must notify the site PI and DCC as soon as possible, or within 7 calendar days, of any unexpected fatal or immediately life-threatening suspected adverse reaction.

11.6 Reporting of Other Safety Information

Study personnel should promptly notify the DCC when an unanticipated problem involving risks to subjects or others is identified, which is not otherwise reportable as an adverse event.

11.7 Annual Reporting

A progress report will be provided to the sponsor on a yearly basis.

11.8 Study Monitoring

The PI for each site and the study personnel under the direction of each site PI will be responsible for performing the human subjects research and collecting all relevant data for the protocol. As data are collected, safety is monitored during and after all study procedures, which include MRI examinations, study drug administration, PFTs and blood draws. During visits and phone calls, subjects will be asked to report any interim events they feel may represent adverse events, which will be recorded.

Data and safety monitoring activities for this study will continue until all subjects have completed their participation and all subjects are beyond the time point at which study-related adverse events would likely present.

11.9 Review of Safety Information

11.9.1 Medical Monitor Review

The Medical Monitor (PI) will receive monthly reports from the DCC compiling new and accumulating information on AEs and SAEs recorded by the sites on appropriate CRFs. In addition, the Medical Monitor will review and triage SAE reports received by the DCC.

11.9.2 DSMB Review

The Data and Safety Monitoring Board (DSMB) will review safety data during planned DSMB Data Review Meetings; the frequency of these meetings will be based upon the Charter which is to be finalized at the first meeting. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs. The DSMB will be informed of an Expedited Safety Report in a timely manner.

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair. In addition, the study stopping rules have been established and will be monitored in real-time (Section 9.1, *Study Stopping Rules*). After careful review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

After every DSMB meeting, the DCC will distribute the official DSMB memo to each site for submission to their IRB. The DCC will also record confirmations that each IRB received the memo.

11.9.3 Temporary Halt for Emergency Safety Review

A temporary halt in enrollment will be implemented if an *ad hoc* DSMB safety review is required. In the event that the study temporarily halts enrollment, no new subjects will be consented or start on therapy. Subjects in the screening phase of the study may continue to undergo minimal risk procedures (e.g. blood tests); all other procedures should be deferred. Randomization will not occur until the DSMB review is complete. The health authorities will be notified of any halt in enrollment.

12 STORAGE OF SAMPLES

Research samples will be collected at time points already scheduled for the core mechanistic studies, in order to allow specimens to be stored for use in new assays that have yet to be optimized or conceived, or assays performed by other members for cross-validation studies. Appropriate informed consent will be obtained for both the collection and storing of samples. Any blood samples collected and not immediately used will be centrifuged to collect serum or plasma and stored in freezers at -80°C in a secure lab. Appropriately collected blood for subsequent DNA analysis will be immediately sent to UCLA at room temperature for processing.

13 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

13.1 Clinical Endpoint Analyses

13.1.1 Primary Clinical Endpoint

LV circumferential strain

13.1.2 Secondary Clinical Endpoints

Pulmonary function and osteopontin levels

13.1.3 Biostatistical Modeling

13.1.4

Hypothesis 1.1*: Spironolactone 50 mg qd is noninferior to eplerenone 50mg qd when assessing 12-month change in myocardial strain (Ecc) in DMD patients with preserved ejection fraction.*
Hypothesis 1.2a*: Less myocardial injury by baseline LGE score predicts greater preservation of Ecc in DMD boys treated with mineralocorticoid receptor antagonist (MRA).*
Hypothesis 1.2b*: Higher baseline osteopontin, a muscle injury marker, predicts greater Ecc improvement among DMD patients treated with MRA.*
Hypothesis 1.3*: There is similar decline of forced vital capacity (FVC%) before vs. after treatment with MRA.*
For each study participant, change in myocardial strain (Ecc) from baseline to the one-year follow-up will be calculated. To compare Ecc change between DMD patients receiving eplerenone 50mg qd and those receiving spironolactone 50 mg qd in Hypothesis 1.1, we will conduct both graphical and inferential analyses. A side-by-side boxplot for Ecc change will be constructed to illustrate the observed difference between groups. The comparison of Ecc change will be conducted using an independent two-sample t-test when the normality assumption is not violated. If this assumption does not hold, a nonparametric exact Wilcoxon rank sum test will be conducted. Furthermore, analysis of covariance (ANCOVA) will be used to test the equality of the average change of myocardial strain (Ecc) between DMD patients receiving eplerenone 50mg qd and those receiving spironolactone 50 mg qd after adjusting for the baseline myocardial strain (Ecc).

To determine whether the decline in Ecc for DMD patients treated with MRA is negatively associated with baseline LGE score in Hypothesis 1.2a, we will conduct both graphical and inferential analyses. Scatter plot of baseline LGE score and decline in Ecc will be constructed for DMD patients treated with MRA. Pearson's correlation coefficient will be calculated to quantify the magnitude of association between baseline LGE score and decline in Ecc. Further analyses will be conducted by calculating the Spearman's rank correlation coefficient between baseline LGE score and decline in Ecc and regressing Ecc decline on baseline LGE

score adjusting for possible confounders such as baseline age, baseline myocardial Ecc, use of beta-blocker, mean blood pressure, and EF.

To determine if the decline in Ecc for DMD patients treated with MRA is positively associated with baseline osteopontin in Hypothesis 1.2b, similar graphical and inferential analyses will be conducted as for Hypothesis 1.3a. Scatter plot of the baseline osteopontin and the decline in Ecc will be constructed for DMD patients treated with MRA to assess whether their relationship is linear. Pearson's correlation coefficient will be calculated to quantify the magnitude of the association. Further analyses will be conducted by calculating the Spearman's rank correlation coefficient between the baseline osteopontin and the decline in Ecc and regressing the Ecc decline on the baseline osteopontin by adjusting for possible confounders such as baseline age, baseline Ecc, use of beta-blocker, mean blood pressure, and EF.

To examine the reduction in the rate of decline of FVC% before vs. after treatment with MRA in Hypothesis 1.3, we will conduct both graphical and inferential analyses. Time plots of FVC% over multiple clinical assessments will be constructed for DMD patients treated with MRA. By including a time knot at the initiation of treatment, we will use a linear spline (piecewise linear) mixed effects model to estimate the rates of decline of FVC% before and after treatment and to test whether the reduction in the rate of decline is significant between pre- and post- treatment periods. A random intercept will be included to account for the within-person correlation in outcomes among multiple clinical assessments. In the model, covariates will be included as potential confounders, such as age and 1-year preenrollment FVC%. For the piecewise linear mixed effects model, the normality assumption for FVC% will be assessed to determine whether a certain transformation of the outcome should be applied.

Power Analysis: In the pilot RCT comparing eplerenone 25 mg qd to placebo in boys with DMD, we observed an average increase of 1.15 in Ecc with a standard deviation of 2.38 in the eplerenone group. Using a Type I error rate of 0.05, we conducted a power calculation using a non-inferiority test on 12-month change in myocardial strain (Ecc) for Hypothesis 1.1.

If there is truly no difference in the 12-month Ecc change between the spironolactone and eplerenone groups, 50 patients (25 in each group) will result in at least 80% power to ensure that the lower limit of a one-sided 95% confidence interval for the true difference between the spironolactone and eplerenone groups to be above the non-inferiority limit (largest difference that is clinically acceptable) of -1.7. Anticipating 5% dropout, comparable to what we observed in the pilot RCT, **26 boys will be enrolled in each group of the RCT**.

For Hypotheses 1.2a and 1.2b, we wish to estimate the correlations between baseline LGE score/osteopontin and decline in Ecc for DMD patients treated with MRA. The pilot RCT showed a correlation coefficient of 0.6061 between decline in Ecc and baseline LGE score and 0.3785 between decline in Ecc and baseline osteopontin in the eplerenone group. We expect that the correlation between the baseline LGE score/ osteopontin and decline in Ecc for DMD patients treated with MRA to range from -0.3 to -0.7 and 0.3 to 0.7. We calculated the power based on the required sample size under various scenarios of the smallest detectable Pearson's correlation coefficient (Table 2).

Table 2. Power analysis with a Type I error of 0.05 (one-sided)

		Power
Smallest detectable Pearson's correlation coefficient	± 0.3	59.40%
	± 0.4	82.44%
	± 0.5	95.51%
	± 0.6	99.49%
	± 0.7	99.99%

Thus, we have a power of more than 95.51% to detect a Pearson's correlation coefficient of -0.5 between the baseline LGE score and the decline in Ecc and a Pearson's correlation coefficient of 0.5 between the baseline osteopontin and the decline in Ecc for DMD patients treated with MRA.

13.2 Demographic Data

13.2.1 Baseline Characteristics and Demographics

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner:

- Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.
- Categorical data (i.e., sex and race) will be presented as enumerations and percentages.

Statistical presentation for baseline and demographic characteristics may be further summarized in the statistical analysis plan (SAP).

Medical history will be collected, including the existence of current signs and symptoms and clinical significance for each body system.

Statistical presentation for baseline and demographic characteristics will be further defined in the statistical analysis plan (SAP).

13.2.2 Use of Medications

All medications used will be coded using the World Health Organization (WHO) drug dictionary. The number and percentage of participants receiving concomitant medications or therapies will be presented. Prior exposure to corticosteroids will also be presented. Statistical presentation of concomitant medications or therapies may be further summarized by subsets of cohort subjects as appropriate (e.g. by SAR outcome status).

Medications will be defined by each site's standard of care.

13.3 Interim Analyses

Upon completion of 50% of 12 month follow-ups, an interim analysis of primary and secondary endpoints will be performed for efficacy.

13.4 Reporting Deviations from Original Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol and in the subsequent SAP. Any changes in these principal features will require a protocol or an SAP amendment, which would be subject to review by the independent DSMB, the study sponsor, and the health authorities. These changes will be described in the final report as appropriate.

13.5 Final Study Analysis

The final study analysis will report the results of the original SAP. Any additional analyses will be identified as ad hoc. A final study report will be prepared summarizing the results of analyses.

14 SOURCE DATA

14.1 Source Data

Data will be collected from each site utilizing an electronic CRF (Redcap). Source documentation will be maintained at each site.

14.2 Access to Source Data

Only those involved with the research study will have access to the source data. Source data will be collected, stored and protected in accordance with the institutional policies and procedures.

15 QUALITY CONTROL AND QUALITY ASSURANCE

15.1 Data Handling

All data will be collected in accordance with institutional policies and procedures.

Any data transfer will be done using secure online file transfer sites or by mailing copies on CDs or USB drives. All patient identifiers will be removed before any such transfers take place. Data will not be shared with anyone outside of the research staff and PHI will not be shared with anyone outside of the site that recorded it.

15.2 Data Monitoring

Each site will submit a set of qualifying images prior to the start of enrollment in order to ensure they are capable of completing all MRI scans at a quality acceptable for analysis. If any site does not return images with acceptable quality, the primary site (OSU) will work with that site to determine what is necessary to obtain acceptable images. All types of MRI scanners used will also be tested using the same phantom to ensure reliability of scans. Once enrollment begins, all images will be sent to OSU on the day of the visit. Along with data analysis, OSU will do a quality analysis for each set and provide sites with feedback within one week of the visit. This will help sites continue to produce good quality images throughout the study and to quickly repeat any scans if necessary. If any site continually sends poor image data, they will be required to go through a re-qualification process before continuing enrollment. This may include re-testing with phantoms, checking equipment and protocols, training technicians, etc. To ensure data reliability, subsets of all outcome measures will undergo both inter-observer and intra-observer testing by expert reviewers.

Data blinded to assignment will be continually monitored by DCC staff at OSU. Only the biostatistician working independently of the investigators will evaluate any unblinded data before all follow-up has been completed.

15.3 Data Storage Systems

All electronic data will be stored on secure servers at each site and any hard copies will be stored in locked rooms or cabinets, in accordance with institutional policies and procedures.

15.4 Site Management and Oversight

Sites will undergo a rigorous screening process prior to inclusion in the trial that includes quality review of clinical, imaging, blood collection and processing, pulmonary function testing, drug storage and dispensing, and all other aspects of the study protocol.

16 ETHICAL CONSIDERATIONS AND GOOD CLINICAL PRACTICE

16.1 Statement of Compliance

The study will be conducted in adherence with the requirements set forth by the NIH (A-110 (2 CFR Part 215)) as well as those of the respective institutions.

16.2 Informed Consent Process

The process of obtaining informed consent will be in adherence with the policies and procedures of the internal review board of each participating site.

16.3 Privacy and Confidentiality

The privacy interests of human subjects and confidentiality of data will be in adherence with the policies and procedures of the internal review board of each participating site. All study-related information will remain confidential and secured in a coded fashion in a password-protected system for clinical research data management.

17 PUBLICATION POLICY

The PI and site PIs along with key contributors will all be involved in manuscript preparation and publication.

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APPENDIX 1. GENERAL SCHEDULE OF VISITS

VISIT TIME POINTS		Pre-[1]	Baseline[1]	WK 2	MO 1	MO 2	MO 3	MO 6	MO 9	MO 12
VISIT NUMBER		1	2	3	4	5	6	7	8	9
VISIT WINDOWS in DAYS					± 7 days	± 7 days	± 14 days	± 14 days	± 14 days	± 14 days
VISIT	DETAILS									
Informed Consent	Consent to evaluate for study entry	X								
Assess Eligibility Criteria	Study Entry if all inclusion/exclusion criteria met	X								
Demographics	Age, Race, Ethnicity	X								
Medical History	Past Medical History	X								
Physical Exam	Height and Weight	X								
Concomitant Medications	Drug Names, Start & End Dates	X		X	X	X	X	X	X	X
Randomization			X							
Follow Up telephone calls	Medication adherence/Diary review, AE/SAE, Hospitalizations, Cardiovascular events or Death; Confirm continued participation			X	X	X	X	X	X	X
Study medication	Medication training, family/patient education, Dispensing and returns with diary review		X				X	X	X	X
Follow up	Follow up with AE/SAE to subject's primary physician if consented			X	X	X	X	X	X	X
Blood test for safety	Cystatin C / GFR		X							X
	Potassium		X		X	X	X	X	X	X
Other blood tests	Osteopontin, Hematocrit, additional study-related tests		X							X
	Research blood for DNA		X							
Cardiac MRI	Scans without and with contrast (may be ± 7 days at baseline)		X							X
Pulmonary Function Test	May be clinically-acquired PFTs if ± 14 days of visit		X							X
Questionnaires	Quality of Life and Upper Extremity Function		X							X

[1] Pre and Baseline visits may be concurrent.