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Treatment Of Preserved Cardiac function heart failure with an Aldosterone an Tagonist

TOPCAT

IND Number: 71,883

Version 1.7 – June 04, 2009

Funded by the National Heart, Lung, and Blood Institute, NIH/DHHS

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PROTOCOL SIGNATURE PAGE

I have read the following protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the study drug and the conduct of the study.

I will use the current informed consent form version approved by the National Heart, Lung and Blood Institute and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol and as specified in the Manual of Procedures (MOP) and, in particular, I agree to report any adverse events, serious adverse events, and unanticipated adverse drug effects (UADEs) as defined in Sections C.5.4 – C.5.6 of this protocol.

I further agree that the National Heart, Lung and Blood Institute, the appropriate regulatory authorities and staff from the regional coordinating centers have access to any source documents from which case report form information may have been generated.

I also agree to handle all clinical supplies (including study drug) provided by the National Heart, Lung and Blood Institute and collect and handle all clinical specimens in accordance with the protocol.

The below signed confirm herewith to have read and understood this trial protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance with the protocol and Good Clinical Practice guidelines, as well as local regulations; and to accept respective revisions conducted by authorized personnel of National Heart, Lung and Blood Institute and by competent authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Principal Investigator(s)		
Principal Investigator(s)		

TRIAL OF ALDOSTERONE ANTAGONIST THERAPY IN ADULTS WITH PRESERVED EJECTION FRACTION CONGESTIVE HEART FAILURE

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PROTOCOL OVERVIEW (ABSTRACT)

This Phase III trial is a multicenter, international, randomized, double blind placebo-controlled trial of the aldosterone antagonist, spironolactone, in 3515 adults with heart failure and left ventricular ejection fraction of at least 45%, recruited from over 200 clinical centers. The primary endpoint is a composite of cardiovascular mortality, aborted cardiac arrest or hospitalization for the management of heart failure. Secondary endpoints include all-cause mortality, new onset of diabetes mellitus, atrial fibrillation, and quality of life. The trial duration is approximately 6 years, with approximately 4 years for subject enrollment and an additional 2 years of follow-up, with an average subject follow-up of 3.45 years. Dynamic balancing by clinical center at the time of randomization will be used to ensure that the distribution of clinical centers is similar in the two treatment groups. The study population will include those who meet the inclusion criteria, some of which are:

- Male or female age 50 years or older;
- Heart failure defined as one symptom at screening and one sign present in the last 12 months (described in protocol);
- Left ventricular ejection fraction $\geq$ 45% (per local reading);
- Controlled systolic blood pressure (SBP), defined as: SBP < 140 mm Hg or SBP from 140-160 mm Hg if subject is being treated with 3 or more medications to control BP;
- Serum potassium < 5.0 mmol/L prior to randomization;
- At least one hospitalization in the last 12 months for which heart failure was a major component of the hospitalization OR elevated BNP or N-terminal pro-BNP within the last 60 days;
- Willing to comply with scheduled visits, as outlined in the protocol;
- Signed informed consent form.

Exclusion criteria can be found in Section C.1.2.

Study drug dosing will start at 15 mg/day and may be titrated up to 45 mg according to subject tolerance, safety parameters, and symptoms, and will be continued throughout the trial. Following each change in the dosing regimen, subjects will have blood drawn for safety labs 1 week later. Subjects will take study medication every day according to specific instructions provided by the study staff at the clinical site. All other treatments will follow accepted local standards for medical care for specific morbidities as described by the American College of Cardiology (ACC), the American Heart Association (AHA), and the European Society of Cardiology (ESC) Practice Guidelines, as appropriate. Such treatments may also be adjusted by the local medical practitioner, if necessary. All randomized subjects will be followed even if study drug is discontinued ahead of schedule, except in the case that the subject refuses to participate further in the study.

Follow-up study visits to monitor symptoms, medications, and events and to dispense study drug will occur every 4 months during the first year and every 6 months thereafter. Quality of life will be assessed three times in the first year of the trial and annually thereafter. An electrocardiogram (ECG) will be performed at baseline only. Blood, DNA, and urine samples will be collected from a subset of subjects and stored in a repository for later use in ancillary studies. Clinical endpoints of pre-specified types will be adjudicated by a clinical events committee in a blinded fashion. Continual safety surveillance has been built into the study by means of the proposed dosing and safety assessment regimen described in the protocol. The 15 mg dose of spironolactone was formulated to reduce the risks and side-effects associated with this drug. The Data and Safety Monitoring Board (DSMB) will meet regularly, at least twice

a year. The DSMB chair will be notified of any events considered probably or definitely related to study drug. At the time of notification, he/she will determine if an additional DSMB meeting is required. The study will be conducted according to the provisions of the Declaration of Helsinki, the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), and applicable national and local regulations.

A. SPECIFIC AIMS

A.1 Primary Aim

To determine if treatment with spironolactone can produce a clinically meaningful reduction in cardiovascular mortality, aborted cardiac arrest or hospitalization for the management of heart failure, compared with placebo, in adults with heart failure and left ventricular ejection fraction of at least 45%.

Primary Outcome Measure: Cardiovascular mortality, aborted cardiac arrest or hospitalization for the management of heart failure, as a composite. Treatment arms will be compared using time-to-event analysis.

Secondary Outcome Measures:

- All-cause mortality
- CV mortality or CV-related hospitalization (i.e. hospitalization for non-fatal MI, non-fatal stroke, or the management of heart failure) composite
- CV-related hospitalization
- Hospitalization for the management of heart failure incidence rate (to account for multiple hospitalizations per subject)
- Sudden death or aborted cardiac arrest

A.2 Secondary Aim #1

To determine if treatment with spironolactone can produce a clinically meaningful reduction in new clinical diagnoses compared with placebo, in adults with heart failure and left ventricular ejection fraction of at least 45%.

Secondary Outcome Measures:

- New onset of diabetes mellitus
- Development of atrial fibrillation
- Myocardial infarction (fatal and non-fatal)
- Stroke (fatal and non-fatal)
- Deterioration of renal function
- Sudden death, aborted cardiac arrest, or hospitalization for management of ventricular tachycardia

A.3 Secondary Aim #2

To evaluate the relative impact of spironolactone versus placebo on functional status and quality of life in adults with heart failure and left ventricular ejection fraction of at least 45%.

Secondary Outcome Measures:

- Quality of life, as measured by the:
  - Kansas City Cardiomyopathy Questionnaire (KCCQ) – Primary quality of life outcome measure
  - EuroQOL (EQ5D) visual analog scale

- McMaster Overall Treatment Evaluation (OTE)
- Patient Health Questionnaire (depression scale)

A.4 Secondary Aim #3

To determine if treatment with spironolactone is safe, compared with placebo, in adults with heart failure and left ventricular ejection of at least 45%.

Safety Outcome Measures:

- All-cause mortality
- Hospitalization for any reason
- Laboratory indices of renal and metabolic function.

B. BACKGROUND

B.1 Prior Literature/Studies

Chronic heart failure (CHF) is a broad syndrome characterized by the relative inability of the heart to adequately meet metabolic demands of tissues without an abnormal elevation in filling pressure, which contributes to the clinically recognizable constellation of signs and symptoms. Although the etiologies of CHF are diverse, the premature mortality, incumbent morbidity, and associated healthcare burdens are not cause specific. Regardless of the etiology, CHF represents a progressive disorder that afflicts approximately 10% of the elderly and is the most common reason for hospitalization of patients over 65 years old (Hunt et al., 2001), with a prevalence of 4.9 million people in the United States, and 550,000 new cases diagnosed annually (American Heart Association, 2003). Epidemiologic and hospital-based studies have demonstrated that among patients with newly diagnosed CHF in the community, 43% to 54% of patients have preserved systolic function (PSF) (Senni et al., 1998; Vasan et al., 1999; Ahmed et al., 2002; McDermott et al., 1997). CHF patients without low ejection fractions have been variably described as having HF-PSF, heart failure with preserved ejection fraction, or diastolic heart failure. Although each term has relative merits, they do not completely characterize the complex interactions between systolic and diastolic function, vascular-ventricular coupling, neuroendocrine activation, and cardiorenal adaptations that result in the syndrome of heart failure. Pragmatically, since a quantitative left ventricular ejection fraction (LVEF) is used to define the well-studied systolic dysfunction (LVEF<40%) component of the heart failure population, an LVEF ≥40% can be used to identify the remaining proportion of heart failure patients with relatively PSF.

Relative to systolic dysfunction CHF, HF-PSF has a higher proportion of women and the elderly. The Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) Trials, with concurrent screening for both systolic dysfunction and HF-PSF, found a similar incidence of atrial fibrillation and diabetes mellitus across ejection fraction groups but a lower frequency of prior myocardial infarction in those with HF-PSF (McMurray et al., 2003). In the Cardiovascular Health Study, approximately 67% of women older than 65 years of age had PSF compared with 42% of men (Kitzman et al., 2001). The estimate of the prevalence of this syndrome varies dramatically based upon the study design with a range from 13 to 74% reported among those with heart failure (Ahmed et al., 2002). The annual mortality rate has been estimated to be between 1.3 and 17.5% (Vasan et al., 1995). In the recently completed CHARM-Preserved trial, involving 3025 patients with symptomatic heart failure and an LVEF greater than 40% (median 54%), the mortality rate was 5.5 per 100 person-years, which though less than the approximately 10 per 100 person-years for heart failure with depressed LVEF, was still threefold higher than age-matched subjects without heart failure (Yusuf et al., 2003). These

patients also have significant morbidity. CHF patients with PSF (HF-PSF) have a high risk of re-hospitalization for HF and functional decline, reduced exercise performance, and worse quality of life than non-HF patients (Hundley et al., 2001; Kitzman et al., 2002; Smith et al., 2003).

B.2 Rationale for This Trial

B.2.1 Rationale for Investigation of New Renin-Angiotensin-Aldosterone System (RAAS) Inhibitors in CHF Patients with PSF

This randomized double-blind placebo-controlled trial is designed to test the hypothesis that the addition of a mineralocorticoid receptor blocker to conventional therapy would improve clinical outcomes as assessed by reduced risk of death and hospitalizations for major cardiovascular events in patients with symptomatic heart failure and a quantitative LVEF at or above 45%. Despite the persistent advances over the past two decades in the treatment and prevention of cardiovascular diseases, the incidence of heart failure continues to increase. In some respects, this increase is a consequence of successes in the management of other life-threatening cardiovascular disorders, producing a larger reservoir of older individuals surviving with coexisting major cardiovascular comorbidities. Moreover, patients with heart failure and PSF have a particularly high rate of recurrent hospitalizations for a variety of major cardiovascular complications. The efficacy demonstrated with two separate mineralocorticoid receptor blockers, reducing the risk of death and hospitalizations for heart failure in patients with symptomatic heart failure and reduced ejection fraction, and acute MI complicated by heart failure, (spironolactone and eplerenone, respectively), provides a strong rationale for testing a mineralocorticoid receptor blocker in patients with heart failure and relatively preserved systolic ejection fraction. In addition to the potential reductions of individual risks of cardiovascular morbidity and mortality, the benefits achieved in this understudied population that utilizes considerable health care resources, would have major public health implications – reductions in both mortality and in costly hospitalizations.

B.2.2 Rationale for Use of Spironolactone

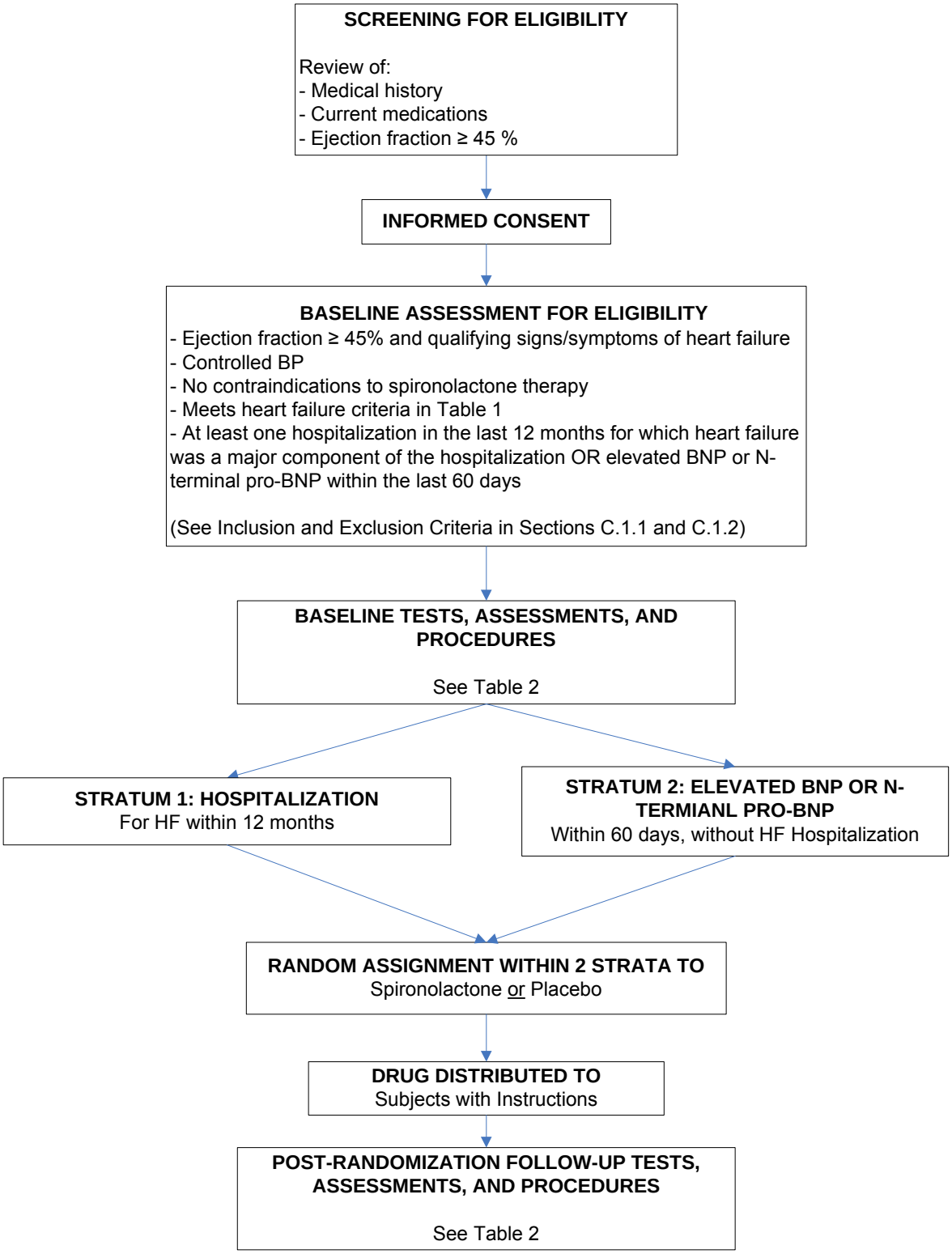
There are two candidates for aldosterone inhibition: the more familiar generic drug spironolactone and the newer eplerenone (owned by Pfizer). The important clinical benefits of these two mineralocorticoid receptor blockers are supported by mechanistic animal studies demonstrating that these agents reduce interstitial fibrosis, ventricular remodeling, vascular oxidative stress, improved endothelial function and have other favorable actions that could be anticipated to translate into clinical benefits in patients with heart failure and PSF. Both drugs have demonstrated improvement in survival in high-risk cardiovascular patients by mechanisms that likely go well beyond the renal effects of aldosterone inhibition. Spironolactone has an associated 10% rate of gynecomastia in males, which is not a side effect of eplerenone. However, from the Randomized Aldactone Evaluation Study (RALES) trial experience, this side effect resulted in negligible discontinuance of the drug. In the TOPCAT trial, gynecomastia is not anticipated to be a major issue as the population recruited for the trial will include a large number of females, many of whom are postmenopausal.

C. STUDY DESIGN AND METHODS

Next page.

Figure 1

SUBJECT FLOW IN TRIAL



C.1 Participants

C.1.1 Inclusion Criteria

In order for a subject to be eligible for inclusion in the trial, all of the following criteria must be met:

1. Male or female; Age 50 years or older;
2. Heart failure as defined in Table 1. One symptom must be present at the time of screening and one sign must be present in the last 12 months. Heart failure eligibility should be carefully monitored and documented in the subject's medical records.
3. Left ventricular ejection fraction (ideally obtained by echocardiography, although radionuclide ventriculography and angiography are acceptable) ≥ 45% (per local reading). The ejection fraction must have been obtained within 6 months prior to randomization and after any MI or other event that would affect ejection fraction;
4. Controlled systolic BP, defined as a target systolic BP < 140 mm Hg. Subjects with BP up to and including 160 mm Hg are eligible for enrollment if on 3 or more medications to control BP.
5. Serum potassium < 5.0 mmol/L prior to randomization;
6. At least one hospital admission in the last 12 months for which heart failure was a major component of the hospitalization. Transient heart failure in the context of myocardial infarction (MI) does not qualify.

   OR

   Brain natriuretic peptide (BNP) in the last 60 days ≥ 100 pg/ml or N-terminal pro-BNP ≥ 360 pg/ml and not explained by another disease entity;
7. Women of child-bearing potential must have a negative serum/urine pregnancy test within 72 hours prior to randomization, must not be lactating, and must agree to use an effective method of contraception during the entire course of study participation.
8. Willing to comply with scheduled visits, as outlined in Table 2;
9. Informed consent form signed by the subject prior to participation in the trial.

TABLE 1. Criteria for Diagnosing Heart Failure

SYMPTOMS (at least one must be present at the time of screening)	SIGNS (at least one in last 12 mos.)
• Paroxysmal nocturnal dyspnea • Orthopnea • Dyspnea on mild or moderate exertion	• Any rales post cough • Jugular venous pressure (JVP) ≥ 10 cm H_2O • Lower extremity edema • Chest x-ray demonstrating pleural effusion, pulmonary congestion, or cardiomegaly

C.1.2 Exclusion Criteria

If a subject meets any one of the following criteria then he/she is ineligible for enrollment in the trial:

1. Severe systemic illness with life expectancy judged less than three years;
2. Chronic pulmonary disease requiring home O_2, oral steroid therapy or hospitalization for exacerbation within 12 months, or significant chronic pulmonary disease in the opinion of the investigator;
3. Known infiltrative or hypertrophic obstructive cardiomyopathy or known pericardial constriction;

4. Primary hemodynamically significant uncorrected valvular heart disease, obstructive or regurgitant, or any valvular disease expected to lead to surgery during the trial;
5. Atrial fibrillation with a resting heart rate > 90 bpm;
6. Myocardial infarction in past 90 days;
7. Coronary artery bypass graft surgery in past 90 days;
8. Percutaneous coronary intervention in past 30 days;
9. Heart transplant recipient;
10. Currently implanted left ventricular assist device;
11. Stroke in past 90 days;
12. Systolic blood pressure (SBP) > 160 mm Hg;
13. Known orthostatic hypotension;
14. Gastrointestinal disorder that could interfere with study drug absorption;
15. Use of any aldosterone antagonist or potassium sparing medication in last 14 days or any known condition that would require the use of an aldosterone antagonist during study participation;
16. Known intolerance to aldosterone antagonists;
17. Current lithium use;
18. Current participation (including prior 30 days) in any other therapeutic trial;
19. Any condition that, in the opinion of the investigator, may prevent the subject from adhering to the trial protocol;
20. History of hyperkalemia (serum potassium ≥ 5.5 mmol/L) in the past six months or serum potassium ≥ 5.0 mmol/L within the past two weeks;
21. Severe renal dysfunction, defined as an estimated glomerular filtration rate (GFR) < 30 ml/min (per the Modification of Diet in Renal Disease (MDRD) 4-component study equation). Subjects with serum creatinine ≥ 2.5 mg/dl are also excluded even if their GFR is ≥ 30 ml/min;
22. Known chronic hepatic disease, defined as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels > 3.0 times the upper limit of normal as read at the local lab.

C.1.3 Human Subjects Considerations

C.1.3.a Informed Consent

A waiver of consent may be requested from the Institutional Review Board/Ethics Committee (IRB/EC) of each clinical center in order to submit to the Clinical Trial Coordinating Center (CTCC) a completed screening form on non-randomized subjects. Written informed consent will be obtained from all potentially eligible trial subjects. Consent from a surrogate will not be permitted.

The repository will be a side-arm study of the main protocol. All sites participating in the side-arm study will approach all potentially eligible trial subjects for consent. A separate informed consent for each specimen type collected will be obtained prior to randomization. There are two portions to the repository sub-study: (1) DNA portion and (2) blood and urine portion. Random codes will be assigned to the repository samples and subjects may request to have their repository samples withdrawn and destroyed at any time while the trial is ongoing. At the completion of the trial, the repository samples and the clinical database will be double-coded. The clinical dataset will be anonymized such that it could not be linked back to the study subjects. Once the link between the subject ID and the repository sample code has been destroyed, subjects will no longer have the option to withdraw and/or destroy their repository samples. Repository samples with associated clinical data will not be made available for future research studies until the database and samples have been anonymized at the end of the trial.

The repository samples will be stored for future testing in a central repository maintained by NHLBI and may be kept for up to 30 years after the close of the study.

Other than random assignment to either spironolactone or placebo, all subjects will undergo routine care for heart failure with PSF.

Before the first trial-related procedure for a subject is performed, the investigator will obtain informed consent from the study subject by means of a dated and signed consent approved by the local IRB/EC in his/her country.

The informed consent process will be performed in accordance with the ICH guidelines for Good Clinical Practice (GCP), local laws and regulations.

Potential study subjects will be provided the current informed consent form and be given adequate time to study the information. The informed consent form will be provided to the subject in the local language. Informed consent may only take place after the potential study subject has had adequate time to study the informed consent form, ask any questions and decide whether or not to participate in the trial.

The informed consent process includes individual discussion with the subject about what study participation will involve. The information to be discussed will include all the information provided in the TOPCAT trial informed consent form. The discussion process includes informing the study subject both verbally and in writing that:

-if he/she refuses to participate in the study, the quality of medical care he/she receives will not be affected and
-he/she may withdraw at any time without giving reason and without affecting future care and
-without disclosing his/her name, relevant medical and personal data will be disclosed to the sponsor and regional coordinating centers who are obliged to use the information anonymously and solely for scientific purposes and
-his/her medical records may be reviewed during on-site monitoring, and may be inspected by auditors and/or regulatory authorities who are obliged to confidentiality and
-confidentiality will be maintained at all times according to local data protection laws.

Both the date a potential study subject is given the informed consent form and the date the study subject gives informed consent must be recorded. The study subject will be given a copy of the signed informed consent form.

After informed consent has been provided by the study subject, the original informed consent form will be kept in the patient file at the clinical site and will be made available for audit purposes. If the filing of the original signed consent form in the subject's hospital file is not permitted by the hospital or clinical setting, it must be filed in the investigator files and an indication that consent was obtained (with the date specified) should be noted in the medical files.

C.1.3.b Patient Confidentiality

Patient confidentiality will be maintained according to ICH guidelines for GCP and applicable local and national data protection laws. A study identification number will be assigned to each subject. The link between patient name and I.D. number will be stored only at the clinical center where the subject receives his/her care, thereby ensuring that all data transferred from a

subject's medical records to a study report form and any process derived from the study report form is handled confidentially.

C.1.3.c DNA Confidentiality

Blood samples prepared for DNA extraction will be sent to the repository. The sample will not have the original study I.D. number, the patient's name, or any other information that could identify the subject. The specific procedures are detailed in the Manual of Procedures (MOP) and the Repository Instruction Manual.

C.1.3.d Potential Risks

Spironolactone has been licensed for the treatment of heart failure in all of the countries participating in the TOPCAT trial for many years. The most common risks of taking spironolactone include hyperkalemia (observed at < 1.0% in the RALES trial with no serious consequences), hyponatremia, headache, drowsiness, lethargy, diarrhea, cramps, bleeding, gastritis, vomiting, anorexia, nausea, rash, pruritis, and urticaria. Gynecomastia, erectile dysfunction, and post-menopausal bleeding are less common. Hirsutism, agranulocytosis, and hyperchloremic metabolic acidosis have also been reported.

Although breast tenderness and gynecomastia have been reported in up to 10% of male patients treated with spironolactone, the risk of this side effect is dose-related and uncommon in patients treated with daily doses of 50 mg or less (as planned in this trial). In the RALES trial, gynecomastia resulted in negligible discontinuance of the drug and the condition is expected to be less of a problem in the TOPCAT trial as the study will be investigating patients with HF-PSF, a large proportion of whom are post-menopausal women.

A potentially serious side effect sometimes seen in patients treated with spironolactone is hyperkalemia. People with impaired renal function are considered to be at higher risk of hyperkalemia - an observation used to define the exclusion criteria of first the RALES trial and now TOPCAT. The investigators in the RALES trial attributed the observed incidence of hyperkalemia (1% in the placebo group and 2% in the spironolactone-treated group) to the exclusion of patients with elevated serum creatinine and potassium at baseline (and also to the relatively low treatment dose of spironolactone: the mean dose was 26 mg). Similar exclusion criteria will be used in the TOPCAT trial; however, the starting dose of spironolactone will be lower and renal function will be more accurately and reliably defined at baseline by estimated GFR. By careful evaluation of the pre-disposing factors for hyperkalemia and use of close monitoring of serum potassium during the study, it is anticipated that the rate of clinically significant hyperkalemia seen in TOPCAT will be similar to or possibly lower than that observed in the RALES trial.

Therapeutic trials investigating heart failure have been performed to date almost exclusively on patients with systolic dysfunction. However, now there is a growing awareness that a large proportion of patients with heart failure have preserved systolic function and that survival of these patients is also adversely affected. While treatment has been shown to be useful in patients with heart failure with systolic dysfunction, this is an area which has been understudied in those heart failure patients with PSF. Consequently much still remains to be learned about HF-PSF and its treatment.

C.1.3.e Potential Benefits

Subjects enrolled in this trial who are receiving active drug may receive a benefit. Also, there may be considerable benefit to future patients with HF-PSF as a result of the medical knowledge obtained from this study.

C.2 Trial Enrollment

C.2.1 Recruitment Protocol

The Principal Investigator at each private practice or clinical center, his or her designee, and the coordinator will have the responsibility for case finding and subject recruitment. The coordinator will conduct a chart review, while complying with local institution Health Insurance Portability and Accountability Act (HIPAA) requirements, to identify potentially eligible subjects. The coordinator will contact the subject per local guidelines to assess interest in the trial and to schedule an office or clinic visit for determination of full eligibility. Subjects may also be approached for participation while in-hospital if the subject is potentially eligible based on chart review. It should be noted that a subject may be screened for trial eligibility more than once during the accrual period.

C.2.2 Stratification

Due to the large number of clinical centers and potentially small number of enrolled subjects at some sites, dynamic balancing (Zelen, 1974) rather than stratified randomization across sites will be utilized to ensure that the distributions of clinical centers are similar in the two treatment groups. This approach will prevent the creation of excessively small stratum sizes. In addition, subjects will be stratified on inclusion criterion #6. Stratum I will include subjects selected based on a hospitalization in the 12 months prior to enrollment with a heart failure diagnosis and stratum II will include those subjects not reporting a hospitalization in the prior 12 months for which heart failure was a major component (for whom elevated BNP or Pro-BNP is required).

C.2.3 Blinding

Subjects and treating physicians will be blinded to whether subjects are receiving spironolactone or placebo. Because the trial has a double-blind design, safety laboratory tests will be performed for each subject for the duration of the trial, regardless of treatment arm. Similarly, monitoring of potential side effects will be continuous and irrespective of treatment assignment. While unmasking of the drug assignment for an individual subject is expected to be very rare, given the proposed dosing and safety-monitoring regimen described in Section C.3, a procedure for unblinding is included in the Manual of Procedures (MOP).

C.2.4 Baseline Visit and Randomization

After written informed consent is obtained, a baseline visit will occur, during which confirmation of eligibility will be obtained and baseline labs will be drawn. The maximum allowable timeframe between study baseline visit and the randomization date is 14 days. The baseline visit and randomization may occur on the same day. If baseline laboratory values were collected more than 14 days before the date of randomization, the clinic sites should repeat baseline labs, update any changes in the subject's medical history and concomitant medications, and confirm that the subject still meets all the study inclusion/exclusion criteria prior to randomization. Laboratory values obtained within the 14 day interval are acceptable as long as there were no inter-current change in medications and no borderline laboratory values. Subjects will be randomly assigned in a 1:1 ratio using permuted blocks to receive either spironolactone or placebo. Randomization will be accomplished over the Internet using randomization software accessed via a secure website. After verifying key eligibility criteria and supplying clinical center information, the randomization software will return a Treatment Allocation Code (A thru L) corresponding to either spironolactone or placebo. Labels containing treatment allocation code will be on the drug packet to verify correct assignment.

C.3 Treatment

C.3.1 Description of Study Medication

Study drug supplies will be provided by the Department of Health and Human Services (DHHS) Program Support Center in Perry Point, MD. Shipments will consist of the following:

1. Bottles containing 150 spironolactone 15 mg tablets
2. Bottles containing 150 placebo tablets, identical in size and appearance to the 15 mg spironolactone tablets.

Both the spironolactone 15 mg tablets and matching placebo are manufactured by URL Mutual Pharmaceutical in Philadelphia, PA, USA in accordance with federal regulations and ICH guidelines for Good Manufacturing Practices.

C.3.2 Randomization Procedures

Subjects will be assigned in the order they are enrolled into the study, to receive the allocated treatment according to a computer-generated randomization plan using NERI's Verandi software package. Once a subject has been assigned a Treatment Allocation Code, the subject will remain on the same study drug treatment allocation code for the duration of the study.

C.3.3 Study Drug Administration

Study medication will be dispensed at Randomization, 4 Month visit, 8 Month visit, 12 Month visit, and every 6 months thereafter. Previously dispensed study drug supplies are to be brought in at each subsequent visit to verify drug compliance. The volume of unused tablets or number of tablets will be recorded on the appropriate case report form (CRF), and the tablets will be returned to the subject. Site personnel will instruct the subject on the importance of compliance. A guideline for study drug dispensing is in the Manual of Operations.

The first dose of study drug will be administered as soon as possible after written informed consent has been obtained, baseline procedures have been performed, there is confirmation that laboratory results are within acceptable parameters, and randomization has occurred. Initial dosing should occur on the same date as randomization.

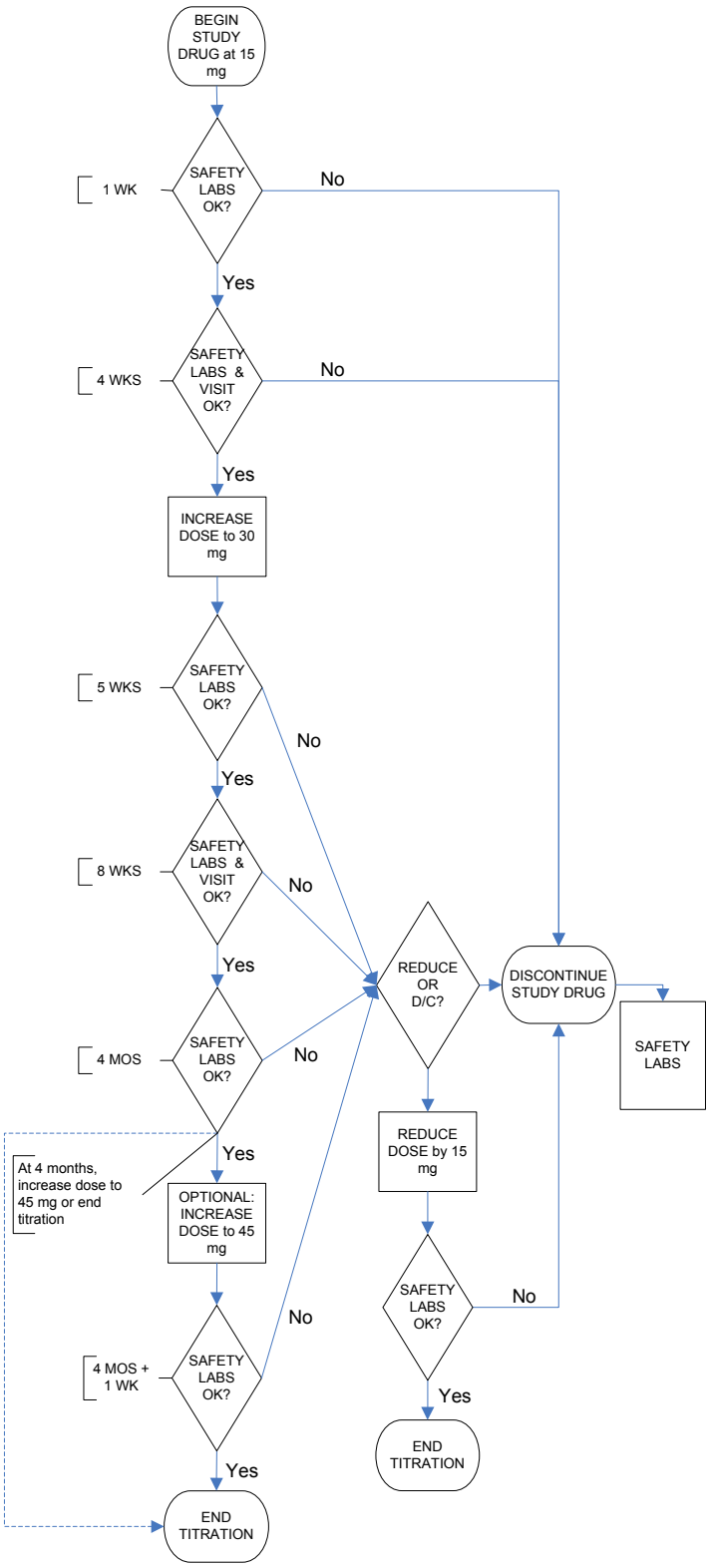
C.3.4 Study Drug Titration and Dosing Regimen

All subjects randomized into the study will begin on an initial dose of 15 mg daily (i.e. one tablet by mouth every day). The titration schedule and safety assessment intervals are illustrated in Figure 2. After 4 weeks, the dose should be increased to 30 mg daily (i.e. two tablets by mouth every day) if all safety parameters are acceptable. In the event that the subject continues to have ongoing heart failure symptoms, the treating physician has the option to increase the dose to 45 mg daily at 4 months. Study drug may only be increased after a subject has remained at a constant dose level for 4 weeks. Study drug may not be titrated to less than 15 mg daily or greater than 45 mg daily. Safety labs (i.e., electrolytes and chemistries) will be collected at 1 week after each change in the dosing regimen (i.e., either increased, decreased, or stopped). Electrolytes will include sodium, potassium, chloride, and bicarbonate/total CO_2. Chemistries will include BUN and creatinine.

Once the subject is appropriately titrated, the dosing regimen (i.e., 15 mg, 30 mg, or 45 mg by mouth every day) should remain stable **unless** scheduled laboratory results exceed the safety parameters, and the potassium value is confirmed by a non-hemolyzed sample. The flowchart in Figure 2 illustrates the various pathways for dose titration of the study drug. Also included in Figure 2 are descriptions of when to reduce, discontinue and/or reinitiate study drug. :

Figure 2. Study Drug Titration and Safety Assessment Schedule

The study drug titration and safety assessment schedule is illustrated in this figure.



Titration: Initial dose: **15 mg/day.** After 4 weeks, the dose should be increased to **30 mg/day** if all safety parameters are acceptable. In the event that the subject continues to have ongoing heart failure symptoms, the investigator has the option to increase the dose to **45 mg/day** at 4 months. Study drug may only be increased after a subject has remained at a constant dose level for 4 weeks. Study drug may not be titrated to less than 15 mg daily or greater than 45 mg daily.

Reduce drug: Reduce the dosing regimen if potassium ≥ 5.5 mmol/L. If the subject is on 45 mg, the dose should be reduced to 30 mg; if the subject is on 30 mg, the dose should be reduced to 15 mg; if the subject is already on the lowest dose (i.e. 15 mg), and if there no alternative explanations for the elevated potassium level (e.g. subjects are taking potassium supplements), the study drug should be permanently discontinued if deemed appropriate by the treating physician and/or TOPCAT Medical Monitors. Once a downward dose adjustment has been made, the study drug should not be uptitrated beyond this level for the trial duration.

Discontinue drug: Permanently discontinue study drug if potassium ≥ 6.0 mmol/L on a non-hemolyzed sample, regardless of the dosing regimen, if there no alternative explanations for the elevated potassium level.

NOTE: Treating physicians may consult the TOPCAT Medical Monitors prior to discontinuing any subjects on study drug as a result of elevated potassium levels. Since there is some room for clinical judgment, subjects could potentially continue to take study drug as long as they are properly monitored. Treating physicians may opt to control a subject's potassium level by adjusting his/her potassium supplement intake (if deemed appropriate and safe) or by recommending a low potassium diet.

Safety labs: Collect safety labs (i.e., electrolytes and chemistries) at 1 week after each change in the dosing regimen (i.e., either increased, decreased, or stopped).

Reinitiation (after non-compliance): If the dosing regimen is interrupted due to non-compliance, study drug may be reinitiated at the discretion of the treating physician. If a subject is eligible for drug reinitiation, the physician should choose from one the following three options:

- Reinitiate study drug at the highest previously tolerated dose (dose just prior to drug discontinuation); or
- Reinitiate study drug at a lower dose; follow-up labs at 1 week, then resume scheduled study visits if lab work is acceptable; or
- Do not reinitiate study drug

If possible, study drug should be reinitiated within one week of drug discontinuation. The number of times study drug may be reinitiated after non-compliance is at the discretion of the treating physician.

C.3.5 Concomitant Medication

Subjects will be treated with other medications at the discretion of their cardiologist and/or primary care provider. At study visits, current medications will be recorded on the study forms. If a subject begins open-label use of any aldosterone antagonist or potassium-sparing diuretic at any time during the study, withdrawal from study drug is required.

The following drug interactions have been observed with spironolactone:

- ACE inhibitors or ARB – may be associated with hyperkalemia
- Alcohol, barbiturates, or narcotics – may be associated with hypokalemia
- Corticosteroids, ACTH – may be associated with hypokalemia
- Pressor amines (e.g. norepinephrine) – may reduce vascular responsiveness
- Skeletal muscle relaxants – may amplify muscle relaxant responsiveness
- Lithium – may lead to lithium toxicity
- NSAIDs – may be associated with hyperkalemia
- Cardiac glycosides (e.g. digoxin) – may lead to digoxin toxicity
- Anticoagulants (e.g. warfarin, heparin) – may reduce the effects of anticoagulation

C.3.6 Indications for Permanent Discontinuation of Study Drug

- Persistent hyperkalemia (potassium ≥ 6.0 mmol/L, based on a non-hemolyzed sample)
- Potassium ≥ 5.5 mmol/L, based on a non-hemolyzed sample, and subject on lowest dose of study drug (15 mg). Other explanations for the elevated potassium level should be ruled out.
- Anaphylactoid reaction or intolerance
- Serum creatinine ≥ 3.0 mg/dl, or at a lower threshold per local physician judgment
- Open label use of any aldosterone antagonist or potassium-sparing diuretic that cannot be discontinued for valid clinical reason
- Other adverse events that require discontinuation of study drug in the judgment of the study investigator, such as a medical course that is incompatible with the concomitant use of spironolactone.

The reason and the circumstances for permanent discontinuation of study drug will be documented. If study drug is permanently discontinued, the subject will continue to be followed until the end of the trial period.

C.3.7 Indications for Withdrawal From the Study

- Subject refusal to continue in the study
- Heart transplantation

All protocol-specified visits and follow-up procedures should be performed for every subject enrolled in the trial, even if the study drug is discontinued. If the subject refuses to continue with the study visits, every attempt should be made to continue contact by telephone, written communication, or record review to determine if outcome events have occurred, unless the subject specifically refuses such follow-up. The reason for withdrawal will be documented for all subjects withdrawn from the study. If the withdrawing subject is unwilling to have his/her medical records reviewed until the end of the trial period (to document vital status and cause of death), he/she must submit a written refusal. Subjects may withdraw consent from the repository sub-study but continue participating in the main study. Subjects who withdraw consent from the main study are automatically withdrawn from the sub-study.

C.3.8 Study Completion

A subject will be considered to have completed the study if he/she has completed follow-up until the end of the trial period, undergoes heart transplantation, or dies. All subjects will be followed for a minimum of 2 years and a maximum of 6 years.

Clinic sites must complete all the necessary "End of Study" CRFs for all study subjects even if the end of study visit falls in-between the study scheduled clinic visits. Please refer to the MOP and ADEPT user guide for additional information.

C.3.9 Subject Compliance

Study drug compliance will be assessed at each study visit by comparing the expected vs. actual consumption of study drug tablets. The subject will bring all remaining study drug to the follow-up visit. The study coordinator will measure and record the volume or count and record number of remaining tablets, and a new 4 or 6 month supply (depending on the visit schedule) will be dispensed.

C.3.10 Drug Accountability Log

All study drug supplies (i.e. spironolactone 15 mg and corresponding placebo tablets and bottles) provided by the DHHS Program Support Center to the investigator for use in the clinical study must be accounted for in written documentation that must be maintained by the investigator and that will be monitored by the CTCC.

Forms to record dispensing of study medication will be provided with the initial shipment of the study medication. A copy of the complete records of study drug accountability for all supplies received for the study must be provided to the CTCC as part of the close-out procedure for the study. The drug accountability records must be retained by the investigator along with the subjects' study records.

C.3.11 Remote Monitoring for Eligibility

To ensure patient eligibility, the CTCC may perform regular remote monitoring "visits" on all clinic sites by requesting specific source documents from a random group of subjects throughout the study. Source documents for study eligibility monitoring purposes may include ECHO reports, lab data, and hospital discharge summaries.

C.3.12 Code Break

The Treatment Allocation Code may be broken if an emergency situation arises that in the Investigator's opinion requires knowledge of the code.

A request for unblinding should only be made in situations where knowledge of the treatment assignment will actually affect the subsequent care or decision-making process for care of the trial subject. It should be assumed that the trial subject will remain in the trial and will continue adherence to the trial protocol after the event is resolved. Therefore, every effort should be made to maintain trial participation in a blinded nature. It is anticipated that code breaks will be very rare and that all subjects will be appropriately monitored for safety.

Refer to the Manual of Procedures (MOP) for a description of the process for code break.

C.4 Measurements

C.4.1 Schedule of Measurement

See next page.

Table 2. Schedule of Trial Measurements

	Record Screening	Baseline Screening	Randomization and Drug Distribution	1 Week	4 Weeks	5 Weeks	8 Weeks	4 Months	8 Months	12 Months	18 Months	24, 36, 48, 60, 72 Months	30, 42, 54, 66 Months
Medical History	X	X											
Current Medications	X	X			X		X	X	X	X	X	X	X
Echocardiogram*	X												
Physical Exam, Wt., Vital Signs		X			X		X	X	X	X	X	X	X
Assessment of Study Drug Compliance					X		X	X	X	X	X	X	X
Blood Studies**		X		X ***	X	X***	X	X	X	X	X	X	X
ECG		X											
Adverse Event and Study Outcome Monitoring					X		X	X	X	X	X	X	X
Urine Microalbuminuria		X								X		X	
QOL****		X						X		X		X	
Repository Specimens													
Urine Specimen		X								X			
Blood Specimen		X								X			
DNA Specimen		X								X			

* Ejection$_{fraction\ obta}$ ined within 6 months prior to randomization and after any MI or other event that would affect ejection fraction.

** Blood Studies (local lab):

- Baseline blood studies include: CBC, electrolytes, BUN, creatinine, blood glucose, and LFTs and should be done within 14 days prior to the randomization date CBC will include WBC$_{count,}$ hematocrit, hemoglobin, and platelet count. Electrolytes will include sodium, potassium,$_{chlo}$ ride, and bicarbonate/total CO_2. LFTs will include alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin, and albumin.
- Follow-up safety blood studies include: electrolytes, BUN and creatinine. Electrolytes will include sodium, potassium, chloride, and bicarbonate/total CO_2.

*** Safety labs will be collected at 1 week after each change in the dosing regimen (i.e., either$_{incr}$ eased, decreased, or stopped).

**** OTE instrument will only be administered at the 4 and 12 month visits; KCCQ and EQ-5D instruments will only be administered at Baseline, 4 and 12 month visits and annually thereafter; Patient Health Questionnaire instrument will only be administered at Baseline and 12 month visits and annually thereafter.

C.4.1.a Record Screening (Table 2)
Record screening will include review of past medical history and current medications. The most recent echocardiogram from the past 6 months will be evaluated to determine if ejection fraction is ≥ 45% (per local reading). It is preferred that the qualifying ejection fraction be obtained by echocardiography. Ejection fraction obtained by radionuclide ventriculography or angiography is also acceptable in instances where an echocardiogram suitable for quantification is not available. A subset of the echocardiograms (video copy or digital image is acceptable) utilized for screening must be submitted to the Brigham and Women's Hospital Echocardiography Core Laboratory for QC purposes. Each site is required to submit the first 2 echos used to determine eligibility to the Echocardiography Core Laboratory which will read these pre-eligibility echocardiograms for a central QC of ejection fraction. Subjects may withdraw or decline to release their echocardiograms to the Echocardiography Core Laboratory at any time during the study. Clinic sites should notify the CTCC immediately of a subject's request to withdraw his/her echocardiogram from the core lab.

C.4.1.b Baseline Screening (Table 2)
At the baseline screening visit, the subject will have a physical examination, including vital signs. Blood will be drawn for CBC, electrolytes, BUN, creatinine, blood glucose, and liver function tests (LFTs). CBC will include WBC count, hematocrit, hemoglobin, and platelet count. Electrolytes will include sodium, potassium, chloride, and bicarbonate/total CO_2. LFTs will include alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total bilirubin, and albumin. A urine test for microalbuminuria will be conducted. Creatinine, potassium, and LFTs, as well as the blood pressure measurements will be used to confirm eligibility. Current medication use will be reviewed to confirm that the subject does not meet exclusion criteria. Age, gender, race, and serum creatinine concentration will be obtained in order to calculate an estimated GFR using the 4-component MDRD Study prediction equation. The GFR estimate will be used to determine whether a subject has acceptable renal function to be enrolled in this study (see exclusion criterion 21). The initial medical history will focus on demographics, cardiac risk factors, and the prior 12 months for recent hospitalizations and procedures. An electrocardiogram (ECG) will be obtained at baseline. The subject will be asked to complete the first quality of life questionnaires. Procedures for the physical examination, blood draw, and urine test will be detailed in the Manual of Procedures (MOP). After randomization, two bottles of study drug will be dispensed with instructions.

All subjects from sites participating in the repository sub-study will be approached for consent to provide blood and urine samples for the repository, including a whole blood sample for DNA extraction.

C.4.1.c Follow-Up Visits (Table 2)
Health status and study drug compliance will be evaluated at scheduled visits throughout the study. Subjects must plan to have blood drawn for safety labs at 1 week post drug initiation/dose change. They will be scheduled to have an office visit and safety labs at 4 weeks post drug initiation. If the study drug is increased at this time, they will have blood work one week after dose change (week 5), and then full evaluation at 8 weeks. Subsequent planned visits will be scheduled every four months for the first year and every six months thereafter. Specifics for study drug titration are described in Section C.3.4 and Figure 2. Unplanned visits will be determined by the treating physician for symptoms, abnormal lab work, or other reasons.

At each office visit, the following will be obtained by short interview: current signs/symptoms consistent with HF and with administration of study drug, and current medications (subjects will be asked to bring these to each visit for accurate inventory). Blood pressure will be taken and

recorded. Every effort should be made to control blood pressure throughout the course of follow-up. Body weight will be recorded. Electrolytes, BUN, and creatinine, will be drawn to assess study drug safety. Electrolytes will include sodium, potassium, chloride, and bicarbonate/total CO_2. A urine test for microalbuminuria will be conducted annually.

Four quality of life instruments will be administered to trial subjects in the appropriate language according to the Schedule of Measurement (Table 2).

Blood and urine specimens for the repository will be obtained at baseline and 12 months from a subset of subjects.

If drug reduction/discontinuance is indicated by the chemistry panel results, a follow-up visit will be scheduled within one week at which time the subject will be evaluated for change in course of therapy.

Towards the end of the trial follow-up period, the Social Security or National Death Index will be searched for any subjects of unknown vital status in the U.S. Similar procedures will be implemented as feasible in other countries, with the assistance of the Regional Leaders.

C.4.1.d Windows for Visits

The acceptable windows for study visits are shown in Table 3. Safety monitoring during the titration period must be conducted at the study site. If for some reason a subject is unable to complete a study visit in person for a visit at Month 4 or later, the QOL instruments will be mailed to the subject along with a hospital-addressed stamped envelope for return of the completed questionnaires to the clinical site. The QOL instruments will be assigned for analysis to the nearest available window based on completion date.

Table 3. Acceptable Windows for Study Visits

Visit	Window
Week 1, 4, 5, 8	$\pm$ 3 days
Month 4	$\pm$ 2 weeks
Month 8, 12	$\pm$ 2 weeks
Later Visits	$\pm$ 4 weeks

C.4.2 Outcome Variables

Outcome variables have been chosen that will best capture the multi-faceted impact of spironolactone on heart failure with relatively PSF, a disease with significant morbidity, mortality, and associated costs. The primary trial endpoint is **a composite of** cardiovascular mortality, aborted cardiac arrest or hospitalization for the management of heart failure. Table 4 provides a summary of all outcome measures for the trial. In addition, all components of composite endpoints will be reported.

Table 4. Trial Outcome Measures

Primary Outcome

- Cardiovascular (CV) mortality, aborted cardiac arrest or hospitalization for the management of heart failure, as a composite.

Secondary Outcomes

Morbidity and Mortality

- All-cause mortality
- CV mortality or CV-related hospitalization (i.e. hospitalization for non-fatal MI, non-fatal stroke, or the management of heart failure) composite
- CV-related hospitalization
- Hospitalization for the management of heart failure incidence rate (to account for multiple hospitalizations per subject)
- Sudden death or aborted cardiac arrest

New Clinical Findings

- New onset of diabetes mellitus
- Development of atrial fibrillation
- Myocardial infarction (fatal and non-fatal)
- Stroke (fatal and non-fatal)
- Deterioration of renal function (see Section C.4.2.b)
- Sudden death, aborted cardiac arrest, or hospitalization for management of ventricular tachycardia

Quality of Life

- Kansas City Cardiomyopathy Questionnaire (KCCQ)
- EuroQol (EQ5D) visual analog scale
- McMaster Overall Treatment Evaluation (OTE)
- Patient Health Questionnaire (depression scale)

Safety Measures

- All-cause mortality
- Hospitalization for any reason
- Laboratory indices of renal and metabolic function

C.4.2.a Morbidity and Mortality

Vital and hospitalization status will be monitored through subject contacts and by interview and medical record review at the clinic site. If a death occurs, the nurse coordinator will complete a death form indicating the date, time, and official cause of death, as well as a description of events leading up to the death.

Selected outcome forms and supporting documentation will be forwarded from the CTCC to the Clinical Endpoints Committee (CEC) for review as described in the TOPCAT Manual of Procedures (MOP).

C.4.2.b New Clinical Findings

New onset of diabetes mellitus will be assessed by physical exam, symptoms, and defined by measurement of blood glucose and introduction of anti-diabetic medication. New diagnosis of atrial fibrillation will be made by reported symptoms and clinically indicated monitoring of heart rhythm. Deterioration of renal function is defined as a twofold increase in baseline serum creatinine level that at a minimum exceeds the upper limit of normal. Stroke and MI are centrally adjudicated and defined in the CEC Manual of Procedures (MOP).

C.4.2.c Quality of Life

The primary goals of heart failure management are improving patient function, slowing disease progression, and improving quality of life. The quantification of this latter treatment goal requires the use of a health-related quality of life instrument, typically including a range of domains of health status. Four instruments will be administered to trial subjects in the appropriate language, if a validated version is available, according to the Schedule of Measurement (Table 2). The overall quality of life assessment at each visit typically will not exceed 12-15 minutes per subject.

The Kansas City Cardiomyopathy Questionnaire (KCCQ) will be used as the primary endpoint for evaluation of functional status and quality of life in this trial. The KCCQ is a self-administered 23-item questionnaire taking approximately 4-6 minutes that measures physical limitation, symptoms (frequency, severity and recent change over time), quality of life, social interference, and self-efficacy. The KCCQ has been used in several recent and ongoing heart failure trials, including the EPHESUS trial.

In addition to the KCCQ, a brief generic health status measure, the “feeling thermometer” from the EuroQOL Health Status Questionnaire (EQ-5D; Brazier et al., 1993), which is a visual analog (0-100) scale, ranging from the worst imaginable health state (0) to the best imaginable health state (100) will be administered, as well as the McMaster Overall Treatment Evaluation (OTE) (Juniper et al. 1994). The OTE has 3 items addressing the overall effect of the treatment according to whether a subject has improved or deteriorated with respect to symptoms related to heart failure since the treatment started (therefore this instrument will not be part of the baseline QOL battery). If subjects indicate an improvement or deterioration, they will be asked to score the magnitude and the importance of the perceived change on a 7-point scale. The items will be combined to form a 15-graded scale, ranging from the worst deterioration (-7) to the highest improvement (+7) with “No change” (0) as the middle score. The OTE will be administered only at the 4 and 12 month follow-up visits.

Finally, the Patient Health Questionnaire, a 9-item health scale derived from the PRIME-MD that includes a measure of depression severity, will be administered.

C.4.3 Event Adjudication

New England Research Institutes, Inc. (NERI) as the CTCC will serve as the primary liaison to the sites for reporting of study endpoints and will be responsible for ensuring the required endpoint-related data and source documents are collected. The Clinical Endpoint Committee at the Brigham and Women’s Hospital in Boston will serve as the CEC and will be responsible for reviewing and adjudicating all suspected study endpoints consisting of cardiovascular vs. non-cardiovascular death, hospitalization for congestive heart failure, cardiac arrest, myocardial infarction, stroke, new onset of diabetes mellitus, new onset of atrial fibrillation, and hospitalization for the management of ventricular tachycardia.

The primary objective of the CEC is consistent and unbiased review and adjudication of study endpoints throughout the course of the trial. At the CEC, each event will be assigned and reviewed by a Physician Reviewer. The Physician Reviewer will document key details of the event, make a preliminary decision, and present his/her findings at the CEC meeting. In certain instances, the Chairman will generate a case precedent, an internal consistency measure, for difficult or noteworthy events that set a precedent for how future events should be regarded.

For each endpoint, the Physician Reviewers are responsible for providing a final adjudication for each event along with appropriate chart documentation describing the key details related to the event as well as rationale supporting their adjudication. The CEC maintains strict internal quality assurance measures in order to maintain the high-level quality of adjudicated data and in addition, all operations are conducted under the International Conference on Harmonization Good Clinical Practices (ICH/GCP) and Code of Federal Regulations (21 CFR 312, 21 CFR 50, 21 CFR 56). The CEC maintains Standard Operating Procedures for all functions and procedures and is subject to review and audit by the sponsor, or their representatives, and regulatory authorities. A 10% sample for re-adjudication will be randomly and blindly inserted in the review process by the CTCC and the results will be reported at CEC meetings. Details of CEC procedures will be included in the TOPCAT trial Manual of Procedures (MOP).

C.4.4 Repository

The repository will be a sub-study of the main protocol and subjects will be asked to provide additional informed consent to participate. For those subjects who consent, urine and blood specimens will be collected at baseline and 12 months, spanning an interval when most events and physiological changes are likely to occur. A whole blood sample for DNA extraction will also be collected for those subjects who consent. The proposed collections are summarized in Table 5. SeraCare BioServices currently serves as the long term NHLBI repository. All pre-barcode labeled collection and shipping containers will be provided to the clinical centers. The repository specimens will be stored for later use in ancillary studies yet to be approved and funded. Details of sample handling, storage, and shipping procedures are included in the TOPCAT MOP.

TABLE 5. Specimen Collection

Serum	• Up to three 10 ml tubes whole blood, collected and processed for storage of plasma and serum r as detailed in the Manual of Procedures (MOP) • Aliquot into pre-labeled cryovials and store at -20^{o}C • Shipment to repository when shipping rack filled
Urine	• 20 ml urine (mid-stream, time of day recorded but unrestricted)Aliquot into pre labeled cryovials and stored at -20 ^{o}C • Shipment to repository as above
DNA	• Packed cells from whole blood collected in EDTA tubes will be used for the DNA extraction.

C.5 Adverse Events

C.5.1 Definition

For purposes of this study, an adverse event (AE) is any untoward medical occurrence in a subject which occurs after the subject signs the informed consent form for the trial and no later than 30 days after a subject has permanently discontinued the study medication. Except for the study outcomes (see Table 4 Trial Outcome Measures) any untoward medical occurrences beginning more than 30 days after a subject has permanently discontinued study drug will not

be collected. Clinic sites must report all AEs (related and not related to study drug) to the CTCC in a timely manner. AEs are automatically reported to the CTCC when the sites complete the AE CRFs in ADEPT.

C.5.2 Classification of Adverse Events

C.5.2.a Severity

The severity (intensity) of each AE will be assessed according to the following definitions:

Mild: Symptom(s) barely noticeable to the subject or does not make the subject uncomfortable. The AE does not influence performance or functioning. Prescription drugs are not ordinarily needed for relief of symptom(s).

Moderate: Symptom(s) of a sufficient severity/intensity to make the subject uncomfortable. Performance of daily activities is influenced. Treatment of symptom(s) may be needed.

Severe: Symptom(s) of a sufficient severity to cause the subject severe discomfort. Severity may cause cessation of treatment with the drug. Treatment for symptom(s) may be given.

Life-threatening: Symptom(s) of a sufficient severity/intensity to cause the subject to be at immediate risk of death. Treatment for symptom(s) may be given.

C.5.2.b Relationship

The temporal/causal relationship between the study drug (spironolactone or placebo) will be determined by the investigator according to the following definitions:

Definite: Clearly related to the study drug.

Probable: Likely (high suspicion) related to the study drug.

Possible: May be related to the study drug.

Unrelated: Clearly not related to the study drug.

C.5.3 Data Collection Procedures for Adverse Events

Adverse events will be recorded according to the date and time of first occurrence, severity, and duration, as well as any treatment prescribed. Following the subject's signing of the informed consent form, all adverse events that were not present at enrollment will be recorded. Any medical condition present at the signing of the informed consent form, which remains unchanged or improves, will not be recorded as an adverse event. However, worsening of a medical condition that was present at the time of the informed consent form signing will be considered a new adverse event and reported. Abnormal laboratory values, if felt by the investigator to be clinically significant, will also be recorded on the AE Form and assessed in terms of severity and relationship to study drug. Laboratory values that are abnormal prior the signing of the informed consent form and that do not worsen will not be recorded on the AE Form. AEs will not be collected after a subject has been permanently discontinued from the study drug for 30 days.

C.5.4 Serious Adverse Events (SAEs)

The term "Serious Adverse Event" is defined to serve as a guide for regulatory reporting requirements and should not be confused with the severity (intensity) of an event. An AE is considered serious for this trial if it meets one or more of the following criteria:

- Fatal
- Life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/ birth defect
- Results in permanent impairment/damage of a body function/structure
- Requires intervention to prevent permanent impairment of a body function/structure

Clinic sites must report all SAEs to the CTCC within 48 hours of learning of the event. SAEs are automatically reported to the CTCC when the sites complete the SAE CRFs in ADEPT. The subject must be monitored carefully until the condition disappears and/or the etiology is defined. SAEs will not be collected after a subject has been permanently discontinued from study drug for 30 days.

C.5.5 Unanticipated Adverse Drug Effects (UADEs)

An Unanticipated Adverse Drug Effect (UADE) is any serious adverse effect on health or safety, or any life-threatening problem or death caused by or associated with the study drug, if that effect, problem, or death was:

- Not previously identified in nature, severity, or degree of incidence in the protocol, informed consent template, investigator brochure, or package insert (including any revisions to these materials)
- Any other unanticipated serious problem that relates to the rights, safety, or welfare of subjects.

We anticipate UADEs to be rare events as this study drug is well-documented.

C.5.6 Reporting Procedures

All study outcome events should be reported to the TOPCAT CTCC within 48 hours. All SAEs and UADEs will be considered time-sensitive events reportable to the TOPCAT CTCC within 48 hours of learning of the event to meet regulatory (e.g. FDA) reporting guidelines as specified by regulations. A summary of all other adverse events will be reported to regulatory agencies (e.g. FDA) at the time of the annual report and semi-annually to the DSMB.

Sponsor reporting of UADEs and other safety information requiring reporting to regulatory authorities and ethics committees in other participating countries will occur according to the local requirements of that country.

The sponsor will also inform all investigators concerned of relevant information about UADEs that could adversely affect the safety of study subjects.

C.6 Statistical Methods

C.6.1 Sample Size and Power

The primary composite endpoint of CV mortality, aborted cardiac arrest, or hospitalization for the management of heart failure will be analyzed as the time to first occurrence of any such event, utilizing all follow-up data (censored at trial end) and a two-sided log rank test (.05 Type I error). At least 80% power is desired to detect a 20% relative decrease in the 3-year event rate.

The power calculations assume 3515 subjects with an average of 3.45 years follow-up (minimum 2 years and maximum of 6 years). After a 24-month ramp-up period, enrollment rates are assumed to average 88 subjects per month, with 2 additional years of follow-up.

The CHARM-Preserved trial data suggested that the 3.0 year rate of CV deaths combined with heart failure hospitalization would be approximately 24% in the TOPCAT placebo group. Few patients are expected to have aborted cardiac arrest as their first event for the composite endpoint, so placebo event rates in the TOPCAT study were initially expected to be very similar to those in CHARM. A range of 3-year event rates were initially considered for the TOPCAT placebo group, ranging from 17.41% to 30.87%.

The I-PRESERVE study results were published in December 2008 (Massie et. Al, 2008). Mean follow-up in I-PRESERVE was 49.5 months. The primary outcome for I-PRESERVE was a composite of all-cause mortality and cardiovascular hospitalization, which is somewhat broader than the TOPCAT primary outcome because it includes all mortality, not just cardiovascular mortality, and it includes all cardiovascular hospitalizations, not just heart-failure-related hospitalizations. Of the 2061 subjects in the I-PRESERVE placebo group, 763 experienced the I-PRESERVE primary outcome, which corresponds to a 3-year event rate of approximately 28.6%. One of the secondary outcomes for I-PRESERVE was a composite of mortality due to heart failure and hospitalization due to heart failure. This is somewhat narrower than the TOPCAT primary outcome, because it only includes heart-failure related mortality rather than all cardiovascular mortality. In the I-PRESERVE placebo group, 438 of 2061 subjects experienced this secondary outcome. This corresponds to a 3-year event rate of approximately 15.8%. The eligibility criteria for TOPCAT are expected to produce a study population with somewhat higher event rates than I-PRESERVE, which did not require that all subjects have either a recent heart-failure hospitalization or elevated BNP or pro-BNP.

Therefore, the 3-year event rate in the TOPCAT placebo group is expected to be at least 17.41%.

The 3-year loss-to-follow-up rate is expected to be between 15% and 20%.

Table 6 shows the statistical power available to detect a 20% relative decrease in the 3-year event rate, for a range of placebo event rates, assuming 3515 subjects with an average of 3.45 years follow-up. The power was calculated using Shih's macro (Shih, 1995), after taking into account a sample size inflation of 3% to account for interim monitoring.

Table 6. Achievable statistical power for N=3515, assuming equal number of subjects in each treatment arm, Type I error = .05, two-sided test, 2.0 additional years of follow-up, 15.00% to 26.34% event rate in the placebo group over 3.0 years follow-up, 15% to 20% loss rate over 3.0 years follow-up, and 3% sample size inflation for interim monitoring.

Event rates						Power	
At 3 years follow-up			At 3.45 years follow-up			15% loss rate	20% loss rate
Placebo	Treatment	Relative Reduction	Placebo	Treatment	Relative Reduction		
15.00%	12.00%	20.0%	17.05%	13.67%	19.8%	73.8%	72.4%
16.00%	12.80%	20.0%	18.17%	14.57%	19.8%	76.9%	75.5%
17.41%	13.93%	20.0%	19.75%	15.85%	19.8%	80.9%	79.5%
19.63%	15.70%	20.0%	22.22%	17.83%	19.8%	86.0%	84.9%
21.85%	17.48%	20.0%	24.69%	19.82%	19.7%	90.1%	89.1%
24.09%	19.27%	20.0%	27.16%	21.82%	19.7%	93.2%	92.4%
26.34%	21.07%	20.0%	29.64%	23.82%	19.6%	95.5%	94.9%

Because quality of life is a continuous measure, there will be high power to detect moderate to small differences in the change scores of the two treatment groups using a sample size of 3515.

C.6.2 Primary Endpoint Analysis Plan

C.6.2.a Primary Analysis of the Primary Endpoint

The primary analysis of all study endpoints will be conducted according to intention-to-treat (with no covariate adjustment). The primary endpoint, a composite of CV mortality, aborted cardiac arrest, or hospitalization for the management of heart failure, at the end of the 6 year subject accrual and follow-up period, will be compared by trial arm (spironolactone vs. placebo) using a logrank test of time to first event from the time of randomization. For this composite endpoint the time to event will be the time at which the first observed event component of the composite endpoint is observed. This method will utilize all available follow-up (ranging from 2 to 6 years for subjects who complete the trial) to provide the most powerful treatment comparison.

For all time-to-event analyses, subjects will be censored at the time of their last contact, unless they undergo a heart transplant. If a patient undergoes a heart transplant, their time-to-event measurement for any trial outcome will be censored at the date of heart transplant or last contact, whichever occurs earlier. Every effort will be made to obtain vital status on all trial subjects whose last contact was earlier than planned (dropouts), initially through telephone tracking by site staff, and at the end of the trial using National Death Index and/or Social Security Death Index search (for U.S. subjects).

C.6.2.b Secondary Analysis of the Primary Endpoint

Secondary analyses of the primary study endpoint will be of three types:

1) Comparison of spironolactone vs. placebo will be made as a function of treatment compliance (randomized treatment taken at correct current dose on at least 80% of study days vs. less than 80% of study days). This method attempts to better estimate the magnitude of the true treatment effect although parameter estimates are at risk due to subject selection bias created by evaluation of treatment outside of the original randomization structure.

2) Cox proportional hazards regression (Cox, 1972) will be used to most efficiently estimate the treatment effect after adjustment for important covariates that are known to impact the outcome of patients with PSF heart failure (Pocock, 2002). For this analysis, age, diabetes at baseline (insulin-treated vs. non-insulin-treated vs. no diabetes), and hospitalization for the management of heart failure in the 6 months prior to enrollment will be used for covariate adjustment, based on risk factor analyses of CHARM-Preserved trial data.
3) A descriptive dose response analysis, using currently prescribed mg/kg as a time-varying covariate in a Cox proportional hazards model, will be performed for subjects randomized to the active treatment. (Subjects randomized to the active treatment but currently taken off study drug will be assigned a current dose of 0 mg/kg.) The dose per kilogram may be confounded with how well a patient's CHF responds to the drug, and also confounded with how a patient's safety markers respond to the drug. Therefore, descriptive analyses of safety markers by currently prescribed mg/kg will also be performed.

C.6.2.c Interim Analyses

A group sequential analysis plan is proposed, with four looks at the data including the final analysis. However, the DSMB may decide to change the number or timing of interim looks. Conditional power will be calculated at each look. Asymmetric stopping boundaries are proposed in Table 7, using an alpha-spending approach (DeMets et al., 1994). These boundaries are designed to accommodate a possible change in the number of looks that the DSMB chooses to have, and to accommodate any reasonable spacing of looks. The proposed boundaries will facilitate early stopping of the trial if there are safety concerns, i.e. if the event rate is much higher in the spironolactone treatment arm than in the placebo treatment arm. Early halting for efficacy, if the event rate is much higher in the placebo arm than the spironolactone arm, may also occur. However, stronger statistical evidence will be required to halt early for efficacy than for safety.. Note that if the study continues to its planned sample size, a more extreme p-value will be needed to declare spironolactone to be worse than placebo, compared to the p-value needed to declare spironolactone to be better than placebo. This is because more of the "safety alpha" than the "efficacy alpha" will have been spent during the interim looks.

Table 7 Proposed interim monitoring boundaries for safety and efficacy.

	P-value boundaries for early stopping (two-sided p-values based on log-rank test)	
Look	For safety (observed spironolactone event rate higher than observed placebo event rate)	For efficacy (observed placebo event rate higher than observed spironolactone event rate)
Any interim look with ≤ half the expected events observed	.001	.0001
Any interim look with > half the expected events observed	.01	.001
Final look	2-sided p-value such that the overall Type I error is 5%, evenly split between declaring placebo better and declaring spironolactone better, when the true difference is 0	2-sided p-value such that the overall Type I error is 5%, evenly split between declaring placebo better and declaring spironolactone better, when the true difference is 0

The stopping boundaries for analysis of the primary endpoint, in conjunction with secondary endpoint comparisons and evaluation of safety (adverse event rates, including abnormal laboratory findings, all-cause mortality, and hospitalization for any reason) will all be considered by the DSMB to determine whether to recommend stopping the trial early. The TOPCAT trial will actively recruit subjects for 4 years. Maximum length of time on study will be 6 years, minimum 2 years.

C.6.2.d Subgroup Analyses

In order to identify the subject subgroups for whom spironolactone may be most or least beneficial, several pre-specified subgroup analyses will be conducted based on the subject's status at the time of randomization, namely:

- Randomization stratum: Hospitalized for heart failure in the year prior to study enrollment, vs. not hospitalized for heart failure during that time period
- Ejection fraction based on local reading, above vs. below the median
- Age 50-64 vs. 65-74 vs. $\geq$ 75 years
- Male vs. female
- Racial category: Black vs. White vs. All Others
- Ethnicity: Hispanic vs. Non-Hispanic
- History of hypertension vs. no history of hypertension
- Diabetes mellitus (insulin-treated) vs. diabetes mellitus (non-insulin-treated) vs. no diabetes mellitus
- New York Heart Association congestive heart failure class II vs. (III or IV)
- Systolic blood pressure below vs. above median
- Systolic blood pressure < 140 mm Hg vs. systolic blood pressure $\geq$ 140 mm Hg (entry into trial with controlled vs. uncontrolled blood pressure)
- Use vs. no use of cardiac medications, specifically beta-blockers, ACE inhibitors, aspirin, angiotensin receptor blockers, lipid-lowering agents, and diuretics
- Use vs. no use of blood pressure lowering medication
- Pulse pressure above and below median
- Estimated GFR above and below median
- BMI above and below median
- Analysis by region: Americas and E. Europe
- Prior MI vs. no prior MI

Covariate by treatment group interaction tests will be performed to test whether the treatment effect is homogenous across subgroups. Statistical testing within subgroups will not be conducted unless the interaction test p-value is < 0.05.

C.6.3 Secondary Endpoints Analysis Plan

Secondary endpoints further characterizing the morbidity and disease-specific mortality of this patient population will also be analyzed using time-to-event methods as described in Section C.6.2.a for the primary trial endpoint. These secondary endpoints include: all-cause mortality, CV mortality and CV hospitalization composite, CV hospitalization, all components of composite endpoints, hospitalization for any reason, new onset of diabetes mellitus, development of atrial fibrillation, deterioration of renal function (twofold increase in baseline serum creatinine to a value above the upper limit of normal), myocardial infarction, stroke, sudden death and/or aborted cardiac arrest. To account for multiple hospitalizations per subject, an incidence rate for hospitalization for heart failure in the two groups will be compared using a two-sample test based on the binomial distribution.

An interim monitoring plan for all-cause mortality is proposed, using the same approach and p-value boundaries as described in Section C.6.2.c for interim monitoring of the primary endpoint.

Laboratory indices of renal and metabolic function to assess drug safety will be analyzed using longitudinal linear regression methods, with normalizing transformations as appropriate.

Two general approaches to the analysis of quality of life and health status data will be taken. Analyses examining the influence of treatment on quality of life outcomes at specific follow up time points will be carried out through the use of analysis of covariance, adjusting for baseline status and other covariates. In order to utilize all available data describing the trajectory of subjects' functioning during the follow-up period, statistical models developed specifically for the analysis of longitudinal repeated measures data will also be used in secondary analyses to analyze the repeated quality of life measurements.

In addition to the general linear model described above, a generalized estimating equation model for ordinal multinomial data will be used to analyze repeated NYHA functional status measurements.

A challenge in the analysis of quality of life data relates to the unavoidable problem of missing data (due to death, incapacity, subject refusal, or loss to follow up). The proposed analytic strategy assumes that measurements are missing at random (Rubin, 1976); however, it is possible that subjects with impaired quality of life may be less likely to complete the interviews. We will examine the sensitivity of our results to a variety of alternative assumptions regarding the relationship between quality of life and the likelihood of completing the instruments. Potential approaches will include imputing missing values with the natural "worst case" score for each of the quality of life endpoints and application of multiple imputation techniques (Schafer, 1997).

The Kansas City Cardiomyopathy Questionnaire (KCCQ) will be the primary measure of quality of life (QOL). However, each QOL measure captures somewhat different aspects of QOL. Each QOL measure will be analyzed in a similar fashion. Qualitative agreement or disagreement in the direction of spironolactone's effect on each QOL measure will be described.

C.6.4 Site and Cohort Differences

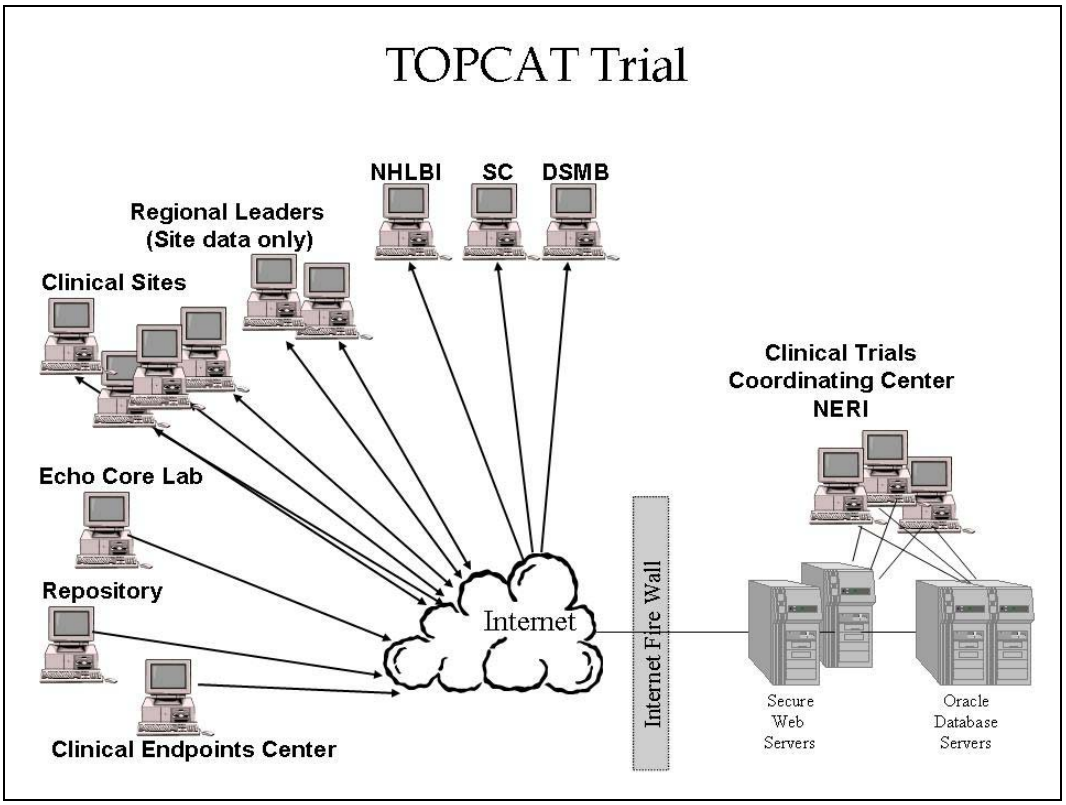
During the ongoing trial, analyses will be conducted on a periodic basis to assess geographic and site differences in protocol violation rates, enrollment rates, subject characteristics and adverse event rates. Differences identified may lead to a site visit to review subject data. The characteristics of subjects who are screened for but do not participate in the trial will also be compared with enrolled subjects. This analysis will allow assessment of the generalizability of trial findings and whether the enrolled subject cohort is representative of the entire patient population.

C.7 Data Management

C.7.1 Information Flow

Data will be sent to and received from several sources, including the clinical sites, the repository, the CEC, and the Echocardiography Core Laboratory. The flow of data among the units in this trial is illustrated in Figure 3. Clinical sites will enter data over the Internet using the Advanced Data Entry and Protocol Tracking (ADEPT) software, a customized and secure Web application. Sites will send blood and urine specimens directly to the repository for central processing, and records of receipt of such samples and final volumes stored will be electronically transmitted to the CTCC and stored in the ADEPT Data Management System (DMS). Echocardiograms stored on videotape or CD-ROM will be submitted to the Echocardiography Core Laboratory by FedEx. Results of interpretations/analyses performed by the Echocardiography Core Laboratory will be entered electronically using the ADEPT DMS.

Figure 3. Information Flow



C.7.2 Overview of Data Management System

ADEPT uses a "browser-based" user interface together with an Oracle relational database engine which allows direct data entry from multiple study sites or at the CTCC, and then stores these data centrally at the CTCC. Information entered into the data entry system will be by study I.D. number; names will not be linked with subject data in the database. Clinical sites will maintain records linking the patient name with the I.D. assigned for the study in locked files. Sites will have full access to their own data and be able to view this data remotely, over the Internet.

All study data will be stored on NERI's Oracle server. Access to data on this server (from both inside and outside the data center) is controlled by Oracle's extensive security features. Oracle archiving and backup system ensures minimal data loss.

C.7.3 Protocol Management and Reporting

In addition to providing robust data entry capabilities, ADEPT includes numerous features to streamline field operations and facilitate protocol adherence. Specifically, information regarding the study protocol and relative order of study events (e.g., medical exams, questionnaires) are programmed into ADEPT. Web-based, real time reports in both graphical and tabular format are available to the funding agency, Executive Committee, DSMB, and site management staff to track participant accrual and data quality. Standard ADEPT reports include:

- Upcoming appointments;
- Study Instruments pending entry;
- Study Instruments pending edit resolution;
- Missing data rates;
- Time (minimum, maximum, and average) to data enter each study CRF;
- Audit logs for all edits to study data;
- Subjects with overdue visits;
- Protocol violations

In addition to these standard reports, custom reports can be readily developed within the ADEPT system. The CTCC will provide sites, laboratories and the sponsor on-line access to a variety of reports designed to summarize recruitment, retention and compliance with the study protocol.

C.8 Quality Assurance

C.8.1 Site Certification

C.8.1.a Regulatory Documentation

The investigator(s) who are responsible for the conduct of this study, in compliance with this protocol, are identified on the FDA Form 1572 Statement of Investigator. The following regulatory documentation will be collected from each site prior to study initiation:

- IRB or EC approval of the protocol and informed consent form
- FDA Form 1572 Statement of Investigator ensuring compliance with 21 CFR 312 Investigational New Drug Application (or country equivalent)
- Curriculum vitae and current medical licenses from all investigators (PI and Sub-investigators)
- IRB/EC membership list and Federal Wide Assurance (FWA) certification ensuring compliance with 21 CFR 50 Protection of Human Subjects and 21 CFR 56 Institutional Review Boards
- Laboratory certification(s) as appropriate, and list of normal ranges
- Financial Disclosure and Conflict of Interest forms for all investigators (PI and Sub-investigators)
- Protocol Signature Page

C.8.1.b Site Contracts

Two contracts are required per site. One is legally binding and includes references to any insurance policy. This is signed by a Clinical Center Administrator or by the Regional Leader. The second is the Investigator contract, signed by all Clinical Investigators. This contract obligates the Investigator to follow trial protocol and protocol related documents, adhere to GCPs, properly store and control study drug, accommodate and assist with site monitoring

visits, complete any required reporting and make the best effort to recruit a minimum number of subjects at the site. All contracts will be translated as required.

C.8.1.c Training
Training will be completed on-line via a website established by the CTCC, or via a CD-ROM from the CTCC. Each training module will be followed by exercises to be completed by each individual to be certified for that module.

C.8.2 Site Monitoring
All sites will be visited at least once during the trial by representatives from the CTCC, Regional leader teams, and/or the sponsor. For monitoring purposes, "All sites" refers to all sites that enroll three or more subjects in the trial. Sites not meeting this criterion may not have an in-person visit; however a for-cause visit may be warranted. Additional visits will generally be reserved for sites with problems (audits for cause). The monitoring visit consists of reviewing and evaluating three separate components: conformance to IRB/EC and consent form requirements, compliance with trial protocol, and source document data verification. Any site found to be Unacceptable or Acceptable/Needs Follow-up on any monitoring visit is required to submit a written response and/or corrective action plan to the CTCC within 21 days of the receipt of the final monitor findings. Sites that fail to meet the standards for acceptable performance will undergo follow-up action, which will be determined by the severity of the discrepancies and may include repeat on-site monitoring, probation, or suspension. Procedures for the termination/closure of a clinical site are provided in the Manual of Procedures (MOP).

C.9 Close Out Procedures

C.9.1 Site Close Out Procedures
The CTCC will be responsible for notifying the regulatory authorities and ethics committees in the participating countries that the clinical trial has ended according to the laws and regulations of those countries. The trial may terminate at the planned target of 6 years after recruitment begins or at an earlier date if circumstances warrant. Details regarding the study closeout period will be provided in the Manual of Procedures (MOP). The objectives of the closeout phase are to:

1) Resolve all missing and inconsistent data to the extent possible
2) Evaluate the data as fully as possible to permit assessment of the effect of spironolactone on the primary endpoint.
3) Fulfill ethical obligations to trial participants.
4) Exploit the scientific value of study data as fully as possible.

C.9.2 Study Related Closeout Procedures
Closeout procedures will be developed by the Steering Committee and disseminated by the CTCC. Regardless of the timing and circumstances of the end of the study, closeout will proceed in two stages: An interim period for analysis and documentation of study results, and a final reporting of the main study results:

1) Interim - About 3-4 months will be needed to complete data collection and to prepare a manuscript for submission to an appropriate journal, reporting on the trial's main results.
2) Reporting of study results - The study results will be released to participating physicians, referring physicians, subjects, and the general community.

D. STUDY ORGANIZATION & POLICIES

D.1 Organization

The trial is sponsored by the National Heart, Lung, and Blood Institute (NHLBI). The NHLBI is responsible for the overall direction of the trial. Day-to-day management of the study will be the responsibility of the NHLBI Project Office, the CTCC, and the Executive Committee. The Executive Committee (EC) consists of the Steering Committee Chair, the NHLBI, and the CTCC Principal Investigators. In addition to day-to-day management of the trial, their role is to make recommendations to the Steering Committee regarding study conduct. The Steering Committee (SC) has as its voting members the SC Chair, the NHLBI project officer, the CTCC PI, and other investigators appointed by NHLBI. The SC oversees all aspects of the study, including monitoring trial progress and review of trial results. The SC may also establish subcommittees to facilitate the conduct of the trial. The SC will meet at least twice a year.

The Clinical Trial Coordinating Center has responsibility for contracting clinical centers for the trial, developing the Manual of Procedures (MOP), data collection forms, and all related systems. The CTCC is responsible for all reports needed for Committee meetings, and for interim and final statistical analyses.

The Data and Safety Monitoring Board (DSMB) is composed of independent experts in cardiology, biostatistics, and ethics who are appointed by the Director of the NHLBI to monitor the conduct of the trial including enrollment, safety, and efficacy outcomes. The DSMB will meet regularly, at least twice a year. Between these meetings, the DSMB chair will be notified of any events considered probably or definitely related to study drug. At the time of notification, he/she will determine if an additional DSMB meeting is required.

The Drug Distribution Center is based in the U.S. and provides tablets of spironolactone and placebo. They are responsible for the packaging and distribution of study drug in collaboration with the CTCC.

The Regional Leaders for the trial are based in Boston, Montreal, Russia, Republic of Georgia, Argentina, and Brazil. The leaders will coordinate approximately 100 trial sites in the US, 50 sites in Canada, 60 sites in Russia and Republic of Georgia, and 60 sites in South America.

Each Leader organization will be responsible within its Region for:

- Identification of country leaders (HF specialists) as required;
- Site recruitment and support of site certification (the CTCC will provide the materials and database access);
- Support and triage of site queries – especially clinical;
- Disbursement of site payments (funds and instructions provided by the CTCC);
- Site monitoring as requested by the CTCC;
- Region C: All data entry and editing.

D.2. Conflict of Interest Policy

A Financial Conflict of Interest form will be filled out by each investigator at least annually, and also at any time that a new significant financial conflict of interest is identified.

The Investigators include the Executive Committee Members, the Steering Committee Members, the Principal Investigator at each site, and any other person who is responsible for the design, conduct, or reporting of TOPCAT research, including sub-grantees, contractors, or collaborators. For purposes relating to conflict of interest, the definition of Investigator also includes the Investigator's spouse and dependent children.

D.3 Publications Policy

The Steering Committee will review all publications following the guidelines given below.

D.3.1 Data Analysis and Release of Results

The scientific integrity of the project requires that data from all of the sites be analyzed study-wide and reported as such. An individual center is expected not to separately report its data. The development of reports of data from individual sites for the determination of institutional variability is the prerogative of the Steering Committee. Additionally, all presentations and publications are expected to protect the integrity of the major study objectives. With the exception of interim analyses for the DSMB, endpoint data will not be presented prior to the release of the main study results. Recommendations as to the timing of presentation of endpoint data and the meetings at which they are presented will be provided by the Steering Committee.

D.3.2 Review Process

Each manuscript or abstract must be submitted to the Steering Committee for review of its scientific merit and appropriateness for submission. The Steering Committee may recommend changes to the authors and will make a final decision about submission. Each manuscript or abstract should also be sent to the NHLBI for review prior to submission.

D.3.3 Primary Outcome Papers, Abstracts and Presentations

The primary outcome papers are defined as those that present outcome data for the entire trial cohort. The determination of whether or not a particular analysis represents a primary outcome report will be made by the Steering Committee. Authorship on the baseline and primary outcome papers will be "The TOPCAT TRIAL Investigators." For such manuscripts, there will be an appendix containing the names of all participating site investigators and their organizational affiliation. Papers and abstracts that are not primary outcome papers will have named authors based upon involvement and ending with the phrase "for the TOPCAT TRIAL Investigators." The same appendix will be appended to non-primary outcome manuscripts as for primary outcome papers. All manuscripts for submission must be approved by the Steering Committee.

D.4 Substudies

D.4.1 Introduction

Two types of substudies will be considered: ancillary studies and databank studies. Ancillary studies are those that require data collection beyond the primary protocol and/or propose using specimens in the trial repository, while Databank studies are based solely upon data collected as part of the main study. Participation in the substudies is open to all study investigators. In order to assure that all substudies are of high scientific merit, the DSMB will review applications for ancillary studies and make recommendations regarding merit to the Steering Committee. Databank studies will be considered directly by the Steering Committee or a designated subcommittee.

D.4.2 Ancillary Studies

An ancillary study uses trial participants in an investigation that is not described in the trial protocol and involves collecting new data that are not part of the trial data set or that use repository samples. Such studies must be carried out by applicant investigators or in conjunction with trial investigators. In general, any such study will require an independent consent form, IRB/EC approval, and an independent funding source. Ancillary studies must be

approved by the Steering Committee and any external review committees. All applications for ancillary studies must be submitted in writing to the Steering Committee. The scientific merit of the application, and any possible impact of the sub-study on the parent TOPCAT study, will be reviewed and assurance provided that the timing of the resulting publication(s) will not interfere with the main publications of the study.

D.4.3 Databank Studies

A databank study utilizes data that have been collected as part of the main trial in order to answer a question different from that posed by the main protocol. It usually involves only data analysis and generally does not require supplemental funding because it uses the resources of the CTCC. Such studies require the approval of the Steering Committee, are based on scientific merit of the application, assurance that reporting of the databank study will not interfere with the main publications of the study, and availability of CTCC resources.

D.4.4 Application Review Process

The Steering Committee (or designated subcommittee) will review applications for substudies in a timely fashion. If several applications for similar substudies are received, collaboration and joint resubmission will be encouraged. Applications from non-trial investigators will be entertained but will be assigned lower priority than similar applications from trial investigators.

D.4.5 Other Competing Studies

Simultaneous participation by trial subjects in other prospective investigations requires the prior approval of the Steering Committee and is generally to be discouraged. It is recognized that the exigencies of patient care may require that the subject be entered into a compassionate use protocol. If this occurs, the CTCC should be notified within 10 days.

D.4.6 Data Storage and Analysis

Data collection forms for ancillary studies will be stored at the sites and the final dataset will be copied to the CTCC for merging into the primary dataset.

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TOPCAT Echo and Vascular Stiffness Substudy Protocol

Central Aortic Stiffening and Diastolic Function in Patients with Heart Failure and Preserved Ejection Fraction: The Impact of Aldosterone Receptor Antagonism

PROTOCOL SIGNATURE PAGE

I have read the following ancillary study protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the study drug and the conduct of the study.

I will use the informed consent form approved by the National Heart, Lung and Blood Institute and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol.

I further agree that the National Heart, Lung and Blood Institute, the appropriate regulatory authorities and staff from the regional coordinating centers have access to any source documents from which case report form information may have been generated.

I also agree to handle all clinical supplies (including study drug) provided by the National Heart, Lung and Blood Institute and collect and handle all clinical specimens in accordance with the protocol.

The below signed confirm herewith to have read and understood this trial protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance with the protocol and Good Clinical Practice guidelines, as well as local regulations; and to accept respective revisions conducted by authorized personnel of National Heart, Lung and Blood Institute and by competent authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Principal Investigator(s)		
Principal Investigator(s)		

TOPCAT Ancillary Study Proposal

Heart failure with preserved ejection fraction (HF-PEF) is a diverse clinical syndrome characterized by signs and symptoms of heart failure despite apparently preserved systolic function, and accounts for nearly half of heart failure cases. Patients with HF-PEF demonstrate a pattern of functional decline similar to patients with heart failure and reduced ejection fraction, with increased hospitalizations, reduced quality of life and increased risk of death compared with an age-matched population without HF. Diastolic dysfunction, secondary to myocardial fibrosis and hypertrophy, has been presumed to be the primary pathophysiologic basis of HF-PEF. Other mechanisms, including abnormalities of renal function, have been implicated as well, and the true contribution of diastolic dysfunction to the pathogenesis of HF-PEF may be variable. *A better understanding of the pathophysiologic mechanisms in this disorder is critical to targeting more effective treatment.*

Current management of patients with HF-PEF is empiric, focused primarily on relief of congestive symptoms and aggressive management of comorbidities; no specific therapies are clearly associated with durable improvements in diastolic function or prognosis. Aldosterone receptor antagonists reduce mortality and morbidity in patients with heart failure and left ventricular dysfunction. The well-documented antifibrotic effects of aldosterone antagonists, combined with the general salutary effects of RAAS antagonism in patients with heart failure, suggest that patients with HF-PEF may derive similar clinical benefit.

The Treatment Of Preserved systolic function Cardiac failure with an Aldosterone anTagonist (TOPCAT) trial is a 3515-patient, placebo-controlled, randomized clinical trial funded by the National Heart Lung and Blood Institute (NHLBI) that will test the hypothesis that treatment with the aldosterone antagonist spironolactone will reduce the risk of cardiovascular death, heart failure hospitalization, or aborted cardiac arrest in patients with HF-PEF. We plan to conduct an ancillary study of the TOPCAT trial with the ***overall goal of characterizing the spectrum of myocardial and vascular stiffness abnormalities in patients with HF-PEF and examining the impact of treatment with an aldosterone antagonist on these abnormalities.***

Background: Diastolic function in HF-PEF

Progress in the understanding and treatment of HF-PEF has been thwarted in part by a lack of consensus regarding the optimal clinical definition. Operationally, the syndrome is defined by the presence of heart failure signs and symptoms in the absence of a prominent abnormality of systolic function. Since these criteria are rather nonspecific, the diagnosis of HF-PEF is applied to a broad range of patients with variable pathophysiology ranging from primary myocardial disease to progressive renal failure. **Characterizing the pathophysiologic abnormalities present in a broad range of patients with HF-PEF is a key preliminary step towards understanding mechanisms and targeting effective therapies.**

The pathophysiologic mechanisms responsible for the development of heart failure in patients with preserved systolic function remain poorly understood, in part because of the heterogenous nature of this disorder. Diastolic dysfunction, implying abnormalities of active myocardial relaxation or passive ventricular compliance, has been described as the *sine qua non* of HF-PEF, leading many to characterize the syndrome as 'diastolic' heart failure. The prevailing model emphasizes primary myocardial abnormalities that impair ventricular performance during diastole, shifting the ventricular pressure-volume relationship upward and to the left, thereby enhancing susceptibility to pulmonary venous hypertension with small changes in circulating blood volume or ventricular afterload. [1,2] Indeed, abnormalities of both passive myocardial stiffness and active myocardial relaxation have been demonstrated in selected patients with HF-PEF, suggesting that aberrant diastolic function plays an important role in heart failure pathogenesis[1].

A variety of cellular mechanisms likely contribute to worsening of diastolic function. Enhanced synthesis of fibrillar (Type I) collagen in the myocardium, a consequence of hemodynamic loading, ischemia, and neurohormonal activation, may play an important role, since myocardial fibrosis is a major determinant of altered diastolic filling and compromised systolic pump function in those with longstanding hypertension.[3,4] Perturbation of cytosolic calcium transients (for example, as a consequence of myocardial ischemia) or the myocardial contractile apparatus (as in hypertrophic cardiomyopathy) may lead to transient or permanent abnormalities of ventricular relaxation.[5,6] Passive chamber compliance may be diminished as a consequence of myocyte hypertrophy, increases in left ventricular mass, and alterations in the interstitial collagen network.[7,8]

While abnormal ventricular relaxation can be documented in a wide range of patients, only a small fraction of those with apparent "diastolic dysfunction" develop exertional dyspnea or other clinical symptoms of congestive heart failure.[9,10,11] Patients with HF-PEF exhibit some of the same pathophysiologic characteristics as those with reduced systolic function heart failure, including elevations in neurohormones, such as norepinephrine and BNP.[12] The relationships between myocardial hypertrophy, fibrosis, neurohormonal activation, and ventricular performance are well documented in experimental models of systolic heart failure, but there is little evidence that the same relationships hold for HF-PEF. **Overall, the extent to which intrinsic abnormalities of myocardial diastolic function play a central role in the pathophysiology of HF-PEF in the broad spectrum of patients with this disorder is unknown. We anticipate that the specific knowledge of structural and functional abnormalities in patients with HF-PEF may help to guide future therapeutic approaches, as it has for patients with heart failure and LV dysfunction.**

The renin-angiotensin-aldosterone system is thought to play a central role in the pathogenesis of diastolic dysfunction. Angiotensin II, both via the stimulation of the type 1 receptor and the stimulation of endothelin, promotes collagen synthesis and cardiac fibrosis[13]. In experimental models of hypertension and compensated left ventricular hypertrophy, the blockade of angiotensin II type 1 receptor can prevent the development of diastolic heart failure[14].

In addition to its well recognized role in sodium and water retention, aldosterone is a major stimulus for myocardial fibrosis[15]. In animal studies, aldosterone administration to salt-fed rats leads to the development of cardiac inflammation, remodeling, and fibrosis[16], while blockade of the aldosterone receptor in experimental models reduces myocardial fibrosis[17]. In patients with essential hypertension, aldosterone levels correlate with LV mass[18] and the severity of LV dysfunction independent of blood pressure[19,20]. Increased expression of aldosterone synthase in the myocardium has been observed in patients with heart failure, in association with increased myocardial fibrosis and left ventricular hypertrophy[21]. The benefits of aldosterone antagonism in patients with heart failure and reduced LVEF have been firmly established in the RALES[22] trial and in post-MI patients in the EPHESUS[23] trial. **The stimulation of myocardial fibrosis by aldosterone may contribute to diastolic dysfunction, and improving diastolic function with an aldosterone antagonist is a rationale for improving outcomes in patients with diastolic dysfunction and HF-PEF.**

Background: Arterial Stiffness and HF-PEF

Arterial stiffness is an important risk factor for the development and progression of heart failure. The abnormal loading sequence created by increased arterial stiffness is associated with impaired ventricular relaxation during early diastole and marked prolongation of the time constant (τ) of left ventricular relaxation. Abnormalities of ventricular-vascular coupling as a consequence of changes in pulsatile load may play an important role in the

pathogenesis of heart failure in patients with preserved ejection fraction (HF-PEF). As a consequence, pharmacologic interventions that reduce the stiffness of the central arteries may be highly beneficial in the prevention and treatment of HF-PEF.

Both aldosterone receptors and an endogenous aldosterone synthetic pathway are present in conduit vessels and play a role in smooth muscle growth, matrix composition and endothelial function. In animal models, aldosterone excess is associated with fibrosis of large vessels and increased vascular stiffness that is reversed following treatment with aldosterone receptor antagonists. **Collectively, these observations suggest an important role for aldosterone as a modulator of arterial stiffness and support the hypothesis that selective aldosterone antagonism may indirectly improve ventricular function in HF-PEF through a favorable effect on arterial function.**

Detailed measurement of vascular stiffness is increasingly possible using noninvasive methods. Analysis of pulse waveforms generated from peripheral arterial applanation tonometry can be used to estimate central aortic pulse pressure, carotid-femoral pulse wave velocity (PWV), aortic augmentation index (AIx), and other key measures of pulsatile load. The Sphygmocor® system (Atcor Medical, Inc., Sydney, Australia) is an FDA-approved software package linked to a tonometer that permits a reliable derivation of central aortic waveforms from the radial arterial pressure wave, and also allows calculation of aortic pulse wave velocity using carotid and femoral waveforms gated to an electrocardiogram. In the recently completed Conduit Artery Function Evaluation (CAFÉ) substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), the Sphygmocor® device was utilized to identify a statistically significant reduction in central aortic systolic blood pressure and central aortic pulse pressure with an amlodipine/perindopril combination relative to a beta-blocker/diuretic combination among 2199 patients with hypertension randomized in the primary ASCOT trial.

Specific Aims

To test the hypothesis that long-term therapy with the aldosterone-receptor antagonist spironolactone will improve diastolic function relative to placebo in patients with HF-PEF. We will noninvasively assess myocardial structure and function using two-dimensional echocardiography with Doppler Tissue Imaging. Specifically, we will exploit the randomization and follow-up scheme of the primary trial to test the primary hypothesis that spironolactone therapy will enhance myocardial relaxation velocity (Tissue Doppler E') relative to placebo in patients with HF-PEF. The impact of spironolactone on other echocardiographic measures of myocardial structure and function (including left ventricular mass, left atrial volume, and systolic function) will also be assessed.

To test the hypothesis that the severity of diastolic dysfunction at baseline will correlate with other markers of disease severity, and independently predict cardiovascular outcomes in patients with HF-PEF. We will examine the relationship between baseline measures of diastolic function and the primary TOPCAT trial outcomes to characterize the spectrum of myocardial abnormalities present in this population and to examine:

a. Do baseline measures of diastolic dysfunction predict cardiovascular outcomes?
b. Can diminished myocardial relaxation velocity at baseline be used to identify subgroups likely to experience the greatest benefit from spironolactone therapy?
c. Do baseline measures of diastolic function correlate with other measures of disease severity, including functional status and quality of life?

To test the hypothesis that long-term therapy with the aldosterone-receptor antagonist spironolactone improves central aortic stiffness relative to placebo in patients with HF-PEF. We will noninvasively assess central pulsatile hemodynamics in a cohort of TOPCAT participants utilizing peripheral applanation tonometry.

We will utilize the randomization and follow-up scheme of the primary trial to test the hypothesis that long-term administration of spironolactone improves carotid-femoral pulse wave velocity (PWV) relative to placebo in patients with HF-PEF. The impact of spironolactone on other key measurements of pulsatile load, including augmentation index, central aortic systolic pressure, central aortic pulse pressure, and systolic ejection period, will also be assessed.

To test the hypothesis that abnormalities in central aortic stiffness predict cardiovascular mortality and heart failure hospitalization in patients with HF-PEF. We will examine variations in measured parameters of central aortic stiffness at baseline (carotid-femoral pulse wave velocity, augmentation index, central aortic systolic pressure, central aortic pulse pressure, and systolic ejection period) and assess their correlation with the primary outcome in the TOPCAT trial.

Methods

TOPCAT Trial

The TOPCAT trial is an international, multicenter, randomized, double-blind, placebo-controlled trial of the aldosterone antagonist, spironolactone, versus placebo in 3515 adults with heart failure and left ventricular ejection fraction of at least 45%, recruited from 200-300 clinical centers. Eligible patients include men and women at least 50 years of age with left ventricular ejection fraction ≥ 45% measured within 6 months prior to enrollment, prior hospitalization for heart failure or elevated BNP, and signs and symptoms of heart failure. Exclusion criteria include known restrictive or infiltrative cardiomyopathy; known constrictive pericarditis; known hypertrophic obstructive cardiomyopathy; concomitant severe medical illness limiting life expectancy to < 3 years; hemodynamically significant, uncorrected valvular heart disease; atrial fibrillation with resting heart rate > 90 beats/min; significant chronic obstructive pulmonary disease; recent coronary revascularization; recent stroke; recent use of an aldosterone receptor antagonist; heart transplant or ventricular assist device; abnormal hepatic function; advanced renal disease, with estimated GFR < 30 cc/min and/or serum creatinine ≥ 2.5 mg/dl; and serum potassium > 5.0 mmol/L. The trial duration is approximately 6 years, with approximately 4 years for subject enrollment and an additional 2 years of follow-up, for an average subject follow-up of 3.45 years. Study visits will occur every 4 months during the first year (more frequently in the first 4 months) and every 6 months thereafter. The primary endpoint is a composite of cardiovascular mortality, aborted cardiac arrest, and hospitalization for heart failure. Enrollment began in August 2006.

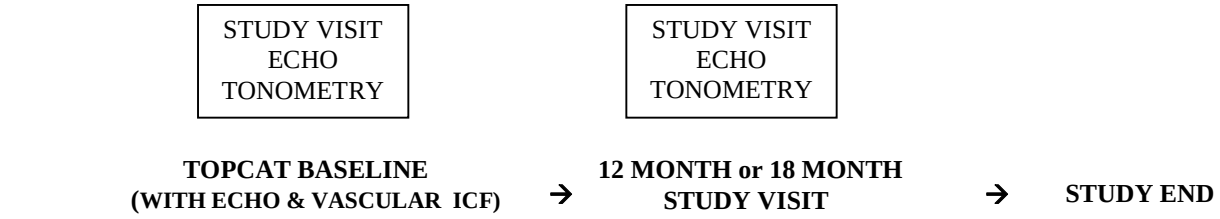
Ancillary Study Design

To achieve the specific aims outlined above, we propose to conduct a nested, mechanistic substudy within the architecture of the primary TOPCAT trial. We will utilize the TOPCAT trial organization to obtain additional non-invasive measurements at the baseline and 12 or 18 month visits in 250 subjects enrolled at selected trial sites. These 250 patients will undergo both Doppler echocardiography and tonometry. Patients already enrolled in TOPCAT, who have not yet reached their 18 month study visit, will also be offered the opportunity to participate in this substudy. In lieu of a formal baseline assessment with echocardiography and tonometry, these patients will be asked to consent for analysis of retrospective data from any available echocardiographic images completed within 60 days prior to their enrollment into TOPCAT. These patients will undergo the Doppler Echocardiography procedure, but will not undergo the tonometry procedure for the vascular stiffness substudy at the 12 or 18 month visit. Priority will be given to those sites experienced in the use of Doppler Echocardiography for diastolic function assessment and to those with prior experience of arterial tonometry and familiarity with the Sphygmocor® system. A detailed arterial tonometry and echocardiography protocol and manual of operations will be developed and distributed to the sites. Standardized on-site training regarding

measurement of stiffness parameters will be provided for all participating centers. Day-to-day technical support and regular surveillance of data quality will be provided by the imaging core laboratory at Brigham and Women's Hospital and by AtCor Medical, Inc.

To be eligible for the substudy, patients must be enrolled in the TOPCAT study at one of these participating centers, and willing to provide informed consent for the additional non-invasive measurements.

Patients who enroll in Echo and Vascular Stiffness Substudy during TOPCAT baseline screening:



Patients who enroll in Echo and Vascular Stiffness Substudy after TOPCAT baseline visit:

Study Visits

Echocardiography

Echocardiographic acquisition

Echocardiograms will be performed at the sites by qualified echocardiographic personnel (technicians or physicians) in accordance with standard echocardiographic clinical practice. The baseline echocardiogram will be performed before administration of spironolactone or placebo. To be enrolled after the baseline visit, the patient must have undergone a suitable echocardiogram within a 60-day time period prior to TOPCAT randomization that could be submitted for core lab review. The required echocardiographic examination will be a modification of the standard echocardiographic examination, and will include the majority of standard echocardiographic views normally obtained for clinical practice, in addition to specific echocardiographic assessments designed to assess diastolic function (Table 0.1).

Table 0.1. Echocardiographic Assessments Obtained at Sites

Echocardiographic View	Images Obtained
• Parasternal long axis view	• 2-D image for septal and posterior wall thickness • M-Mode Images • Colorflow Doppler
• Parasternal Short axis view, papillary muscle level • Apical 4-chamber View	• 2-D images for Septal and posterior wall thickness • 2-D images for volume and ejection fraction measures, LA size and RV function • Colorflow Doppler for assessment of mitral regurgitation • Doppler Tissue Imaging of Mitral annular velocities (primary endpoint)

- Mitral Inflow Pulsed Doppler

Recording of Echocardiographic Studies and Transmission of Echocardiographic Data to Core Laboratory

Echocardiograms will be recorded to videotape (VHS or S-VHS) or digital media in DICOM format (CD, or Magneto-Optical Disc). As possible, study site and patient ids will be recorded with each study – no patient identifiers will be used. For studies obtained retrospectively, every effort should be made to remove identified patient data prior to submission to the core laboratory. If this is not possible, studies will be de-identified on receipt by core laboratory personnel. Echocardiographic studies will be sent to the core laboratory via courier in one bulk shipment on the 15th of every month and logged in upon receipt at the core laboratory. Sites will include an echocardiographic tracking form for each study sent to the core laboratory. All echocardiographic measurements will be made at the Cardiac Imaging Core Laboratory (Brigham & Women's Hospital, Boston, MA: Director Scott Solomon).

Echocardiographic Data Analysis

Echocardiographic assessments will be made in the core laboratory by experienced research echocardiographers utilizing off-line PC-based echocardiographic analysis software. Echocardiograms received in analog (videotape) format will be digitized utilizing industrial quality analog-to-digital video capture equipment, and image loops will be stored on hard disk with weekly backups to DVD-R media.

Echocardiographic assessments are outlined in Table 0.2. All volume measurements will be made by manually tracing the endocardial border at end diastole and end systole. **Left ventricular and left atrial volumes** will be calculated using the modified Simpson's rule method[24]. **Right ventricular function** will be assessed utilizing fractional area change derived from right ventricular area measurements in the apical-4 chamber view[24]. **Left ventricular mass** will be derived from wall thickness measurements utilizing the modified Devereux method[25].

Table 0.2. Echocardiographic Assessments Performed at Core Laboratory

Measure	Method
End-diastolic volume (EDV)	Simpson's Rule, method of discs
End-systolic volume (ESV)	Simpson's Rule, method of discs
Ejection Fraction	Derived from volumes: EF = 100x(EDV-ESV)/ESV
LV wall thickness, septum and posterior wall	m-mode
Left ventricular mass	Derived from m-mode wall thickness measurements based on Modified Devereux method: left ventricular mass (g)= 1.04 × 0.8 [(left ventricular wall thicknesses + internal dimension) – (internal dimension)] + 0.6
Right ventricular fractional area change	Derived from RV diastolic area and systolic area in apical 4-chamber view.
Left atrial volume	Simpson's rule, method of discs
Mitral inflow E wave velocity	Measured directly at from mitral inflow pulsed wave Doppler at leaflet tips
Mitral inflow A wave velocity	Measured directly at from mitral inflow pulsed wave Doppler at leaflet tips
Mitral E/A Ratio	Derived from E and A wave velocities
Mitral E Deceleration time	Measured directly as the time from peak E-wave to the baseline (extrapolated)
Isovolumic contraction time (IVCT)	Measured directly as the time from the beginning of the QRS complex to the start of ejection time
Isovolumic Relaxation Time (IVRT)	Measured directly as the time from end of ejection time to the

Myocardial performance index	beginning of the E-wave Derived from IVCT, IVRT and Ejection Time: MPI = (IVRT+IVCT)/ET

The primary measure of diastolic function for the diastolic function substudy will be change in lateral mitral annular relaxation velocity (E') from baseline to one-year. Mitral annular velocities will be assessed using Tissue Doppler imaging (TDI) of the lateral and septal mitral annuli (See Figure 0.1). Mitral annular relaxation velocities of the lateral and septal mitral annulus will be assessed from the Doppler tissue spectral waveforms utilizing the mean amplitude of the Doppler tissue peak spectral waveform. Mitral inflow early and late diastolic filling velocities will be assessed, as will pulmonary venous Doppler waveforms, from the apical four chamber view. Additional measures of diastolic function will also be performed:

Isovolumic contraction time (IVCT) will be calculated as the time from the beginning of the foot of the QRS complex to start of ejection time;

Isovolumic relaxation time (IVRT) will be measured as time interval from the end of ejection time (measured from the pulsed wave LV outflow tract Doppler) to the beginning of mitral inflow (start of E-wave);

Myocardial performance index (MPI or Tei Index) will be calculated as (Isovolumic relaxation time + isovolumic contraction time) / ejection time;

Mitral deceleration time (DT) will be assessed as the time from the peak of the early mitral inflow wave (E-wave) to the baseline. Derived measures of diastolic function will include **the ratio of early mitral inflow velocity to annular velocity (E/E').** In addition, patients will be categorized as having normal, mild, moderate or severe impairment of diastolic function utilizing a qualitative assessment schema that takes into account a variety of diastolic parameters (Table 0.3, Modification of method proposed by Redfield et al[26]). All echocardiographic measurements will be made and averaged over 3 cardiac cycles.

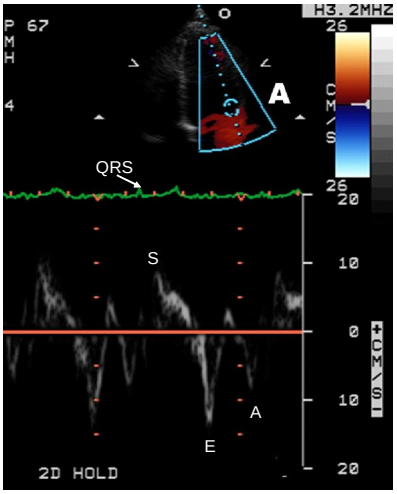


Figure 0.1. Tissue Doppler imaging (TDI) of the lateral mitral annular velocities

Table 0.3. Qualitative Evaluation of Diastolic Function Based on Multiple Parameters

	Normal	Mild	Moderate	Severe
Lateral Mitral Annular Velocity	E' > 12cm/s	E' < 12 cm/s	E' < 10 cm/s	E' < 8cm/s
Mitral inflow to mitral annular velocity ratio	E/E' < 10	E/E' < 10	E/E' ≥ 10	E/E' ≥ 10
Mitral Deceleration Time	DT > 140ms	DT > 140ms	DT > 140ms	DT < 140ms

Anticipated issues with echocardiographic assessment

It is anticipated, based on our prior experience, that not all sites will be able to adequately perform all echocardiographic assessments, particularly Doppler tissue imaging of the mitral annulus. To minimize the number of inadequate studies, we will provide educational material, including a Site Instruction Manual and Echo Pocket Guide. Additionally, the core laboratory will provide specific feedback to the sites to help with individual equipment or operator dependent problems that arise.

Tonometry

Assessment of conduit vessel function will be performed during the baseline screening and during the 12 or 18 months study visit using arterial applanation tonometry in 250 subjects (a subset of the 500 patients undergoing echocardiography) at 20-30 participating clinical centers. For these patients, simultaneous acquisition of ECHO and tonometry data will allow correlation between indices of conduit vessel stiffness with ventricular geometry and function. All subjects will be scheduled in the morning of the scheduled baseline screening visit and the 12 month or 18 month follow up echocardiographic visits. For subjects enrolled retrospectively in the substudy (after they have completed their baseline visit), no tonometry measures will be performed.

On the day of the study visit, all patients who enroll in the Echo and Vascular Substudy prior to randomization will undergo peripheral arterial tonometry using the Sphygmocor® system. All studies will be performed with patients in the supine position for at least 5 minutes, in a temperature-controlled environment, utilizing the right brachial, radial, femoral and carotid arteries. Sphygmomanometric blood pressure will be obtained in the right arm of the patient (using an automated device) in triplicate or until stable within 10 mm Hg for both systolic and diastolic determinations. Pressure waveforms will then be obtained noninvasively by performing arterial tonometry on the radial, femoral, and carotid arteries in rapid succession using a custom tonometer interfaced to the Sphygmocor® workstation, which will simultaneously acquire a single-lead electrocardiographic (ECG) tracing. Two measurements will be recorded at each site (5 minutes apart) to ensure reproducibility and stability. Specific criteria for optimal tonometric waveform morphology will be provided in the training manual and utilized for quality control. Radial waveforms will be utilized to generate central aortic waveforms using a validated transfer function contained within the proprietary software. An upright QRS from the ECG will be used to signal average the tonometry data and establish the delays from peak of QRS to arrival of the pressure waveform at the various pulse recording sites. Distances over the body surface from the suprasternal notch (SSN) to the recording sites at the femoral and carotid arteries will be measured and recorded to permit calculation of pulse-wave velocity. All data will be stored locally on a dedicated laptop computer by the site investigator and copied to the core laboratory at Brigham and Women's Hospital for central adjudication and quality assurance, along with the TOPCAT study patient identification number, date, height, weight, blood pressure, and external transit distances. Pulse waveform analysis and velocity data will be sent in two steps: (1) After the study is performed, the pulse waveform analysis and velocity reports should be faxed to the TOPCAT Cardiac Imaging Core Lab for initial image quality review (2) on the 15th of every month, a hard copy of the entire data file, burned to CD, should be sent in the monthly echo shipment. All TOPCAT study information is entered into the Sphygmocor software system and the basic demographic information (subject id, patient initials, study visit and date of birth) is conveyed on the TOPCAT Vascular Stiffness Tracking Form (which should be included with the shipment of the data file). Regular feedback will be provided to the sites on a patient-by-patient basis to ensure optimal data quality.

Statistical Considerations

Echocardiography

The overall sample size for the echocardiography substudy is based on the interventional study outlined in specific aim #1. We have estimated that 500 subjects will be required to test the primary hypothesis that spironolactone will improve diastolic function in patients with HF-PEF. The sample size is based on the primary endpoint of change in mitral annular relaxation velocity (E') from baseline to one-year. Assuming a two-sided Type I error rate of 0.05, a desired power of 0.90, a conservative estimate standard deviation of the measurement of 3.0 cm/s based on prior population-based assessments[27], a total sample size of 380 subjects is also required to detect a 1.0 cm/s difference in the one-year change scores for diastolic relaxation velocity in the two treatment groups. This calculation assumes that the correlation between baseline and one-year values is 0.5,

which results in a standard deviation of change of 3.0 cm/s. Loss to follow-up is estimated at 10% (based on the expected death rate in this population of approximately 6% per year[28] and expected drop-out for other reasons), therefore 422 patients are required (380/0.9). In addition, it is conservatively estimated that 15% of images may be of insufficient quality for quantitation, leading to an overall sample size of 496 patients (422/0.85). Therefore, the total target sample size for the substudy will be 500 patients enrolled at 50 sites.

The primary efficacy endpoint (and the endpoint used for determining the sample size necessary) for the substudy will be *change in lateral mitral annular relaxation velocity (E') from baseline to one year* (Table 0.4). Additional assessments of diastolic function, including changes in E/E', changes in mitral deceleration time, changes in myocardial performance index, and changes in a composite measure of diastolic function will be secondary endpoints.

For the primary analysis of this primary measure, we will utilize analysis of covariance to compare the placebo and spironolactone groups with respect to changes in the mean baseline and one-year mitral annular relaxation velocity (E'), adjusting for the baseline value of E'. This approach will be preferable to simple comparison of mean changes as it will take into account any chance imbalances in baseline E', and will also take into account regression to the mean[29]. A similar analysis approach will be applied to the secondary outcome measures of diastolic function (change in E/E'; change in mitral deceleration time (DT); change in myocardial performance index (MPI); change in composite measure of diastolic function; change in left ventricular mass; and change in left atrial size).

Table 0.4. Endpoints (Specific Aim #1)

Echocardiographic Endpoints
Primary Endpoint
Change in Lateral Mitral annular relaxation velocity (E'), baseline to 1 year
Secondary Echocardiographic Endpoints
Change in E/E', baseline to 1 year
Change in mitral (E-wave) deceleration time, baseline to one year
Change in myocardial performance index (MPI), baseline to one year
Change in composite measure of diastolic function (see below), baseline to one year
Change in left ventricular mass, baseline to one-year
Change in left atrial size, baseline to one-year

Arterial Tonometry

There is limited data available to guide power calculations for the vascular stiffness substudy, since no studies of vascular stiffness using the Sphygmocor® device are available in patients with diastolic heart failure. Data is available from several hypertension studies utilizing other commercial systems for measurement of pulse wave velocity. White, et al.[30] randomized 269 hypertensive patients to amlodipine or eplerenone for a period of 24 weeks and noted a statistically significant decrease in carotid-femoral pulse wave velocity from 15.5 +/- 2.0 m/s to 13.4 +/- 2.0 m/s in the eplerenone-treated patients (difference 2.1 m/s from baseline to 24 weeks, no standard deviation reported for the change). Mahmud, et al.[31] treated 24 patients with hypertension using spironolactone 50 mg and noted an average decrease of 1.54 +/- 0.2 m/s in carotid femoral pulse wave velocity at 4 weeks. In

the REASON study of amlodipine/perindopril versus atenolol in 471 patients with hypertension, carotid femoral pulse wave velocity was decreased by a mean of 0.9 +/- 2.0 m/s in both treatment arms at 12 months.[32]

Conservatively assuming zero correlation between baseline and follow-up measures, a standard deviation of 2.5 m/s for the change in carotid-femoral pulse wave velocity at 1 year, and a two-sided alpha of 0.05, we made the following sample size estimates (Table 0.5):

Table 0.5. Sample Size Estimates

	Detectable Difference in ΔCF-PWV		
Power	1.0 m/s	1.25 m/s	1.5 m/s
80%	**100 per arm**	64 per arm	45 per arm
90%	**132 per arm**	86 per arm	60 per arm

Thus, a sample size of 264 patients (132 per arm) would provide 90% power to detect a 1.0 m/s difference in the change in carotid-femoral pulse wave velocity at 1 year between patients treated with spironolactone or placebo. A sample size of 200 patients (100 per arm) would permit 80% power to detect a 1.0 m/s difference in the change in carotid-femoral pulse wave velocity at 1 year (or >90% power to detect a 1.25 m/s difference). In actuality, since there is likely to be a nonzero correlation between baseline and follow up measurements of pulse wave velocity, we anticipate the standard deviation of the change will be lower than predicted above and that overall study power will accordingly be greater than calculated here. Nonetheless, assuming that 25% of enrolled subjects will have unusable or poor quality data at one or both time points (due to heart rate irregularity, pacing artifact, or low quality waveforms), we anticipate needing to recruit a minimum of 250 patients for the trial (and as many as 330 patients to ensure 90% power *a priori*). Since we assume that 10 to 15 patients will be recruited per site, it is anticipated that we will need to enlist ~ 20-30 sites from the primary trial for the ancillary study.

The primary statistical analysis for this substudy will be conducted according to intention to treat in the primary TOPCAT trial. The primary endpoint, change in carotid-femoral pulse wave velocity from baseline, will be compared by trial arm (spironolactone vs. placebo) using an Analysis of Covariance (ANCOVA) adjusted for age, gender, heart rate, and mean arterial pressure at baseline.

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TOPCAT Echo Substudy Protocol

Diastolic Function in Patients with Heart Failure and Preserved Ejection Fraction: The Impact of Aldosterone Receptor Antagonism

PROTOCOL SIGNATURE PAGE

I have read the following ancillary study protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study in accordance with the design and specific provisions outlined herein; deviations from the protocol are acceptable only with a mutually agreed upon protocol amendment.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure they are fully informed regarding the study drug and the conduct of the study.

I will use the informed consent form approved by the National Heart, Lung and Blood Institute and will fulfill all responsibilities for submitting pertinent information to the Institutional Review Board or Ethics Committee responsible for this study.

I also agree to report all information or data in accordance with the protocol.

I further agree that the National Heart, Lung and Blood Institute, the appropriate regulatory authorities and staff from the regional coordinating centers have access to any source documents from which case report form information may have been generated.

I also agree to handle all clinical supplies (including study drug) provided by the National Heart, Lung and Blood Institute and collect and handle all clinical specimens in accordance with the protocol.

The below signed confirm herewith to have read and understood this trial protocol and/or amendment and appendices; furthermore, to accomplish this study in accordance with the protocol and Good Clinical Practice guidelines, as well as local regulations; and to accept respective revisions conducted by authorized personnel of National Heart, Lung and Blood Institute and by competent authorities.

PRINTED OR TYPED NAME(S)	SIGNATURE	DATE
Principal Investigator(s)		
Principal Investigator(s)		

TOPCAT Ancillary Study Proposal

Heart failure with preserved ejection fraction (HF-PEF) is a diverse clinical syndrome characterized by signs and symptoms of heart failure despite apparently preserved systolic function, and accounts for nearly half of heart failure cases. Patients with HF-PEF demonstrate a pattern of functional decline similar to patients with heart failure and reduced ejection fraction, with increased hospitalizations, reduced quality of life and increased risk of death compared with an age-matched population without HF. Diastolic dysfunction, secondary to myocardial fibrosis and hypertrophy, has been presumed to be the primary pathophysiologic basis of HF-PEF. Other mechanisms, including abnormalities of renal function, have been implicated as well, and the true contribution of diastolic dysfunction to the pathogenesis of HF-PEF may be variable. *A better understanding of the pathophysiologic mechanisms in this disorder is critical to targeting more effective treatment.*

Current management of patients with HF-PEF is empiric, focused primarily on relief of congestive symptoms and aggressive management of comorbidities; no specific therapies are clearly associated with durable improvements in diastolic function or prognosis. Aldosterone receptor antagonists reduce mortality and morbidity in patients with heart failure and left ventricular dysfunction. The well-documented antifibrotic effects of aldosterone antagonists, combined with the general salutary effects of RAAS antagonism in patients with heart failure, suggest that patients with HF-PEF may derive similar clinical benefit.

The Treatment Of Preserved systolic function Cardiac failure with an Aldosterone anTagonist (TOPCAT) trial is a 3515-patient, placebo-controlled, randomized clinical trial funded by the National Heart Lung and Blood Institute (NHLBI) that will test the hypothesis that treatment with the aldosterone antagonist spironolactone will reduce the risk of cardiovascular death, heart failure hospitalization, or aborted cardiac arrest in patients with HF-PEF. We plan to conduct an ancillary study of the TOPCAT trial with the ***overall goal of characterizing the spectrum of myocardial abnormalities in patients with HF-PEF and examining the impact of treatment with an aldosterone antagonist on these abnormalities.***

Background: Diastolic function in HF-PEF

Progress in the understanding and treatment of HF-PEF has been thwarted in part by a lack of consensus regarding the optimal clinical definition. Operationally, the syndrome is defined by the presence of heart failure signs and symptoms in the absence of a prominent abnormality of systolic function. Since these criteria are rather nonspecific, the diagnosis of HF-PEF is applied to a broad range of patients with variable pathophysiology ranging from primary myocardial disease to progressive renal failure. **Characterizing the pathophysiologic abnormalities present in a broad range of patients with HF-PEF is a key preliminary step towards understanding mechanisms and targeting effective therapies.**

The pathophysiologic mechanisms responsible for the development of heart failure in patients with preserved systolic function remain poorly understood, in part because of the heterogenous nature of this disorder. Diastolic dysfunction, implying abnormalities of active myocardial relaxation or passive ventricular compliance, has been described as the *sine qua non* of HF-PEF, leading many to characterize the syndrome as 'diastolic' heart failure. The prevailing model emphasizes primary myocardial abnormalities that impair ventricular performance during diastole, shifting the ventricular pressure-volume relationship upward and to the left, thereby enhancing susceptibility to pulmonary venous hypertension with small changes in circulating blood volume or ventricular afterload. [1,2] Indeed, abnormalities of both passive myocardial stiffness and active myocardial relaxation have been demonstrated in selected patients with HF-PEF, suggesting that aberrant diastolic function plays an important role in heart failure pathogenesis[1].

A variety of cellular mechanisms likely contribute to worsening of diastolic function. Enhanced synthesis of fibrillar (Type I) collagen in the myocardium, a consequence of hemodynamic loading, ischemia, and neurohormonal activation, may play an important role, since myocardial fibrosis is a major determinant of altered diastolic filling and compromised systolic pump function in those with longstanding hypertension.[3,4] Perturbation of cytosolic calcium transients (for example, as a consequence of myocardial ischemia) or the myocardial contractile apparatus (as in hypertrophic cardiomyopathy) may lead to transient or permanent abnormalities of ventricular relaxation.[5,6] Passive chamber compliance may be diminished as a consequence of myocyte hypertrophy, increases in left ventricular mass, and alterations in the interstitial collagen network.[7,8]

While abnormal ventricular relaxation can be documented in a wide range of patients, only a small fraction of those with apparent "diastolic dysfunction" develop exertional dyspnea or other clinical symptoms of congestive heart failure.[9,10,11] Patients with HF-PEF exhibit some of the same pathophysiologic characteristics as those with reduced systolic function heart failure, including elevations in neurohormones, such as norepinephrine and BNP.[12] The relationships between myocardial hypertrophy, fibrosis, neurohormonal activation, and ventricular performance are well documented in experimental models of systolic heart failure, but there is little evidence that the same relationships hold for HF-PEF. **Overall, the extent to which intrinsic abnormalities of myocardial diastolic function play a central role in the pathophysiology of HF-PEF in the broad spectrum of patients with this disorder is unknown. We anticipate that the specific knowledge of structural and functional abnormalities in patients with HF-PEF may help to guide future therapeutic approaches, as it has for patients with heart failure and LV dysfunction.**

The renin-angiotensin-aldosterone system is thought to play a central role in the pathogenesis of diastolic dysfunction. Angiotensin II, both via the stimulation of the type 1 receptor and the stimulation of endothelin, promotes collagen synthesis and cardiac fibrosis[13]. In experimental models of hypertension and compensated left ventricular hypertrophy, the blockade of angiotensin II type 1 receptor can prevent the development of diastolic heart failure[14].

In addition to its well recognized role in sodium and water retention, aldosterone is a major stimulus for myocardial fibrosis[15]. In animal studies, aldosterone administration to salt-fed rats leads to the development of cardiac inflammation, remodeling, and fibrosis[16], while blockade of the aldosterone receptor in experimental models reduces myocardial fibrosis[17]. In patients with essential hypertension, aldosterone levels correlate with LV mass[18] and the severity of LV dysfunction independent of blood pressure[19,20]. Increased expression of aldosterone synthase in the myocardium has been observed in patients with heart failure, in association with increased myocardial fibrosis and left ventricular hypertrophy[21]. The benefits of aldosterone antagonism in patients with heart failure and reduced LVEF have been firmly established in the RALES[22] trial and in post-MI patients in the EPHESUS[23] trial. **The stimulation of myocardial fibrosis by aldosterone may contribute to diastolic dysfunction, and improving diastolic function with an aldosterone antagonist is a rationale for improving outcomes in patients with diastolic dysfunction and HF-PEF.**

Specific Aims

To test the hypothesis that long-term therapy with the aldosterone-receptor antagonist spironolactone will improve diastolic function relative to placebo in patients with HF-PEF. We will noninvasively assess myocardial structure and function using two-dimensional echocardiography with Doppler Tissue Imaging. Specifically, we will exploit the randomization and follow-up scheme of the primary trial to test the primary hypothesis that spironolactone therapy will enhance myocardial relaxation velocity (Tissue Doppler E') relative

to placebo in patients with HF-PEF. The impact of spironolactone on other echocardiographic measures of myocardial structure and function (including left ventricular mass, left atrial volume, and systolic function) will also be assessed.

To test the hypothesis that the severity of diastolic dysfunction at baseline will correlate with other markers of disease severity, and independently predict cardiovascular outcomes in patients with HF-PEF. We will examine the relationship between baseline measures of diastolic function and the primary TOPCAT trial outcomes to characterize the spectrum of myocardial abnormalities present in this population and to examine:

a. Do baseline measures of diastolic dysfunction predict cardiovascular outcomes?
b. Can diminished myocardial relaxation velocity at baseline be used to identify subgroups likely to experience the greatest benefit from spironolactone therapy?
c. Do baseline measures of diastolic function correlate with other measures of disease severity, including functional status and quality of life?

Methods

TOPCAT Trial

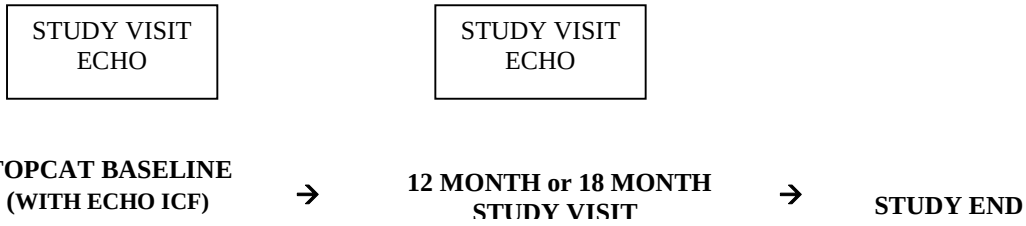
The TOPCAT trial is an international, multicenter, randomized, double-blind, placebo-controlled trial of the aldosterone antagonist, spironolactone, versus placebo in 3515 adults with heart failure and left ventricular ejection fraction of at least 45%, recruited from 200-300 clinical centers. Eligible patients include men and women at least 50 years of age with left ventricular ejection fraction ≥ 45% measured within 6 months prior to enrollment, prior hospitalization for heart failure or elevated BNP, and signs and symptoms of heart failure. Exclusion criteria include known restrictive or infiltrative cardiomyopathy; known constrictive pericarditis; known hypertrophic obstructive cardiomyopathy; concomitant severe medical illness limiting life expectancy to < 3 years; hemodynamically significant, uncorrected valvular heart disease; atrial fibrillation with resting heart rate > 90 beats/min; significant chronic obstructive pulmonary disease; recent coronary revascularization; recent stroke; recent use of an aldosterone receptor antagonist; heart transplant or ventricular assist device; abnormal hepatic function; advanced renal disease, with estimated GFR < 30 cc/min and/or serum creatinine ≥ 2.5 mg/dl; and serum potassium > 5.0 mmol/L. The trial duration is approximately 6 years, with approximately 4 years for subject enrollment and an additional 2 years of follow-up, for an average subject follow-up of 3.45 years. Study visits will occur every 4 months during the first year (more frequently in the first 4 months) and every 6 months thereafter. The primary endpoint is a composite of cardiovascular mortality, aborted cardiac arrest, and hospitalization for heart failure. Enrollment began in August 2006.

Ancillary Study Design

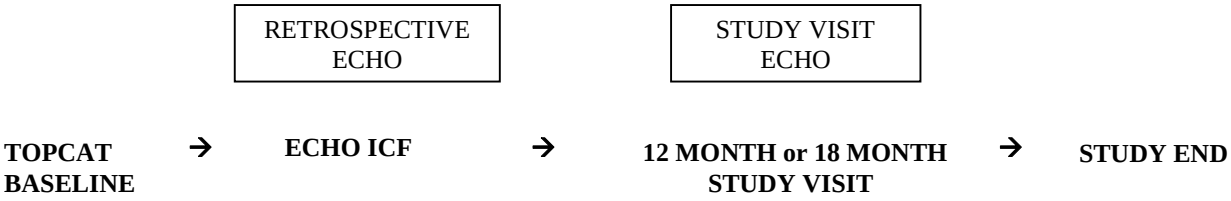
To achieve the specific aims outlined above, we propose to conduct a nested, mechanistic substudy within the architecture of the primary TOPCAT trial. We will utilize the TOPCAT trial organization to obtain additional non-invasive measurements at the baseline and 12 or 18 month visits in 500 subjects enrolled at selected trial sites. Patients already enrolled in TOPCAT who have not yet reached their 18 month visit will also be offered the opportunity to participate in this ancillary study. In lieu of a formal baseline assessment with echocardiography assessment, these patients will be asked to consent for retrospective analysis of retrospective data of any available echocardiographic images completed within 60 days prior to their enrollment in TOPCAT. Priority will be given to those sites experienced in the use of Doppler Echocardiography for diastolic function assessment. Day-to-day technical support and regular surveillance of data quality will be provided by the imaging core laboratory at Brigham and Women's Hospital.

To be eligible for the substudy, patients must be enrolled in the TOPCAT study at one of these participating centers, and willing to provide informed consent for the additional non-invasive measurements.

Patients who enroll in the ECHO Substudy during TOPCAT baseline screening:



Patients who enroll in the ECHO substudy after TOPCAT baseline screening visit:



Study Visits

Echocardiography

Echocardiographic acquisition

Echocardiograms will be performed at the sites by qualified echocardiographic personnel (technicians or physicians) in accordance with standard echocardiographic clinical practice. The baseline echocardiogram will be performed before administration of spironolactone or placebo. To be enrolled after the baseline visit, the patient must have undergone a suitable echocardiogram within a 60-day time period prior to TOPCAT randomization that could be submitted for core lab review.The required echocardiographic examination will be a modification of the standard echocardiographic examination, and will include the majority of standard echocardiographic views normally obtained for clinical practice, in addition to specific echocardiographic assessments designed to assess diastolic function (Table 0.1).

Table 0.1. Echocardiographic Assessments Obtained at Sites

Echocardiographic View	Images Obtained
• Parasternal long axis view	• 2-D image for septal and posterior wall thickness • M-Mode Images • Colorflow Doppler • 2-D images for Septal and posterior wall thickness
• Parasternal Short axis view, papillary muscle level • Apical 4-chamber View	• 2-D images for volume and ejection fraction measures, LA size and RV function • Colorflow Doppler for assessment of mitral regurgitation • Doppler Tissue Imaging of Mitral annular velocities (primary endpoint) • Mitral Inflow Pulsed Doppler

Recording of Echocardiographic Studies and Transmission of Echocardiographic Data to Core Laboratory

Echocardiograms will be recorded to videotape (VHS or S-VHS) or digital media in DICOM format (CD, or Magneto-Optical Disc). As possible, study site and patient ids will be recorded with each study – no patient identifiers will be used. For studies obtained retrospectively, every effort should be made to remove identified patient data prior to submission to the core laboratory. If this is not possible, studies will be de-identified on receipt by core laboratory personnel. Echocardiographic studies will be sent to the core laboratory via courier in one bulk shipment on the 15th of every month and logged in upon receipt at the core laboratory. Sites will include an echocardiographic tracking form for each study sent to the core laboratory. All echocardiographic measurements will be made at the Cardiac Imaging Core Laboratory (Brigham and Women's Hospital, Boston, MA: Director Scott Solomon).

Echocardiographic Data Analysis

Echocardiographic assessments will be made in the core laboratory by experienced research echocardiographers utilizing off-line PC-based echocardiographic analysis software. Echocardiograms received in analog (videotape) format will be digitized utilizing industrial quality analog-to-digital video capture equipment, and image loops will be stored on hard disk with weekly backups to DVD-R media.

Echocardiographic assessments are outlined in Table 0.2. All volume measurements will be made by manually tracing the endocardial border at end diastole and end systole. **Left ventricular and left atrial volumes** will be calculated using the modified Simpson's rule method[24]. **Right ventricular function** will be assessed utilizing fractional area change derived from right ventricular area measurements in the apical-4 chamber view[24]. **Left ventricular mass** will be derived from wall thickness measurements utilizing the modified Devereux method[25].

Table 0.2. Echocardiographic Assessments Performed at Core Laboratory

Measure	Method
End-diastolic volume (EDV)	Simpson's Rule, method of discs
End-systolic volume (ESV)	Simpson's Rule, method of discs
Ejection Fraction	Derived from volumes: EF = 100x(EDV-ESV)/ESV
LV wall thickness, septum and posterior wall	m-mode
Left ventricular mass	Derived from m-mode wall thickness measurements based on Modified Devereux method: left ventricular mass (g)= 1.04 × 0.8 [(left ventricular wall thicknesses + internal dimension) – (internal dimension)] + 0.6
Right ventricular fractional area change	Derived from RV diastolic area and systolic area in apical 4-chamber view.
Left atrial volume	Simpson's rule, method of discs
Mitral inflow E wave velocity	Measured directly at from mitral inflow pulsed wave Doppler at leaflet tips
Mitral inflow A wave velocity	Measured directly at from mitral inflow pulsed wave Doppler at leaflet tips
Mitral E/A Ratio	Derived from E and A wave velocities
Mitral E Deceleration time	Measured directly as the time from peak E-wave to the baseline (extrapolated)
Isovolumic contraction time (IVCT)	Measured directly as the time from the beginning of the QRS complex to the start of ejection time
Isovolumic Relaxation Time (IVRT)	Measured directly as the time from end of ejection time to the beginning of the E-wave
Myocardial performance index	Derived from IVCT, IVRT and Ejection Time: MPI = (IVRT+IVCT)/ET

The primary measure of diastolic function for the diastolic function substudy will be change in lateral mitral annular relaxation velocity (E') from baseline to one-year. Mitral annular velocities will be assessed using Tissue Doppler imaging (TDI) of the lateral and septal mitral annuli (See Figure 0.1). Mitral annular relaxation velocities of the lateral and septal mitral annulus will be assessed from the Doppler tissue spectral waveforms utilizing the mean amplitude of the Doppler tissue peak spectral waveform. Mitral inflow early and late diastolic filling velocities will be assessed, as will pulmonary venous Doppler waveforms, from the apical four chamber view. Additional measures of diastolic function will also be performed:

Isovolumic contraction time (IVCT) will be calculated as the time from the beginning of the foot of the QRS complex to start of ejection time;

Isovolumic relaxation time (IVRT) will be measured as time interval from the end of ejection time (measured from the pulsed wave LV outflow tract Doppler) to the beginning of mitral inflow (start of E-wave);

Myocardial performance index (MPI or Tei Index) will be calculated as (Isovolumic relaxation time + isovolumic contraction time) / ejection time;

Mitral deceleration time (DT) will be assessed as the time from the peak of the early mitral inflow wave (E-wave) to the baseline. Derived measures of diastolic function will include **the ratio of early mitral inflow velocity to annular velocity (E/E').** In addition, patients will be categorized as having normal, mild, moderate or severe impairment of diastolic function utilizing a qualitative assessment schema that takes into account a variety of diastolic parameters (Table 0.3, Modification of method proposed by Redfield et al[26]). All echocardiographic measurements will be made and averaged over 3 cardiac cycles.

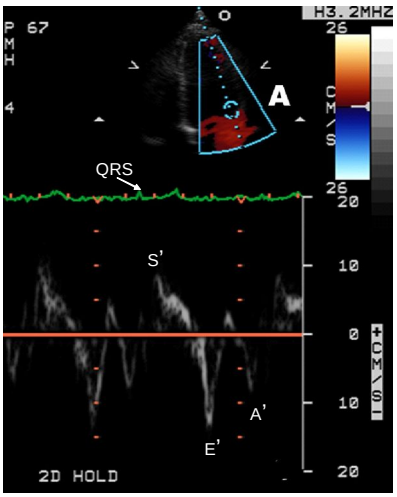


Figure 0.1. Tissue Doppler imaging (TDI) of the lateral mitral annular velocities

Table 0.3. Qualitative Evaluation of Diastolic Function Based on Multiple Parameters

	Normal	Mild	Moderate	Severe
Lateral Mitral Annular Velocity	E' > 12cm/s	E' < 12 cm/s	E' < 10 cm/s	E' < 8cm/s
Mitral inflow to mitral annular velocity ratio	E/E' < 10	E/E' < 10	E/E' ≥ 10	E/E' ≥ 10
Mitral Deceleration Time	DT > 140ms	DT > 140ms	DT > 140ms	DT < 140ms

Anticipated issues with echocardiographic assessment

It is anticipated, based on our prior experience, that not all sites will be able to adequately perform all echocardiographic assessments, particularly Doppler tissue imaging of the mitral annulus. To minimize the number of inadequate studies, we will provide educational material, including a Site Instruction Manual and Echo Pocket Guide. Additionally, the core laboratory will provide specific feedback to the sites to help with individual equipment or operator dependent problems that arise.

Statistical Considerations

Echocardiography

The overall sample size for the echocardiography substudy is based on the interventional study outlined in specific aim #1. We have estimated that 500 subjects will be required to test the primary hypothesis that spironolactone will improve diastolic function in patients with HF-PEF. The sample size is based on the primary endpoint of change in mitral annular relaxation velocity (E') from baseline to one-year. Assuming a two-sided Type I error rate of 0.05, a desired power of 0.90, a conservative estimate standard deviation of the measurement of 3.0 cm/s based on prior population-based assessments[27], a total sample size of 380 subjects is also required to detect a 1.0 cm/s difference in the one-year change scores for diastolic relaxation velocity in the two treatment groups. This calculation assumes that the correlation between baseline and one-year values is 0.5, which results in a standard deviation of change of 3.0 cm/s. Loss to follow-up is estimated at 10% (based on the expected death rate in this population of approximately 6% per year[28] and expected drop-out for other reasons), therefore 422 patients are required (380/0.9). In addition, it is conservatively estimated that 15% of images may be of insufficient quality for quantitation, leading to an overall sample size of 496 patients (422/0.85). Therefore, the total target sample size for the substudy will be 500 patients enrolled at 50 sites.

The primary efficacy endpoint (and the endpoint used for determining the sample size necessary) for the substudy will be *change in lateral mitral annular relaxation velocity (E') from baseline to one year* (Table 0.4). Additional assessments of diastolic function, including changes in E/E', changes in mitral deceleration time, changes in myocardial performance index, and changes in a composite measure of diastolic function will be secondary endpoints.

For the primary analysis of this primary measure, we will utilize analysis of covariance to compare the placebo and spironolactone groups with respect to changes in the mean baseline and one-year mitral annular relaxation velocity (E'), adjusting for the baseline value of E'. This approach will be preferable to simple comparison of mean changes as it will take into account any chance imbalances in baseline E', and will also take into account regression to the mean[29]. A similar analysis approach will be applied to the secondary outcome measures of diastolic function (change in E/E'; change in mitral deceleration time (DT); change in myocardial performance index (MPI); change in composite measure of diastolic function; change in left ventricular mass; and change in left atrial size).

Table 0.4. Endpoints (Specific Aim #1)

Echocardiographic Endpoints
Primary Endpoint
Change in Lateral Mitral annular relaxation velocity (E'), baseline to 1 year
Secondary Echocardiographic Endpoints
Change in E/E', baseline to 1 year
Change in mitral (E-wave) deceleration time, baseline to one year
Change in myocardial performance index (MPI), baseline to one year
Change in composite measure of diastolic function (see below), baseline to one year
Change in left ventricular mass, baseline to one-year
Change in left atrial size, baseline to one-year

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TOPCAT SPECIMEN REPOSITORY PLAN

May 3, 2007

Version 1.3

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Overview of the TOPCAT study (abstract):

This trial is a multicenter, international, randomized, double blind placebo-controlled trial of the aldosterone antagonist, spironolactone, in 4500 adults with heart failure and left ventricular ejection fraction of at least 45%, recruited from over 150 clinical centers. The primary endpoint is a composite of cardiovascular mortality, aborted cardiac arrest or hospitalization for the management of heart failure. Secondary endpoints include all-cause mortality, new onset of diabetes mellitus, atrial fibrillation, and quality of life. The trial duration is 4.25 years, with 2.0 years for subject enrollment and an additional 2.25 years of follow-up, with an average subject follow-up of 3.0 years. Dynamic balancing by clinical center at the time of randomization will be used to ensure that the distribution of clinical centers are similar in the two treatment groups. The study population will include those who meet the inclusion criteria, some of which are:

- Male or female age 50 years or older;
- Heart failure defined as one symptom and one sign present in the last 12 months (described in protocol);
- Left ventricular ejection fraction $\geq$ 45% (per local reading);
- Controlled systolic blood pressure (SBP), defined as: SBP < 140 mm Hg or SBP from 140-160 mm Hg if subject is being treated with 3 or more medications;
- Serum potassium < 5.0 mmol/L prior to randomization;
- At least one hospitalization in the last 12 months for which heart failure was a major component of the hospitalization OR elevated BNP or N-terminal pro-BNP within the last 30 days;
- Willing to comply with scheduled visits, as outlined in the protocol;
- Signed informed consent form.

Study drug dosing will start at 15 mg/day and may be titrated up to 45 mg according to subject tolerance, safety parameters, and symptoms, and will be continued throughout the trial. Following each change in the dosing regimen, subjects will have blood drawn for safety labs 1 week later. Subjects will take study medication every day according to specific instructions. All other treatments will follow accepted local standards for medical care for specific morbidities as described by the American College of Cardiology (ACC), the American Heart Association (AHA), and the European Society of Cardiology (ESC) Practice Guidelines, as appropriate. Such treatments may also be adjusted by the local medical practitioner, if necessary. All randomized subjects will be followed even if study drug is discontinued ahead of schedule, except in the case that the subject refuses to participate further in the study.

Follow-up study visits to monitor symptoms, medications, and events and to dispense study drug will occur every 4 months during the first year and every 6 months thereafter. Quality of life will be assessed three times in the first year of the trial and annually thereafter. An electrocardiogram (ECG) will be performed at baseline only. Blood, DNA, and urine samples will be collected from a subset of subjects and stored in a repository for later use in ancillary studies. All clinical endpoints will be adjudicated by a clinical events committee in a blinded fashion. Continual safety surveillance has been built into the study by means of the proposed dosing and safety assessment regimen described in the protocol. The 15 mg dose of spironolactone was formulated to reduce the risks and

side-effects associated with this drug. The Data and Safety Monitoring Board (DSMB) will meet regularly, at least twice a year. The DSMB chair will be notified of any events considered probably or definitely related to study drug. At the time of notification, he/she will determine if an additional DSMB meeting is required. The study will be conducted according to the provisions of the Declaration of Helsinki, the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP), and applicable national and local regulations.

Overview of the TOPCAT repository:

The TOPCAT study plans to collect specimens at a subset of sites from patients who opt to participate. This plan outlines how specimens will be collected, who will have access to the specimens, the types of analyses planned for the specimens, process for determining specimen allocation, and a timeline for access by TOPCAT PIs as well as investigators outside the study (non-TOPCAT PIs approved by NHLBI). The purpose of the repository specimens is to provide a bank of specimens for future studies collected from the study population of preserved ejection fraction heart failure patients that are either on spironolactone or placebo. Repository blood and urine samples for the TOPCAT trial will be collected at the baseline and 1-year visits from consenting patients according to the sample collection protocol outlined in the section below**.** It is anticipated that between 1,500 and 3,000 patients will opt into the repository from the U.S., Canada and Russia. Each patient participating would have samples collected at the baseline and 12 month visits.

How specimens will be collected, stored and shipped:

Repository blood and urine samples for the TOPCAT trial will be collected at the baseline and 1-year visits from consenting patients according to the sample collection protocol outlined in detail in the TOPCAT manual of operations. All collection and storage tubes, as well as approved shipping containers will be provided in advance to participating centers by the NHBLI central repository. Sites will be asked to process the samples locally and store temporarily at -70°C or colder for shipment in batch on dry ice. Sites without -70°C storage should store specimens at in a -20 °C freezer or on dry ice.

Samples will be shipped to the repository in dry ice shipping boxes:

- Every 6 months or
- When they accumulate a full shipper box (3 full cryoboxes)

whichever comes first.

Samples will be stored at the NHLBI central repository at -70°C for the trial duration, then released to ancillary study investigators for processing. Aliquots will be created at Seracare when samples are distributed for funded ancillary studies. This global “standard” aliquoting and labeling of aliquots will be funded by ancillary studies at the time of sample retrieval. NERI and Seracare will come to an agreement on a “cost per sample” such that the expense is not overly burdensome on investigators requesting small aliquots. Ancillary studies must also plan to fund the cost of deriving the specimen list and shipping costs to the ancillary study investigator.

Specimen access:
TOPCAT PIs who meet the criteria outlined in the Ancillary Study Policy are eligible to submit proposals for use of repository samples. The repository will be a shared resource and will be stored at the NHLBI repository (currently Seracare Biosciences).

How samples will be used:
The plan for sample usage is detailed in the table below. At a minimum, 1 aliquot from each subject of each sample type will remain as a shared resource in the NHLBI repository at Seracare at the end of the contract period. At the earliest, samples may be withdrawn from the bank after the last 12 month patient visit for the last patient that has consented to participate in the repository. If samples are withdrawn prior to the end of the study, they may be tested but will not have any clinical data associated with them. In addition, to adhere to the repository ICF, any laboratory that accepts and processes samples prior to the end of the study must have clear documented procedures for sample and data destruction as patients may opt out of the repository at any time prior to database lock.

Planned Biomarker TOPCAT Repository Analyses (if funding separate from the main TOPCAT study is obtained)

Measurement	Baseline sample needed?	Follow-up sample needed?	Aliquot size needed
Markers of Neurohormonal Activation			
Plasma Renin Activity	Yes		100 µL EDTA plasma
Aldosterone	Yes		100 µL serum
B-type natriuretic peptide	Yes	Yes	100 µl EDTA plasma
N-terminal fragment of the BNP prohormone (Nt-proBNP)	Yes	Yes	100 µl EDTA plasma
Markers of Sodium Balance + Renal Function			
Sodium (Urine)	Yes		25 µL serum/urine
Creatinine (Urine + Serum)	Yes		25 µL serum/urine or EDTA plasma
Microalbumin (Urine)	Yes		25 µL urine
Markers of Inflammation			
C-Reactive Protein (CRP)	Yes	Yes	40 µL EDTA plasma
Markers of Wall Stress			
ST2	Yes	Yes	500 µL serum
Markers of Collagen Metabolism			
MMP-1, MMP-9, TIMP1	Yes	Yes	

Procollagen Type I Carboxy Terminal Peptide (PIP)	Yes	Yes	
N-terminal type III procollagen (PIIINP)	Yes	Yes	0.5 cc serum

Planned SNP Analysis - Selected genes to be included as part of the genetic panel (if funding separate from the main TOPCAT study is obtained)

Gene description	Gene name	Gene map locus
Primary genes		
11-beta-hydroxysteroid dehydrogenase type 2	HSD11B2	16q22
Angiotensinogen	AGT	1q42-q43
Aldosterone synthase	CYP11B2	8q21-q22
Angiotensin I converting enzyme	ACE	17q23.3
Angiotensin II receptor, type 1	AGTR1	3q21-q25
Collagen type III	COL3A1	2q31
Nuclear receptor subfamily 3, group C, member 2	NR3C2	4q31.1
Renin	REN	1q32
Proposed exploratory genes		
11-beta-hydroxysteroid dehydrogenase type 1	HSD11B1	1q32-q41
Adrenergic, alpha-2A-receptor	ADRA2A	10q24-q26
Adrenergic, alpha-2B-receptor	ADRA2B	2p13-q13
Adrenergic, alpha-2C-receptor	ADRA2C	4p16.1
Adrenergic, beta-1-receptor	ADRB1	10q24-q26
Adrenergic, beta-2-receptor	ADRB2	5q31-q32
Adrenergic, beta-3-receptor	ADRB3	8p12-p11.2
Adducin 1	ADD1	4p16.3
Angiotensin I converting enzyme 2	ACE2	Xp22
Angiotensin II receptor, type 2	AGTR2	Xq22-q23
Angiotensin II receptor-associated protein	AGTRAP	1p36.22
Atrial natriuretic peptide	NPPA	1p36.21
Bradykinin receptor B1	BDKRB1	14q32.1-q32.2
Bradykinin receptor B2	BDKRB2	14q32.1-q32.2
Natriuretic peptide precursor B	NPPB	1p36.2
Carnitine Transporter type 2	SLC22A5	5q31
Chloride channel Kb	CLCNKB	1p36
Chymase-1	CMA1	14q11.2
Collagen, type I, alpha 1	COL1A1	17q21.33
Corin	CORIN	4p13-p12
Cytochrome P450, family 17, subfamily A, polypeptide 1	CYP17A1	10q24.3
Natriuretic peptide precursor C	NPPC	2q24
GNAS complex locus	GNAS	20q13.3
Guanine nucleotide binding protein (G protein),	GNB3	12p13

beta polypeptide 3		
Kallikrein 1	KLK1	19q13.3
Kininogen	KNG	3q27
Matrix metalloproteinase-1	MMP-1	11q22.3
Matrix metalloproteinase-2	MMP-2	16q13-q21
Matrix metalloproteinase-3	MMP-3	11q22.3
Matrix metalloproteinase-9	MMP-9	20q11.2-q13.1
Matrix metalloproteinase-13	MMP-13	11q22.3
Multidrug resistance 1	MDR1	7q21.1
Natriuretic peptide receptor A	NPR1	1q21-q22
Natriuretic peptide receptor C	NPR3	5p14-p13
Nitric oxide synthase 3	NOS3	7q36
Paraoxonase 3	PON3	7q21.3
Potassium inwardly-rectifying channel, subfamily J, member 1	KCNJ1	11q24
Protein kinase c-beta1	PRKCB1	16p11.2
Serum/glucocorticoid regulated kinase	SGK	6q23
Sodium channel, nonvoltage-gated 1, gamma	SCNN1G	16p12
Sodium channel, nonvoltage-gated 1, beta	SCNN1B	16p12.2-p12.1
Solute carrier family 9 sodium/hydrogen exchanger), member 1 (antiporter, Na+/H+, amiloride sensitive)	SLC9A1	1p36.1-p35
TIMP metallopeptidase inhibitor 1	TIMP-1	Xp11.3-p11.23
TIMP metallopeptidase inhibitor 2	TIMP-2	17q25
TIMP metallopeptidase inhibitor 3	TIMP-3	22q12.3
Transforming growth factor, beta 1	TGFB1	19q13.1
WNK lysine deficient protein kinase 1	WNK1	12p13.3

Timeline for submission:

Date	Milestone	Who
May 14, 2007	Round 1 - Application deadline for NHLBI ancillary study RFA	Approved TOPCAT PIs submit electronic application
July 31, 2007	Deadline for returning Round 2 proposals for TOPCAT SC	TOPCAT PIs submit to TOPCAT SC
August 10, 2007	Round 2 - Decision on which proposals should proceed to secure funding. Sites that have recruited ≥ 2 patients will be given highest priority.	
August 17, 2007	Round 2 - Letter of intent deadline for NHLBI ancillary study RFA http://grants.nih.gov/grants/guide/rfa-files/RFA-HL-07-009.html	Approved TOPCAT PIs submit LOI for NHLBI Ancillary studies RFA if funding to be secured via NIH
Sep 17, 2007	Round 2 - Application deadline for NHLBI ancillary study RFA	Approved TOPCAT PIs submit electronic application
Sep 30, 2008	Last patient randomized	
Sep 30, 2009	Last 12 month visit sample for repository collected	
Jul 1, 2010	Funding must be secured for proposals for first round of sample allocation, TOPCAT PIs confirm funding to SC	TOPCAT PIs with approved proposals
Aug 1, 2010	First round specimen allocation lists based on funded studies identified by NERI and sent to NHLBI repository	NERI / NHLBI repository
Dec 31, 2010	Last patient follow-up visit	
Jan 1, 2011	Database locked	NERI
Jan, 2011	Dataset de-identified and anonymized; dataset linked to anonymized samples	NERI
Jan, 31, 2011	De-identified dataset corresponding to samples given to TOPCAT PIs and samples aliquoted and sent to PIs of funded ancillary studies	NERI / NHLBI repository
Dec,31, 2013 (latest)	Limited access dataset available (3 years after the last patient follow-up)	NERI
Dec, 31, 2013 (latest)	Repository specimens become a shared resource	NHLBI repository

Kit components, specimens collected at each patient visit and barcode labeling information :

4 collection tubes will be used and 12 tubes stored at each patient visit that repository specimens are collected as per the table below. All collection tubes and aliquot tubes will be pre-labeled with barcode labels provided by Seracare. Each kit (used for 1 patient visit) will contain labels with the same parent barcode and a different suffix for specimen type and aliquot. The parent barcode is randomly assigned and unrelated to the subject ID.

Treatment Of Preserved Cardiac function heart failure with an Aldosterone an Tagonist
TOPCAT
Funded by the NHLBI

COLLECTION TUBES

TUBE #	Descriptor	vol (mL)	Label Line 1	Label Line 2
1	BD Plastic Serum separator Vacutainer tube (tiger top, 7.5mL)	7.5	Barcode	Serum separator
2	BD Plastic Serum separator Vacutainer tube (tiger top, 7.5mL)	7.5	Barcode	Serum separator
3	EDTA tube (lavender top, 10 mL)	10	Barcode	EDTA
4	Urine collection tube (10 mL)	10	Barcode	Urine collection
STORAGE TUBES				
5	Serum cryovial	2	Barcode	TOPCAT serum 1
6	Serum cryovial	1	Barcode	TOPCAT serum 2
7	Serum cryovial	1	Barcode	TOPCAT serum 3
8	Serum cryovial	1	Barcode	TOPCAT serum 4
9	Plasma cryovial	1.8	Barcode	TOPCAT plasma 1
10	Plasma cryovial	1.8	Barcode	TOPCAT plasma 2
11	Packed cells (for DNA) cryovial	1.8	Barcode	TOPCAT pkd cells 1
12	Packed cells (for DNA) cryovial	1.8	Barcode	TOPCAT pkd cells 2
13	Urine cryovial	2	Barcode	TOPCAT urine 1
14	Urine cryovial	2	Barcode	TOPCAT urine 2
15	Urine cryovial	2	Barcode	TOPCAT urine 3
16	Urine cryovial	2	Barcode	TOPCAT urine 4
17	form label			

Appendix A: Anonymization plan for specimens

TOPCAT SPECIMEN REPOSITORY AND ANONYMIZATION PROCESS

BACKGROUND INFORMATION:

Please see attached TOPCAT repository ICF section 3.
3. How will my samples be collected, stored, and anonymized?

Repository and Database Setup Procedures

Up to 12 Repository specimens will be collected during 1 the baseline patient visit, and again during the 12 month patient visit. Specimens include 10 blood and urine specimens and 2 DNA specimens.

	Descriptor	Label Line 1	Label Line 2
1	Serum cryovial	Specimen code + unique tube suffix	Serum aliquot
2	Serum cryovial	Specimen code + unique tube suffix	Serum aliquot
3	Serum cryovial	Specimen code + unique tube suffix	Serum aliquot
4	Serum cryovial	Specimen code + unique tube suffix	Serum aliquot
5	Plasma cryovial	Specimen code + unique tube suffix	Plasma aliquot
6	Plasma cryovial	Specimen code + unique tube suffix	Plasma aliquot
7	Packed cells cryovial	Specimen code + unique tube suffix	Packed cells
8	Packed cells cryovial	Specimen code + unique tube suffix	Packed cells
9	Urine cryovial	Specimen code + unique tube suffix	Urine aliquot
10	Urine cryovial	Specimen code + unique tube suffix	Urine aliquot
11	Urine cryovial	Specimen code + unique tube suffix	Urine aliquot
12	Urine cryovial	Specimen code + unique tube suffix	Urine aliquot

- All specimens will be barcoded
- An example of the barcode string is: AA12345 001 where the AA12345 is called the sample ID and the 001 is the suffix #.
- The barcode is random and unrelated to the subject ID or site number.
- All samples from a subject during 1 visit will have the same “random parent specimen code” and unique suffixes.

Procedures During Trial – collection and storage of repository samples

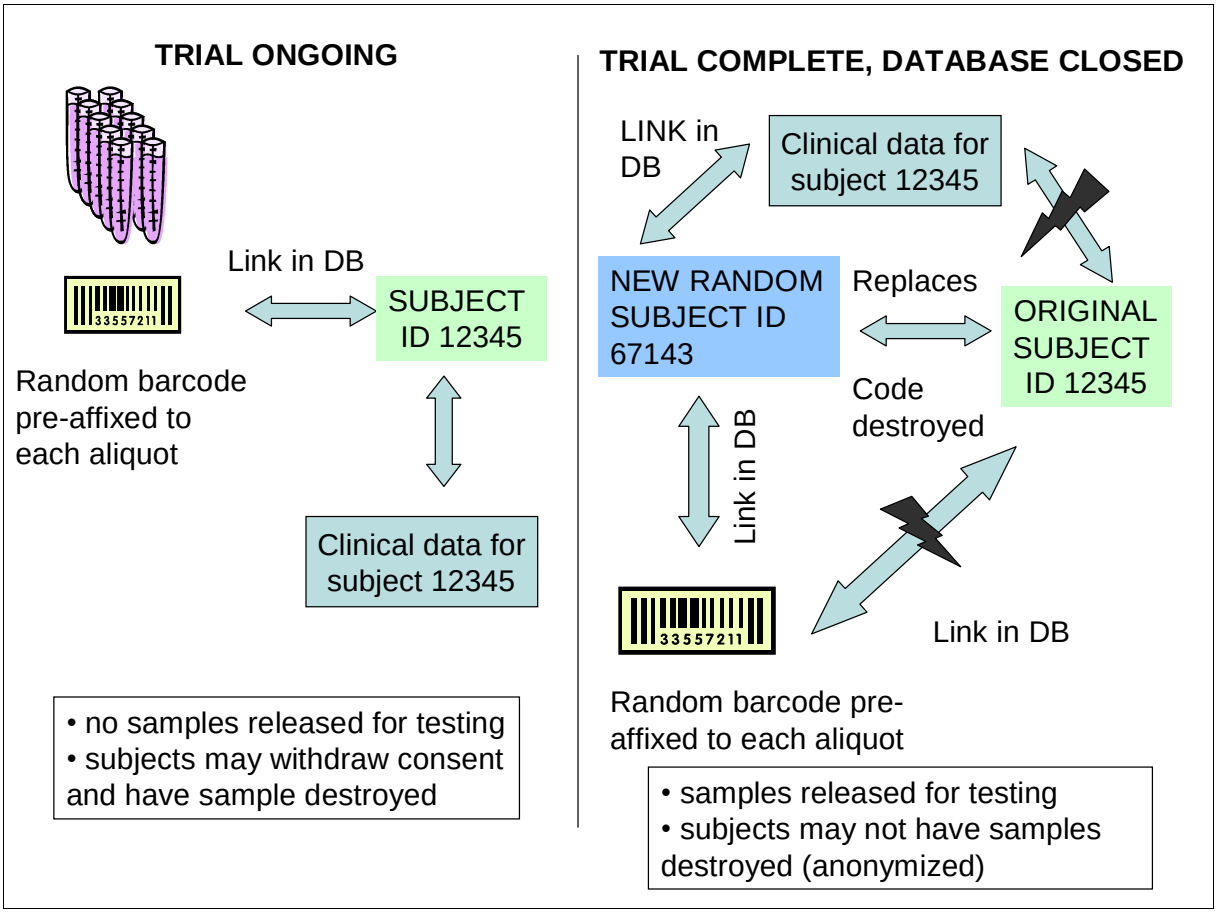
- Site procures specimens in pre-labeled barcoded tubes.
- Site logs all specimens into ADEPT using the specimen acquisition form.
- Shipping manifest is automatically generated in ADEPT.
- ADEPT will perform an automatic check comparing ICF to sample acquisition.
- Samples will not be released to investigators with any associated clinical data while trial is ongoing.
- Subjects may withdraw their consent and have samples destroyed until the database is locked and anonymized.

Anonymization Procedures Post-trial (after database closed)

- At NERI, generate new random subject IDs and link to original subject ID

- Anonymize dataset according to NIH guidelines for anonymizing limited access public datasets (see Appendix 1 for how these guidelines will be implemented for TOPCAT)
- Link the barcode with the new subject ID generated by the anonymized dataset.
- Destroy key linking original subject ID with new random subject ID
- Subjects may no longer withdraw consent and have samples destroyed since the link between the barcode and subject has been broken.

Figure A, Schematic for Anonymization process



1. All participants who refused to permit sharing their data with other researchers will be deleted from this Limited access Data Set.

2. Participant identifiers:
    a. New random identification numbers will replace original identification numbers.
    b. The key linking the original and new ID numbers will be destroyed.

3. Variables that might lead to the identification of participants and of centers:
    a. Clinical center identifier -- the data set will not contain center identifiers.
    b. Interviewer or technician identification numbers will be recoded or deleted.
    c. Regional variables with little or no variation within a center because they could be used to identify that center will be deleted.
    d. Unedited, verbatim responses that are stored as text data (e.g., specified in "other" category) will be deleted.

4. Dates: All dates will be coded relative to a specific reference point (e.g., date of randomization). This provides privacy protection for individuals known to be in a study who are known to have had some significant event (e.g., a myocardial infarction) on a particular date. Birth and other milestone dates will also be recoded relatative to a specific reference date.

5. Variables with low frequencies for some values, that might be used to identify participants, may be recoded. These might include:
    a. Socioeconomic and demographic data (e.g., marital status, occupation, income, education, language, number of years married).
    b. Household and family composition (e.g., number in household, number of siblings or children, ages of children or step-children, number of brothers and sisters, relationships, spouse in study).
    c. Numbers of pregnancies, births, or multiple children within a birth.
    d. Anthropometry measures (e.g., height, weight, waist girth, hip girth, body mass index).
    e. Physical characteristics (e.g., missing limbs).
    f. Prior medical conditions with low frequency (e.g., group specific cancers into broader categories) and related questions such as age at diagnosis and current status.
    g. Parent and sibling medical history (e.g., parents' ages at death).

6. Race/ethnicity and sex information when very few participants are in certain groups or cells.
    a. Polychotomous variables: values or groups will be collapsed so as to ensure a minimum number of participants (e.g., at least 20) for each value within each race-sex cell.
    b. Continuous variables: distributions will be truncated if needed to ensure that a minimum number of participants (e.g., at least 20) have the same highest and lowest values in each race-sex cell.
    c. Dichotomous variables: data should either be grouped with other related variables so as to ensure a minimum number of participants (e.g., at least 20) in each race-sex cell or deleted.

Appendix B: Repository ICF

See next page

Treatment Of Preserved Cardiac function heart failure with an Aldosterone anTagonist (TOPCAT)
Protocol Number: TOPCAT
CONSENT TO COLLECT DNA, BLOOD AND URINE SAMPLES FOR REPOSITORY SUB-STUDY (OPTIONAL)

Name of Subject: _____ Date: _____	
Principal Investigator:	
Institution:	
Funded by:	National Heart, Lung, and Blood Institute, which is part of the National Institutes of Health and the Department of Health and Human Services

The nature of the repository sub-study and the potential risks and benefits are discussed below. Please discuss any questions you have about this study with your doctor or the medical staff explaining it to you.

1. Why is this repository sub-study being done?

The purpose of this sub-study is to establish a repository of genetic material (DNA), blood and urine samples from TOPCAT study participants for use in future studies. This repository sub-study is an add on to the main research study, sponsored by the National Heart, Lung, and Blood Institute (NHLBI), National Institute of Health (NIH), Department of Human and Health Services (DHHS), of the United States. Participants enrolled into the main research study are eligible to participate in this optional repository sub-study if they are enrolled at a participating site. There are two portions to this optional sub-study: (1) DNA or genetic portion and (2) blood and urine portion. An Ethics Committee (or Review Board) will review (and approve or reject) each sub-study proposal before any of your blood samples can be used by researchers, based on the pertinence of the proposed studies. Studies that can be proposed could, for example, answer questions regarding the impact of the medication used in this trial on your kidney function, on some hormones in your blood, or on markers of inflammation. The DNA samples will be used to determine if some genes might predict your response to heart failure medications (in this case spironolactone). Alternatively, some genes may make individuals more susceptible to various side effects of heart failure medications, and these could also be identified in order to refine heart failure therapy in the future. Your samples are not limited to research on cardiovascular disease. Using your DNA samples, investigators may look at inherited factors which are related to diseases, responses to medications, or other factors that are thought to pass from parents to children. This DNA may be used to test genes across the genome, including those that may regulate hormones, growth factors and other processes or substances that

may influence disease. You will not be told of these possible tests, nor will you receive results of any of these tests. You may continue to participate in the main research study even if you choose not to participate in any portion of the repository sub-study. If you decide not to participate in this repository sub-study, this will not affect your ability to receive any benefit to which you are otherwise entitled.

2. What will my participation involve?

If you choose to participate in the DNA repository portion of the sub-study, a blood sample containing your DNA, will be collected once at the beginning of the study. You will not be required to make an extra visit in order to provide this sample.

If you choose to participate in the blood and urine portion of the repository sub-study, urine (approximately two tablespoons) and blood samples (approximately two tablespoons) will be collected at the beginning of the study and at twelve months. You will not be required to make an extra visit in order to provide these samples.

You may choose to participate in either the DNA portion of the repository, the blood and urine portion of the repository or both portions of the repository.

3. How will my samples be collected, stored, and anonymized?

Random codes will be assigned to your blood, urine and DNA samples. The samples will not be labeled with your name or any other information that could identify you. While the trial is ongoing, Dr. *insert name of PI* will know which code numbers belong to your samples. You may request to have any of your samples withdrawn and destroyed at any time while the trial is ongoing. At the completion of the trial, the clinical database will be double-coded. This means that your original subject ID will be recoded as a new anonymous code that could never be linked back to you. This new anonymous subject ID is linked to your clinical data and your samples. You will not be able to have your samples destroyed once the database has been locked and anonymized. The samples you provide will be stored for future testing in a repository maintained by the National Heart Blood and Lung Institute (NHLBI). The samples will be kept for a minimum of the duration of the study, and up to 30 years after the close of the study. No identifying information will be sent with your samples and your samples will not be sold to anyone.

4. What will it cost me?

You and/or your health insurer are responsible for paying the costs of the routine standard of care. There is no cost to participate in this sub-study. You will not receive any payment for your participation in any portion of this repository sub-

study. It is possible that future research done on your samples may help to develop something that is commercially valuable. You will not receive payment for any commercial activity that results from research with your samples.

5. What are the possible risks of taking part in the repository sub-study?

Risks associated with drawing blood from a vein in your arm include momentary discomfort and/or bruising at the site where the blood is drawn. Although unlikely, infection, excess bleeding, clotting, or fainting may occur.

There is an extremely rare chance that a breach of confidentiality could occur as it is possible to identify a person by use of DNA if a comparison DNA sample is available or provided by you.

6. What are the potential benefits of taking part in the repository sub-study?

There are no direct benefits to you for participation in this sub-study. However, the medical knowledge obtained from this sub-study may help treat patients in the future.

7. What about confidentiality?

All documents and information pertaining to this research will be kept confidential in accordance with all applicable federal, state, and local laws and regulations. You should understand that medical records and data generated by the study may be reviewed by *insert name of institution* Institutional Review Board (IRB), the Data Safety Monitoring Board (DSMB), the Office for Human Research Protection (OHRP) of the Department of Health and Human Services, the Sponsor, New England Research Institute, Inc., the United States Food and Drug Administration (FDA), the National heart, Lung and Blood Institute (NHLBI), and the National Institute of Health (NIH) to assure proper conduct of the study and compliance with federal regulations.

Your name or other information that might reveal your identity will not be given. Your samples will not be made available for research studies until the database has been locked and anonymized at the completion of the trial.

This data is being collected with public funds available from the National Heart, Lung and Blood Institute (NHLBI). Therefore, “limited access data” which refers to study data that is changed in such a way so that no identifying information remains, may be released in this public access database. This data will not contain your name or other identifying information. It is possible that samples you provide, or data that is generated using your samples may be released by the NHLBI to qualified investigators at non-profit or for-profit organizations for research purposes.

8. What other options do I have?

You may choose not to participate in any or all parts of this repository sub-study. If you decide not to participate in the repository sub-study, this will not affect your ability to receive any benefit to which you are otherwise entitled. There is no penalty for not participating in this sub-study.

9. What if I get ill or injured as a result of participation?

In the event of injury resulting from your participation in the repository sub-study, the facilities at *insert name of institution* and medical/professional attention will be made available to you at your expense. Financial compensation from *insert name of institution* will not be provided. If you believe that you have suffered an injury related to this research as a participant in this study, you should contact Dr. _____ at telephone number _____.

10. What are my rights as a research participant?

Your participation in this sub-study is entirely voluntary, and refusal to participate will involve no penalty or loss of benefits to you. If you decide not to participate, this will not affect your ability to receive medical care at *insert name of institution* or to receive any benefits to which you are otherwise entitled.

If you decide that you want to withdraw any of your samples during the trial, please contact *insert name of PI*, the Principal Investigator, at *insert contact number of PI*.

Since the repository samples and the clinical database are anonymized at the end of the trial, you will not be able to withdraw your samples once the database has been locked and double-coded at the end of the trial.

You may discontinue participation in the repository study at any time without penalty or loss of benefits to which you are otherwise entitled. Any new information that develops during this study, which might affect your decision to participate, will be given to you immediately.

Your participation in the repository sub-study may be stopped by your doctor, even without your consent, for medical reasons. The sponsor of this study, the National Heart Lung and Blood Institute can end the study at any time, for any reason.

A signed copy of this consent form will be given to you.

11. Whom may I contact with questions?

If you have any questions, at any time, about the repository sub-study, or want to discuss any possible study-related injuries please contact Dr. _____(PI NAME), at telephone number _____(PI PHONE) . If you still have questions regarding the study or your rights as a participant in the study you may discuss them with an administrator of the Institutional Review Board_____at telephone number _____.

PATIENT'S AGREEMENT FOR THE DNA PORTION OF THE REPOSITORY

By signing this form, you are giving consent for any future studies of genes that we may perform in the laboratory. Your DNA sample will remain under the custodianship *of the NHLBI. Your DNA obtained from your blood is stored and tested with an identifying number, and your name will not appear on the stored samples. You will not be told of these possible tests, nor will you receive results of any of these tests.*

__ *I agree to participate in the DNA portion of the repository*

__ *I decline participation in the DNA portion of the repository*

If you agreed to participate in the DNA portion of the repository, please check ONE of the following regarding the diseases to be studied:

__*I agree to allow my DNA sample to be studied for genes related to any disease, health condition or risk factors.*

__*I agree to allow my DNA sample to be study ONLY for genes related to heart disease, stroke, kidney diseases, other cardiovascular diseases, or risk factors associated with these diseases.*

PATIENT'S AGREEMENT FOR THE BLOOD AND URINE PORTION OF THE REPOSITORY

By signing this form, you are giving consent for any future studies using your blood and urine samples that we may perform in the laboratory. Your samples will remain the under the custodianship of the NHLBI. Your blood and urine samples will be stored and tested with an identifying number, and your name will not appear on any of the stored samples. You will not be told of these possible tests, nor will you receive results of any of these tests.

__ *I agree to participate in the blood and urine portion of the repository*

__ *I decline participation in the blood and urine portion of the repository*

I have fully explained to the participant the nature and purpose of this research and the risks involved in participation. I have answered all of his/her questions to the best of my ability.

Date	Research staff signature
	Print name

I have read this consent form or it has been read to me. I have a copy. I understand the research study. All of my questions have been answered.

I know I am free to quit at any time during the study. I understand that if I have any questions at any time, they will be answered.

Date	Subject signature
	Print name