Design and Rationale of the Phase 3 Tafamidis in Transthyretin Cardiomyopathy

Clinical Trial (ATTR-ACT)

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Transthyretin cardiomyopathy (TTR-CM) is a rare, life-threatening disease characterized by the accumulation of amyloid fibrils of misfolded transthyretin protein in the heart.[1] The natural history of TTR-CM is not well characterized with only one small multicenter longitudinal trial conducted[2] along with a few single-center studies in larger but still relatively small populations (~100–300 patients).[3-5] Given the relative paucity of data available on the natural history of TTR-CM, large-scale trials to study the efficacy and safety of a treatment must be designed with little prior knowledge or precedent for choosing endpoints, duration of study, or statistical considerations in a relatively rare disease. The purpose of this review is to provide a brief overview of TTR-CM and to present the rationale behind the design of the Transthyretin Cardiomyopathy Tafamidis clinical trial (ATTR-ACT; NCT01994889), a Phase 3 double-blind, placebo-controlled study of tafamidis meglumine in the treatment of TTR-CM that began enrollment in December 2013.

Transthyretin and TTR Amyloidosis

Transthyretin (TTR; also referred to as pre-albumin) is a 127-amino acid protein, primarily synthesized in the liver, that forms a 55 kDa homotetramer to transport thyroxine and retinol-binding protein-retinol (vitamin A) complex.[6,7] The native TTR homotetramer can dissociate into monomers that can misfold and ultimately form amyloid fibrils.[8] Various mutations of the TTR gene are associated with an increased rate of tetramer dissociation, which is thought to be the rate-limiting step in amyloidogenesis.[9,10]

TTR amyloidosis is a rare disease that occurs when TTR amyloid fibrils aggregate and deposit in various tissues.[11,12] Presenting symptoms and disease course are highly variable and influenced by the underlying TTR mutation, age of the affected individual, gender, and geographic location.[13-15] Typical disease manifestations include severe disabling autonomic and sensorimotor disturbance, cardiomyopathy, glaucoma, vitreous opacities, and gastrointestinal, renal, and urogenital dysfunction. Two phenotypic presentations of the disease predominate: transthyretin familial amyloid polyneuropathy (TTR-FAP), which primarily affects the peripheral nerves, and TTR-CM, which primarily affects the heart, although there is a wide overlap of these phenotypes.

Transthyretin Cardiomyopathy

TTR-CM can be hereditary or non-hereditary. More than 100 TTR mutations have been identified, at least 22 of which are mostly associated with TTR-CM.[16-18] The valine 122 to isoleucine (Val122Ile) is the most common TTR-CM variant, with a prevalence of about 3–4% in the African-American population.[19] The non-hereditary form of the disease is caused by aggregation of the wild-type TTR protein and has been previously referred to as senile systemic amyloidosis. In this non-hereditary form, wild-type TTR forms amyloid fibrils, and deposits primarily in heart tissue. The epidemiology of TTR-CM is not well characterized but the disease is increasingly diagnosed at referral centers, which may be related to an aging population and the greater use of non-invasive cardiac imaging techniques such as cardiac magnetic resonance imaging and 99mTc-phosphate scintigraphy.[20] Based on the frequency of the Val122Ile TTR allele and US census data, up to 100,000 to 150,000 older African-Americans are estimated to be affected by TTR-CM.[21] Recent studies in patients with heart failure with preserved

ejection fraction (HFpEF) found that TTR amyloid deposits accounted for a significant number of HFpEF cases.[22,23] The clinical significance of subclinical wild-type TTR amyloid deposits in the heart in elderly patients with HFpEF has yet to be determined, but these deposits could be contributing to the overall HFpEF syndrome in some elderly patients with HFpEF who do not have overt, clinically apparent cardiac amyloidosis.

TTR-CM is a late-onset disease with symptoms typically manifesting in patients aged 60 years or older.[2] Cardiac symptoms include dyspnea on exertion, fatigue and effort intolerance, orthostatic hypotension, and syncope.[24] Conduction abnormalities including bundle branch block, atrioventricular block, sinoatrial disease, and atrial fibrillation are frequent.[24] Objective measures of cardiac involvement include an abnormal electrocardiogram with low QRS voltage, pseudoinfarction patterns, and discordance between left ventricular wall thickness and QRS voltage; left and right ventricular wall thickening, severely reduced systolic and diastolic longitudinal tissue velocities, and severely reduced absolute global longitudinal strain (with relative apical sparing) demonstrated on echocardiography; predominant subendocardial delayed enhancement with difficulty nulling the myocardium on cardiac magnetic resonance imaging; and elevated plasma troponin and brain natriuretic peptides.[25,26]

Until recently, TTR-CM was definitively diagnosed by histological identification of amyloid deposits in a cardiac biopsy sample, but non-invasive diagnostic methods are being explored.[24] Nuclear scintigraphy using 99mTechnetium (Tc)- labeled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD), 99mTc-labeled pyrophosphate (PYP), or 99mTc-labeled hydroxymethylene diphosphonate (HMDP) to accurately

diagnose TTR-CM has been proposed as a non-invasive method for diagnosing suspected amyloidosis.[27] A study of patients with diagnosed cardiac amyloidosis showed that 99mTc-PYP cardiac imaging was able to differentiate between light-chain (AL) cardiac amyloidosis and TTR-CM in patients with normal light-chains and high 99mTc-PYP uptake.[28] A quantitative heart to contralateral lung ratio, which can be calculated from 99mTc-PYP imaging, of ≥1.6 was associated with worse survival in patients with TTR-CM.[29] This non-invasive diagnostic method is able to identify early-stage TTR-CM in asymptomatic carriers of variant TTR[30] and thus is able to diagnose TTR-CM without the complications that can arise with cardiac biopsy, and may be contributing to the increasing recognition of this disease.

Prognosis is poor in patients with TTR-CM. In patients with wild-type TTR-CM, estimates of median survival time range from approximately 26 months to 67 months from diagnosis[2-4,31,32] and 72 months from symptom onset.[5] Patients with Val122Ile TTR-CM have median survival time from diagnosis ranging from approximately 36 months to 43 months.[2,31,32] Death in most patients with cardiac amyloidosis is from sudden death and progressive pump failure.[24]

Current Treatments for TTR-CM

Symptom management has been the mainstay of therapy for TTR-CM patients with the use of diuretics for symptoms of heart failure and pacemaker placement for bradycardia.[24] The only disease-modifying treatment option currently available for TTR-CM patients is organ transplantation in patients with genetic forms of the disease.[24] Liver transplantation removes the primary production site of the TTR protein and,

theoretically, removes the source of amyloidogenic TTR. For some patients with wild-type TTR-CM, heart transplants may be appropriate depending on the severity of cardiac involvement.[33,34] This treatment is generally not feasible given the scarcity of organs and advanced age of affected patients in addition to the surgical risk and need for life-long immunosuppression.[24] Additionally, evidence suggests that the disease can progress after liver transplant due to deposition of wild-type TTR amyloid fibrils.[35] Diflunisal, a nonsteroidal anti-inflammatory drug, was shown to increase serum TTR stability in 37 patients with TTR-FAP[36] and, in a subsequent clinical trial in 130 patients, to reduce the rate of progression of neurological impairment over 2 years.[37] In a cohort of 13 patients with ATTR-CM, a single-arm, open-label cohort study suggested that at a low dose (250 mg, orally twice a day), and with careful monitoring, diflunisal along with a prophylactic proton pump inhibitor (PPI) could be safely administered to selected patients, although an association was observed between chronic diflunisal use and adverse changes in renal function, suggesting that severe renal dysfunction may be prohibitive to diflunisal administration.[38] Diflunisal is not approved to treat TTR-FAP or TTR-CM and, as a nonsteroidal anti-inflammatory drug, its chronic use may present a safety concern for patients at risk of renal, gastrointestinal, or cardiovascular disease. At present, there are no approved pharmacologic therapies that have been shown to improve survival in TTR-CM.

Mechanism and Rationale for Tafamidis Therapy in TTR-CM

Tafamidis is an oral-administered small molecule approved in parts of Europe, Asia, and Latin America to delay neurologic progression of TTR-FAP. Tafamidis binds to TTR at the thyroxine binding sites and inhibits TTR tetramer dissociation, the rate-limiting step

in the amyloidogenic process.[39] By stabilizing the tetrameric native state of TTR, tafamidis increases the activation barrier associated with tetramer dissociation. It has been shown to slow fibril formation *in vitro*[40] and in TTR variants associated with cardiac phenotypes, selectively binds and stabilizes TTR tetramers from plasma.[41,42] Using a validated immunoturbidimetric assay, an analysis of TTR from patient plasma found that tafamidis stabilized wild-type and Val122Ile TTR tetramer in 34 of 35 (97.1%) patients at the end of 6 weeks and 28 of 35 (88%) at the end 12 months of treatment.[41]

Clinical trial data have demonstrated long-term efficacy and safety of tafamidis in the treatment of TTR-FAP.[42-46] In a randomized, placebo-controlled trial in patients with TTR-FAP, those in the intent-to-treat population who received tafamidis demonstrated 52% less neurologic deterioration after 18 months of treatment than patients who received placebo.[45] Based on these and other data, it was hypothesized that tafamidis might also slow or halt the progression of TTR-CM and in a Phase 2 open-label, interventional study, 12 months of tafamidis 20 mg given once daily to patients with Val122Ile and wild-type TTR-CM stabilized disease progression with an acceptable safety profile.[41]

Design and Rationale of ATTR-ACT

The Phase 3 ATTR-ACT study is an international, multicenter, double-blind, placebo-controlled, randomized clinical trial. Enrollment began in December 2013 and was completed in August 2015; the study is ongoing. The primary objective is to evaluate the efficacy, safety, and tolerability of tafamidis (20 mg or 80 mg orally once daily) in comparison with placebo for the treatment of TTR-CM. Patients completing this study

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will be given the option of continuing into a long-term extension study of tafamidis. The appropriate Institutional Review Board and Independent Ethics Committee approvals were secured before start of the study.

Study Design and Dose Selection

This study utilizes a three-arm, parallel, placebo-controlled, randomized study design with a 30-month double-blind treatment phase (Figure 1). The duration of the treatment period was based on the reported median survival time from diagnosis for TTR-CM patients available at the time of study initiation.[2-4,31,32]

The 20 mg dose of tafamidis has been successfully used previously in both polyneuropathy and cardiomyopathy clinical trials.[11,42,45,47] While this dose was shown to stabilize wild-type and Val122Ile TTR tetramer in the majority of patients after 12 months treatment using an immunoturbidity assay,[41] a greater range of tafamidis exposures have been used to assess TTR stabilization in healthy volunteers and in ATTR cardiac amyloid subjects using a subunit exchange assay,[48] demonstrating the potential for a greater degree of stabilization at TTR:tafamidis stoichiometry values greater than those achieved at the 20 mg dose.[49] The inclusion of an 80 mg dose, which results in near maximal TTR stabilization, permits exploration of a higher dose to assess efficacy and safety of adequately separated doses.[50] In the event that patients experience adverse events that could impact dosing adherence, they will receive a blinded dose reduction. Patients receiving the 80 mg dose will be reassigned to the 40 mg dose, which was empirically selected in an effort to allow patients who could not tolerate the higher dose to potentially remain in the study at a dose higher than 20 mg.

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Patients receiving either placebo or the 20 mg dose of tafamidis will be maintained on that dose.

Patient Population

The inclusion criteria were chosen to select patients with a predominant cardiac phenotype; specifically, documented TTR-CM with either wild-type TTR or a variant TTR genotype (assessed by genotyping, with patients with concurrent monoclonal gammopathy of undetermined significance requiring a confirmatory test using mass spectrometry or immunohistochemistry), the presence of TTR amyloid deposits in biopsy tissue, age ≥18 to ≤90 years, and a history of heart failure evidenced by at least one prior hospitalization for heart failure or clinical evidence of heart failure (without hospitalization) requiring diuretics. TTR precursor protein identification can also be made by immunohistochemistry or mass spectrometry. Nuclear scintigraphy using 99mTc-labeled PYP, HMDP, or DPD is used as a confirmatory test of TTR involvement.[27]

A 6-minute walk test (6MWT) of >100 meters is also required for inclusion, so patients with advanced stage disease (New York Heart Association [NYHA] functional class IV) who are unlikely to benefit are not enrolled. As patients with variant TTR-CM are potentially more likely to have a mixed neurologic/cardiac phenotype, they may also be more likely to experience difficulties completing this test. As such, and also to account for any other variation between groups, the study intends to enroll a minimum of 30% variant TTR-CM patients and 30% wild-type TTR-CM patients. To ensure patients included in the study have a cardiac cause for their symptoms and to ensure a sufficient

event rate within the 30-month duration of the study, a plasma N-terminal prohormone of brain natriuretic peptide (NT-proBNP) concentration ≥600 pg/mL is required for enrollment.

Major exclusion criteria include a confirmed diagnosis of light chain (AL) amyloidosis, previous treatment with tafamidis, an estimated glomerular filtration rate of <25 mL/min/1.73 m^2, and concurrent treatment with non-steroidal anti-inflammatory drugs, tauroursodeoxycholate and doxycycline, calcium channel blockers, or digitalis. Additional exclusion criteria include modified-body mass index (mBMI) of <600 kg/m^2•g/L, and heart failure not due to TTR-CM.

Study Assessments

The efficacy assessments (Table 1) include all-cause mortality and frequency of cardiovascular-related hospitalization, which includes heart failure, arrhythmia, myocardial infarction, and stroke as well as other cardiovascular-related events. Quality of life assessments include the Kansas City Cardiomyopathy Questionnaire (KCCQ) to assess the functional and clinical limitations of patients with heart failure; the EuroQOL-5 Dimension-3 Level, a self-administered generic health status instrument to assess quality of life; and the Patient Global Assessment to assess overall health status. In addition, disease progression is measured using the 6MWT and the NYHA functional classification. These assessments are conducted at baseline, at 6-month intervals, and at the end of the study or at the time of patient discontinuation. For subjects who discontinue prior to Month 30, a vital status follow-up is conducted at Month 30 to obtain their mortality status for use in the analysis. Other efficacy assessments include TTR

stabilization, plasma concentrations of TTR oligomer and troponin I and mBMI which

serves as an index of nutritional status. NT-proBNP and troponin T are measured since

they are associated with survival in wild-type TTR-CM.[4] To monitor compliance to the

protocol, pharmacokinetic samples to determine tafamidis and diflunisal concentrations

are also collected. Diflunisal is a nonsteroidal anti-inflammatory drug, not approved to

treat TTR-FAP, but has been shown to slow TTR-FAP progression.[37]

Safety assessments (Table 1) include incidence of adverse events, vital signs,

echocardiograms, and clinical laboratory tests. These assessments are conducted at

baseline and at clinic visits until the end of the study or patient discontinuation.

Study Endpoints

The primary analysis will use a hierarchical combination of all-cause mortality and

frequency of cardiovascular-related hospitalizations (defined as a non-elective

admission to an acute care setting for medical therapy for cardiovascular-related

morbidity resulting in at least a 24-hour stay). Key secondary endpoints comprise the

change from baseline to Month 30 in the 6MWT and KCCQ-overall score. Additional

secondary and exploratory endpoints will be analyzed. This protocol utilizes an Endpoint

Adjudication Committee (EAC) to determine whether certain investigator-reported

events meet the definition of cardiovascular-related efficacy endpoints, using pre-

defined endpoint criteria. The EAC is independent of the study sponsor.

Statistical Considerations

The sample size is based on findings from previous studies and an understanding of

current clinical outcomes in this population, and the uncertainty of the assumptions

derived from these limited data available at the time of study initiation.[2,45] Sample size assumptions include an all-cause mortality rate of 12.5% for the tafamidis group and 25.0% for placebo group (i.e. 50% reduction in mortality with treatment) and an average cardiovascular-related hospitalization frequency of 1.5 for the tafamidis group and 2.5 for the placebo group over 30 months. For a significance level of 0.05 (two-sided test), a sample size of 300 (assuming pooled tafamidis 20 mg and tafamidis 80 mg versus placebo) yields a power >90%. To mitigate the uncertainty around the study design assumptions due to the lack of data, target sample size was increased from 300 to 400 planned.

Patients were allocated to the 3 arms of the study in a 2:1:2 ratio (placebo:20 mg:80 mg). Patients were stratified by baseline NYHA functional class (NYHA Class I and NYHA Classes II and III combined) and TTR genotype (variant or wild-type) with a minimum of 30% variant and 30% wild-type across the overall study. The stratification factors were utilized at randomization.

The primary analysis uses a hierarchical combination of all-cause mortality and frequency of cardiovascular-related hospitalizations over the duration of the trial. Heart transplant, heart and liver transplant, and implantation of a cardiac mechanical assist device are treated as death for this analysis. The primary analysis pools the patients in the tafamidis 20 mg and tafamidis 80 mg groups (including patients in the 80 mg group that may have had a dose reduction to 40 mg) into one group. This pooled tafamidis group is to be compared with the placebo group using the Finkelstein-Schoenfeld method.

The Finkelstein-Schoenfeld method[51] (Table 2), increases the sensitivity and power of the analysis while also preserving the importance of the all-cause mortality endpoint. The test is based on the principle that each patient in the clinical study is compared with every other patient within each stratum in a pair-wise manner. The method recognizes the higher importance of all-cause mortality. The pair-wise comparison proceeds in a hierarchical fashion using all-cause mortality first, assigning a +1 to the "better" patients and a –1 to the "worse" patients. The test statistic is based on the sum of these scores within each strata and then summed across the 4 strata. The null hypothesis for the primary analysis is that neither all-cause mortality nor frequency of cardiovascular-related hospitalizations is different between the tafamidis and placebo treatment groups. The corresponding alternative hypothesis is that at least one and possibly both mortality and frequency of cardiovascular-related hospitalizations are different between the tafamidis and placebo treatment groups.

The key secondary endpoints are evaluated using a mixed model repeated measures analysis of covariance with an unstructured covariance matrix (or as appropriate); center and patient-within-center as random effects; treatment, visit, TTR genotype (variant and wild-type), and visit-by-treatment interaction as fixed effects and baseline score as covariate. As a secondary analysis, the components of the primary endpoint (i.e. all-cause mortality and frequency of cardiovascular-related hospitalizations) are analyzed separately.

All randomized patients who receive at least 1 dose of study treatment are included in the safety analysis. All adverse events observed from the time of first dosing

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with study medication (at randomization) until the end of study participation are included in the safety analysis. Adverse events that occur during treatment are reported separately if the event occurred prior to randomization. All adverse events are coded according to the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by treatment group.

Discussion

This trial is the largest multicenter investigation of a treatment for TTR-CM, a condition associated with high mortality and symptom burden for which there are no approved disease-modifying pharmacotherapies. The primary objective of the study is to evaluate the efficacy of tafamidis treatment compared with placebo in patients with biopsy-confirmed TTR-CM. The safety and tolerability of 2 tafamidis doses, 20 mg and 80 mg, also will be assessed.

Enrollment for this trial began in December 2013 and was completed in approximately 1.5 years, which is relatively rapid compared with other heart failure studies.[52,53] There are a few potential reasons for this expedited enrollment, including a low screen failure rate, clear objective criteria for diagnosis of TTR-CM, a lack of available treatments, and high mortality rate in this disease. Access to the study drug in the long-term extension study upon completion of this study also may have encouraged patient enrollment.

The progressive nature of some rare diseases, like TTR-CM, makes clinical requirements of a disease-modifying therapy more relevant to the stage of disease (i.e. early stage versus late stage).[54] Since tafamidis is predicted to delay disease

progression by stabilizing the TTR tetramer, it is necessary to balance selection of a cohort at an early disease stage with one that has event rates sufficient to compare outcomes with the placebo group. It is anticipated that the stringent inclusion and exclusion criteria of this trial combined with the high enrollment rate provide sufficient data to allow meaningful interpretation of the results.

Well-characterized endpoints to determine efficacy of a pharmacologic treatment are difficult to define for rare diseases. Additionally, endpoints that are clinically meaningful to patients and regulators must be chosen. The endpoints for the ATTR-ACT study were chosen using data from the available natural history studies and clinical trials.[2-5,41] In rare disease studies where there are small patient populations, the Finkelstein-Schoenfeld method can increase the sensitivity and power of the analysis while also preserving the importance of the all-cause mortality endpoint. This method is used in the present study and will help ensure that the selected endpoints allow clear conclusions about the effectiveness of tafamidis in treating TTR-CM.

Limitations of this study include the following: the limited availability of information on the natural history of cardiac amyloidosis which poses challenges in designing a robust clinical trial to evaluate treatment, the impact of multiple chronic conditions prevalent in an aging study population on outcomes of interest (QOL and submaximal functional capacity), and mobility limitations may lead to high dropout rates. The mixed neurologic/cardiac phenotype of some TTR-CM patients, especially those with mutations, may also impact efficacy endpoints such as mobility measures like the 6MWT. The study design aims to minimize this potential limitation by ensuring a

minimum of 30% variant and 30% wild-type patients in the study. Patients with NYHA

Class II/III may also be overrepresented in the study and preclude treatment effects

given the extent of disease progression. The clear inclusion criteria may reduce the

impact of this limitation.

Summary

The ATTR-ACT study is designed to evaluate clinically meaningful endpoints for

patients, families, and regulators and will provide important insights into the efficacy and

safety of tafamidis for the treatment of TTR-CM. The design of this study will also inform

other studies in rare disease that must manage similar challenges in design and

recruitment.

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[[Figure Legend]]

Figure 1 Study design

[[TABLES]]

Table 1. Assessments for Efficacy and Safety

Efficacy
All-cause mortality and cardiovascular-related hospitalization
Kansas City Cardiomyopathy Questionnaire
6-Minute Walk Test
EuroQOL-5 Dimensions-3 Level
Patient Global Assessment
Modified Body Mass Index
TTR Stabilization, oligomer concentration, and concentration measurements
New York Heart Association Functional Classification
N-Terminal Prohormone of Brain Natriuretic Peptide
Troponin I
Echocardiograms
Electrocardiograms
Safety
Adverse Events
Medical History
Physical Examinations
Vital Signs
Clinical Laboratory Tests

TTR indicates transthyretin.

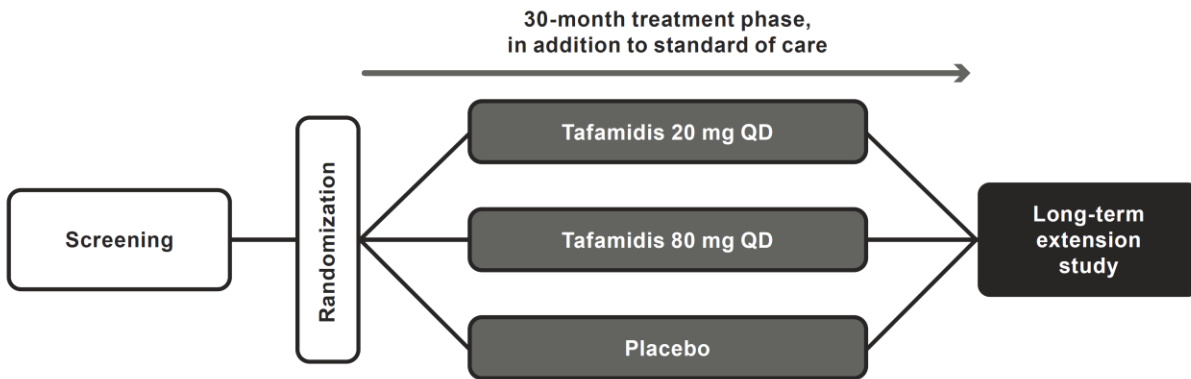**Table 2. Finkelstein-Schoenfeld Scoring Algorithm**

In each scenario, a pair-wise comparison of patients is made by first taking mortality into account. If there is a clear difference (scenario 1), then a score is assigned. If there is no difference (scenario 2), then survival time is considered and a score is assigned if there is a clear difference between the patients. If there is no difference between the 2 patients in survival time, then the frequency of cardiovascular hospitalization (scenario 3) is considered and a score is assigned. If there is no difference in cardiovascular hospitalization frequency between the 2 patients (scenario 4), then no score is assigned. If there is missing information (scenario 5 and 6), then the next assessment in the hierarchy is considered.

Scenario	Mortality	Survival Time	Cardiovascular Hospitalization Frequency	Score
1	Dead	-	-	−1
	Alive	-	-	+1
2	Dead	Low	-	−1
	Dead	High	-	+1
3	Dead	tied	High	−1
	Dead	tied	Low	+1
4	Dead	tied	tied	0
	Dead	tied	tied	0

5	Alive	-	High	−1
	Alive	-	Low	+1
6	Alive	-	tied	0
	Alive	-	tied	0

[[FIGURE]]

Figure 1 Study design

QD indicates once daily.