

Clinical Protocol

- Ad5.hAC6 Gene Transfer for CHF -

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CLINICAL PROTOCOL

6.1 INTRODUCTION & RATIONALE

6.1.1. **Specific Aim: To determine the safety and potential efficacy of intracoronary delivery of an adenovirus encoding adenylyl cyclase 6 in patients with stable but severe heart failure in a Phase 1 / Phase 2 clinical trial**

We propose to conduct a randomized, placebo-controlled, double-blinded Phase 1 / Phase 2 clinical trial using adenylyl cyclase type 6 (AC6) gene transfer in patients with stable but severe congestive heart failure (CHF). We will use intracoronary delivery of a replication incompetent human adenovirus-5 (E1/E3-deleted) encoding human AC6 (Ad5.hAC6). The clinical trial will involve a single administration of increasing doses of Ad5.hAC6 (3.2×10^9 - 10^{12} vp). Patients will receive intracoronary Ad5.hAC6 or phosphate buffered 3% sucrose (placebo) in a 3:1 randomization ratio (Ad5.hAC6 : placebo). Intracoronary nitroprusside will be infused during test substance administration in both groups. We propose to enroll 56 patients — 14 will receive intracoronary placebo and 42 will receive graded doses of intracoronary Ad5.hAC6. The highest Dose Group, which will include 12 Ad5.hAC6 patients, will then be compared to a cumulative placebo group of 14 patients. In addition, the two highest doses will be combined (Ad5.hAC6, n=24) and compared to placebo (n=14) for an additional comparison, which will increase statistical power. These comparisons will enable a rational selection of doses to use in subsequent trials. Patients will be recruited at the VA San Diego Healthcare System in La Jolla, CA, the Minneapolis Heart Institute Foundation in Minneapolis, MN, the Northwestern Memorial Hospital in Chicago, IL, and Fletcher Allen Healthcare in Burlington, VT. Safety will be monitored intensively and beneficial effects evaluated by exercise performance, echocardiography, hemodynamic evaluation, and symptoms.

6.1.2. **Rationale**

There are three elements that provide a rationale for a new approach for the treatment of CHF using gene transfer. These, together with substantial preclinical data regarding safety and efficacy, justify a carefully conducted clinical trial. **1) CHF is a serious life-threatening disease.** Patients with Class III and IV heart failure (symptoms at rest or with minimal activity) even when optimally treated, have a 4-year mortality of 50% — a prognosis worse than many cancers. **2) CHF is a prevalent disease.** CHF is the only cardiovascular disease that is increasing in prevalence. It is the major non-elective admitting diagnosis in patients over 65 years of age in the United States, where it is estimated to affect 5 million people. **3) There is an unmet medical need in the area of CHF therapy.** Although life prolongation is observed with angiotensin converting enzyme inhibitors, β -adrenergic receptor antagonists, and implanted cardiac defibrillators, no CHF treatment has resulted in life prolongation beyond months to a few years, prognosis remains dismal despite optimal therapy, and side effects of available treatments are protean and substantial. Current optimal therapy has not resulted in reversing this inexorable disease. Patients, physicians, the American College of Cardiology and the American Heart Association all recognize the need for better therapeutic options for patients with CHF.

6.2. BACKGROUND

6.2.1. Adenylyl Cyclase and Heart Failure

Adenylyl cyclase (AC), the effector enzyme that mediates cAMP synthesis, is the central focus in the current proposal. Molecular cloning studies indicate that there are at least ten different isoforms of AC, each with different structure, tissue, and chromosomal distribution and regulation. Such differences in regulation can include stimulation or inhibition by $G\beta\gamma$, $G\alpha_i$, Ca^{2+} and various protein kinases. Cardiac myocytes appear to express AC5 and AC6 predominantly,¹⁻³ two AC isoforms that are related in structure and function, but which fulfill unique biological roles.

AC plays a pivotal role in contractile responsiveness, is tightly linked to left ventricular (LV) function, and is functionally impaired in heart failure. Cardiac-directed expression of AC6 has favorable effects on LV contractility.^{4,7} Among AC isoforms, AC6 has features that make it a logical candidate to increase LV function in the setting of heart failure. First, the inhibitory GTP-binding protein, $G\alpha_i$, inhibits cAMP generation *via* AC5 and AC6,³ the dominant AC isoforms in cardiac myocytes. This feature may provide protection against β -adrenergic receptor (β AR) overstimulation. Second, submicromolar levels of intracellular calcium — which stimulate several AC isoforms — either have no effect or inhibit AC6 and AC5,³ thereby providing an additional modulation of β AR activation through AC. Finally, recent studies have shown that increased AC6 content has favorable effects on cardiac myocytes that are independent of cAMP generation but which would increase contractile function.⁸ For example, AC6 gene transfer increases sarco(endo)plasmic reticulum Ca^{2+} -ATPase 2a (SERCA2a) calcium uptake,⁹ reduces phospholamban (PLB) expression¹⁰ and phosphorylation, and increases Akt activation through inhibition of an Akt-specific phosphatase, PH domain and leucine rich repeat protein phosphatase (PHLPP).¹¹ These unique features make the prospect of AC6 gene transfer fundamentally different from other agents that increase intracellular cAMP (dobutamine, milrinone), but which have failed to prolong life in CHF.

Our group and others have reported down regulation of myocardial AC5¹ and AC6^{1,2} in animal models of heart failure. Might restoration of cardiac AC6 content increase contractile function in CHF? AC6 may circumvent the deleterious features of stimulation of proximal elements of this pathway by remaining under regulation by $G\alpha_i$ and intracellular calcium, and through increased calcium handling, PLB phosphorylation, and reduced apoptosis (Akt activation), effects which are not dependent on cAMP. The relative advantages of AC5 vs AC6 in this regard are not precisely known. However, when these transgenes are expressed in murine cardiomyopathy, only AC6 increases heart function and prolongs life,^{5,6,12} and we recently found that targeted deletion of AC5 is associated with increased basal LV dP/dt,¹³ indicating that increased cardiac AC5 content may decrease LV function. In contrast, AC6 deletion is associated with marked reduction in calcium handling and reduced LV function.¹⁴

Two hallmarks of low ejection fraction heart failure of diverse etiologies are decreased ability of cardiac myocytes to generate cAMP and depressed myocardial contractility. Previous treatments for clinical heart failure have focused on increasing myocardial cAMP content using pharmacological agents that stimulate the β AR (dobutamine) or decrease the breakdown of cAMP through phosphodiesterase inhibition (milrinone). In general these efforts have failed, perhaps because of deleterious consequences of unrelenting stimulation of the β AR pathway. Indeed current approaches embrace the use of β AR antagonists over agonists for management of compensated heart failure. Paradoxically, increasing AC6 in cardiac myocytes does not result in sustained cAMP generation.^{4,7,15-18} A goal of our laboratory has been to determine how AC6

confers protective effects to the heart while other elements in this signaling pathway do not.^{8-11,19-21} Our preclinical data^{5,6,17,18} supports the idea that AC6, unlike other elements in this pathway, will be beneficial to patients with heart failure. These data and additional unpublished data provide a sound rationale for a clinical trial of AC6 gene transfer in CHF and will be reviewed in detail in the subsequent sections of this document.

6.2.2. Clinical Congestive Heart Failure

Patients with heart failure associated with symptoms at rest or with the activities of daily living (Class III and Class IV) have a poor long-term outcome with current optimal therapy, with up to 40% of patients dying within 3-4 years after the onset of symptoms.²² We need new treatments for severe heart failure. Recent advances show prolongation of life with angiotensin converting enzyme inhibitors, and, in selected patients, β AR antagonists. Even with optimal medical management, heart failure is an inexorable disease and is associated with excessive morbidity and substantial short-term mortality. In addition to high mortality, dilated systolic heart failure is associated with reduced LV contractile function, LV dilation, reduced ejection fraction, and elevations in LV filling pressure and pulmonary artery wedge pressure. Hallmarks of CHF include exercise intolerance and increased plasma levels of norepinephrine and B-type natriuretic peptide (BNP), often used as an indicator of successful therapy.²³

The proposed study will focus on exercise tolerance, hemodynamic measurements and serial samples of serum to assess BNP levels — all of these measures will reflect the overall status of CHF. There will be some variation in these measurements. We have included a control group to estimate the variability. This will assist in our interpretation of clinical indicators regarding the safety and potential efficacy of our proposed gene transfer studies. The protocol is designed to determine whether Ad5.hAC6 can attenuate the progression of disease and reverse some of the manifestations of disease in patients with stable but severe CHF. We think it unlikely that global heart geometry will return to normal, particularly since many of the patients enrolled are likely to have heart failure due to previous myocardial infarction. Although myocardial scar (infarction) may not directly be affected, Ad5.hAC6 may increase intrinsic contractile function of uninfarcted (viable) LV and thereby reduce symptoms and increase cardiac reserve and exercise tolerance. Finally, recent data show that increased expression of AC6 in the failing heart reduces myocardial apoptosis, which would preserve LV function over time.²⁴

Alternative therapies for CHF include coronary artery bypass grafting (CABG) or percutaneous coronary interventions (PCI) for patients with severe myocardial ischemia, medical therapy, biventricular pacing, implantable cardiac defibrillators (ICD) and cardiac transplantation. Alternative therapeutic options for CHF indicated for specific patients (according to AHA/ACC Guidelines) will not be excluded during our study. Patients that are appropriate candidates for PCI or CABG will not be recruited, and if their physician decides that the indications for these procedures exist after recruitment into the study, such patients will undergo revascularization and not remain in the proposed trial. Patients who are to receive cardiac transplantation and those requiring intravenous inotropes will not be recruited into the study; should transplantation become desirable during the study, this will be accommodated. Patients that are taking digitalis, diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, β AR antagonists, spironolactone, and other pharmaceutical agents for the treatment of heart failure will be enrolled and will not be asked to discontinue these medications. Patients in whom biventricular pacemaker leads have been deployed to enable cardiac resynchronization therapy (CRT) will not be enrolled unless they have persistent Class III or IV

CHF and meet all other criteria. In the proposed trials, all enrolled patients must have an ICD in place. Medical therapy is somewhat successful in reducing symptom severity, and, in some cases, may prolong life. But even with optimal therapy the long-term outlook for patients with severe CHF is dismal. Heart transplantation has an 80% 5-year survival rate, an improvement over medical therapy, but very few patients are eligible for transplantation, and donor hearts are not readily available. Only 3000 procedures are performed in the US each year, which represents <0.1% (1 per 1000) of 4-5 million patients with severe CHF.

Several means of increasing intracellular cAMP exist. Most provide short-term improvement of heart function in patients with severe CHF. However, none of the agents that increase intracellular cAMP are similar to AC6. For example, constant infusion of a β AR agonist (like dobutamine) or oral or intravenous administration of a phosphodiesterase inhibitor (like milrinone) increases cAMP and myocardial contractile function. However, previous trials that used dobutamine or milrinone have not been associated with life prolongation, although their utility in symptom relief is recognized.²⁵ These agents, in contrast to the therapy we propose, provide unremitting stimulation of the heart or sustained elevations in cAMP. Increased AC6 expression does not result in sustained β AR stimulation and, in preclinical studies, appears to be safe and efficacious.^{4-7,16-18} β AR antagonists have been successful in patients with compensated CHF. AC6 therapy, we believe, will be safe for patients with both Class III and Class IV symptoms. Patients on β AR antagonists who are enrolled in the proposed trial will continue such therapy—indeed, we believe that AC6 will provide additive beneficial effects in these patients due to increased contractility *via* cAMP-independent mechanisms,⁸ including reduced expression and function of phospholamban,¹⁰ improved calcium handling,⁹ and Akt activation.¹¹

6.2.3. Intracoronary Adenovirus Vector Delivery in Patients

6.2.3.1. Rationale. The adenovirus vector will be delivered into the coronary arteries to expose the heart to the highest possible concentration of vector.^{16,17} We believe that the portal of entry into the cardiac interstitium is through the coronary capillary endothelial cells *via* vesicular transport (transcytosis). By volume, the majority cell type in the heart is the cardiac myocyte. Gene transfer is necessary for the proposed studies because the rationale is to increase cardiac content of a specific AC that will then increase contractile function through cAMP-dependent and cAMP-independent effects.^{4,9-11,15-17} This cannot be achieved by infusion of the protein itself, which, at 150 kD, would not gain entry into the cell.

6.2.3.2. Safety. There is no previous experience with intracoronary delivery of Ad5.hAC6 in patients. There have been clinical trials in which an E1-deleted adenovirus encoding human fibroblast growth factor-4 (Ad5.hFGF4), an angiogenic gene, was delivered into the coronary arteries of 450 patients with angina (aggregate number from four randomized, double-blind clinical trials),²⁶⁻²⁸ in doses ranging from 3.3×10^8 to 3.3×10^{10} vp.

6.2.3.3. Previous Clinical Trials Using Intracoronary Adenovirus Vectors

6.2.3.3.1. AGENT-1 enrolled 19 PBS-treated and 60 Ad5.hFGF4-treated patients (3.3×10^8 to 3.3×10^{10} vp) in a randomized, double-blind clinical trial.²⁶ Two patients that received Ad5.FGF4 at a dose of 3.3×10^9 vp had transient elevations in serum glutamic oxaloacetic transaminase (SGOT). One week after Ad5.hFGF4 administration, one patient had an SGOT increase 2-fold the upper limit of normal; the other patient showed a 20-fold elevation. Both patients had normal SGOT values by 4 weeks, and these values remained normal 12 months later. SGOT

elevations were not observed in the two higher dose groups. Transient mild temperature elevation occurred in 3 of 11 patients in the highest dose group (3.3×10^{10} vp).

6.2.3.3.2. AGENT-2 enrolled 17-treated and 35 Ad5.hFGF4-treated patients (10^{10} vp) in a randomized, double-blind clinical trial.²⁷ Transient, mild (<2-fold upper limit of normal) elevations were noted in SGOT or SGPT. SGOT levels increased in 3 of 17 PBS-treated (18%) and 10 of 34 Ad5.hFGF4-treated patients (29%). SGPT levels increased in 5 of 17 PBS-treated (29%) and 10 of 35 (29%) Ad5.FGF4-treated patients. Transient reductions in platelet count were observed in 1 PBS-treated and 5 Ad5.hFGF4-treated patients—in only 1 patient was the value <100,000/μl. One patient who received Ad5.FGF4 developed transient fever, myalgia, and a transient rise in total bilirubin to 1.8 mg/dl (direct 0.6 mg/dl)—2w after Ad5.hFGF4 administration the bilirubin level had returned to normal.

6.2.3.3.3. AGENT-3 & AGENT-4 (combined) enrolled 177 PBS-treated, and 355 Ad5.hFGF4-treated patients (10^9 vp, n=166; 10^{10} vp, n=165) in a randomized, double-blind clinical trial.²⁸ The occurrence of adverse events from initial administration to a minimum of 23 months of follow-up was no different between PBS-treated and Ad5.hFGF4-treated patients, with the exception of transient fever (PBS: 3.4%; Ad5.hFGF4: 7.6%; p<0.038). Fevers occurred within the first few days of Ad5.hFGF4 administration and resolved with no treatment or with antipyretic medication. In all studies (AGENT 1-4), hemodynamics during and after product administration were stable²⁸ and no complications were associated with intracoronary administration. Serial measurements of cardiac troponin — the best serum indicator of myocarditis²⁹ — were normal, indicating that clinically significant myocardial injury did not occur.

It is important to emphasize that these trials (AGENT 1-4) were conducted in a different patient population than proposed in the current study (angina vs CHF) and used a different transgene (hFGF4 vs hAC6).²⁶⁻²⁸ In addition, our highest proposed dose of Ad5 is 30-fold higher than the highest dose previously used (10^{12} vs 3.3×10^{10} vp). Even so, it is reassuring that 450 patients have received intracoronary adenovirus and the rate of complications, except for transient mild fever, has been similar to patients that received intracoronary PBS.

6.2.3.3.4. Outcome. The initial trials using intracoronary Ad5.hFGF4 resulted in indications of efficacy;^{26,27} but were not sufficiently powered to conclude that treadmill time was increased (primary endpoint). Large-scale Phase 2b / Phase 3 clinical trials were then conducted (AGENT-3, AGENT-4).²⁸ However, despite a favorable safety profile, interim analyses showed that patients who received intracoronary Ad5.hFGF4 did not have increased exercise duration, and the trials were discontinued. However, retrospective analysis uncovered a significant increase in exercise duration in women,²⁸ and a randomized clinical trial enrolling women with myocardial ischemia was initiated in early 2008.

Nevertheless, the efficacy endpoint in the AGENT-3 trial was not met. Why proceed with another trial using a similar delivery method?

There are three differences between the recent Phase 2b/3 clinical trial of Ad5.hFGF4 (AGENT-3) and the proposed trial (**Table 1**): 1) We will use a higher maximal dose of

Table 1. Proposed Clinical Trial vs AGENT-3		
	AGENT 3²⁸ Ad5.hFGF4	Proposed Trial Ad5.hAC6
Highest Dose (vp)	10^{10}	10^{12}
Nitroprusside?	No	Yes
CV Disease	Angina	CHF

adenovirus vector; 2) we will use intracoronary nitroprusside, which increases the extent of cardiac gene transfer;³⁰ and 3) we will be treating a different disease with a different transgene. These differences, combined with our preclinical data, provide a rationale for the proposed clinical trial.

6.2.4. Selection of Vector — Why Adenovirus?

In selecting from among adenovirus, AAV or lentivirus, the delivery method dictates the selection of vector. Lentivirus vectors are unsuitable for intracoronary delivery because they do not readily cross endothelial cells and therefore do not gain access to the cardiac interstitium.³¹ Using indirect intracoronary delivery, we obtain up to 50% transgene expression in murine heart with adenovirus vectors,³² but we are unable to detect any gene expression with very high dose HIV1-based lentivirus vectors using the same methods (unpublished data). The direct intracoronary delivery method, as we will use in the proposed clinical trial, provides 50-100 fold higher gene transfer efficiency with adenovirus than with an AAV2 vector³³⁻³⁵ (**Table 2**). Newer AAV vectors, especially AAV9, may provide increased cardiac gene transfer efficiency.³⁶ However, insert size is limited in AAV vectors —only 4.7 kb— enough for the AC6 cDNA (3.4 kb), but insufficient to include tet-regulated expression elements (an additional 2.0 kb) to enable termination of transgene expression, which we feel is important when using vectors that provide perpetual transgene expression.

	Dose - vp -	Efficiency - % LV -	Citation
Adenovirus	10 ¹¹	30% (rabbit)	Gene Ther 1: 51, 1994 ³³
	10 ¹²	39% (pig)	Circ Res 96: 767, 2005 ³⁴
AAV2	5x10 ¹³	2% (pig)	J Gene Med 7: 316, 2005 ³⁵

Of paramount importance in considering vectors is the degree to which the vector will incite an inflammatory response in the tissue and host into which it is delivered. Most virus vectors have been modified so that they are unlikely to cause a clinical infection. However, proteins encoded by adenovirus vectors are recognized as foreign antigens by the host with resultant cell-mediated and humoral immune responses. In some applications this has been an insurmountable problem with adenovirus vectors. Recent modifications, including deletions of virus DNA so that virus protein expression is reduced, have provided vectors that may be associated with less immune response. Route of delivery, dose, and targeted tissue also are important determinants of an inflammatory response. For example, with intracoronary delivery of first and second generation adenovirus vectors (**Table 3, next page**), myocardial inflammation is not reported except when very high amounts of adenovirus (7.5x10¹² vp per gram of LV perfused) are used.^{16,17,26-28,33,34,37-48} The highest dose proposed in our current clinical trial is 10¹² vp, which represents 1.3x10¹¹ vp per gram of LV perfused. In contrast, when adenovirus vectors are directly injected in the LV wall, a dose-dependent inflammatory response is seen.⁴⁹ This may reflect high local concentrations of adenovirus and a direct cytopathic effect with subsequent inflammation. The relative absence of myocarditis after intracoronary delivery of adenovirus (**Table 3, next page**) differs from studies that have shown that adenovirus vectors induce cellular immune responses in other target organs, which may account, at least in part, for observed transient gene expression.⁵⁰⁻⁵³ The cellular immune response generally is directed toward capsid proteins and involves antigen presentation by major histocompatibility complex (MHC) class I molecules,⁵³ which play a fundamental role in foreign antigen presentation to the cellular

Table 3. Direct Intracoronary Delivery of Adenovirus

Ref	Species	Vector	vp per gram LV	Day Post-Rx	Efficacy	Myocarditis
26	Human	Ad.FGF4 (-E1)	2.2x10 ⁶ -2.2x10 ⁸	28-96	Yes*	No
27	Human	Ad.FGF4 (-E1)	7.1 x 10 ⁷	56	Yes*	No
28	Human	Ad.FGF4 (-E1)	7.1 x 10 ⁷	365	No*	No
37	Pig	Ad.FGF4 (-E1)	6.9x10 ⁷ -1.4x10 ⁹	28	Yes	No
38	Pig	Ad.FGF5 (-E1)	1.7 x 10 ⁹	14	Yes	No
39	Pig	Ad.FGF2 (-E1)	3.5 x 10 ⁹	14	Yes	No
40	Pig	Ad.FGF4 (-E1)	8.3x10 ⁷ -8.3x10 ⁹	14-96	Yes	No
41	Pig	Ad.FGF4 (-E1)	8.3 x 10 ⁹	21	Yes	No
16	Pig	Ad.AC6(-E1)	1.2 x 10 ¹⁰	10-60	Yes	No
17	Pig	Ad.AC6(-E1)	1.2 x 10 ¹⁰	14-126	Yes	No
42	Rabbit	Ad.VEGF(-E1/-E3)	6 x 10 ¹⁰	17	Yes	No
34	Pig	Ad.FGF5 (-E1)	6.7 x 10 ¹⁰	14	Yes	No
43	Minipig	Ad.lacZ (-E1)	9 x 10 ¹⁰	31	NR	No
33	Rabbit	Ad.lacZ (-E1)	10 ¹⁰ -10 ¹¹	5-60	NR	No
44	Rabbit	Ad.β2AR (-E1/-E3)	2 x 10 ¹¹	21; 2d steroid	Yes	Unknown
45	Rabbit	Ad.βARKct (-E1/-E3)	2 x 10 ¹¹	3-6; 2d steroid	Yes	Unknown
46	Rabbit	Ad.βARKct (-E1/-E3)	2 x 10 ¹¹	5	Yes	Unknown
47	Rabbit	Ad.V2 Rec (-E1)	4 x 10 ¹¹	Unclear vs im	Yes	Unclear
48	Pig	Ad.Gi (-E1/-E3)	7.5 x 10 ¹²	7	Yes	Yes (mild)

Published studies using direct intracoronary delivery of adenovirus vectors — those procedures using thoracotomy and aortic cross clamping (indirect intracoronary delivery) or other methods are not reviewed. Studies are listed in increasing order of amount of adenovirus delivered per gram of myocardium served by the vessel(s) injected. Unless specifically stated in the paper, certain assumptions were made regarding left ventricular (LV) weight: rabbits weighing 3.0 kg have left ventricular (LV) weights of 10 grams; pig LV weight is 3 grams per kilogram body weight for normal pigs and 3.5 grams per kilogram for minipigs; average human LV weight 140 grams; AV nodal artery perfuses 1 gram myocardium; 1 pfu = 100 vp. Ad, adenovirus; im, intramuscular; Rec, receptor; d, day; -E1, E-1 deleted vector; NR, not relevant. * new randomized clinical trials recently initiated in women

immune system. Several elements may contribute to the apparent “immune privilege” of heart relative to other organs: 1) Antigen presenting cells are not abundant in heart;⁵⁴ 2) MHC class I molecules are expressed only at low levels in myocardium;⁵⁵ 3) Cardiac myocytes *per se* do not appear to express MHC class I or class II molecules.⁵⁵

For gene transfer to be successful in clinical settings, the simplest and safest technique that is effective will prevail as the method of choice. Emphasis on simplicity and safety will be particularly relevant for application of such procedures to patients with severely compromised hearts in whom invasive procedures such as thoracotomy, cross clamping of major vessels, and cardiopulmonary bypass would be high risk. These concerns, combined with the unknown

effects of persistent expression of exogenous genes, provides a rationale for the use of shorter term expression vectors such as adenovirus as a safety measure in these early days of clinical gene transfer trials. Having said this, our data indicate a persistent increase in cAMP generating capacity in LV samples of animals that received intracoronary Ad5.AC6 eighteen weeks prior, suggesting that the transgene does in fact persist for a substantial period of time.¹⁶ Indeed, we have documented persistent LV functional improvement twelve weeks after intracoronary delivery of adenovirus vectors.^{38,40} Finally, Ad5.hAC6 DNA, in doses similar to those proposed in the clinical trial, persists and AC6 transgene is expressed in LV samples 12 weeks after delivery (**Section 8 of IND: Pharmacology and Toxicology**).

6.2.5. Cell-Based Approaches. Cell-based approaches provide a promising alternative.^{56,57} The prospect of generating new functioning cardiac myocytes is exciting. However, we have twelve years experience with cardiac gene transfer, but are just initiating studies with cell-based approaches. We have examined the sequelae of increased cardiac gene transfer of AC6 in twelve different animal models and documented the safety and efficacy of the approach. The increase in LV function that we have documented with AC6 gene transfer matches or exceeds results so far published from studies using cell-based approaches.

6.3. CLINICAL STUDY PROTOCOL

6.3.1. General Description. We propose to conduct a Phase 1 / Phase 2 randomized, placebo-controlled, and double-blinded clinical trial of intracoronary Ad5.hAC6 in patients with stable but severe CHF. Patients will receive intracoronary Ad5.hAC6 in increasing doses (3.2×10^9 – 10^{12} vp) or intracoronary phosphate buffered 3% sucrose (placebo). Intracoronary nitroprusside will be infused during test substance administration in both groups.

6.3.1.1. Gender and Minorities. Prospective subjects will be identified from the cardiomyopathy clinics at the participating hospitals (San Diego VA Healthcare System in La Jolla, CA, the Minneapolis Heart Institute Foundation in Minneapolis, MN, the Northwestern Memorial Hospital in Chicago, IL, and Fletcher Allen Healthcare in Burlington, VT and referral centers through advertising approved by the Human Research Protection Program of our institutions. There will be more males than females because the San Diego VA Hospital (rare female patients) will be a major enrollment site. The goals, approved by the NIH, are to enroll 20% minority patients and 32% women.

6.3.1.2. Dose Groups. Patients will receive ascending doses of intracoronary Ad5.hAC6 beginning at 3.2×10^9 vp with 8 patients per group in a 3:1 randomization: 6 Ad5.hAC6-treated vs 2 placebo-treated patients for Dose Groups 1-3 (**Table 4, next page**). Dose Groups 4 and 5 will include 16 patients per Dose Group (12:4 Ad5.hAC6 vs placebo randomization). The maximal dose (10^{12} vp) was selected for three reasons: 1) the conservative design of the trial enables careful assessment for toxicity in a gradual dose-escalation trial. If toxicity were to occur, it would be quickly observed and appropriate measures taken (see Stopping Rules, **Appendix 6.1**). Therefore, the 10^{12} vp dose would only be initiated if all previous doses showed no serious toxicity; 2) Ad5.hAC6 at 10^{12} vp was not associated with vector-related toxicity in the GLP Toxicology and Biodistribution Study; and 3) Ad5.hAC6 at 10^{12} vp was associated with a high level of cardiac hAC6 expression 70 days after delivery in the GLP Toxicology and Biodistribution Study (**Section 8 of IND**). Extracardiac hAC6 expression had markedly declined at 70 days in other organs at 70 days. The NHLBI mandates that their Data Safety Monitoring Board (DSMB) follow this clinical gene transfer trial. Treatment group allocation will be

randomized and blinded. Although the study is a double-blinded trial, the DSMB will be unblinded as necessary to evaluate safety and other data in individual patients. After receiving test substance, patients will remain in the hospital, in a monitored bed for one night. Patients will be re-evaluated at Weeks 1, 2, 4, and 12 and Months 6 and 12 (**Table 5**).

Table 4. Dose Groups

Dose Group	vp	Ad5.hAC6 (n)	Placebo (n)
1	3.2 x 10 ⁹	6	2
2	3.2 x 10 ¹⁰	6	2
3	10 ¹¹	6	2
4	3.2 x 10 ¹¹	12	4
5	10 ¹²	12	4
<i>Total Subjects</i>		42	14

Table 5. Clinical Tests and Frequency

	Pre	D1 ¹	D4 ²	W1	W2	W4	W12	M6	M12
Exercise Treadmill Test	••					•	•		
Interview	•	•		•	•	•	•	•	•
Physical Exam	•	•		•	•	•	•	•	•
Urine Sample	•			•	•	•	•		
Blood Sample	•	•	•	•	•	•	•	•	•
Chest Radiogram	•						•		
ECG	•	•		•	•	•	•	•	•
Stress Echo (dobutamine)	•					•	•		
ICD Interrogation	•	•			•	•		•	•
RHC, LV dP/dt (± dobutamine)		•				•			
Ad5.hAC6 or Placebo (intracoronary)		•							

D1, the day of test substance administration; **W**, week; **M**, month; **LVdP/dt**, first derivative of LV pressure before & during dobutamine infusion; **ICD**, implantable cardiac defibrillator; **Stress Echo** (to determine ejection fraction) performed before and during dobutamine infusion

¹Patients will be monitored overnight after the initial cardiac catheterization & test substance administration

²Cardiac troponin I & CK-MB measurement only

Note: 1) the two exercise treadmill tests and the dobutamine stress echocardiogram required prior to test substance administration (“Pre”) should be performed on three separate days; 2) 4w Studies: the exercise treadmill test & stress echocardiogram should be performed on separate days, and the exercise test performed prior to the right heart catheterization (to avoid femoral bleeding); 3) 12w Studies: the exercise treadmill test & stress echocardiogram should be performed on separate days. The minimal number of visits to the hospital or clinic during the course of the trial will be thirteen.

6.3.2. Inclusion and Exclusion Criteria

Patients with severe but stable low ejection fraction heart failure (see inclusion criteria) are candidates for enrollment in the proposed trial. LV ejection fraction can be determined by echocardiography, radionuclide or LV angiography within 12-months of enrollment, and should be assessed when the patient is on optimal therapy (referring cardiologist opinion) for at least 14 days. Clinical indications for coronary angiography prior to enrollment, based on AHA/ACC Guidelines, include: 1) patients with active ischemia (Class I indication); and 2) patients in whom coronary disease is suspected (Class IIa indication).

6.3.2.1. Inclusion Criteria

1. Male or non-pregnant female patients aged 18-80 years of age
≥3-month history of heart failure
3. Compensated (stable) CHF not on intravenous inotropes, vasodilators or diuretics, on optimal medical and device therapy as defined by AHA/ACC Guidelines
4. LV ejection fraction (on optimal therapy) of ≤40%
5. Implanted cardiac defibrillator
6. At least one major coronary artery (or graft) with <50% proximal obstruction
7. Women of child-bearing capacity must have a negative pregnancy test within 2 days of test substance administration, and female and male patients must be willing to use birth control during sex for 12w after test substance administration if the female partner is of child-bearing capacity.
8. Subjects willingly provide informed consent consistent with ICH-GCP guidelines

6.3.2.2. Exclusion Criteria

1. Unstable or Class IV angina
2. Coronary revascularization planned or predicted in next 6 months
3. Ischemic myocardium in 3 or more regions of a single perfusion bed, as assessed by stress echocardiography or jeopardized viable myocardium >15% on perfusion imaging.
4. ≥50% occlusion of an “unprotected” left main coronary artery. If arterial or venous conduits provide blood flow to the distal left coronary circulation (ie, patent bypass grafts) then left main disease is “protected” and such patients are not excluded. The cardiologist performing the cardiac catheterization will make these decisions.
5. 2° AV Block (Mobitz 2) or 3° AV block unless pacemaker is present
6. Hospitalization for CHF requiring intravenous inotropes or vasodilators in the past 4 weeks
7. History of biopsy proven myocarditis
8. Myocardial infarction in previous 6 months
9. Restrictive, hypertrophic or infiltrative cardiomyopathy or chronic pericarditis
10. Previous or planned organ transplant recipient or donor.
11. Thrombocytopenia (<100,000 platelets/μl) or bleeding diathesis
12. COPD requiring supplemental oxygen at home

13. AST > 2 times upper limit of normal or chronic liver disease such as cirrhosis or Hepatitis C Virus (HCV). Patients with HCV are eligible only if both of two conditions are met: a) liver function tests are normal; AND b) liver biopsy is normal or shows only mild fibrosis.
14. Current or predicted hemodialysis within 12 months or estimated glomerular filtration rate (EGFR) <30 ml/min. On online EGFR calculator that uses sex, age, body weight and serum creatinine is available at: www.kidney.org/professionals/kdoqi/gfr_calculator.cfm
Use the higher of two EGFR results, which are based upon MDRD and CKD-EPI formulas.
15. CVA or TIA <6 months prior to enrollment
16. Patients who are immunosuppressed by medicines (corticosteroids, methotrexate, cyclophosphamide, cyclosporine), illnesses (AIDS, HIV), or neutrophil count <1000/mm³
17. Patients receiving other investigational drug therapy within 30 days of enrollment including gene transfer
18. Patients with diseases other than CHF that, in the opinion of the investigator, put the subject at risk or adversely affect the results

6.3.3. Screening and Pre-Cath Data Acquisition from Enrolled Subjects. Patients considered for enrollment will first be screened (chart review) by research nurses of the participating hospitals to determine if inclusion and exclusion criteria are met. Patients with active myocardial ischemia will undergo a clinically indicated evaluation to define the extent of jeopardized viable myocardium. Those patients found to have large areas of ischemic dysfunction (≥ 3 regions in a single vascular bed on stress echocardiography or >15% reversible perfusion defect on stress perfusion imaging) will not be enrolled. A coronary angiogram will be performed in those patients with clinical indications. Patients passing these initial screens will have the clinical trial described to them in plain English. A consent form will be provided (written in plain English). Consenting patients will then undergo initial clinical testing, and, if criteria are met, will undergo cardiac catheterization for test substance administration within 60d. Screening procedures, shown in **Table 5 (above)** will include:

- History, Kansas City Cardiomyopathy Questionnaire and physical examination including vital signs (temperature, HR, BP, and respiratory rate), examination of skin, eyes, ears, nose and throat, auscultation of heart, lungs and abdomen, palpation of abdomen, and brief neurological evaluation.
- ECG
- Echocardiography to assess LV size and function
- Determination of LV ejection fraction using stress echocardiography, performed before and during intravenous dobutamine infusion to assess LV function and contractile reserve. The subject must not have taken any β -adrenergic receptor antagonist drugs (“ β -blockers”) in the previous 24 hr before the test, and must not have cigarettes, consumed caffeine-containing beverages or eaten in the previous 4 hr. Patients who have not adhered to these restrictions should be re-scheduled for their test on another day. Standard procedures for the stress echocardiography will be followed according to standard protocols. After basal echocardiographic images are acquired, patients will receive intravenous delivery of 5, 10 and then 20 $\mu\text{g}/\text{kg}/\text{min}$ while ECG, blood pressure and oximetry are monitored. Infusions will be cumulative and last 5 min per dose. At 4.0 min after initiation of each dose, echocardiographic images will again be acquired. Subsequently these data will be analyzed,

providing volume determinations of the LV, LV ejection fraction, and ejection fraction response to stress.

- Serum analysis (See **6.3.6.**)
- Chest X-ray (PA only)
- ICD interrogation, a routine part of care in patients with such devices, will be followed serially. This will document the number of ICD therapies for ventricular tachycardia or ventricular fibrillation. To ensure consistency of detection, we will collaborate with the electrophysiology service to ensure that ICDs are programmed to detect tachycardia above 160-170 beats/minute. In addition, we will program a detection zone at >150 beats/min (lasting >5 sec) to evaluate non-sustained ventricular tachycardias. Periodic manual review will be used to adjudicate detected ICD events as appropriate or inappropriate, and to determine if slower or non-sustained rhythms are atrial or ventricular.
- Exercise Treadmill Test (symptom-limited). Patients unable to walk (spinal injury, orthopedic problems) can be enrolled if all other criteria are met.
- A screening log will be recorded that will include information regarding patients that were screened but who did not qualify for enrollment. The percent of screened patients that were subsequently enrolled, and the reasons for exclusion of screened patients will provide useful data.

6.3.4. Randomization and Test Substance Preparation. The VA San Diego Research Pharmacist, Stephen Funk, will enter the unique identifying number of each vial of test substance within a given Dose Group into a randomization Table and thereby assign the order in which each vial will be dispensed within each Dose for the entire 56-subject trial. These vials are 5 ml capacity and will contain 2.0 ml of test substance. The contents of the vials will be coded on the label so that the Research Pharmacists at participating centers and others involved with the trial will be blinded as to whether the vial contains placebo or Ad5.hAC6. Cornell will provide the vial numbering system and code for placebo versus Ad5.hAC6 to Dr. Funk, who will keep it on file to enable rapid identification of vial contents in the case of serious adverse events, whereupon the appropriate local and federal institutions will be notified. In no other instance is Dr Funk to be unblinded. Cornell has also provided emergency contact numbers for unblinding if required. The Research Pharmacist will store vials (-80°C) until 2 hr before delivery. The vial will be removed from the freezer, and immediately placed in crushed ice (4°C). When the contents of the vial are no longer frozen (this will take 90 min), its contents (2.0 ml) will be drawn into a sterile 12 ml polypropylene syringe. This will be diluted with 8 ml of sterile **ice-cold** saline so that the total volume in the 12 ml syringe is 10 ml. This syringe will be placed inside a sterile polypropylene open end conical tube, placed in an ice-bucket, and delivered, by the Research Pharmacist to the cardiac catheterization laboratory. The vials must be kept as cold as possible, the saline diluents must be ice-cold, and the syringe containing test substance must immediately be placed in crushed ice, because adenovirus activity decays at temperatures higher than 0°C.

6.3.5. Routine Care vs Protocol-Related Procedures. Many of the procedures proposed (blood work, exercise tests, ECG, chest X-Ray, echocardiography, initial diagnostic cardiac catheterization) are part of recommended evidence-based procedures for the diagnosis and care of patients with heart failure. Diagnostic cardiac catheterization is clinically indicated in patients

with heart failure and suspected coronary disease (Class IIa indication), and in patients with active ischemia (Class I indication). All patients will receive either placebo (physiologically buffered 3% sucrose) or Ad5.hAC6 delivered into their coronary arteries, and this clearly is an experimental procedure. Intracoronary delivery of Ad5.hAC6 or placebo will be accompanied by intracoronary infusion of nitroprusside. Nitroprusside has been infused in the coronary arteries of patients with heart disease, but it cannot be considered a routine procedure. Right heart catheterization is routine in elective cardiac catheterization to assess patients with heart failure. However, the repeat right and left heart pressure measurement study 4 weeks after test substance administration is part of the experimental protocol *per se*, and not part of routine patient care.

6.3.6. Coronary Angiography, Right Heart Catheterization, LV Contractile Function & Test Substance Administration

6.3.6.1. Coronary Angiography. Consenting patients passing the initial screen (**Section 6.3.3. and Table 5**) will undergo coronary angiography using standard practices. The assurance of airtight seals at all junctions between catheters, manifolds and stopcocks, constant visual vigilance, and experience will prevent the injection of air bubbles. Systemic heparinization and frequent flushing of catheters will serve to prevent formation and injection of thrombi. Each of the 3 major coronary arteries & arterial or venous conduits in patients with surgical revascularization, will be visualized (using Omnipaque contrast or equivalent) to assess the degree of coronary artery stenosis. If the coronary angiogram confirms that the subject is a suitable candidate to receive test substance, the cardiologist will proceed with right heart catheterization (next section).

6.3.6.2. Right Heart Catheterization. Subjects with suitable coronary anatomy (see above) will undergo right heart catheterization. This will be repeated 4w after test substance administration. The following measures obtained:

- Pressures from: right atrium, right ventricle, pulmonary artery, pulmonary artery wedge
- Oxygen content of blood samples from aorta and pulmonary artery
- Cardiac output by thermodilution (at least 3 separate measurements)

6.3.6.3. LV Contractile Function. After acquisition of data from right heart catheterization and heparin (ACT >200 sec) or bivalirudin administration, a micromanometer catheter (Millar Instruments, Houston, Texas) will be advanced retrograde through a guide catheter and placed in the LV chamber. The high-fidelity LV pressure signal and its first derivative (dP/dt) will be displayed along with pressure signal from the fluid-filled guide. As recommended in the package insert for the Millar catheter, the guide catheter should be flushed frequently with heparinized saline while Millar catheter is in place.

Pressure will be recorded before and during intravenous delivery of 5, 10 and then 20 µg/kg/min dobutamine. Infusions will be cumulative and last 5 min per dose. Data acquisition during infusion will be recorded 2.5 min after the initiation of each dose. A disposable external transducer (Baxter, Irvine, California or equivalent) will be used to calibrate the micromanometer signal. The high-fidelity LV pressure signal, arterial blood pressure, and electrocardiogram will be acquired simultaneously and digitized at 500 Hz using a 12-bit analog-to-digital converter interfaced to a personal computer. The digitally obtained first derivative of LV pressure will be used in all analyses of LV pressure development and relaxation. Methods will follow those described previously in studies conducted at the San Diego VA.⁵⁸ Assessment

of LV contractile function, assessed by this same method, will be repeated 4w after test substance administration. The following measures will be obtained or derived from the acquired signals, before and during dobutamine infusion:

- LV end diastolic pressure
- LV pressure development (peak $+dP/dt$) and relaxation (peak $-dP/dt$)
- Arterial blood pressure
- Heart rate
- Cardiac output (thermodilution)
- Pulmonary artery and arterial blood oxygen contents

6.3.6.4. Test Substance Administration. Test substance (Ad5.hAC6 or placebo) will be administered by the interventional cardiologist after coronary angiography and right heart catheterization are complete. Intracoronary Ad5.hAC6 or placebo will be administered into all major coronary vessels — left anterior descending (LAD), left circumflex (LCx) and right coronary artery (RCA) or corresponding conduit if the patient has patent arterial or venous conduits from coronary artery bypass grafting. An effort will be made to keep cardiovascular medication constant from the time of test substance administration until the end of the trial. However, the patient's care-provider will determine whether changes are made in medical therapy.

6.3.6.4.1. Catheter Placement. A guide catheter (6F Guiding Catheter, Medtronic or equivalent) will be inserted through the arterial access sheath and directed retrograde into the aorta and into the ostium of the left and, subsequently, right coronary arteries (in either order). Other coronary guide catheters may be used. The guiding catheter should be placed securely into the coronary ostium. With the 6F guiding catheter in the left coronary ostium, an infusion catheter with polytetrafluoroethylene (PTFE; Teflon[®]) coating on its inner surface (Renegade[®] Hi-Flo Microcatheter, Boston Scientific or equivalent) will be inserted into the catheter and advanced until the end of the infusion catheter emerges from the distal end of the coronary guide catheter. The infusion catheter is then advanced 1.0 cm into the origin of the LAD. A soft-tipped wire (BMW Wire, 0.014," Guidant, or equivalent) may be required to place the perfusion catheter. The ideal position of the infusion catheter is 1 cm distal to the origin, avoiding placing the infusion catheter beyond any proximal branches of the vessel. If there is a proximal branch of the LAD that is <1 cm from its origin, the infusion catheter should be placed proximal to the branch origin. When the infusion catheter is properly placed, 3-5 ml of contrast (Omnipaque[®]) is injected and recorded for subsequent review to document correct catheter placement. This is repeated after test substance administration to document catheter position after infusion. This same procedure is then followed for the subsequent infusions into the LCx, RCA, and other vascular conduits (vein or arterial grafts). The specified catheters were used in the toxicology and biodistribution study and are biocompatible with Ad5 vector (see **Section 7, Chemistry, Manufacturing and Controls**).

6.3.6.4.2. Infusion. The Research Pharmacist will bring to the catheterization laboratory, when requested by the PI, previously dispensed test substance (10.0 ml in a sterile 12.0 ml syringe, on ice). Subsequently, 5.0 ml will be delivered to the LAD, 3.0 ml into the LCx and 2.0 ml into the RCA perfusion beds. Delivery rate will be 4.0 ml/min. Vector delivery will occur through the infusion catheter appropriately placed in the origin of each of the three major vessels. A

continuous intracoronary infusion of nitroprusside (50 µg/min) will be initiated 1 minute before and continue for the duration of test substance delivery. Intracoronary nitroprusside in this dose has been used in clinical settings in patients with heart disease.⁵⁹⁻⁶¹ Infusion of nitroprusside and test substance will be initiated only if the systolic blood pressure is ≥85 mmHg and will continue for the duration of test substance delivery. Intracoronary nitroprusside in this dose has been used in clinical settings in patients with heart disease.⁵⁹⁻⁶¹

Intracoronary nitroprusside infusion (50 µg/min) is initiated via automated infusion pump. One minute after initiation of intracoronary nitroprusside delivery, test substance infusion will begin (manually delivered at 4.0 ml/min). When the appropriate volume of test substance has been completely delivered, 2.0 ml of saline is manually delivered into the infusion catheter at a rate of 4.0 ml/min. Meanwhile the nitroprusside infusion has continued uninterrupted. When the manual saline flush is completed, the nitroprusside infusion is terminated and test substance administration is complete. In this manner, the entire volume of test substance volume is delivered and no significant amount remains in the infusion catheter.

6.3.6.4.3. Post-Procedure. Patients will remain hospitalized overnight (in a monitored bed) after test substance administration and will be discharged the next morning, after an additional blood sample is obtained.

Table 6. Acceptable Intervals for Visits

Follow-up Visits	Acceptable Range
4d	± 1d
1w	± 2d
2w	± 3d
4w	± 3d
12w	± 5d
6m	± 1w
12m	± 2w
d, day; m, month	

6.3.7. Safety Monitoring (Table 5 and Table 6)

Safety monitoring (and evaluation of efficacy, see **6.3.8**) will be performed through regularly scheduled visits. Ideally these visits will occur at 4d, 1w, 2w, 4w, 6m and 12m. However, to accommodate for scheduling challenges due to weekends and holidays, some flexibility is required. The acceptable intervals for these visits are displayed in **Table 6**.

Safety monitoring (Table 5) will include:

- Adverse events, serious adverse events (continuous)
- Physical examination (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- Interview & Kansas City Cardiomyopathy Questionnaire⁶² (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)

- Medication usage, hospitalizations, survival (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- Arrhythmia: Monitoring in hospital after initial test substance administration, and ICD interrogation (Before, 1d, 2w, 4w, 6m, 12m)
- ECG (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- Serum analysis (Before, 1d, 1w, 2w, 4w, 12w, 6m and 12m)
 - Hematology: HGB, HCT, PLT, RBC, WBC, PT, INR, PTT
 - Electrolytes/Renal: K, Na, HCO₃, Cl, , BUN, Creatinine, glucose
 - Heart: CPK-MB, cardiac troponin I (these tests must be performed within 24 hr of test substance administration, the morning after test article administration, and on Day 4 after test substance administration); B-Type natriuretic peptide (BNP); sedimentation rate
 - Liver: AST, ALT, ALP, LDH, bilirubin (total and direct), albumin, total protein
 - Ad5.hAC6 DNA by qPCR (1hr, 1w and 2w after intracoronary infusion). Serum samples will be stored at -80°C until the trial is unblinded before the PCR assay is performed—otherwise the results would break the blind.
 - Prior to test article administration, serum anti-adenovirus-5 antibody titer will be assessed by antibody neutralization assay, performed at Fred Hutchinson cancer center.

- Urinalysis (Before, 1w, 2w, 4w, 12w)

- Chest radiogram (Before, 12w)

6.3.8. Efficacy Evaluation (Table 5)

6.3.8.1. Primary Endpoints

- Exercise Treadmill Testing (Before, 4w, 12w)
- LV ejection fraction (stress echocardiography) before and during dobutamine infusion (Before test substance administration and 4w and 12w later)
- LV peak +dP/dt (assessed by cardiac catheterization) before and during dobutamine infusion (Before test substance administration and 4w later).

6.3.8.2. Secondary Endpoints

- Physical Examination (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- Interview & Kansas City Cardiomyopathy Questionnaire (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- Medication usage, hospitalizations, survival (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- BNP (Before, 1d, 1w, 2w, 4w, 12w, 6m, 12m)
- ICD discharge frequency and episodes of ventricular tachycardia

6.3.9. Dose Advancement

All patients in a specified Dose-Group will be treated before advancement to the next higher Dose Group. In addition, no patients will be treated on consecutive days. For example, if a patient receives test substance on Monday, the next patient would not receive test substance until Wednesday of the same week, or later. These intervals will enable the evaluation of toxic effects related to the procedure and to initial exposure to the test substance.

- DSMB examines data obtained 2w after test substance administration in the first 3 patients of **Dose Group 1** (n=8). If no safety issues are present, the remaining patients will receive test substance. Two weeks after the last patient in Dose Group 1 is treated, the trial will advance to **Dose Group 2**, upon approval of the DSMB.
- DSMB examines data obtained 2w after test substance administration in the first 3 patients of **Dose Group 2** (n=8). If no safety issues are present, the remaining patients will receive test substance. Two weeks after the last patient in Dose Group 2 is treated, the trial will advance to **Dose Group 3**, upon approval of the DSMB.
- DSMB examines data obtained 2w after test substance administration in the first 3 patients of **Dose Group 3** (n=8). If no safety issues are present, the remaining patients will receive test substance. Two weeks after the last patient in Dose Group 3 is treated, the trial will advance to **Dose Group 4**, upon approval of the-DSMB.
- DSMB examines data obtained 2w after test substance administration in the first 3 patients of **Dose Group 4** (n=16). If no safety issues are present, the remaining patients will receive test substance. Two weeks after the last patient in Dose Group 4 is treated, the trial will advance to **Dose Group 5**, upon approval of the DSMB.
- DSMB examines data obtained 2w after test substance administration in the first 3 patients of **Dose Group 5** (n=16). If no safety issues are present, the remaining patients will receive test substance. If the first 3 patients of **Dose Group 5** show severe adverse effects, the remaining 13 patients that were to receive this dose will instead be added to **Dose Group 4**. The same procedure would be applied for any Dose Group in which toxicity was observed in the initial patients of the higher dose.

6.3.10. Statistical Methods

6.3.10.1. General Features. A biostatistician, who assisted with this section of the proposal, will conduct data analyses for this study. Test substance administration assignment and Dose Group will guide tabulation of data regarding safety. Clinical parameters will be compared within individual dose groups and primary endpoints evaluated by pooling the two highest dose groups versus placebo. The design of the trial reflects the likelihood that the FDA will approve a limited number of patients in this first trial of Ad5.hAC6 in CHF, for example, <60 active treatment patients. The proposed initial dose (3.2×10^9 vp) is >2 log units below the effective dose in pigs with CHF.¹⁷ Dose escalation will be 1.0 to 0.5 log units per dose, and we will treat 8 patients in each of Dose Groups 1-3, and 16 patients in each of Dose Groups 4 and 5. Based on our preclinical data, we anticipate a favorable effect at the highest doses—Dose Group 4 (3.2×10^{11} vp and Dose Group 5.

6.3.10.2. Endpoints for Efficacy. Primary Endpoints used to evaluate efficacy will include: improvements in: a) exercise capacity assessed by a symptom-limited treadmill; b) LV ejection fraction (stress echocardiography, before and during dobutamine infusion); c) LV peak +dP/dt before and during dobutamine infusion. Compared with the use of a single primary endpoint —

such as the 6-Minute Walk Test — multiple primary endpoints will enable: a) including patients who cannot walk due to spinal or orthopedic problems; b) circumventing limitations of the 6-Minute Walk Test in predicting long term benefits of a treatment;⁶³ c) global assessment of efficacy that combines exercise capacity, and two independent measures of LV contractile function: LV ejection fraction, and the rate of pressure development (dP/dt). A statistically significant increase in any of the 3 primary endpoints will constitute efficacy. To guard against Type I error inflation, a conservative α criterion of 0.025 will be used for these comparisons. Once all data up to and including 12w has been collected, the statistician at the San Diego site will perform statistical analysis regarding the primary endpoints.

6.3.10.3. Statistical Power. We evaluated statistical power for primary endpoints by expressing effect sizes in standard deviation units. Thus, the same power analysis applies equally to the 3 measures of improvement. For these primary endpoint tests we will combine the highest 2 Dose Groups which will enable comparing 24 Ad5.hAC6-treated vs 14 cumulative placebo-treated patients, thereby achieving adequate statistical power. A comparison between 24 Ad5.hAC6-treated patients and 14 control patients, assuming a β -error of 0.2 (power of 0.8) and an α -error of 0.025, these primary endpoint tests will be sensitive to a difference of 1.08 SD unit between groups. For exploratory analysis of single dose groups, **Table 4** shows that Dose Group 5, for example, will include 12 Ad5.hAC6-treated patients that would be compared to a cumulative 14 placebo-treated patients. These analyses will be made without Type I error correction using a 0.05 α criterion, and will have a β -error of 0.2 for effects of 1.15 SD units. Within-group change in the primary endpoints (Exercise Treadmill Test, and LV ejection fraction and LV peak +dP/dt before and during dobutamine infusion) before and after test substance administration will be compared using 2-tailed paired t-tests with a 0.05 α criterion. Efficacy, when based upon such a small number of patients, would primarily give confidence for selecting a dose or doses for the conduct of a larger scale Phase 2 trial.

6.3.10.4. Placebo Group. We have stated in the consent forms that there will be randomization, so patients will know, in advance of agreeing to participate, that there is a 25% chance of receiving placebo. We will not suggest that participation in the trial will lead to improved symptoms. Since there are no clinical data indicating that Ad5.hAC6 is superior to placebo in the treatment of CHF, the two groups, from an ethical viewpoint, are identical. The risks of the trial are stated in the consent form, and participation is voluntary. Our preclinical data and clinical data using a different adenovirus vector but with a similar delivery method indicates that conducting the clinical trial may be relatively safe, and that the potential gain from the proposed research outweighs the risks.

The proposed trial will be randomized, double blinded, and placebo controlled. Without the proposed design, patients would be placed at risk for data that would be of questionable value. Not only would trends indicating efficacy be uninterruptible (because of the placebo effect), but whether an adverse event was due to treatment or underlying disease also would be difficult to determine. We have designed the trial to include the minimal number of placebo patients required to detect a 25% treatment effect with 80% statistical power. This conservative design will enable us to conduct a double-blinded placebo-controlled trial, which will provide optimal data on safety, and will permit us to identify doses that can be subsequently tested for efficacy in larger Phase 2 trials. With the proposed design we have attempted to obtain the maximal amount of information — both safety and dose-finding — that is possible within the

constraints of a small randomized trial with a starting dose 2 logs below minimal efficacy in preclinical studies.

6.3.11. Risks

6.3.11.1 Procedure Risks and Measures to Reduce Risk

6.3.11.1.1. Procedure Risks. Cardiac catheterization is associated with a 1% complication rate. The majority of these events are related to bleeding at the site of catheter insertion (femoral artery and vein) and do not usually require transfusion. Other risks are rare and include infection, thrombus formation or embolization, renal compromise that is usually reversible, and arrhythmia, sometimes requiring DC cardioversion. The risk of death is 0.1% (once in 1000 procedures) and is usually associated with severe left main coronary artery disease or unstable angina.

The highest risk patients are those with unstable angina, independent of LV function. Patients with unstable or severe angina will not be enrolled in the proposed trial. However, the most common complication of cardiac catheterization is bleeding. It is conceivable that patients with CHF may have more complications after bleeding than patients without CHF, but bleeding complications are usually easy to manage and not life-threatening. Two complications of cardiac catheterization that have been associated with a mild increase in risk in patients with CHF vs patients with normal LV function merit consideration: 1) contrast nephropathy; and 2) worsening CHF. In 450 patients with angina but normal LV ejection fractions], intracoronary injection of Ad5.hFGF4 was not associated with adverse events during vector delivery²⁶⁻²⁸

The infusion of sodium nitroprusside into human coronary arteries is currently being performed in other clinical studies,⁵⁸⁻⁶⁰ and is not expected to result in adverse events. The potential side effects of intracoronary infusion of nitroprusside include a feeling of flushing of the skin and mild light-headedness. The risks are low blood pressure that could cause *angina* if severe coronary artery disease is present. However, if these side effects occur they will be very brief, and cardiologists do use intracoronary infusion of nitroprusside in patients with severe coronary artery disease and it appears to be safe.⁵⁸⁻⁶⁰

6.3.11.1.2. Measures to Reduce Procedure Risks. These will be minimized by having an experienced cardiac catheterization team performing the studies, and eliminating patients with unstable or severe angina and poor renal function. By carefully choosing the patients for the trial using these guidelines, we expect that the risks will not exceed that observed with routine cardiac catheterization.

Contrast-associated nephropathy. In the proposed trial, we have excluded patients with calculated creatinine clearances of <30 ml/min, to circumvent this potential complication. In addition, low-osmolar nonionic contrast agents such as Omnipaque[®] greatly reduce the incidence of nephropathy and now are used routinely in our cardiac catheterization laboratories. These two precautions should allow us to avoid post-cardiac catheterization contrast-associated nephropathy.

Contrast-induced precipitation of decompensated CHF. This occurs very rarely, because patients with high pulmonary artery wedge pressures will be treated with diuretics before proceeding to angiography. The enrollment of patients with stable symptoms, the use of low-osmolar contrast agents, and not performing left ventriculography (reduces contrast load 30-40%) are additional measures that will be used to avoid this complication.

6.3.11.2. Vector Risk and Measures to Reduce Risk

6.3.11.2.1. Vector Risks. The primary concern using replication-incompetent adenovirus vectors is inflammation due to expression of immunogenic virus proteins or an acute cytopathic effect. The heart appears to be less susceptible to these events than the liver. In the proposed study, Ad5.hAC6 is delivered into the coronary arteries and therefore the highest exposure would be in the heart. There is no evidence of inflammation in liver, heart or elsewhere 7, 28 or 70d after intracoronary delivery of Ad5.hAC6 3.2×10^{12} vp in pigs (see **Section 8 of IND: Pharmacology and Toxicology**). Similarly, no elevations in CPK-MB or troponin were found, indicating that cardiac injury was unlikely, even at the highest dose. In addition, we have not seen differences (vs control) in inflammation on histological inspection of the heart in pigs that received 1.4×10^{12} vp of intracoronary Ad5.mAC6 with¹⁶ or without CHF,⁶ and no differences were seen in CPK-MB or troponin levels in pigs with CHF that received Ad5.mAC6, or in patients that received up to 3.2×10^{10} vp of Ad5.hFGF4.²⁶⁻²⁸

An additional concern is infection of testis or ovary with adenovirus. In pigs that have received intracoronary injection of 3.2×10^{12} vp Ad5.hAC6, low levels of Ad5.hAC6 mRNA was detected in ovary in 2 of the 6 females studied, which persisted for 70d in one case (see **Section 8 of IND: Pharmacology and Toxicology**). However, it is extremely unlikely that the transgene would enter the ova and be passed on to a second generation, due to the transient and extrachromosomal nature of adenovirus incorporation and expression. A recent study showed that injection of Ad5.lacZ (10^8 vp/g) into the LV chamber of mice did not result in germ line transmission.⁶⁴ This dose (10^8 vp/g) is the equivalent of 10^{13} vp delivered to a 100 kg human. Indeed, even when adenovirus infection and transgene expression are very high in ovary, through direct injection in mice, expression does not appear in oocytes, but is limited to the thecal portion of the ovary. Furthermore, when such females are mated, none of the fetuses have detectable adenovirus DNA.⁶⁵ An analogous study was conducted in male mice, with identical outcomes.⁶⁶ Although a risk of replication competent Ad5 (E1-deleted) adenovirus exists at doses in excess of 10^{11} vp, we are using an E1/E3-deleted Ad5, which reduces the risk of replication competent adenovirus considerably.

6.3.11.2.2. Measures to Reduce Vector Risks. The principal manner in which vector risks will be assessed is by serum levels of parameters used to assess hepatic and myocardial toxicity (**Section 6.3.6**), which will be performed on samples obtained at baseline and 1, 2, 4 and 12w and 6 and 12m after treatment. The preclinical data support the safety of these doses, but we will be vigilant for adverse events and stop the trial if they are seen. We will assess myocarditis by serially following CPK-MB and cardiac troponin I serum levels, tests that are exquisitely sensitive for cardiac injury.²⁹ These tests will be obtained at baseline, 1, 2, 4, and 12w and 6 and 12m after treatment. In addition, all patients will be monitored overnight for cardiac arrhythmias. Fertile women will be asked to use barrier or medicinal birth control during intercourse for 7d prior to test substance administration and to be tested for pregnancy 48h prior to test substance administration to reduce the risk of gene transfer to the fetus in the event of pregnancy. Fertile men and women will be advised to use barrier or medicinal birth control for 12 weeks following test substance administration, to reduce even further the already remote risk of germ line transmission of the transgene. There is a very small chance that the Ad5.hAC6 vector may be excreted for a short period of time in body fluids. Family members and others should avoid contact with all body fluids of the subject for two weeks after randomization.

6.3.11.3. Transgene Risks and Measures to Reduce Risk

6.3.11.3.1. Transgene Risks. The transgene promotes intracellular cAMP production in response to β -adrenergic receptor stimulation. This should serve to increase cardiac contractile function. Because the protein does not result in sustained adrenergic activation, we do not expect provocation of arrhythmias or exacerbation of heart failure. Indeed, our experience with AC6 shows that it does not provoke arrhythmias and *prolongs* life in heart failure and acute myocardial infarction.^{6,16,18} Extracardiac gene expression of AC6 must be considered, particularly in lung, liver, and peripheral arteries, but also in other cells of the heart such as fibroblasts.

Lung. In the bronchial tree, an increase in cAMP would lead to bronchodilation. Since bronchoconstriction is frequently seen in heart failure, this peripheral effect would be expected to be favorable. In the pulmonary vasculature, an increase in cAMP would be anticipated to reduce pulmonary vascular resistance and thereby increase pulmonary blood flow. Indeed, increasing intracellular cAMP levels in pulmonary artery vascular smooth muscle cells from patients with pulmonary hypertension results in favorable effects linked with pulmonary artery vasodilation.^{67,68} Histological inspection of the lung showed no abnormalities compared to the control group (**Section 8, Pharmacology and Toxicology**).

Liver. In hepatocytes, a very large amount of AC6 expression would be anticipated to increase gluconeogenesis and glucose output—increases in serum glucose levels would be offset by increased insulin secretion. Fatty acid oxidation may increase which would result in elevation in circulating ketone bodies. These effects are similar to what would be seen in a fasting subject. Sustained increases in liver cAMP generation appears to protect against ischemic injury⁶⁹ and apoptosis.⁷⁰ In smooth muscle of hepatic vessels, increased cAMP generation would be predicted to reduce vascular resistance and increase hepatic and portal blood flow, which would be an advantage for patients with hepatic congestion, which often is associated with clinical CHF. Histological inspection of the liver showed no evidence of histological changes (**Section 8, Pharmacology and Toxicology**). *Spleen.* In the biodistribution study (**Section 8, Pharmacology and Toxicology**) we saw substantial Ad5.hAC6 distribution to the spleen, which decreased over time. At dose of 3.2×10^{12} vp, Ad5.hAC6 mRNA levels, which were 1066 copies per μg total RNA 7d after vector administration, were reduced by 90% at 70d. The high Ad5.hAC6 DNA copy number early after Ad5.hAC6 delivery likely represents RBC-Ad5.hAC6 complexes, which are filtered by the spleen, and phagocytic cells that contain Ad5.hAC6 DNA. Histological inspection of the spleen showed no abnormalities.

Smooth muscle cells. In smooth muscle cells comprising the arteriolar vessels that regulate peripheral vascular resistance, increased AC6 expression would be anticipated to reduce vascular resistance. This is unlikely, however, to result in a drop in blood pressure in patients with heart failure since peripheral resistance in such patients is abnormally high. Indeed, a mainstay of medical therapy for patients with heart failure is the use of vasodilators like angiotensin converting enzyme inhibitors. The blood pressure would not be expected to drop, because increased cardiac output offsets reduced vascular resistance so that blood pressure does not change or even increases somewhat.

Fibroblasts. AC gene transfer in fibroblasts is associated with reduced collagen matrix generation.⁷¹ Since cardiac fibrosis is often seen in heart failure, this potential side effect might also have favorable results.

6.3.11.3.2. Measures to Reduce Transgene Risks. The possibility of an unexpected unbridled stimulation of β -adrenergic responsiveness leading to increased heart rate must be considered. This could, potentially, unmask clinically undetected severe coronary artery disease and result in angina or worsening CHF. We have shown that intravenous esmolol returns the heart's contractile responsiveness to normal following gene transfer with AC6, and this would be instituted to treat such an untoward event should it occur. The principle manner in which extracardiac gene toxicity will be assessed is by liver enzymes that are obtained at baseline and 1, 2, 4, and 12 weeks and 6 and 12 months after treatment. The preclinical data support the safety of these doses, but we will be vigilant for adverse events and stop the trial if they are seen.

6.3.11.4. Dobutamine Risks and Measures to Reduce Risk

6.3.11.4.1. Dobutamine Risks. Intravenous dobutamine in doses of 5, 10 and 20 $\mu\text{g}/\text{kg}/\text{min}$ will be used during the cardiac catheterizations and during measurement of EF by stress echocardiography. Each dose will be infused for 5 min, during a cumulative 15 min infusion for the 3 doses. Intravenous dobutamine is safely used in the treatment of CHF to increase cardiac output. Moreover, there is a long and well-reported history of using short-term high dose intravenous dobutamine infusion (30-50 $\mu\text{g}/\text{kg}/\text{min}$) in patients with severe CHF with a remarkably safe profile. The safety of intravenous dobutamine during in patients with ICDs and impaired LV function (mean EF = 32%) recently was determined in 87 patients who underwent intravenous dobutamine at 5, 10, 20, 30, 40 and 50 $\mu\text{g}/\text{kg}/\text{min}$ (cumulatively infused at 3 min per dose).⁷² Infusions were stopped when 85% of age-predicted HR was attained. The tests were well tolerated in all patients and there were no serious complications. Dobutamine infusion was associated with: a) nonsustained ventricular tachycardia (3 or more consecutive PVCs) (n=6); b) angina (n=3); c) hypotension (SBP <90 mmHg) (n=1); d) supraventricular arrhythmias (n=1). The majority of these events resolved upon termination of the infusion. In contrast to this study, which used a mean maximal dobutamine dose of 36 $\mu\text{g}/\text{kg}/\text{min}$, we propose to use a maximal dose of 20 $\mu\text{g}/\text{kg}/\text{min}$.

6.3.11.4.2. Measures to Reduce Dobutamine Infusion Risks. Dobutamine infusions will be conducted with full hemodynamic monitoring with a cardiologist present (cardiac catheterization laboratory) or with ECG, BP, and oximetry monitoring with a registered nurse or physician's assistant present and a physician immediately available (stress echocardiography). Dobutamine infusions will be terminated in the following circumstances: 1) a sustained systolic blood pressure <85 mmHg or >230 mmHg; 2) HR within 10 bpm of the rate the ICD is programmed to discharge; 3) ventricular tachycardia or provocation of ICD discharge; 4) new onset atrial fibrillation; 5) moderate or severe angina; 6) subject requests termination of the infusion. If required, intravenous esmolol will be used for persistent problems, should they arise.

6.3.12. Data and Safety Monitoring Board. The Institutional Review Board for participating hospitals have reviewed and approved the proposed clinical trial, consent forms, data and safety monitoring plans, and initiation of the clinical trial, which has been approved by the FDA. As required by NIH policy, the NHLBI DSMB will monitor the clinical trial. The DSMB will monitor data for safety, make recommendations regarding advancing the trial to higher dose levels, and determine when the trial should be terminated. The DSMB will have access to unblinded data for rapid evaluation of all serious adverse events. The DSMB will meet regularly to review adverse events and general progression of the clinical trial. It is important to stress, however, that the DSMB will review serious and unexpected adverse events as they arise. In addition to notification of the DSMB for serious and unexpected adverse events, these events

will be reported also to the IRBs and other federal authorities as mandated by the FDA. Finally, deaths will be reported in an expedited manner, within 24 hr of occurrence.

6.3.13. Toxicity Endpoints Limiting Progression of the Trial. The DSMB will make a decision regarding whether the trial should continue to higher doses. These decisions will depend on the incidence, amplitude, and consequence of the adverse event. Death, hospitalization due to worsening heart failure, and serum liver enzyme elevations are potential complications that may arise during the course of this trial. Some (but not all) of these events may be associated with intracoronary Ad5.hAC6 administration. Proximity to time of administration and dose-related toxicity are features that may indicate vector-related events. However, toxicities related to the procedure per se (unrelated to vector) may also occur, especially after the initial cardiac catheterization in which the test substance is administered and during which contrast media will be used—contrast nephropathy or worsening CHF for example. Similarly, bleeding at the site of arterial and venous access is one of the more common complications of cardiac catheterization, occurring in 1 of 100 patients undergoing the procedure.

The following situations (bulleted items below) define dose-limiting toxicity. Dose escalation would not be recommended in the first 3 Dose Groups if dose-limiting toxicity were observed in 2 of the 6 patients that received Ad5.hAC6. In Dose Groups 3 and 4, dose escalation would not be recommended if dose-limiting toxicity were seen in 3 of the 12 patients that received Ad5.hAC6.

- Any Grade 3 or 4 or persistent (>7d) Grade 2 adverse events including (but not limited to) abnormalities in serum chemistry values (especially liver enzymes), hematological abnormalities, life-threatening cardiac arrhythmias, precipitation of angina
- Excessive hospitalization rates for worsening heart failure after test substance administration
- Increased mortality rates

6.3.13.1. Mortality. Death is likely to occur in patients with severe heart failure independent of Ad5.hAC6 administration and the procedures proposed, because patients with severe CHF have high mortality rates, even when optimally treated. Therefore, it will be expected that mortality will occur during the conduct of the proposed study. This is an important reason to include a control group, so that the DSMB have a means to evaluate whether mortality is due to the disease treated or, conversely, due to Ad5.hAC6. In the small study proposed, it will be difficult to determine differences between groups by statistical testing. Therefore, in addition to the placebo group, the DSMB should rely on expected vs observed event rates over time. Patients will be on optimal therapy in this trial. Based on mortality data on such patients (Class III-IV compensated CHF), the expected annualized mortality is 15-20%.^{22,73} In the proposed trial of 56 patients, approximately 4 patients would be expected to die in 12 weeks. Obviously, any death would be a cause for scrutiny. A greater than expected death rate in treated patients would be a reason to terminate the trial.

6.3.13.2. Worsening CHF Requiring Hospitalization. Based on a similar analysis as above, the DSMB should be guided by the expected rates of hospitalization for worsening heart failure in this population. In the BEST trial,⁷² the control group (optimal pharmacological therapy, no bucindolol) had a hospitalization rate of 40% over the two year trial, or 20% per year. In the proposed trial, the expected hospitalization rate, based on these data, would be 4 patients in 12 weeks. If a rate substantially higher than this were detected the DSMB would be advised to intervene.

6.3.13.3. Hepatotoxicity. In the AGENT-1²⁶ and AGENT-2²⁷ trials (combined), the incidence of >2-fold increase in SGOT was 14% in Ad5.hFGF4 patients and 8% in PBS patients. In AGENT-3 and AGENT-4²⁸ there were no differences between groups in SGOT elevation among 177 PBS patients and 355 Ad5.hFGF4 patients. Transient reversible SGOT (ALT) elevations may follow intracoronary adenovirus administration, although it is not clear that this effect is dose-related. All Grade 3 and 4 (and persistent Grade 2) elevations in liver enzymes will be examined carefully to determine if: a) the incidence is higher than that seen in placebo-treated patients; b) the occurrence is related to Ad5.hAC6 administration by proximity to treatment day or is related to dose. More detailed guidelines for terminating the trial based on liver toxicity (and other parameters) have been approved by the NHLBI DSMB and can be found in **Appendix 6.1** in the IND submission.

6.3.13.4. Study Stopping Rules. If the incidences (per 56 subjects) of the following complications of cardiac catheterization occur, whether related to Ad5.hAC6 administration or not, the Sponsor will notify the DSMB and consider stopping the clinical trial.

- Contrast nephropathy with irreversible and severe decline in creatinine clearance in 3 patients
- Worsening CHF related to contrast load in 6 patients
- CVA or TIA related to cardiac catheterization in 2 patients
- Femoral artery or vein hematoma at the site of arterial or venous access requiring vascular surgery intervention or blood transfusion 3 patients
- Myocardial infarction with both troponin I elevation and a 2-fold increase (above the upper limit of normal) in CPK-MB in the first 24 hr after initiation of cardiac catheterization in 2 patients
- Isolated elevation of troponin I with CPK-MB <2-fold upper limit of normal, with either chest pain or electrocardiographic changes diagnostic for myocardial ischemia or infarction in the first 24 hr after initiation of cardiac catheterization in 3 patients

6.3.13.5. Timing for Reporting. Life threatening complications and deaths will be reported to the IRB, DSMB, FDA and all other required agencies within 24 hr. Serious and unexpected adverse events will be submitted to all these bodies as expedited reports.

6.3.13.6. Long Term Follow-up. The proposed trial will closely evaluate patients by physical examination, patient interview, laboratory assessment, EKG and ICD interrogation sequentially for 12 months. Patients then will be contacted by phone 24 and 36 months after Ad5.hAC6 administration. If any concerns are identified during the telephone interviews, the patients will be asked to report to the outpatient cardiology clinic for direct assessment by a cardiologist who will then direct additional care and testing as indicated.

The questions that will be asked at the 24 month and 36 month telephone interviews:

Since the subject's last visit:

1. Is the subject alive? Yes No
2. Has the subject been diagnosed with cancer? No Yes
3. Has the subject been admitted to the hospital for worsening heart failure? No Yes
4. Has the subject received heart transplantation or insertion of a left ventricular assist device (LVAD) No Yes

5. Has the subject received coronary artery bypass graft surgery or valve replacement?
 No Yes
6. Has the subject experienced a myocardial infarction or unstable angina? No Yes
7. Has the subject experienced a stroke or been diagnosed with any other neurologic disorder?
 No Yes
8. Has the subject experienced any other significant illness (including but not limited to hematologic disorders and autoimmune diseases)? No Yes
9. Has the subject participated in another gene therapy trial since the last follow-up period?
 No Yes
10. Additional comments, if any (e.g. additional information related to patient's general health).

The efficacy and safety end-points of the study end 12 months after randomization. The answers to the questions posed in the 24 and 36-month telephone calls are focused on whether or not the subject remains alive, and whether there has been interval hospitalizations related to worsening heart failure. The CRF for these calls will serve as a record of such events.

The SAE reporting requirements are the same for the 24-month and 36-month phone calls as for SAEs occurring during the first 12-month interval after randomization. If a previously unidentified SAE is uncovered during the phone call (for example, a previous hospitalization) the SAE should be recorded on the CRF and unless the SAE meets the requirements for submission of an expedited report, then no additional report needs to be sent to the FDA. For such a case, the SAE would appear as part of the safety data contained in the subsequent annual report. If the SAE meets the requirements for an expedited safety report, then the amendment that is submitted should make reference to the date on which you were notified of the SAE and also the date of the actual occurrence of the SAE.

6.3.14. Potential Benefits. It is possible that AC6 gene transfer may improve the patient's symptoms of heart failure beyond what the patient may already be experiencing with standard therapy. It is unknown how long such an effect would last, if it were to occur at all. It is also possible that survival may be improved, but the trial proposed is not powered to determine this. Because there will be patients that receive placebo and others that receive very low amounts of Ad5.hAC6 (Dose Groups 1-3), beneficial effects are not anticipated. Patients in Dose Groups 4 and 5 may show a clinical benefit of treatment, but this is not suggested in the consent forms. Higher doses may include sufficient numbers of patients to detect a clinical benefit of treatment, but this would require confirmation in larger Phase 2 trials. The proposed therapy may be safer than surgical interventions would be in this patient population. Finally, the proposed studies will provide new data regarding a previously untested approach for the treatment of clinical CHF. Our proposed study will test this new potential therapy in an optimally safe and scientifically rigorous manner.

6.3.15. Reimbursement for Time and Travel for Participation. Each patient that completes the trial will receive up to \$1010 (total) for the hospital and clinic visits required. Reimbursement will be as follows:

- before test substance administration: up to 5 visits at \$30/visit (\$150)
- a minimum of 24 hr in hospital for the initial cardiac catheterization and test substance administration. Loss of 1 work-day (\$180)

- a follow-up right heart catheterization that will take $\frac{3}{4}$ of a day (\$140)
- 11 follow up visits for clinic, DSE and ETTs : \$60/visit, except for Week 4 and 12 and Month 6 and 12, (\$30/visit): \$540

TOTAL (per patient): \$1010

Patients that enroll but do not complete the entire study will be reimbursed in a manner to reflect time lost as indicated by the number of visits adjusted to the number of visits, catheterizations, exercise tests, and dobutamine stress echocardiograms that they complete, according to the above schedule. This amount of reimbursement for travel and time lost is similar to that provided by studies of similar complexity conducted in our community. Funding for lodging will be provided for subjects living >100 miles away who otherwise could not participate in the trial.

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