Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Detailed Methodology

Inclusion Criteria

- Male or non-pregnant female patients aged 18-80 years of age ≥3-month history of HF
- Compensated (stable) HF not on intravenous inotropes, vasodilators or diuretics, on optimal medical and device therapy as defined by AHA/ACC Guidelines
- LV ejection fraction (on optimal therapy) of ≤40%
- Implanted cardiac defibrillator
- At least one major coronary artery (or graft) with <50% proximal obstruction
- Women of childbearing 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 childbearing capacity.
- Subjects willingly provide informed consent consistent with ICH-GCP guidelines

Exclusion Criteria

- Unstable or Class IV angina
- Coronary revascularization planned or predicted in next 6 months
- 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
- ≥50% occlusion of an left main coronary artery
- 2° AV Block (Mobitz 2) or 3° AV block unless pacemaker is present
- Hospitalization for HF requiring IV inotropes or vasodilators in the past 4 weeks
- History of biopsy proven myocarditis
- Myocardial infarction in previous 6 months
- Restrictive, hypertrophic or infiltrative cardiomyopathy or chronic pericarditis
- Previous or planned organ transplant recipient or donor.
- Thrombocytopenia (<100,000 platelets (μ L) or bleeding diathesis
- COPD requiring supplemental oxygen at home
- AST > 2 times upper limit of normal or chronic liver disease such as cirrhosis or Hepatitis C Virus (HCV).
- Current or predicted hemodialysis within 12 months or estimated glomerular filtration rate (EGFR) <30 ml/min
- CVA or TIA <6 months prior to enrollment
- Patients who are immunosuppressed by medicines (corticosteroids, methotrexate, cyclophosphamide, cyclosporine), illnesses (AIDS, HIV), or neutrophil count <1000/mm3

- Patients receiving other investigational drug therapy within 30 days of enrollment including gene transfer
- Patients with diseases other than CHF that, in the opinion of the investigator, put the subject at risk or adversely affect the results

Dose Groups, See eTable 1

Screening and Clinical Testing. See eTable 2

Randomization and Blinding. Vials were labeled with dose to enable randomization within each dose and included a unique number that provided a means to later identify whether the vial contained Ad5.hAC6 or placebo. A document containing this information was kept at the manufacturer's facility (Weill Cornell). Subjects care givers and those acquiring and analyzing data (echocardiographers and others) were blinded to group assignment for the entirety of the trial. Unblinding occurred only after data were locked. The core research pharmacist (also blinded) randomly selected a vial from within a given dose group to send to a participating center when a subject was ready for randomization.

Study Product Delivery. Ten ml of study product was divided in order to deliver 5 ml into the left anterior descending, 3 ml into the left circumflex, and 2 ml into the right coronary artery perfusion beds at a rate of 4 ml/min. Nitroprusside (50 μg/min IC) was also delivered through the infusion catheter, starting 1 minute before and continuing for the duration of each administration of study product, to increase gene transfer efficiency.[18] Correct catheter placement was documented before and after each infusion by contrast injection.

LV dP/dt: LV pressure data were acquired before study product administration and 4w after randomization. A 5F JR4 guide catheter was placed into the LV cavity and a 2F Millar catheter was advanced through the guide 2 cm beyond its tip. LV pressures from fluid filled guide and Millar catheters along with ECG were recorded before and during graded intravenous infusions of dobutamine (5, 10, and 20 μg/kg/min, 5 min each, 15 min cumulative). LV pressure (via guiding catheter) was used to calibrate the Millar transducer pressure. Data were digitally acquired at 500 Hz and analyzed during the last 30s of each infusion stage for peak positive (+dP/dt) and peak negative (-dP/dt), which are estimates of LV systolic and diastolic function. Premature (PVC) and post-premature (post-PVC) beats were excluded and dP/dt values for all other beats (usually 30 to 40 beats) in the 30-second interval were averaged. Core laboratory personnel conducted acquisition and analysis of LV dP/dt to ensure uniformity

Symptoms. Subjects completed the Kansas City Cardiomyopathy Questionnaire (KCCQ) before and 4w and 12w after randomization. A single composite score, the arithmetic mean of graded responses (exclusive of the two questions that reflect knowledge of HF), where higher values denote worse symptoms underwent statistical analysis. All 14 placebo and 22 subjects of D4+5 completed baseline and 4w questionnaires, one subject from each group did not complete the 12w questionnaire and one Ad5.hAC6 subject did not return the form.

PCR Assay. Blood samples were obtained 1 hour, 1 week and 2 weeks after study product delivery and examined for detection of serum Ad5.hAC6 DNA by PCR. The Genomic Core at UCSD extracted Ad5.hAC6 DNA using a DNA extraction kit from Qiagen following manufacture's instruction and detected using real-time quantitative PCR. The transgene-specific primers include: Forward primer overlapping the CMV promoter: (CMV_AC6-167F: 5′ CCG

TCA GAT CCG CTA GAG ATC T3'), Reverse primer in the AC6 gene (CMV_AC6-247R: 5' GGG ACC AGG AGG CCA CTA AA3'), and probe in between CMV-AC6 gene: (CMV_AC6-199T: 5'AGA ATT CGC CCT TCG GCA GCA TGT3'). The Ad5.hAC6 plasmid DNA was used as standard in the PCR for determining copy number. The internal control was 18S rRNA (Forward primer: 948 F: CGC CGC TAG AGG TGA AAT TC, Reverse primer: 1009 R: TTG GCA AAT GCT TTC GCT C, and probe: 969 P: TGG ACC GGC GCA AGA CGG AC).

Anti-Ad5 Antibody Assay. Blood samples were obtained prior to randomization and sent to Fred Hutchinson Laboratory (Seattle WA) where a neutralizing antibody assay for titer quantification of anti-human-adenovirus 5 antibodies was conducted. The anti-Ad5 antibody assay is based on the ability of the test serum to block adenovirus infection of 293 cells. The adenovirus used in the Ad5 neutralizing antibody (NAB) assay was genetically engineered to carry a reporter gene for secreted alkaline phosphatase (SEAP). To allow for neutralization of the virus by the test serum, equal volumes of a fixed amount of virus and the test serum at final dilutions ranging from 1:18 to 1:4608 were incubated for one hour. The mix was added to 293 cells that had been seeded at $3x10^4$ cells/well in 96 well plates two days prior to the neutralization step of the assay. After one hour, the infection mix was removed and the wells are re-fed. One day after infection, 50 ml of media was removed and the amount of SEAP in this supernatant was detected in a reporter gene assay using the chemiluminescent substrate (CSPD$^{\text{Ò}}$) (Life Technologies, Grand Island, NY) and read on a luminometer (Dynex, Chantilly, VA). The neutralization titer was defined as the dilution at which a 50% reduction of SEAP activity from the serum sample was observed relative to SEAP activity from virus infection alone.

Right Heart Catheterization. Measurements of heart rate, cardiac output (thermodilution) and mean pressures in right atrium, pulmonary artery, pulmonary artery wedge, and aorta were recorded before and 4w after study product administration.

eResults. Findings

EF, LV dPdt, and ETT Duration. The differences in EF response between those participants with ischemic vs non-ischemic etiology are shown in **Figure e1**. Dose group data for the three key efficacy endpoints at all time points are listed in **eTable 3 (EF), eTable 4 (LV dP/dt) and eTable 5 (ETT Duration).**

Ad5.hAC6 Dose-Response Relationship. The summary data for the Ad5.hAC6 dose-response relationship is in **eTable 6.**

LV Volume. The summary data for LV end-diastolic and end-systolic volume and end-diastolic volume, measured by echocardiography, are shown in **eTable 7.**

Hemodynamic Measurements. The summary data obtained during right heart catheterization are shown in **eTable 8.**

Quantitative PCR Assay on Serum. We obtained blood samples 1 hour after study product delivery to detect Ad5.hAC6 DNA using PCR. The mean number of Ad5.hAC6 DNA copies per μl serum were: placebo (n=14): 0·014±0·011; Dose 1 (n=6): 0·002±0·0; Dose 2 (n=6): 0·003±0·001; Dose 3: (n=5): 1·311±0·80; Dose 4 (n=12): 0·006±0·001; Dose 5 (n=11): 0·272±0·159. Dose 3 was significantly increased vs placebo (p=0·011) and Dose 5 tended to be increased (p=0·08 vs placebo). Of the 14 subjects that had detectable Ad5.hAC6 DNA in serum 1 hr after the completion of vector delivery, 4 (3 Dose 3, 1 Dose 5) continued to have detectable Ad5.hAC6 DNA in serum at 1 week. Three of the four were Dose 3 subjects who showed Ad5·hAC6 DNA copies per μl serum 1hr vs 1w: 0·013 (down from 3·30); 0·234 (down from 3·234); 0·011 (down from 0·061). The fourth subject (Dose 5) had persistent elevation (0·227 at 1w, 0·213 at 1 hr). All four of these subjects had no detectable Ad5.hAC6 DNA at 2w after vector deliver.

Symptom Evaluation. eFigure specifies incomplete data in the key group analyses for the key endpoints. Listed here is supplemental information including incomplete data on symptom evaluation using a modification of KCCQ. All 14 placebo and 22 subjects of D4+5 completed baseline and 4w questionnaires, one subject from each group did not complete the 12w questionnaire and one Ad5.hAC6 subject did not return the form.

Serious Adverse Events. One Dose 5 subject (subsequently determined to have received Ad5.hAC6) had fever the morning after study product infusion and was kept in hospital an extra day. There was one femoral hematoma that required one day of hospitalization. One subject had a cerebral vascular event 1 hour after the 4w catheterization study.

Assessment of Cardiac and Hepatic Injury. Although there was a non-significant increase in TnI shortly after initial study product delivery (**eTable 9**), whether this was vector or procedure related was not clear. There were no statistical differences in these values within or between groups, and no subject had an elevation in CPK-MB (**eTable 9**). The procedure itself, which included IC manipulation with wires and infusion catheters, combined with the use of dobutamine and IC nitroprusside may have provoked a small TnI release. The disappearance of TnI elevation and the absence of elevation in AST or ALT (**eTable 8**) make clinically important adenovirus-associated inflammation, which would have been expected to worsen rather than improve over a two-week period, unlikely.

Frequency of Non-Sustained VT and ICD Therapy Events. See eTable 11.

eTable 1. Dose Groups			
Dose Group	**vp**	**Ad5.hAC6 (n)**	**Placebo (n)**
1	3.2×10^{9}	6	2
2	3.2×10^{10}	6	2
3	10^{11}	6	2
4	3.2×10^{11}	12	4
5	10^{12}	12	4
Total Subjects		42	14
vp, virus particles			

eTable 2. Clinical Tests and Frequency									
	Pre	**D1**	**D4**	**W1**	**W2**	**W4**	**W12**	**M6**	**M12**
Exercise Test	⊙					⊙	⊙		
History & KCCQ	⊙	⊙		⊙	⊙	⊙	⊙	⊙	⊙
Physical Exam	⊙	⊙		⊙	⊙	⊙	⊙	⊙	⊙
Urine Sample	⊙			⊙	⊙	⊙	⊙		
Blood Sample	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙	⊙
Chest X-Ray	⊙						⊙		
ECG	⊙	⊙		⊙	⊙	⊙	⊙	⊙	⊙
Echocardiography	⊙					⊙	⊙		
ICD Interrogation	⊙	⊙			⊙	⊙		⊙	⊙
RHC, LVEDP, LV dP/dt		⊙				⊙			
IC Ad5.hAC6 or Placebo		⊙							

KCCQ, Kansas City Cardiomyopathy Questionnaire; ICD, implantable cardiac defibrillator; RHC, right heart catheterization; LVEDP, LV end-diastolic pressure; LV dP/dt, rate of LV pressure development; IC Ad5.hAC6, intracoronary delivery of human adenovrus-5 encoding human adenylyl cyclase type 6

eTable 3. Ejection Fraction						
Group (mean±SE)	Baseline (%)	After Randomization (%)				n
		4w	Change 4w - Baseline	12w	Change 12w - Baseline	
Placebo	29.6±2.4	33.7±3.0	4.1±2.2	31.6±2.0	0.8±1.2	13-14
Dose 1	31.8±2.7	32.5±3.0	0.7±1.9	36.5±2.8	4.7±1.6	6
Dose 2	29.3±3.0	29.2±3.6	-0.2±2.6	28.5±3.3	-0.8±2.2	6
Dose 3	41.2±2.7	35.3±4.7	-3.0±2.9	37.3±4.2	-0.2±1.5	5-6
Dose 4	30.3±2.5	34.1±2.1	3.5±2.0	33.2±3.3	2.8±3.6	11-12
Dose 5	29.1±3.5	38.8±3.9	7.7±2.7	35.3±3.5	4.2±3.3	10-11
Dose 4+5	29.7±2.1	36.3±2.2	5.5±1.7	34.2±2.4	3.5±2.4	21-23

eTable 4. Left Ventricular Peak dP/dt							
Group (mean±SE)	LV Peak +dP/dt (mmHg/sec)			LV Peak -dP/dt (mmHg/sec)			n
	Baseline	4w	Change 4w - Baseline	Baseline	4w	Change 4w - Baseline	
Placebo	929±52	931±48	2±44	1068±76	975±75	-93±51	14
Dose 1	1023±76	1038±78	16±48	1194±128	1329±125	135±89	6
Dose 2	1045±137	1083±116	-15±98	1102±116	1134±118	-6±68	4-6
Dose 3	1214±45	956±81	-266±54	1179±41	1078±88	-137±89	4-5
Dose 4	946±70	1045±90	99±69	1041±89	1088±85	47±56	11
Dose 5	941±84	965±79	9±54	1087±85	1142±76	29±36	11
Dose 4+5	943±53	1005±59	56±45	1064±60	1115±56	39±33	22

eTable 5. Exercise Treadmill Test Duration						
Group (mean±SE)	Baseline (sec)	After Randomization (sec)				n
		4W	Change 4w - Baseline	12W	Change 12w - Baseline	
Placebo	416±56	446±70	27±36	501±65	61±24	12-14
Dose 1	516±70	575±102	59±38	529±106	13±54	5-6
Dose 2	365±47	392±39	27±42	549±56	185±40	5-6
Dose 3	348±80	477±87	129±75	517±73	168±73	5-6
Dose 4	411±74	465±75	54±24	484±61	74±53	7
Dose 5	440±53	503±68	37±40	514±65	47±35	10-12
Dose 4+5	429±42	487±49	44±25	501±45	58±29	17-19

eTable 6. Ad5.hAC6 Dose-Response Relationship							
	Placebo (13-14)	*Ad5.hAC6 Response (EF Unit increase vs Baseline)*					P
		D1 (6)	D2 (6)	D3 (5)	D4 (11)	D5 (10)	
4 Weeks	4.8 ± 2.0	0.7 ± 1.9	-0.2 ± 2.6	-3.0 ± 2.9	3.5 ± 2.0	7.7 ± 2.7	0.036
12 Weeks	0.8 ± 1.2	4.7 ± 1.6	-0.8 ± 2.2	-0.2 ± 1.5	2.8 ± 3.6	4.2±3.3	0.84[A]

Values are change in EF vs baseline value (mean ± SE); group size in parenthesis. The relationship between Ad5.hAC6 dose and change in EF from baseline (mean ± SE) was examined using a log-linear regression analysis.

[A] At 12w, D 2-5 (excluding D1) showed a dose-response effect (P<.01)

eTable 7. LV Volume				
LV End Systolic Volume				
	4W Change (ml/M^2)	**12W Change (ml/M^2)**	**4w Change (ml)**	**12w Change (ml)**
Placebo (13)	-0.4 ± 3.4	-0.6 ± 4.7	- 1	- 1
D 4+5 (21)	-9.0 ± 4.8	-9.5 ± 5.1	- 19	- 20
D 4+5 (NI) (10)	-12.9 ± 9.0[A]	-16.5 ± -7.6[B]	- 27	- 35
LV End-Diastolic Volume				
Placebo (13)	2.3 ± 4.6	0.5 ± .8	+ 1	+ 1
D 4+5 (21)	-4.2 ± 5.0	-6.9 ± 5.3	- 9	- 14
D 4+5 (NI) (10)	-6.3 ± 8.2	-8.9 ± 7.8	- 13	- 19
NI, subjects with non-ischemic HF etiology; [A]P<.16 vs placebo; [B]P<.08 vs placebo				

eTable 8. Hemodynamic Measurements

	Placebo (10-14)		Ad5.hAC6 D4+5 (19-24)	
	Pre	4w	Pre	4w
HR (bpm)	72 ± 4	69 ± 3	67 ± 6	68 ± 6
MAP (mmHg)	87 ± 3	85 ± 3	81 ± 8	84 ± 8
Mean RA (mmHg)	10 ± 1	10 ± 1	9 ± 3	9 ± 3
Mean PA (mmHg)	28 ± 3	27 ±3	26 ±2	26 ± 2
Mean PAWP (mmHg)	19 ± 2	20 ± 3	16 ± 8	16 ± 8
LVEDP (mmHg)	24 ± 2	23 ± 2	22 ± 2	23 ± 2
COTD (L/min)	5.4 ± 0.5	5.1 ± 0.4	4.7 ± 0.2	4.5 ± 0.2
Cardiac Index (L/min/M^2)	2.6 ± 0.2	2.4 ±0.2	2.3 ±0.1[A]	2.3 ±0.1

No between-group differences in response at 4w were seen.

HR, heart rate; bpm, beats per minute; MAP, mean arterial pressure; RA, right atrial pressure;

PAWP, pulmonary artery wedge pressure; LVEDP, left ventricular end-diastolic pressure; COTD, cardiac output by thermodilution method

[A] P=.14 vs Pre value for placebo. Normal cardiac index: 2.6 - 4.2 L/min/M^2

eTable 9. Assessment of Cardiac Injury

	Baseline			D2			D4			W2			W4			W12		
	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5
TnI	.03±.01	.04±.02	.04±.01	.17±.08	.25±.17[A]	.18±.1	.10±.05	.21±.12[B]	.15±.1	.03±.01	.04±.02	.03±.01	.04±.01	.06±.03	.05±.02	.04±.01	.04±.02	.04±.01
CPK-MB	2.6±.3	2.2±.2	2.2±.2	2.7±.4	1.7±.2[C]	2.4±.3	2.5±.3	2.1±.4	2.5±.3	2.5±.3	2.0±.3	2.1±.2	2.2±.22	2.1±.2	2.1±.2	2.5±.4	2.4±.2	2.4±.2

TnI and CPK-MB values are in µg/L; P, placebo (n=9-14); D4+5, Ad5.hAC6 Doses 4+5 (n=20-24); D1-5, Ad5.hAC6 Doses 1-5 combined (n=30-41)

[A] P=.77 vs placebo; [B] P=.50 vs placebo; [C] P=.02 vs placebo (note that placebo is higher than D4 + 5, and both are in the normal range)

eTable 10. Assessment of Hepatotoxicity

	Baseline			W1			W2			W4			W12		
	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5	P	D4+5	D1-5
AST	26±3	55±33	40±19	28±5	26±2	24±1	23±2	25±3	23±2	24±2	22±2	21±1	22±2	21±2	21±1
ALT	28±4	25±3	24±2	76±46	28±4[A]	27±2	35±9	29±4	27±2	27±3	23±2	23±2	24±4	23±2	23±2
Sed Rate	17±3	13±2	16±2	21±3	21±3	25±3	20±3	18±3	22±3	15±2	17±3	20±3	15±2	14±3	20±3

AST and ALT in Units/L; sedimentation rate in mm/hr

P, placebo (n=10-14); D4+5, Ad5.hAC6 Doses 4 + 5 combined (n=20-24); D1-5, Ad5.hAC6 Doses 1-5 combined (n=38-42); [A] p=0.17 vs placebo

eTable 11. Frequency of Non-Sustained VT and ICD Therapy Events					
	Placebo 14	**Ad5.hAC6 42**	**Confidence Interval**	**Relative Risk AC6/Placebo**	p
ICD Shocks	1 (7.1%)	(1) 2.4%	0.01 – 11.10	0.33	0.44 [A]
ICD ATP	1 (7.1%)	2 (4.8%)	0.06 – 18.10	0.67	1.0 [A]
Subjects w NSVT	5 (35.7%)	9 (21.4%)	0.24 – 1.90	0.60	0.30 [A]

Both ICD shocks occurred 6m after study product delivery, I in a placebo subject, 1 in a Dose 3 subject. Anti-tachycardia pacing (ATP) occurred at Month 6 in a placebo subject, and at months 6 & 12 in two Ad5.AC6 subjects (Dose 1 & Dose 2). ICD events and NSVT episodes were not dose-related.

[A] *Fisher's exact test*

eFigure. Cause of Heart Failure and Ejection Fraction Response. Cause of heart failure determines ejection fraction (EF) response in Dose 4 + 5 subjects. Shown is EF change at 4 and 12 weeks comparing D4 + 5 subjects with ischemic (n=11) vs non-ischemic heart failure (n=10) vs placebo subjects (n=13). All 3 groups showed an EF increase at 4 weeks, but only subjects with non-ischemic heart failure showed an effect at 12 weeks, a mean +8.2±3.3 EF unit increase vs a mean +0.8±1.2 EF unit increase in the 13 placebo subjects (p=0.024). Participants with non-ischemic heart failure also fared better than those with ischemic heart failure (p=0.02) and better than placebo subjects with ischemic heart failure (P<0.02; n=6; not shown). P values from analysis of variance; symbols denote mean ±SE.