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A Phase II, Randomized, Double-Blind, Multicenter, Based on Standard Therapy, Placebo-Controlled Study of the Efficacy and Safety of Recombinant Human Neuregulin-1 in Patients With Chronic Heart Failure

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Objectives The purpose of this study was to assess the safety and efficacy of recombinant human neuregulin-1 (rhNRG-1) in chronic heart failure (CHF) patients.

Background Neuregulin-1 plays important roles in maintaining cardiomyocyte structure and cardiac pumping functionality and physiology. Previously, rhNRG-1 was proven to be effective in treating heart failure in animals by reducing end-diastolic volume (EDV) and end-systolic volume (ESV) and increasing left ventricular ejection fraction (LVEF%).

Methods A total of 44 CHF patients designated as New York Heart Association functional class II or III were enrolled in a double-blind, randomized manner and treated with a placebo or rhNRG-1 (0.3, 0.6, or 1.2 μg/kg/day) for 10 days, in addition to standard therapies. The follow-up period was 90 days; left ventricular function and structure measured by magnetic resonance imaging were the primary end points.

Results Although not statistically different from placebo, the LVEF% was significantly increased by 27.11 $\pm$ 31.12% ($p = 0.009$) at day 30 after rhNRG-1 treatment in the 0.6-μg/kg group, whereas it was only increased 5.83 $\pm$ 25.75% in the placebo group ($p = 0.49$). In addition, there were decreases in ESV ($-11.58 \pm 12.74\%$, $p = 0.002$) and EDV ($-5.64 \pm 10.03\%$, $p = 0.05$) in the 0.6-μg/kg/day group at day 30; more importantly, both ESV and EDV levels continued to decrease at day 90 ($-20.79 \pm 17.03\%$ and $-14.03 \pm 13.17\%$, respectively), accompanied by a sustained increase in LVEF%. This suggests that short-term treatment with rhNRG-1 results in a long-term reversal of remodeling. The effective dose was proven to be tolerable and safe for CHF patients.

Conclusions rhNRG-1 improved the cardiac function of CHF patients by increasing the LVEF% and showed the capability of antire-modeling by decreasing ESV and EDV compared with pre-treatment. (A Randomized, Double-Blind, Multi-Center, Placebo Parallel controlled, Standard Therapy Based Phase II Clinical Trial to Evaluate the Efficacy and Safety of Recombinant Human Neuregulin-1 for Injection in Patients with Chronic Heart Failure; ChiCTR-TRC-00000414) (J Am Coll Cardiol 2010;55:1907–14) © 2010 by the American College of Cardiology Foundation

Chronic heart failure (CHF) is a common syndrome with a prognosis worse than most forms of cancer. Despite advances that have been made in heart failure (HF) drug development, the overall prognosis remains poor, with high mortality rates. Approximately 80% of men and 70% of women younger than 65 years of age with HF are estimated to die within 8 years of diagnosis (1).

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Abbreviations and Acronyms

AE = adverse event
CHF = chronic heart failure
EDV = end-diastolic volume
ESV = end-systolic volume
HF = heart failure
LVEF = left ventricular ejection fraction
MRI = magnetic resonance imaging
NRG = neuregulin
NT-proBNP = N-terminal pro–B-type natriuretic peptide
NYHA = New York Heart Association
rhNRG = recombinant human neuregulin

Neuregulin (NRG)-1, a member of the neuregulin family, is wildly expressed, particularly in the heart. NRG-1 has an indispensable role in cardiac development and in the maintenance of adult heart structural and functional integrity (2). Conditional NRG-1/ErbB–deficient mice show dilated cardiomyopathy, suggesting that NRG-1, together with its receptors ErbB2 and ErbB4, plays a prominent role in the pathogenesis of CHF (3–5). NRG-1/ErbB signaling is activated in the early stages of CHF, together with a profound upregulation of NRG-1 expression in the left ventricular chamber. However, in the later stages of pump failure, both NRG-1 expression and NRG-1/ErbB signaling are inhibited (6). Evidence of NRG-1's potential involvement in human HF was first shown in the clinical cardiotoxicity of the ErbB2 antibody-based compound trastuzumab, used to treat breast cancer (7).

Enlightened by these findings, we began to investigate the possibility of converting NRG-1 to an HF treatment drug. First, we manufactured a recombinant human neuregulin-1β2 protein (rhNRG-1), which contains 61 amino acid residues and covers the epidermal growth factor-like domain (the domain necessary for ErbB2/ErbB4 activation). Although Zhao et al. (8) suggested that NRG-1 had a hypertrophic effect on cultured rat neonatal cardiac muscle cells, the unique effects of rhNRG-1 were observed in an in vitro experiment in 1998, as reported by Zhou et al. (9). Cardiomyocytes maintained in serum-free cultures displayed a disrupted sarcomere arrangement, whereas rhNRG-1 treatment stimulated both sarcomeric structure reorganization and cardiac contractile unit assembly. This phenomenon was also confirmed by Baliga et al. (10).

Our pre-clinical work demonstrated that short-term intravenous administration of rhNRG-1 attenuated pathological changes and prolonged survival in different types of CHF animal models (including CHF dogs and rats by ligation of the left anterior descending coronary artery, rapid ventricular pacing, anthracycline, and myocarditis) (11). However, it did not alter hemodynamic or cardiac contractility in normal animals. These beneficial effects indicated the potential role of rhNRG-1 in addressing HF, thus making rhNRG-1 a promising therapeutic agent for CHF.

Encouraged by these findings, we put rhNRG-1 into a clinical trial. Since 2004, Zensun Ltd. Co. (Shanghai, China) has sponsored and successfully carried out a series of clinical trials. Phase I and IIa trials in China confirmed the safety of rhNRG-1 in both healthy individuals and CHF patients, showing that rhNRG-1 can effectively improve cardiac function by increasing LVEF% in CHF patients. Based on these findings, we embarked on Phase II clinical trials in both China and Australia, simultaneously using several different protocols aimed at generating different indicators or evaluative end points. In this report, we introduce one of these studies, which focuses on investigating the efficacy of rhNRG-1 on cardiac pump functionality and remodeling in CHF patients.

Methods

The Independent Ethics Committee on Human Research of the Cardiovascular Institute and Fuwai Hospital approved the study protocol.

Study design. This study was designed as a randomized, double-blind, multicenter, background therapy-based, placebo-controlled, parallel group study. Target enrollment was 44 patients at 5 clinical research units in Beijing, with 33 patients in the active treatment group and 11 in the placebo group.

Random allocation of patients to each treatment group was made within each center. Specifically, 8 patients were considered to be a block (4 different treatment groups of 2 patients each). Blocks were randomized to each site as a unit with no block divided between sites. The number of blocks at each site depended on the rate of enrollment progress. Therefore, the numbers of patients in the groups were almost equal.

The doses used in this study were selected according to our previous results. The Phase I trial showed that 1.2 μg/kg/day was the tolerable dose for bolus injection. As revealed by animal studies, compared with the bolus injection, an 8-hour intravenous infusion per day for 10 consecutive days could achieve better improvement in cardiac function together with reduced side effects. In addition, the Phase IIa trial further confirmed that 1.2 μg/kg/day was safe for the HF patients. Thus, 1.2 μg/kg/day and 10-h intravenous infusion were chosen as the maximum dose and administration route in this study.

Patients were randomly assigned to 4 groups, treated with placebo or rhNRG-1 (0.3, 0.6, or 1.2 μg/kg/day) for 10 consecutive days. For the patients who might need to discontinue the study prematurely due to intolerance, a comprehensive safety evaluation would be performed on the day after the most recent administration. Both the experimental and placebo groups were allowed to continue therapy for HF with basic standard drugs, including angiotensin-converting enzyme inhibitors and/or beta-blocker or angiotensin II receptor blocker, diuretic, or digoxin.

Patients. Criteria for participation in the trial included patients with CHF (New York Heart Association functional class II or III) between the ages of 18 and 65 years old, echocardiographically measured LVEF $\leq$40% (echocardiography was only used for screening patients), in relatively stable clinical condition (including clinical signs,

symptoms, and accepted standard treatment for CHF at the target dose or maximum tolerated dose for >1 month). Major exclusion criteria included magnetic resonance imaging (MRI) contraindications, claustrophobia, acute myocardial infarction, hypertrophic cardiomyopathy, constrictive pericarditis, significant valve or congenital heart disease, severe pulmonary hypertension, systolic blood pressure <90 mm Hg or >160 mm Hg, severe ventricular arrhythmia, cardiac surgery or a cerebrovascular event within the previous 6 months, and pregnant women. All patients provided witnessed written consent.

End points and follow-up. The primary end point of the trial was a change in LVEF%, ESV, or EDV as measured by MRI at baseline and days 11, 30, and 90. Cardiac function classification (NYHA), the 6-min walk test, quality of life score, and plasma N-terminal pro–B-type natriuretic peptide (NT-proBNP) were viewed as secondary efficacy end points. The entire study period was 90 days.

Data management and quality assurance. Computer programs combined with human review verified data quality. All queries were answered by the investigators, and the inspection procedures were repeated several times to ensure that results were compelling beyond reasonable doubt. All changes and updates were subject to recording and archiving.

Magnetic resonance images were collected in the core laboratory and transported to Cardiolysis, Inc. (Rotterdam, the Netherlands) (a specialist in cardiac MRI analysis) for analysis. Plasma NT-proBNP was tested in the core laboratory with dedicated kit NT-proBNP assays (Biomedica, Vienna, Austria).

Statistical analysis. Distribution of the baseline variables was compared using analysis of variance and chi-square tests (for categorical variables). Differences among groups after randomization were analyzed after each scheduled follow-up visit by comparing the mean percentage of change from the baseline using analysis of variance; multiple comparisons were evaluated with a method of contrast without adjustment. Within-group differences after treatment were evaluated by paired *t* tests without correction for multiple comparisons. Values are reported as mean ± SD; all p values reported are for 2-tailed tests, and $p < 0.05$ was considered statistically significant.

Results

Patients and compliance with the treatment. Between July 2007 and May 2009, a total of 44 patients with NYHA functional class II or III stable CHF were enrolled and randomized to 4 groups: placebo or 0.3, 0.6, and 1.2 μg/kg of rhNRG-1. There were no significant variations in demographics or background therapies among groups (Table 1). All enrolled patients were administered the drug for 10 consecutive days, except patients 6066 and 6085 in the 1.2-μg/kg group, who withdrew on days 5 and 7, respectively, due to adverse reactions, and patient 6050 in the 0.6-μg/kg group, who was eliminated on day 3 due to a protocol violation. Patient 6018 in the 0.6-μg/kg group dropped out after the 30-day follow-up (Fig. 1).

Cardiac functionality assessed by MRI. Cardiac functionality as measured by MRI was the primary end point of this study. Figure 2 shows a dynamic change in LVEF% over the entire period of the study. Although the changes are not statistically significant between the rhNRG-1 and placebo groups, there was an increase in LVEF% in the 0.6-μg/kg group (12.10 ± 22.27%, $p = 0.12$), first observed at day 11; it became more significant at day 30 (increase of 27.11 ± 31.12%, $p = 0.009$). More importantly, even at day 90, 80 days after the treatment, LVEF% remained at a high level (increase of 31.99 ± 44.93%, $p = 0.02$). Throughout

Table 1 Demographics and Background Therapy

	Placebo (n = 11)	0.3 μg/kg (n = 11)	0.6 μg/kg (n = 11)	1.2 μg/kg (n = 10)	p Value Among Groups
Age (yrs)	43 ± 10.0	47 ± 7.5	39 ± 14.5	48 ± 12.4	0.226
Sex, n					
Male	11	9	10	7	0.202
Female	0	2	1	3	
Weight (kg)	80 ± 12.5	72 ± 14.5	77 ± 12.2	70 ± 15.6	0.338
NYHA functional class, n					
II	7	7	9	5	0.508
III	4	4	2	5	
Therapy, n					
Baseline					
Beta-blocker	10	9	10	10	0.892
ACEI	9	5	10	7	0.115
Devices	0	0	0	0	—
Adjusted					
Beta-blocker	3	3	5	4	0.948
ACEI	1	1	0	0	0.721
Devices	0	0	0	0	—

ACEI = angiotensin-converting enzyme inhibitor; NYHA = New York Heart Association.

JACC Vol. 55, No. 18, 2010
May 4, 2010:1907–14

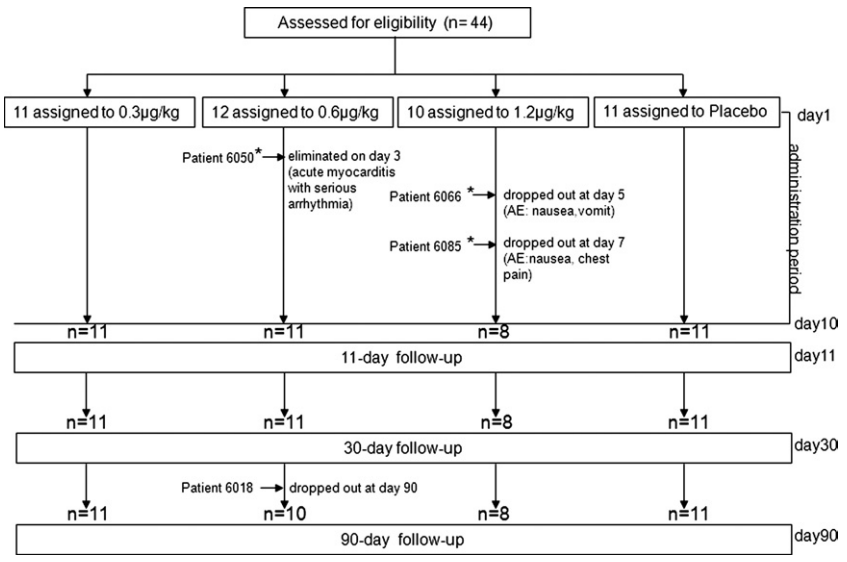
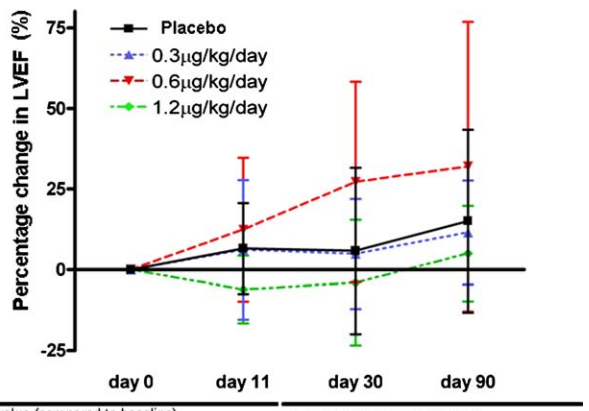


Figure 1 **Flow Diagram of the Study**

*MRI data of patients who dropped out during the administration time were not included in the final analysis. AE = adverse event.

the study, LVEF% in the placebo group did not change significantly compared with the baseline, only showing an increase in LVEF% of 5.83 ± 25.75% (p = 0.49) at day 30 and 15.05 ± 28.29% at day 90 (p = 0.16). Although not as dramatic as what was observed for the 0.6-μg/kg group, the 0.3-μg/kg group experienced an increase in LVEF% at day 90 (11.55 ± 16.11%, p = 0.03). There was no improvement in cardiac pumping functionality in the 1.2-μg/kg group.



P value (compared to baseline)	Day11	Day30	Day90
Placebo	0.16	0.49	0.16
0.3μg/kg	0.63	0.63	0.03
0.6μg/kg	0.12	0.009	0.02
1.2μg/kg	0.16	0.74	0.38

P value (compared to placebo)	Day11	Day30	Day90
0.3μg/kg	0.57	0.67	0.76
0.6μg/kg	0.72	0.11	0.43
1.2μg/kg	0.08	0.42	0.32

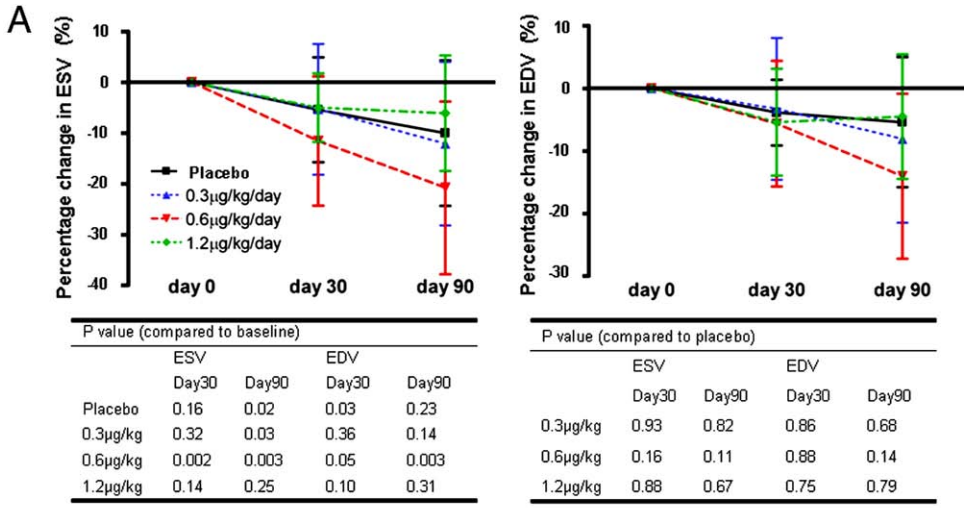
Figure 2 **Percentage of Change in LVEF% After Treatment**

The **graph** shows the percentage of change in left ventricular ejection fraction (LVEF%) (mean ± SD) at days 11, 30, and 90 compared with day 0 (baseline). **Black line** = placebo, **blue line** = 0.3-μg/kg dose, **red line** = 0.6-μg/kg dose, and **green line** = 1.2-μg/kg dose. The **tables at the bottom** show the statistical results shown as the p values.

Together with the increase in LVEF%, there was a tendency for a time-dependent decrease in ESV and EDV in the 0.3- and 0.6-μg/kg groups. By days 30 and 90 in the 0.3-μg/kg group, ESV had decreased by 5.33 ± 12.89% and 12.10 ± 16.12%, respectively, and EDV had decreased by 3.28 ± 11.34% and 8.07 ± 13.38%, respectively. Also at days 30 and 90, in the 0.6-μg/kg group, ESV decreased by 11.58 ± 12.74% and 20.79 ± 17.03%, respectively, and EDV decreased by 5.64 ± 10.03% and 14.03 ± 13.17%, respectively. Consistent with the results of changes in LVEF%, 0.6 μg/kg was the most effective dose for inhibiting cardiac enlargement.

Despite the short period of administration, ESV and EDV were reduced in the rhNRG-1 0.6- and 0.3-μg/kg dose groups even after day 30, when heart pumping functionality had already been greatly improved. Comparing the results of day 90 with those of day 30, ESV and EDV levels were further decreased (Fig. 3B). From this point, the benefit of rhNRG-1 to inhibit or reverse the cardiac remodeling is long-lasting. Further, there are dose-dependent effects exhibited between 0.3 and 0.6 μg/kg.

Secondary efficacy end points. Considering the small sample number, it may be difficult to generate positive results with respect to the 6-min walk test, quality of life, and NYHA functional class in this study. As estimated, there were no significant differences between the placebo and rhNRG-1 treatment groups with respect to improvements in 6-min walk test, quality of life, and NYHA functional class (Table 2).

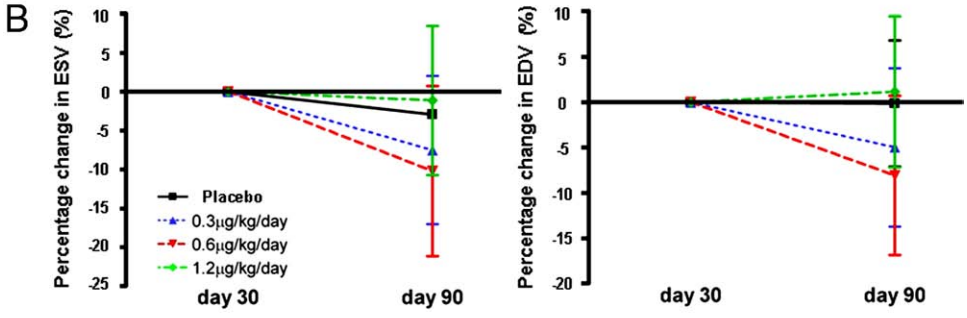


P value (compared to baseline)

	ESV		EDV	
	Day30	Day90	Day30	Day90
Placebo	0.16	0.02	0.03	0.23
0.3μg/kg	0.32	0.03	0.36	0.14
0.6μg/kg	0.002	0.003	0.05	0.003
1.2μg/kg	0.14	0.25	0.10	0.31

P value (compared to placebo)

	ESV		EDV	
	Day30	Day90	Day30	Day90
0.3μg/kg	0.93	0.82	0.86	0.68
0.6μg/kg	0.16	0.11	0.88	0.14
1.2μg/kg	0.88	0.67	0.75	0.79



	ESV			EDV		
	Percentage change	P value compared to day30	P value compared to placebo	Percentage change	P value compared to day30	P value compared to placebo
0 μg/kg	-2.79±9.53%	0.35		-0.15±6.79%	0.82	
0.3μg/kg	-7.50 ±9.51%	0.04	0.37	-5.00 ±8.74%	0.10	0.18
0.6μg/kg	-10.20 ± 10.93%	0.01	0.13	-8.08±8.79%	0.06	0.05
1.2μg/kg	-1.14 ± 9.58%	0.84	0.68	1.13 ±8.31%	0.74	0.81

Figure 3 **Percentage of Change in ESV and EDV After Treatment**

(A, graphs) Percentage of change in end-systolic volume (ESV) and end-diastolic volume (EDV) (mean ± SD) at days 11, 30, and 90 compared with day 0 (baseline). **(A, tables)** Statistical results shown as the p values. **(B, graphs)** Percentage of change in ESV and EDV (mean ± SD) at day 90 compared with day 30. **Black lines** = placebo, **blue lines** = 0.3-μg/kg dose, **red lines** = 0.6-μg/kg dose, and **green lines** = 1.2-μg/kg dose. **(B, table)** Statistical results.

Plasma NT-proBNP, an indicator of the prognosis, was also measured in this study. There was an elevation of NT-proBNP (increase of 26.79 ± 102% compared with baseline) in the placebo group at day 90; this elevation seemed to be inhibited in the 0.6- and 0.3-μg/kg treatment groups (decrease of 3.5 ± 60.07% and increase of 8.66 ± 74.83%, respectively, compared with baseline), as shown in Figure 4. This result indicated that rhNRG-1 might be a favorable treatment for HF patients.

Adverse events (AEs). There were 44 patients enrolled in the study, and the incidence of AEs in the placebo, 0.3-, 0.6-, and 1.2-μg/kg groups was 54.44%, 63.64%, 66.67%, and 100%, respectively. The incidence of AEs did not increase when the drug was administered at a dose <0.6 μg/kg compared with placebo, whereas it increased in the 1.2-μg/kg group. The mostly commonly occurring AEs were gastrointestinal disorders (Table 3), such as nausea, vomiting, poor appetite, dyspepsia, diarrhea, and abdominal discomfort, which were well tolerated in most cases. Other AEs included headache, dizziness, fatigue, and palpitations, none of which worsened over the period of administration. In addition, no serious AEs occurred during this study.

Vital signs such as heart rate, blood pressure, and respiratory rate were tested every day before and after infusion of the drug during the administration period, and no significant changes were observed. Further, rhNRG-1 treatment had no long-term effects on vital signs, as evidenced by data from follow-up visits. Results showed that rhNRG-1 treatment had no effects on the full blood count (FBC) or urine

Table 2 Secondary Efficacy End Points

	Placebo	0.3 μg/kg/day	0.6 μg/kg/day	1.2 μg/kg/day	p Value Among Groups
6-min walk					
Baseline	413 ± 59.9 (n = 11)	394 ± 51.9 (n = 11)	451 ± 72.3 (n = 11)	432 ± 104.7 (n = 10)	>0.05
Day 11	443 ± 61.3 (n = 11)	408 ± 60.7 (n = 10)	475 ± 86.5 (n = 11)	471 ± 123.3 (n = 9)	>0.05
Day 30	474 ± 54.9 (n = 11) (p = 0.002)*	445 ± 53.9 (n = 11) (p = 0.009)*	507 ± 65.4 (n = 11) (p = 0.002)*	488 ± 78.1 (n = 8)	>0.05
Day 90	496 ± 53.7 (n = 11) (p = 0.001)*	466 ± 67.4 (n = 11) (p = 0.004)*	506 ± 51.1 (n = 9) (p = 0.03)*	494 ± 76.4 (n = 9) (p = 0.01)*	>0.05
Quality of life					
Baseline	28 ± 20.1 (n = 11)	31 ± 22.8 (n = 11)	24 ± 13.5 (n = 11)	24 ± 18.1 (n = 10)	>0.05
Day 11	23 ± 17.8 (n = 11)	17 ± 14.4 (n = 11) (p = 0.002)*	24 ± 13.8 (n = 10)	23 ± 21.3 (n = 9)	>0.05
Day 30	16 ± 18.5 (n = 11) (p = 0.004)*	17 ± 16.8 (n = 11) (p = 0.001)*	17 ± 21.0 (n = 11) (p = 0.04)*	18 ± 19.6 (n = 8)	>0.05
Day 90	13 ± 12.9 (n = 11) (p = 0.002)*	16 ± 16.1 (n = 11) (p = 0.009)*	14 ± 12.6 (n = 10) (p = 0.002)*	13 ± 11.6 (n = 9)	>0.05
NYHA functional classification					
Baseline					
Class I	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	>0.05
Class II	7 (63.64%)	7 (63.64%)	9 (81.82%)	5 (50.00%)	
Class III	4 (36.36%)	4 (36.36%)	2 (18.18%)	5 (50.00%)	
Class IV	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Day 11					
Class I	1 (9.09%)	1 (9.09%)	0 (0.00%)	0 (0.00%)	>0.05
Class II	10 (90.91%)	7 (63.64%)	11 (100.00%)	6 (60.00%)	
Class III	0 (0.00%)	3 (27.27%)	0 (0.00%)	4 (50.00%)	
Class IV	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Day 30					
Class I	2 (18.18%)	2 (18.18%)	1 (9.09%)	2 (22.22%)	>0.05
Class II	9 (81.82%)	9 (81.82%)	10 (90.91%)	7 (77.78%)	
Class III	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Class IV	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	
Day 90					
Class I	2 (18.18%)	2 (18.18%)	1 (10.00%)	2 (22.22%)	>0.05
Class II	9 (81.82%)	8 (72.73%)	9 (90.00%)	7 (77.78%)	
Class III	0 (0.00%)	1 (9.09%)	0 (0.00%)	0 (0.00%)	
Class IV	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	

Measured data of 6-min walk and quality of life reported in mean ± SD. Number of patients in each New York Heart Association (NYHA) functional class, value represents the number of patients (percentage). *Statistical significance (compared with the baseline).

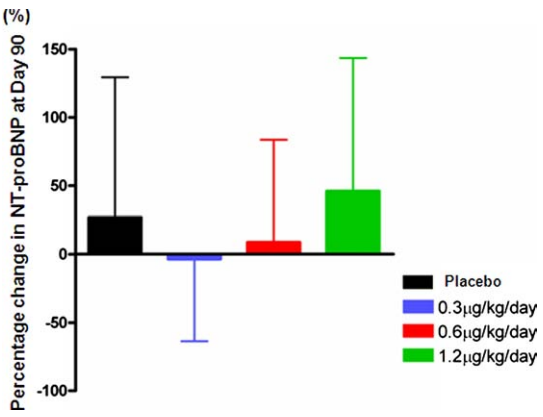


Figure 4 Percentage of Change in NT-proBNP Levels at Day 90

Percentage of change in N-terminal pro–B-type natriuretic peptide (NT-proBNP) (mean ± SD) at day 90 compared with day 0 (baseline). **Black bar** = placebo, **blue bar** = 0.3-μg/kg dose, **red bar** = 0.6-μg/kg dose, and **green bar** = 1.2-μg/kg dose.

test and biochemical markers, including those for myocardium evaluations.

Discussion

NRG-1 has been characterized by its indispensable role during cardiac development and in maintaining functionality. However, whether NRG-1 could be used in treating CHF patients was not apparent. In this study, short-term administration of rhNRG-1 helped increase LVEF% and decreased ESV and EDV levels of the left ventricular chamber even 3 months after treatment, suggesting long-term improvements of cardiac functionality and structure. As a result, we have been able to provide pioneering evidence of the application of NRG-1 in treating CHF.

Related studies. At present, there are no drugs clinically used to treat HF that can directly act on damaged cardiomyocytes and restore heart function. Our previous work obtained significant results with respect to rhNRG-1 potentially rescuing cardiomyocytes from serum-free induced cell structure disarray. At this point, we speculate that rhNRG-1 may be a potential drug for CHF with a novel therapy mechanism, which may directly improve the structure of damaged cardiomyocytes. After the positive impact of rhNRG-1 on the heart was confirmed in both in vitro and in vivo animal studies, rhNRG-1 was put into clinical trials in 2004. Phase I of the rhNRG-1 clinical trial included as many as 70 healthy volunteers, provided a range of safe doses, and showed no significant side effects. Phase IIa trials were conducted in China in 2006 and 2007; the efficacy end points showed that rhNRG-1 could effectively improve cardiac functionality by increasing LVEF% (Li X, Liu X, Gao R, Zhou M, unpublished data, January 2008). Based on these findings, Phase II clinical trials were conducted both in China and Australia simultaneously. The Australia trial varied from the China study in that it was an open-label study without a placebo group and has now reached a conclusion that short-term administration of rhNRG-1 results in acute and sustained improvement in cardiac function.

Efficacy end points. The Chinese Phase II clinical trial was a double-blind, multicenter, placebo-controlled trial to evaluate the efficacy and safety of rhNRG-1 in patients with systolic CHF. The primary end point results showed that there was progressive improvement of LVEF% along with a reduction of EDV and ESV in the 0.6-μg/kg dose group after treatment. However, among the groups, the changes in LVEF%, ESV, and EDV were not statistically significant; this was probably due to the small number of patients enrolled. More encouragingly, in contrast to all other clinical drugs that might favor cardiac remodeling, only 10 days of rhNRG-1 administration resulted in a sustained beneficial effect on the heart. A comparison of ESV and EDV levels on day 90 with those on day 30 showed that both were further decreased in a dose-dependent manner for doses between 0.3 and 0.6 μg/kg. All these results suggest that rhNRG-1 could effectively enhance heart pumping functionality and, most likely, activate a reverse-remodeling process of the ventricular chamber.

It will be very interesting to know how NRG-1 enhances the heart contractility and coupling between contraction and compensation, and thus results in the reverse remodeling. Our recent preliminary studies revealed that rhNRG-1 restored the disturbed intracellular Ca^{2+} homeostasis and enhanced myocardial contractility by suppressing protein phosphatase 1 and 2 expression, which resulted in activation of SERCA2a (an intracellular sarcoplasmic reticulum–based calcium pumper) activities (unpublished data). Furthermore, in animals with CHF, rhNRG-1 activated expression of cardiac-specific myosin light chain kinase (Patent: US11/894,542), which was directly involved in

Table 3 Summary of AEs

Symptoms	% of AEs	Distribution of AEs (%)			
		Placebo	0.3 μg/kg	0.6 μg/kg	1.2 μg/kg
Nausea, vomiting, poor appetite, dyspepsia, diarrhea, abdominal discomfort	48.39	10.3	10.3	27.5	51.7
Chest tightness, dyspnea, ECG changes	20.97	14.2	28.5	42.8	14.2
Headache, fatigue, excitement, hyperhidrosis	25.80	20.0	20.0	46.6	13.3
Skeletal muscle pain, fever, rash	4.84	25.0	50.2	25.0	0

AE = adverse events; ECG = electrocardiogram.

muscle structure and contraction (12). The direct action of rhNRG-1 in cardiomyocyte structure recovery and pumping enhancement may be an initial factor.

Although other stresses that might accelerate the development of HF were controlled by standard HF therapies, the injured cardiac muscles still could not be repaired by these therapies. In contrast, rhNRG-1 treatment caused a fundamental change in cardiomyocytes accompanied by reduced stress with standard therapies, which might lead to the sustained improvement of both left ventricular function and remodeling in the late phase. Interestingly, Bersell et al. (13) recently reported that NRG-1 promoted myocardial regeneration along with decreased hypertrophy around the infarcted area, leading to improved functionality. In the future, we would like to know how this muscle cell proliferation, together with differentiation, works in the heart with CHF to accelerate cardiac muscle function.

An unexpected result was that there was no improvement in cardiac functionality in the high dose (1.2-μg/kg) group. This is probably due to the obvious side effects caused by a high dose of the drug. In vitro studies showed that NRG-1 could balance beta-adrenergic activation by stimulating parasympathetic-like activity (14). This observation was consistent with the most often seen side effects during infusion of rhNRG-1 in our trial, such as nausea and vomiting. In addition, the activation of parasympathetic activity had a negative inotropic effect on the cardiac muscle, which might further inhibit cardiac pumping function.

Safety. All adverse events were reported within 3 months after the initiation of the drug. There was no significant difference in AE incidence between rhNRG-1 (≤0.6 μg/kg) treatment groups and the placebo group. Although an increased incidence of gastrointestinal disorders occurred in the high-dose group, there was no change in biochemical markers after treatment, including myocardial enzymes. Therefore, even in the high-dose group, rhNRG-1 would not cause organ damage or increase the risk of serious AEs.

There was a small change in the blood pressure, but most p values were >0.05, and the changes were irregularly increased or decreased during the study. Although the p values were occasionally <0.05, they were randomly distributed in the different groups including the placebo group, and no dose-dependent effects were observed. Thus, we considered that these were the random events. Changes of other vital signs were similar to the blood pressure (Online Appendix). From all of the above, we concluded that rhNRG-1 treatment had no effect on these indexes.

Conclusions

Short-term administration of rhNRG-1 (0.6 μg/kg) in CHF patients could result in sustained improvement of cardiac pumping and inhibition or reversal of ventricular remodeling compared with baseline, although these changes were not statistically significant between NRG-1 and the placebo groups.

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Key Words: rhNRG-1 ■ cardiac remodeling ■ chronic heart failure ■ magnetic resonance imaging ■ recombinant human neuregulin-1.

APPENDIX

For additional material and the participating investigators and institutions for the ZS-01-206 trial, please see the online version of this article.