

tenuating inflammation in the EAM hearts.

Treatment with ARB or PPAR γ increases the circulating APN concentration in humans,¹³ although these treatments are unlikely to deliver APN efficiently enough to the severely inflamed myocardium to be clinically relevant. Consistent with our results, previous reports demonstrated that the viral gene delivery of APN or HGF endogenously elevates its concentration in autoimmune myocarditis tissue, leading to immunomodulatory effects and the reversal of LV remodeling.^{7,8} In contrast to the *in vivo* viral transfection method, our cell-sheet-based delivery system eliminates concerns related to the use of plasmid vectors and of needle injection into the host myocardium, and more efficiently delivers multiple cardioprotective factors over the long term.^{9,15} After iACS implantation, the expression of cardioprotective factors (APN, HGF and VEGF) in the myocarditis tissues increased significantly, peaking at postoperative day 14, followed by stable and high expression through postoperative day 35. This prolonged and balanced delivery of cardioprotective factors might be more efficient and practical for clinical use than the one-time administration of a single reagent. Moreover, while the transplanted cells and their producing cytokines existed only in the epicardium, functional and pathological recovery by the iACS therapy was detected both in the inflamed and non-inflamed tissues, suggesting that the major therapeutic mechanisms in this study are not direct effects by transplanted cells but paracrine effects by host cardiac cells. The heart is generally formed in contractile myocardium, endocardium and epicardium, and the epicardium is thought to have a rich cardiac progenitor cell niche and to play an important role in cardiac repair.¹⁶

Notably, it has been shown that cell-sheet implantation into the epicardium induces the expression of multiple cardioprotective factors in the heart, and activates host epicardial cells crucial for cardiac repair. Therefore, these therapeutic effects in the study might be associated with the cell-sheet method. We believe that this “cross-talk” between the transplanted cells and the native myocardium activates and/or inhibits multiple pathways, leading to beneficial effects, and therefore that the cell-sheet method is a rational drug-delivery system for cardiac pathologies.

The T-cell-related immune modulatory effects were different between the EAM hearts treated with iACS vs. SVFCS transplantation in this study. While the level of Th1-producing IFN γ in the inflamed area of the heart on day 21 was lower in the iACS group than the SVFCS group, the level of Th17-produced IL17 was not significantly different between them. In addition, regulatory T cells accumulated prominently and to a similar degree in both the iACS and SVFCS groups. Nonetheless, the acute myocarditis severity on day 21 was significantly less after iACS implantation than after SVFCS implantation. The functional assessment also showed that the RWMI increase from day 7 to day 28 of the acute myocarditis phase was less in rats receiving iACS implantation than SVFCS implantation. Thus, the acute myocarditis severity on day 21 might be mainly associated with the Th1-mediated autoimmune response. In contrast, iACS implantation significantly elevated the level of APN in the myocarditis tissue, compared with SVFCS implantation. A T-cell proliferation assay showed that the addition of iACS supernatant, which contained APN and HGF, significantly decreased the level of Th1-producing IFN γ , compared with the addition of recombinant HGF alone. These findings indicated that the greater immunosuppressive effects of iACS implantation on effector Th1 cells compared with SVFCS implantation might be associated with the synergistic paracrine effects of APN and

HGF released by the implanted iACS.

Regulatory T cells and effector Th17 cells might be reciprocally regulated in various autoimmune diseases.³ In our study, some reciprocity between the number of accumulated Foxp3 regulatory T cells and the amount of IL17-producing Th17 in the myocarditis tissues was observed among the groups. ELISA analysis of the myocarditis tissues on day 21 showed that the iACS and SVFCS implantation similarly suppressed Th17 cells and activated the Foxp3 regulatory T cells. Recently, Baldeviano et al. reported that Th17-produced IL17 was dispensable for the severity of the acute myocarditis, but essential for the progression of cardiomyopathy.³ Consistent with this, we found that the cardiac fibrosis related to LV remodeling in the chronic cardiomyopathy phase was similarly attenuated in the iACS and SVFCS implantation-treated rats via the suppression of profibrotic factors: TGF β , MMP2, and MMP9. Thus, this inhibition of morphological deterioration might be associated with the suppression of the Th17-mediated autoimmune response and the induction of immune tolerance. In accordance with this scenario, morphological LV remodeling, such as LV dilatation and LV thinness, on day 42 was similarly suppressed in the groups receiving iACS and SVFCS implantation. In addition, the assessment of RWMI showed that the LV functional deterioration from day 28 to day 56 of the chronic cardiomyopathy phase was similarly suppressed in the rats receiving iACSs and SVFCSs, compared with the Sham operation. However, the cardiac hypertrophy on day 42 was attenuated only in the group receiving the iACSs. Several lines of evidence have indicated that APN directly affects injured myocytes via its receptor, eliciting anti-hypertrophic effects in a pressure-overload hypertrophic model.^{13,17} Thus, the significant suppression of hypertrophy in iACS implantation-treated rats might have resulted from direct and synergistic effects of APN and HGF on the injured myocytes, and not from indirect immune modulatory effects via effector Th17 cells.

This study showed that iACS implantation had beneficial immunologic, pathologic, and functional effects on the heart of rats with autoimmune-associated myocarditis. However, in the clinical setting, fulminant myocarditis is etiologically highly heterogeneous, and thus, the autoimmune activity associated with it varies. The effectiveness of the iACS treatment shown in this study is therefore not directly translatable to the clinical situation. The investigation of T-cell activity by cardiac biopsy or circulating blood samples from patients with fulminant myocarditis might be useful for identifying responders or determining whether iACS treatment is indicated.

Normally, human myocarditis has a sudden onset and it has been known that it often follows a rapidly deteriorating course, leading to severe cardiac dysfunction. It has been reported that early diagnosis and subsequent treatment for fulminant myocarditis might be essential in clinical practice.¹ Therefore, methods need to be developed for promptly generating autologous iACS to maximize its therapeutic effects. The use of allogeneic iACS might be an option for clinical applications. Although there are immunologic concerns associated with the use of allogeneic iACS, this study suggested that iACS treatment upregulated APN and HGF, which attenuated the immunological response by inhibiting macrophages and activating regulatory T cells. Moreover, APN can limit allograft rejection by suppressing the expression of local cytokine/chemokine ligands that mediate inflammation and immune-cell recruitment.¹⁸ Thus, the need for immunosuppressive medications might be minimal for allogeneic iACS treatment, although further study is needed.

Conclusions

This study clearly revealed that adipocyte-produced APN and HGF exert significant immunosuppressive effects, not only on Th1 cells, but also on Th17 cells in a typical model of autoimmune disorders. In addition, this tissue-engineered iACS improved the cardiac performance of autoimmune myocarditis via the suppression of autoimmune cellular activity, induction of immune-tolerance, and reversal of LV remodeling. This strategy of using a tissue-engineered drug-delivery system might be applicable to clinical treatments for fulminant myocarditis.

Acknowledgments

We thank Ms Masako Yokoyama and Mr Akima Harada for their technical assistance. We also thank Mr Norikazu Maeda and Mr Ichihiro Shimomura for helpful discussions. This study was financially supported by a Grant-in-Aid from the Japan Society for the Promotion of Science (A22659251).

Disclosures

There were no competing interests.

References

- Gupta S, Markham DW, Drazner MH, Mammen PP. Fulminant myocarditis. *Nat Clin Pract Cardiovasc Med* 2008; **5**: 693–706.
- Cihakova D, Rose NR. Pathogenesis of myocarditis and dilated cardiomyopathy. *Adv Immunol* 2008; **99**: 95–114.
- Baldeviano GC, Barin JG, Talor MV, Srinivasan S, Bedja D, Zheng D, et al. Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. *Circ Res* 2010; **106**: 1646–1655.
- Yu F, Chen R, Takahashi T, Sumino H, Morimoto S, Nakahashi T, et al. Candesartan improves myocardial damage in obese mice with viral myocarditis and induces cardiac adiponectin. *Int J Cardiol* 2008; **129**: 414–421.
- Choi JO, Yun SH, Sung K, Lee YT, Park JJ, Ju ES, et al. Thioredoxin, adiponectin and clinical course of acute fulminant myocarditis. *Heart* 2011; **97**: 1067–1073.
- Kunita E, Yamamoto H, Kitagawa T, Ohashi N, Utsunomiya H, Oka T, et al. Association between plasma high-molecular-weight adiponectin and coronary plaque characteristics assessed by computed tomography angiography in conditions of visceral adipose accumulation. *Circ J* 2012; **76**: 1687–1696.
- Bobbert P, Scheibebogen C, Jenke A, Kania G, Wilk S, Krohn S, et al. Adiponectin expression in patients with inflammatory cardiomyopathy indicates favourable outcome and inflammation control. *Eur Heart J* 2011; **32**: 1134–1147.
- Futamatsu H, Suzuki J, Mizuno S, Koga N, Adachi S, Kosuge H, et al. Hepatocyte growth factor ameliorates the progression of experimental autoimmune myocarditis: A potential role for induction of T helper 2 cytokines. *Circ Res* 2005; **96**: 823–830.
- Imanishi Y, Miyagawa S, Meaada N, Fukushima S, Kitagawa-Sakakida S, Daimon T, et al. Induced adipocyte cell-sheet ameliorates cardiac dysfunction in a mouse myocardial infarction model: A novel drug delivery system for heart failure. *Circulation* 2011; **124**(Suppl 1): S10–S17.
- Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al. Recommendations for chamber quantification: A report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr* 2005; **18**: 1440–1463.
- Eriksson U, Ricci R, Hunziker L, Kurrer MO, Oudit GY, Watts TH, et al. Dendritic cell-induced autoimmune heart failure requires cooperation between adaptive and innate immunity. *Nat Med* 2003; **9**: 1484–1490.
- Robinson K, Prins J, Venkatesh B. Clinical review: Adiponectin biology and its role in inflammation and critical illness. *Crit Care* 2011; **15**: 221 (1–9).
- Shibata R, Ouchi N, Murohara T. Adiponectin and cardiovascular disease. *Circ J* 2009; **73**: 608–614.
- Yin WH, Wei J, Huang WP, Chen JW, Young MS, Lin SJ. Prognostic value of circulating adipokine levels and expressions of adipokines in the myocardium of patients with chronic heart failure. *Circ J* 2012; **76**: 2139–2147.
- Shimizu T, Sekine H, Yamato M, Okano T. Cell sheet-based myocardial tissue engineering: New hope for damaged heart rescue. *Curr Pharm Des* 2009; **15**: 2807–2814.
- Zhou B, Ma Q, Rajagopal S, Wu SM, Domian I, Rivera-Feliciano J, et al. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. *Nature* 2008; **454**: 109–113.
- Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med* 2004; **10**: 1384–1389.
- Okamoto Y, Christen T, Shimizu K, Asano K, Kihara S, Mitchell RN, et al. Adiponectin inhibits allograft rejection in murine cardiac transplantation. *Transplantation* 2009; **88**: 879–883.

Supplementary Files

Supplementary File 1

Supplementary Methods

Supplementary File 2

Table S1. Hemodynamic indices 5 weeks after the operation

Table S2. PCR primers used in real-time RT-PCR

Figure S1. T-cell proliferation assay.

Figure S2. Capillary formation on postoperative day 35 in each group.

Figure S3. Quantitative reverse transcription polymerase chain reaction (RT-PCR) results for profibrotic markers: TGF β , TIMP1, TIMP2, TIMP3, MMP2, and MMP9, respectively (n=12 each).

Please find supplementary file(s);
<http://dx.doi.org/10.1253/circj.CJ-14-0840>



Safety and Efficacy of Autologous Skeletal Myoblast Sheets (TCD-51073) for the Treatment of Severe Chronic Heart Failure Due to Ischemic Heart Disease

Yoshiki Sawa, MD, PhD; Yasushi Yoshikawa, MD; Koichi Toda, MD, PhD;
Satsuki Fukushima, MD, PhD; Kenji Yamazaki, MD, PhD; Minoru Ono, MD, PhD;
Yasushi Sakata, MD, PhD; Nobuhisa Hagiwara, MD, PhD;
Koichiro Kinugawa, MD, PhD; Shigeru Miyagawa, MD, PhD

Background: Poor survival outcomes for patients with severe heart failure (HF) and the donor shortage for heart transplantation warrant the development of myocardial regenerative therapy. We performed a multicenter, phase II study to evaluate the safety and efficacy of autologous skeletal myoblast sheets (TCD-51073).

Methods and Results: In 3 study sites, we enrolled 7 patients with severe chronic HF due to ischemic heart disease despite maximal therapy, all of whom underwent transplantation of TCD-51073. No serious arrhythmia was reported, and no changes were noted in the frequency of ventricular extrasystole frequency. The primary efficacy endpoint of the change in left ventricular ejection fraction (LVEF) on gated blood-pool scintigraphy at 26 weeks after transplantation showed that 5 subjects were responders (classified as "improved" or "unchanged"). In addition, LVEF on echocardiography improved over time, with a change in LVEF of $7.1 \pm 2.8\%$ at 26 weeks posttransplantation. Among the 7 subjects, 6 showed improvement in New York Heart Association functional class by at least 1 class. The 6-min walk distance was 410.1 ± 136.1 m before transplantation and 455.4 ± 103.7 m at 26 weeks after transplantation.

Conclusions: This study demonstrated the feasibility and safety of the transplantation of TCD-51073 in the patients with severe chronic HF due to ischemic heart disease, suggesting that TCD-51073 might maintain or improve cardiac function, symptoms, and physical function. (*Circ J* 2015; **79**: 991–999)

Key Words: Heart failure; Multicenter study; Myoblast sheets; Regenerative therapy

Over the past 20 years or so, the treatment of chronic heart failure (HF) has been progressed by use of drug therapies such as β -blockers, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and aldosterone antagonists, or device therapy such as cardiac resynchronization therapy. Hence, clinical outcomes of patients with chronic HF are now significantly better, but patients with severe chronic HF who do not respond well to standard drug therapies still have poor outcomes.¹ In addition, although patients with end-stage severe HF benefit from heart transplantation or left ventricular assist system (LVAS) implantation,² these procedures are indicated for only a limited number of such patients worldwide where the number of heart

transplant donors is limited. Thus, the therapeutic strategies available for patients with severe HF are still limited and new treatments need to be developed.

Since 15 years ago, treatment of heart disease with the use of patients' own somatic cells has been reported.³ Among these treatments, using autologous skeletal myoblasts has been investigated in clinical trials, mainly in Western countries, in which cell transplantation was performed using myocardial injection during a surgical procedure through a thoracotomy, such as coronary artery bypass grafting (CABG) or LVAS implantation,⁴ or using myocardial injection of cells through a cardiac catheter.⁵ However, results from a European phase II clinical study (MAGIC trial) demonstrated that transplantation

Received March 1, 2015; revised manuscript received March 26, 2015; accepted April 1, 2015; released online April 24, 2015 Time for primary review: 15 days

Department of Cardiovascular Surgery (Y. Sawa, Y.Y., K.T., S.F., S.M.), Department of Cardiovascular Medicine (Y. Sakata), Osaka University Graduate School of Medicine, Suita; Department of Cardiovascular Surgery (K.Y.), Department of Cardiology (N.H.), Tokyo Women's Medical University, Tokyo; and Department of Thoracic Surgery (M.O.), Department of Therapeutic Strategy for Heart Failure (K.K.), Graduate School of Medicine, University of Tokyo, Tokyo, Japan

This paper was presented at the 79th Annual Scientific Meeting of the Japanese Circulation Society, Late Breaking Clinical Trials 4-6 (April 26, 2015, Osaka, Japan).

Mailing address: Yoshiki Sawa, Professor, MD, Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita 565-0871, Japan. E-mail: sawa@sur1.med.osaka-u.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-15-0243

All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp

of autologous skeletal myoblasts was less effective than CABG as a control procedure.⁶

We developed an autologous skeletal myoblast sheet by using the cell sheet engineering approach developed by Okano et al of Tokyo Women's Medical University, Japan.¹⁰ Following a number of nonclinical studies,^{7–11} we conducted the first-in-human study at Osaka University in subjects with dilated cardiomyopathy who had received an LVAS, and we confirmed the feasibility and safety of the cell sheet, with successful weaning from the LVAS in 2 subjects.¹² We also conducted a phase I study in subjects with severe ischemic cardiomyopathy and dilated cardiomyopathy (UMIN ID; 000003273), and confirmed the safety and feasibility of the sheet in these cohorts. The results supported investigation of the practical use of autologous skeletal myoblast sheets on a commercial basis, leading to the development of TCD-51073 (Terumo Corporation, Tokyo, Japan).

We, therefore, conducted an exploratory, prospective, multicenter, uncontrolled, open-label phase II study of TCD-51073 in subjects with severe chronic HF due to ischemic heart disease, which we designed (1) to validate the method of efficacy evaluation, (2) to collect safety information, and (3) to confirm that transplantation or other procedures at multiple medical institutions was successfully conducted.

Methods

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki, Japanese Pharmaceutical Affairs Law, and Good Clinical Practice. The study was approved by the institutional review board at each study site, and all of the subjects provided prior informed consent to participate in the study.

Patients and Procedures

Patients This study included patients with ischemic heart disease who had impaired left ventricular systolic function and remained in HF status despite maximal oral therapy, including digitalis, diuretics, ACEIs, ARBs, β -blockers, aldosterone antagonists, and oral inotropic agents, and thus were at risk of worsening HF despite standard-of-care therapy. The inclusion criteria were: (1) patients who had chronic ischemic heart disease; (2) patients in New York Heart Association (NYHA) class III or IV HF; (3) patients who remained in HF status despite maximal oral therapy, including digitalis, diuretics, ACEIs, ARBs, β -blockers, aldosterone antagonists, and oral inotropic agents; (4) patients aged 20 years or older at the time of consent; (5) patients who were at risk of worsening HF despite standard-of-care therapy (eg, CABG, mitral valvuloplasty, LV restoration, cardiac resynchronization therapy, and percutaneous coronary intervention) conducted at least 3 months earlier; and (6) patients who had a LV ejection fraction (LVEF) $\leq 35\%$ on resting echocardiography. The exclusion criteria were: (1) patients with evidence of skeletal muscle disease; (2) patients undergoing thyroid hormone treatment; (3) patients with infectious diseases (eg, infections caused by the human immunodeficiency virus, hepatitis B virus, hepatitis C virus, and human T cell leukemia virus type 1); (4) patients who remained in shock because of worsening HF; (5) patients with irreversible non-cardiac organ failure; (6) patients with any malignancy; (7) patients who were pregnant or possibly pregnant; (8) patients with a history of alcoholism or drug addiction within 6 months before the day of consent; and (9) patients with severe pulmonary hypertension.

Procedure At each study site, skeletal muscle (required

amount, 2–5 g) was harvested from the vastus medialis muscle of the subject by aseptic technique, under general anesthesia and with endotracheal intubation. In the Cell Processing Center of Terumo Corporation, skeletal myoblasts were isolated from the harvested skeletal muscle by enzymatic digestion in TrypLE Select™ (Thermo Fisher Scientific Inc, MA, USA) containing collagenase, expanded in culture in MCDB103 medium with fetal bovine serum for approximately 4 weeks, and cryopreserved. At least 7 weeks after the harvest of the skeletal muscle, the skeletal myoblasts were seeded at 6.0×10^7 cells per dish and incubated on temperature-responsive culture dishes (10 cm in diameter) to form a skeletal myoblast sheet (TCD-51073), as described previously.⁷ Transplantation of TCD-51073 was performed through a left thoracotomy under general anesthesia, and 5 sheets of TCD-51073 (representing 3×10^8 cells) were transplanted onto a large area extending from the anterior wall to the lateral wall of the left ventricle. No other concomitant cardiac surgery, such as CABG or mitral valve repair, were performed.

Evaluation Methods

Study Protocol Evaluation for the study was performed up to 26 weeks after transplantation. A 2-year follow-up study is ongoing, which was begun after the evaluation period. Observations included physical examination (subjective and objective), NYHA classification, specific activity scale (SAS) score, and concomitant medications. Examinations included vital signs, body weight, laboratory tests, chest radiography, cardiac computed tomography (CT), echocardiography, resting standard 12-lead ECG, 24-h Holter ECG monitoring, 6-min walk test, cardiopulmonary exercise test (CPX), and gated equilibrium blood-pool scintigraphy. Myocardial perfusion single-photon emission CT and coronary angiography were performed for eligibility screening.

Primary Endpoint The primary endpoint was the change in LVEF on gated equilibrium blood-pool scintigraphy from pretransplantation to 26 weeks posttransplantation. This endpoint was chosen based on the advice provided by the Pharmaceuticals and Medical Devices Agency in Japan.

Secondary Endpoints The prespecified secondary endpoints included success or failure of the transplantation, LVEF, LV end-diastolic volume index, LV end-systolic volume index, NYHA classification, SAS, 6-min-walk distance (6MWD), peak oxygen uptake (peak $\dot{V}O_2$), anaerobic threshold (AT), B-type natriuretic peptide (BNP), and volumetric analysis of echocardiography performed on the long axes of apical 2- and 4-chamber views using the modified Simpson method.

Safety Endpoints Safety endpoints included arrhythmia, adverse events (AEs) and adverse drug reactions (ADRs) other than arrhythmia, serious arrhythmia (requiring inpatient hospitalization or prolonging the existing hospitalization for treatment or monitoring) and serious AEs (SAEs), AEs associated with harvest of skeletal muscle tissues, changes in vital signs over time, and changes in routine hematology and clinical chemistry parameters over time.

Study Administrative Structure The Case Adjudication Committee, which consisted of 2 independent cardiologists and 1 independent cardiac surgeon, was established to provide advice on the determination of patient eligibility and whether the study should be continued, as well as to evaluate efficacy. In addition, the Data and Safety Monitoring Committee, which consisted of 1 cardiac surgeon and 1 medical statistician, was established to review safety data from this study at appropriate intervals or at necessary times to provide recommendations

for continuation, modification, or termination of the study to the sponsor, as necessary. The patients were registered at the patient registration center (EPS Corporation, Tokyo, Japan), and cardiac CT scans were analyzed at the core laboratory (TITAN Inc, Tokyo, Japan).

Statistical Analysis

Primary Endpoint The patients were classified according to the change in LVEF from pretransplantation to 26 weeks posttransplantation as “improved” ($\Delta\text{LVEF} \geq 5\%$), “unchanged” ($5\% > \Delta\text{LVEF} > -3\%$), or “worsened” ($-3\% \geq \Delta\text{LVEF}$). A responder was defined as a subject whose LVEF was classified as improved or unchanged, and the number of responders was determined.

Secondary Endpoints For continuous variables, summary statistical indexes (mean, standard deviation [SD] and 2-sided 95% confidence interval [CI] for the mean, maximum, median, minimum, and number of subjects) were calculated. For discrete variables, frequency distribution tables were constructed.

Safety Endpoints For arrhythmia, and AEs and ADRs other than arrhythmia, the numbers of events and the subjects with events were determined. If an event was assessed as an ADR, the numbers of such events and the subjects with such events were determined. For significant AEs and SAEs, as well as AEs associated with harvest of skeletal muscle tissues, the numbers of events and the subjects with events were determined. For vital signs, routine hematology, and clinical chemistry parameters, summary statistical indexes (mean, SD, and 2-sided 95% CI for the mean, maximum, median, minimum, and number of subjects) were calculated and the time course of the changes was determined.

Results

Classification and Disposition of Subjects

From 3 Japanese study sites, we enrolled 7 patients with severe chronic HF due to ischemic heart disease who continued to be in NYHA class III or higher and have an LVEF $\leq 35\%$ despite maximal therapy between May 2012 and October 2013 (Osaka University Hospital, 4 patients; Tokyo Women's Medical University Hospital, 2 patients; University of Tokyo Hospital, 1 patient). Of the 8 initial subjects who provided informed consent and reviewed by the Case Adjudication Committee, 1 was considered ineligible. All 7 eligible subjects received transplantation of TCD-51073 and completed the study without discontinuations.

Baseline Characteristics of the Patients

The baseline characteristics of the enrolled subjects are shown in Table 1. The mean age at eligibility screening was 56.3 ± 13.2 years (range, 35–71 years), and all of them were male. All the subjects had undergone percutaneous coronary intervention or CABG for ischemic heart disease but presented with symptoms of severe HF, were in NYHA class III, and had a LVEF of $29 \pm 3.7\%$ ($<30\%$ in 3 subjects) on echocardiography. All the subjects were identified as having no indication for further revascularization. In addition, 2 subjects had an implantable cardioverter defibrillator or cardiac resynchronization therapy defibrillator, and 1 subject had undergone mitral valvuloplasty. Mitral regurgitation was reported in 6 subjects, as assessed pretransplantation by echocardiography. One subject who had a history of NYHA class IV symptoms was listed as a status 2 cardiac transplantation candidate by the Japan Organ Transplant Network.

Table 1. Characteristics of Study Patients Undergoing Cell Sheet Transplantation

Demographics	
No. of patients	7
Age, years (mean \pm SD)	56.3 \pm 13.2
>65 years	2 (28.6%)
Male sex	7 (100%)
Body weight (kg) [mean \pm SD]	70.3 \pm 9.5
Height (cm) [mean \pm SD]	168.9 \pm 6.0
Body surface area (m ²) [mean \pm SD]	1.8 \pm 0.1
Cardiac function	
NYHA functional class III	7 (100%)
LVEF (echocardiography) [mean \pm SD]	29.3 \pm 3.7
Risk factor	
Hypertension	3 (42.9%)
Hyperlipidemia	6 (85.7%)
Diabetes mellitus	3 (42.9%)
Oral medication	3 (42.9%)
Insulin	1 (14.3%)
Smoking	7 (100%)
Current or previous (within the past year)	0 (0.0%)
Cardiac history	
PCI or CABG	7 (100%)
PCI	6 (85.7%)
CABG	6 (85.7%)
Pacemaker implant	0 (0.0%)
ICD implant	1 (14.3%)
CRT implant	0 (0.0%)
CRT-D implant	1 (14.3%)
Valve surgery	1 (14.3%)
Left ventricular reconstruction	0 (0.0%)
IABP	1 (14.3%)
LVAD	0 (0.0%)
Myocardial infarction	6 (85.7%)
Nonsustained VT	2 (28.6%)
Atrial fibrillation or flutter	3 (42.9%)
Mitral regurgitation	6 (85.7%)
Medication	
ACEI or ARB	6 (85.7%)
ACEI	5 (71.4%)
ARB	1 (14.3%)
β -blocker	7 (100%)
Aldosterone receptor antagonist	5 (71.4%)
Diuretics	6 (85.7%)
Inotropic	2 (28.6%)
Antiplatelet	6 (85.7%)
Warfarin	5 (71.4%)
Amiodarone	4 (57.1%)
Hypoglycemic	3 (42.9%)
Statins	6 (85.7%)

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CABG, coronary artery bypass grafting; CRT, cardiac resynchronization therapy; CRT-D, cardiac resynchronization-defibrillator therapy; IABP, intra-aortic balloon pump; ICD, implantable cardioverter defibrillator; LVAD, left ventricular assist device; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; SD, standard deviation; VT, ventricular fibrillation; VT, ventricular tachycardia.

Table 2. Summary of Adverse Events Among Patients Undergoing Cell Sheet Transplantation

Adverse event (MedDRA SOC)	Not related*						Cannot be ruled out	Total
	Underlying disease	Surgery	Concomitant medication	Patient's condition	Concurrent conditions	Other		
Congenital blood and lymphatic system disorders				1	1			2
Cardiac disorders	4	3					1	8
Gastrointestinal disorders		1	1			2		3
General disorders and administration site conditions	1	1		1				3
Hepatobiliary disorders		1		1		1		3
Infections and infestations		5		1				6
Injury, poisoning, and procedural complications		9				1		10
Investigations	1	3	3			4		11
Metabolism and nutrition disorders		2	4					6
Musculoskeletal and connective tissue disorders			1			2		3
Neoplasms benign, malignant, and unspecified (including cysts and polyps)					1			1
Nervous system disorders			1					1
Psychiatric disorders		1						1
Renal and urinary disorders		4				1		5
Reproductive system and breast disorders			1					1
Respiratory, thoracic, and mediastinal disorders		4						4
Skin and subcutaneous tissue disorders		4						4
Vascular disorders		2						2

*If there are 2 or more reasons, each is counted.

Table 3. Summary of Arrhythmia Among Patients Undergoing Cell Sheet Transplantation

Patient ID	Adverse event (MedDRA LLT)	Post Tx (days)	Relationship to study drug	Reason for "not related"
T01-01	Nonsustained VT	6	Not related	Underlying disease
T03-01	Atrial fibrillation	2	Not related	Underlying disease
T01-04	Nonsustained VT	4	Not related	Underlying disease, Possibility of concomitant medications
T02-02	Atrial flutter	4	Not related	Surgery
	Atrial fibrillation	5	Not related	Surgery
T01-05	Ventricular extrasystoles	1	Not related	Underlying disease, Possibility of concomitant medications, Surgery

Abbreviation as in Table 1.

Surgical Procedure

Harvest of the skeletal muscle and cell culture were successfully performed in all the enrolled subjects, who were transplanted with 5 sheets of TCD-51073. The proportion of skeletal myoblasts (CD56-positive cells) in the transplanted TCD-51073 was $\geq 60\%$, with a cell viability $\geq 75\%$, indicating evidence of fusion ability in all subjects. This product also complied with sterility, bacterial endotoxins, and mycoplasma tests.

Safety Evaluation

The AEs reported in this study are listed in **Table 2**. AEs associated with the harvest of the skeletal muscle were observed in 2 of the 7 subjects and included 2 events of wound complication in 2 subjects and 1 event of postprocedural swelling in

1 subject. These AEs all resolved without sequelae within 1–14 days. After TCD-51073 transplantation, 74 AEs were observed in the total group. Among these AEs, the most commonly observed were wound complications in 4 subjects, hypokalemia in 3, and postoperative fever in 3. No subject except those with SAEs, as described next, required specific treatment.

Serious arrhythmia, which required inpatient hospitalization or prolonged existing hospitalization for treatment or monitoring, was defined as a significant AE in this study, but no cases occurred. In contrast, 6 arrhythmia events occurred in 5 subjects, including 2 events of ventricular arrhythmia in 2 subjects, 1 event of ventricular extrasystole in 1 subject, 2 events of atrial fibrillation in 2 subjects, and 1 event of atrial flutter in 1 subject (**Table 3**). Among the 2 subjects with

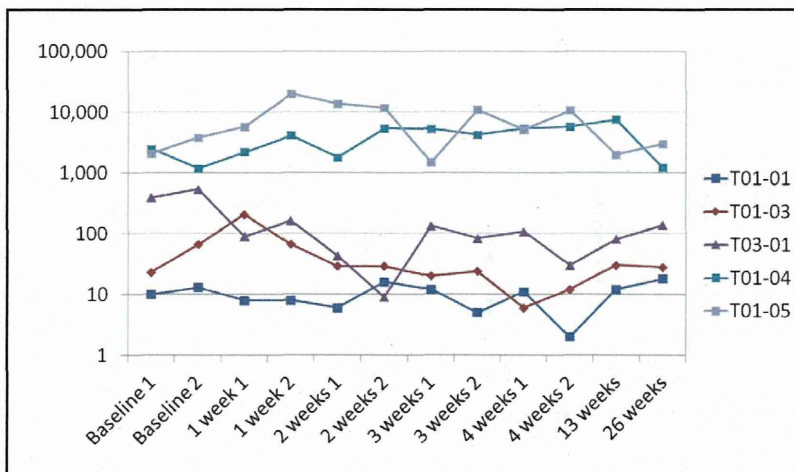


Figure 1. Serial change in the number of premature ventricular contractions on 24-h Holter ECG monitoring in 5 subjects with data available from their respective case report forms performed before cell sheet transplantation; twice weekly at 1, 2, 3, and 4 weeks after transplantation, and once weekly at 13 and 26 weeks after transplantation.

nonsustained ventricular arrhythmia, in subject T01-01 with existing nonsustained ventricular tachycardia, the nonsustained ventricular arrhythmia resolved with drug therapy on the day of onset. In subject T01-04, with existing multiple nonsustained ventricular tachycardias, the nonsustained ventricular arrhythmia did not resolve, but was mild and required no additional drug therapy or treatment. These arrhythmic events were all reported within 1 week after transplantation and were considered attributable to the transplantation procedure or underlying disease.

In addition, the subjects were hospitalized for 4 weeks after transplantation to further investigate whether arrhythmogenic effects were present and 24-h Holter ECG monitoring was performed before transplantation; twice weekly at 1, 2, 3, and 4 weeks after transplantation; and once weekly at 13 and 26 weeks after transplantation. The ECG changes over time in each subject who received TCD-51073 transplantation showed no specific trend, with no significant change in the frequency of ventricular extrasystole (Figure 1).

In this study, 3 SAEs occurred in 3 subjects, comprising colon cancer (subject T02-01, 182 days posttransplantation), prolonged HF (subject T01-04, 31 days posttransplantation), and aggravated HF (subject T03-01, 49 days posttransplantation). The colon cancer was diagnosed as a primary cancer based on pathological findings and was considered unrelated to TCD-51073. This subject underwent right hemicolectomy at 273 days posttransplantation and subsequently recovered. The prolonged HF was attributed to surgery, inappropriate drug administration, and the subject's lifestyle, and was considered unrelated to the transplanted TCD-51073. This subject was discharged from hospital at 141 days posttransplantation, but was re-hospitalized at 159 days posttransplantation. The patient was subsequently discharged from hospital at 172 days posttransplantation, and recovered. The relationship between the event of aggravated HF and the transplanted TCD-51073 was considered unknown. This subject was a Status 2 patient registered for heart transplantation and repeatedly hospitalized and discharged 4 times until 26 weeks posttransplantation, with drugs added and/or modified because of the aggravated HF during 3 of the hospitalizations. For all episodes of hospitalization, the subject recovered with intravenous infusion of inotropic agents and diuretics or modification of diuretics for fluid control and was discharged from hospital.

In the vital signs, physical examination results, and clinical laboratory test results, no serious abnormal changes occurred

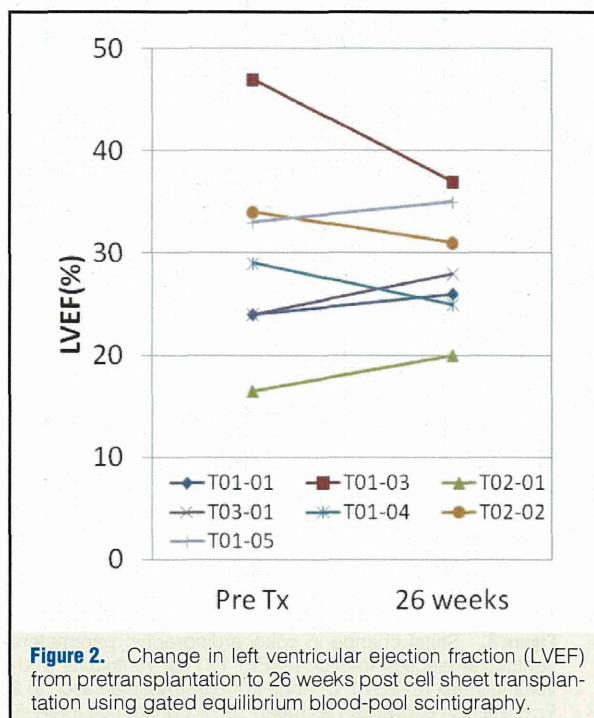


Figure 2. Change in left ventricular ejection fraction (LVEF) from pretransplantation to 26 weeks post cell sheet transplantation using gated equilibrium blood-pool scintigraphy.

in any of the subjects, although effects of the thoracotomy, and concomitant medications or changes associated with observed AEs were noted. For body weight, no serious abnormal changes occurred. No clinically significant abnormal changes or abnormal changes qualifying as SAEs were noted.

Primary Efficacy Endpoint Figure 2 shows the change in LVEF over time according to gated equilibrium blood-pool scintigraphy. The LVEF was found to be "unchanged" in 5 of the 7 subjects. A responder was defined as a subject in whom the LVEF was classified as "improved" or "unchanged" in the present study. Accordingly, 5 subjects were considered to be responders. Among the 2 subject with worsened LVEF, 1 subject (subject T01-04) received continuous administration of drugs that affected LVEF values, including intravenous inotropic agents (eg, dobutamine) and diuretics, for the management of AEs that occurred after transplantation (prolonged

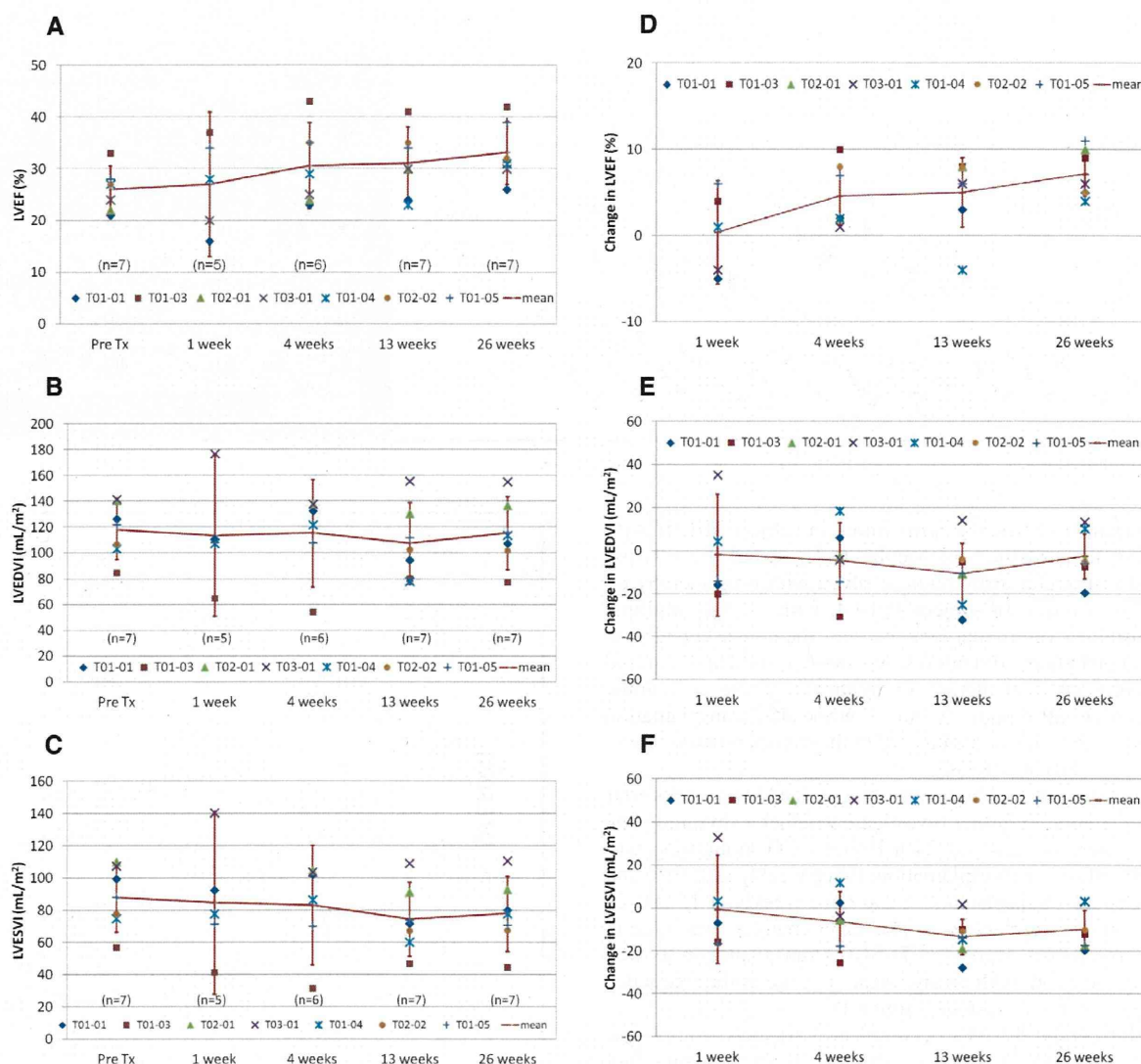


Figure 3. Serial change in echocardiographic parameters from baseline to 26 weeks after cell sheet transplantation: (A) left ventricular ejection fraction (LVEF), (B) left ventricular end-diastolic volume index (LVEDVI) and (C) left ventricular end-systolic volume index (LVESVI). Change from pretransplantation to 26 weeks posttransplantation in (D) LVEF, (E) LVEDVI and (F) LVESVI. Error bar are expressed as mean \pm 95% confidence interval.

HF and renal impairment). The other subject (subject T01-03) became unwell at the time of LVEF measurement before transplantation, and decreased blood pressure associated with administration of a tracer was likely to have affected the LVEF values. These 2 patients showed an increase in LVEF on echocardiography.

Secondary Efficacy Endpoints Results of the echocardiography performed before and at 1, 4, 13, and 26 weeks after transplantation showed LVEF values of $26.0 \pm 4.1\%$, $27.0 \pm 8.9\%$, $30.6 \pm 7.4\%$, $31.0 \pm 6.3\%$, and $33.1 \pm 5.5\%$, respectively (Figure 3A). This is consistent with a trend toward improvement over time, with the changes in LVEF values from pretransplantation to 1, 4, 13, and 26 weeks posttransplantation being $0.4 \pm 4.8\%$, $4.6 \pm 3.6\%$, $5.0 \pm 4.4\%$, and $7.1 \pm 2.8\%$, respectively (Figure 3D). In addition, an increase in LVEF values from pretransplanta-

tion was observed in all subjects, with an increase $>5\%$ from pretransplantation values in 4 subjects. The change in LVEF from pretransplantation to 26 weeks posttransplantation was $2.0 \pm 2.6\%$ in 6 subjects in whom cardiac CT was feasible. No LVEF values could be obtained in 1 subject (subject T01-04) with worsened LVEF by gated equilibrium blood-pool scintigraphy at 26 weeks posttransplantation. In the other subject (subject T01-03), LVEF was unchanged.

Changes in NYHA class over time are shown in Figure 4A. Before transplantation, all subjects were in NYHA class III, but 2, 4, and 1 of the subjects were in NYHA class I, II, and III, respectively, at 26 weeks posttransplantation, indicating improvement in NYHA class by at least 1 class in 6 of the 7 subjects who received a transplant; 2 of the 6 subjects experienced a substantial improvement from class III to class I.

SAS results showed an improvement of at least 1 metabolic equivalent (MET) in 3 subjects and no change in 4 subjects, from pretransplantation (Figure 4B). Subject T01-01 had an increase of at least 2 METs, from 3–4 METs pretransplantation to 6–7 METs at 26 weeks posttransplantation. Subject T01-03 had an increase of at least 2 METs, from 4–5 METs pretransplantation to 6–7 METs at 26 weeks posttransplantation.

The results of the 6MWD and CPX are shown in Table 4. The 6MWD was 410.1±136.1 m pretransplantation and 455.4±103.7 m at 26 weeks posttransplantation. Subjects T01-03 and T03-01 experienced increases of 170 and 214 m, respectively, whereas subject T02-02 had a decrease of 110 m from pretransplantation. Peak $\dot{V}O_2$ was 13.4±5.3 ml·kg⁻¹·min⁻¹ pretransplantation and 14.6±4.9 ml·kg⁻¹·min⁻¹ at 26 weeks posttransplantation. AT was 8.7±2.3 ml·kg⁻¹·min⁻¹ pretransplantation and 9.3±2.2 ml·kg⁻¹·min⁻¹ at 26 weeks posttransplantation. In the 5 subjects in which a comparison before and after transplantation could be made, peak $\dot{V}O_2$ and AT showed a trend toward worsening in 2 subjects and no change or a trend toward improvement in 3 subjects from pretransplantation.

Subject T02-01 was diagnosed with colon cancer at the time of examination at 26 weeks posttransplantation and had physical examination findings such as shortness of breath on exertion and staggering, which may have affected the results of NYHA classification, SAS, 6MWD, and CPX.

Discussion

Overview of Study Results

This study was an exploratory study designed to evaluate the efficacy and safety of TCD-51073 in subjects presenting with severe chronic HF due to ischemic heart disease who were at risk of worsening HF despite maximal therapy. Despite the small sample size, the results demonstrated the following: (1) harvesting of the skeletal muscle and then cell culture were successfully performed in all enrolled subjects; (2) transplantation of TCD-51073 was safe and feasible in multiple medical institutions; (3) no evidence of serious arrhythmia and no other possible SAEs were observed; (4) LVEF was maintained in 5 subjects on gated equilibrium blood-pool scintigraphy and showed improvement over time on echocardiography; (5) 6 subjects showed improvement in NYHA class and almost all subjects showed symptomatic improvement. Some subjects showed improvement in exercise tolerance.

Efficacy Evaluation

This study demonstrated that 5 of the 7 subjects were respond-

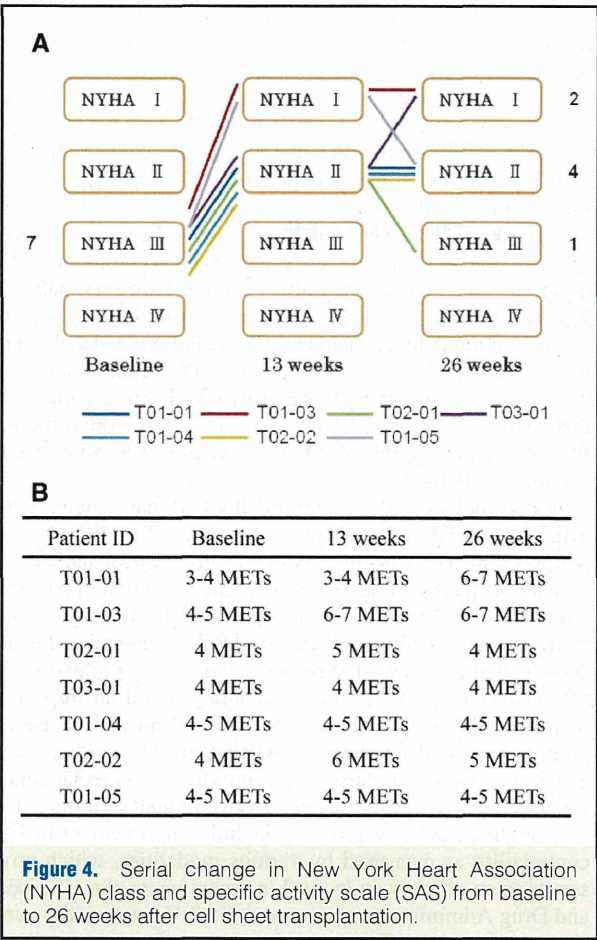


Figure 4. Serial change in New York Heart Association (NYHA) class and specific activity scale (SAS) from baseline to 26 weeks after cell sheet transplantation.

ers in the primary endpoint. Of the 2 subjects with worsened LVEF, 1 subject became unwell at the time of LVEF measurement before transplantation, and the decreased blood pressure associated with administration of a tracer was likely to have affected the LVEF values. The other subject had a variable degree of mitral regurgitation and thus was not suitable for the assessment of changes in systolic function based on LVEF. In addition, the secondary endpoint of LVEF showed improvements in cardiac function over time on echocardiography, suggesting that this product might at least be effective in maintaining cardiac function in patients with severe HF who are at risk of worsening. Overall improvement in the secondary

Table 4. Change in Exercise Tolerance From Baseline to 26 Weeks Transplantation Among Patients Undergoing Cell Sheet Transplantation						
Patient ID	6-min walk distance (m)		Peak $\dot{V}O_2$ (ml·kg ⁻¹ ·min ⁻¹)		AT (ml·kg ⁻¹ ·min ⁻¹)	
	Baseline	26 weeks	Baseline	26 weeks	Baseline	26 weeks
T01-01	485	520	19.4	16.5	10.5	8.6
T01-03	400	570	20.4	21.1	11.3	12
T02-01	486	462*	12.6	7.6*	10.1	7
T03-01	264	478	9.1	13.3	6.7	7.7
T01-04	285	291	7.8	—*	5.4	—*
T02-02	640	530	11	14.5	8.3	11
T01-05	311	337	—*	—*	—	—*

*No examination was performed.

endpoint (NYHA classification) was noted, and a clear improvement in exercise tolerance was observed in some subjects, as measured by 6MWD or Peak $\dot{V}O_2$. As the magnitude of clinically relevant change in these measures has, in some cases, been reported to be $\pm 5\%$ for LVEF, ± 1 class for NYHA classification, ± 50 m for 6MWD, and ± 1.5 ml \cdot kg⁻¹ \cdot min⁻¹ for Peak $\dot{V}O_2$,¹³ not a few subjects experienced a clinically relevant improvement in this study.

Validity of Efficacy Endpoints LVEF is the most common measure used in clinical practice and has been commonly used as an outcome measure in clinical studies. However, patients with severe HF are more likely to have comorbid mitral insufficiency or renal impairment and changes in preload and afterload are caused by various triggers despite administration of diuretics. Assessment of HF status by LVEF alone, a measure that is affected by preload and afterload, is therefore difficult. In addition, the possibility of measurement bias by an assessor cannot be ruled out.

In consideration of the current situation that patients with symptoms of HF in the absence of decreased LVEF, such as patients with HF with preserved EF, account for half of all patients with HF,¹⁴ assessment of cardiac function in patients with HF should include changes in diastolic function. Data from a clinical study conducted by Osaka University, Japan, showed that a review of symptoms and exercise tolerance in patients without LV reverse remodeling found an improvement in pulmonary arterial hypertension and a decrease in pulmonary vascular resistance, as determined by cardiac catheterization. However, data on measures to assess cardiac function needs to be accumulated. Conventional endpoints for regenerative medicine products include improvement in LV contractility as measured by various modalities, which now serves as an efficacy endpoint.³ In recent years, the US Food and Drug Administration has stated that LV contractility cannot be used as an efficacy endpoint in confirmatory studies, which should use endpoints such as mortality and cardiovascular or HF hospitalizations.¹⁵ In addition, the Study Group of the Japan's Ministry of Health, Labour and Welfare reported that improvement in survival outcomes and quality of life should be used as efficacy outcome measures.¹⁶ With regard to study design, whether a double- or single-arm design should be used for evaluation is controversial. However, based on the abovementioned suggestions and the results of this clinical study, the efficacy of regenerative medicine products likely cannot be adequately evaluated using LV contractility alone as an efficacy outcome measure. Therefore, efficacy assessment from diversified perspectives is considered necessary.¹⁷

As described earlier, improvement in survival outcomes or prevention of HF hospitalizations is important in terms of maintenance or improvement of HF status. The previous clinical study conducted by Osaka University reported a survival rate 91.7% at 3 years after transplantation in 17 patients who received transplantation with the skeletal myoblast sheet. The event-free rate at 3 years after transplantation based on Kaplan-Meier curves using the endpoints of death, LVAS implantation for worsening cardiac function, and dependence on catecholamine agents was 85.7%, suggesting a higher event-free rate than that in patients who are registered for heart transplantation at Osaka University and waiting for transplantation as Status 2 patients (54.3%), and these data suggest the efficacy of the skeletal myoblast sheet. Although the evaluation period of 26 weeks in this study still makes any discussion difficult, we await the results from an ongoing follow-up study of up to 2 years after transplantation.

Safety

In this study, no deaths occurred during the 26-week evaluation period and none of the subjects withdrew from the study because of ADRs after transplantation of TCD-51073. In contrast, SAEs included prolonged HF in all the subjects at 31 days after transplantation and, additionally, aggravated HF in 1 subject at 49 days after transplantation. The events of prolonged and aggravated HF were attributed to the transplantation procedure and underlying disease, respectively. Both were reported during hospitalization after transplantation, suggesting that surgical indication in patients with severe HF should be carefully considered, including postoperative management.

Arrhythmia

Since cases of serious arrhythmia were reported in a clinical study of suspension of skeletal myoblasts injected into the myocardium,¹⁸ the potential arrhythmogenic effects of skeletal myoblast transplantation have been discussed. In contrast, animal experiments have demonstrated that skeletal myoblast sheets are free of such side effects.¹⁹ In the present study, no events of serious arrhythmia were observed and no significant change in the incidence of ventricular extrasystole was found on Holter ECG monitoring. Therefore, the arrhythmogenic risk from using TCD-51073 was considered low.

Study Design

As transplantation of TCD-51073 involves invasive surgery, inclusion of a concurrent control group in clinical studies that include subjects with severe disease, such as the present study, is difficult for ethical reasons. Patients with severe symptoms in the present study had already received maximum therapies and had no further treatments for HF. Such patients expect the possible efficacy of this treatment and so all want to receive this treatment and none want to enter the control group. Although a placebo effect is expected to occur when evaluating efficacy before and after therapy in study patients in single-arm efficacy studies, the future development of new efficacy outcome measures that are unaffected by the placebo effect may promote the establishment of an efficacy assessment system that is ethically appropriate and provides highly reliable data.

Significance of TCD-51073 in the Treatment of HF

Although findings from previous clinical research studies and clinical trials suggest that TCD-51073 is unlikely to achieve a marked improvement in cardiac contractility in patients with severe HF who are receiving maximal standard-of-care therapy, this product may be a potential alternative therapy for HF, other than the currently available standardized therapies, such as drug therapy, LVAS implantation, and heart transplantation, if improvement of clinical symptoms, improved exercise tolerance, prolonged survival, and consequent improved cardiac diastolic function are demonstrated in future studies. A current concern in the treatment of HF is the absolute shortage of donors, and we believe that using this product as a bridge to LVAS or heart transplantation to suppress the progress of HF and delay the timing of LVAS implantation while applying various measures to increase donors will contribute to the resolution of the donor shortage. In addition, a reduction in the number of HF hospitalizations and the length of hospital stay because of HF with the use of this product will be beneficial in reducing the escalation of medical costs.