CLINCAL TRACK

Effects of Repetitive Transendocardial CD34$^+$ Cell Transplantation in Patients with Non-Ischemic Dilated Cardiomyopathy

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***Running title*:** Repetitive CD34$^+$ Cell Therapy in Cardiomyopathy

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ABSTRACT

***Rationale*:** Preclinical data in heart failure models suggest that repetitive stem cell therapy may be superior to single-dose cell administration.

***Objective*:** We investigated whether repetitive administration of $CD34^+$ cells is superior to single dose administration in patients with non-ischemic dilated cardiomyopathy (DCM).

Methods and Results: Of 66 patients with DCM, NYHA functional class III, and left ventricular ejection fraction (LVEF)< 40% enrolled in the study, 60 were randomly allocated to repetitive cell therapy (Group A, N=30), or single cell therapy (Group B, N=30). Patients received granulocyte-colony stimulating factor (G-CSF) for 5 days and 80 million $CD34^+$ cells were collected by apheresis and injected transendocardially. In Group A, cell therapy was repeated at 6 months. All patients were followed for 1 year, and the primary end-point was the difference in change in LVEF between the groups. At baseline, the groups did not differ in age, sex, LVEF, NT-proBNP, or 6-minute walk test distance. When directly comparing groups A and B at 1 year, there was no significant difference in change in LVEF (from 32.2±9.3% to 41.2±6.5% in Group A and from 30.0±7.0% to 37.9±5.3% in Group B, P=0.40). From baseline to 6 months, both groups improved in LVEF (+6.9±3.3% in Group A, P=0.001 and +7.1±3.5% in Group B, P=0.001), NT-proBNP (-578±211 pg/ml, P=0.02 and -633±305 pg/ml, P=0.01) and 6MWT (+87±21 m, P=0.03 and +92±25 m, P=0.02). In contrast, we observed no significant changes between 6 months and 1 year (LVEF: +2.1±2.3% in Group A, P=0.19 and +0.8±3.1% in Group B, P=0.56; NT-proBNP: -215±125 pg/ml, P=0.26 and -33±205 pg/ml, P=0.77; 6MWT: +27±11 m, P=0.2 and +12±18 m, P=0.42).

***Conclusions*:** In patients with DCM, repetitive $CD34^+$ cell administration does not appear to be associated with superior improvements in LVEF, NT-proBNP, or 6MWT when compared to single dose cell therapy.

Clinical Trial: NCT02248532

Keywords:
$CD34^+$ cells, dilated cardiomyopathy, heart failure, cellular transplantation,

Nonstandard Abbreviations and Acronyms:

DCM	dilated cardiomyopathy
ESC	European Society of Cardiology
LVEF,	left ventricular ejection fraction
NYHA	New York Heart Association
G-CSF	granulocyte-colony stimulating factor
6MWT	6-minute walk test
NT-proBNP	N-terminal probrain natriuretic peptide
LVESD	left ventricular end-systolic dimension
LVEDD	left ventricular end-diastolic dimension
LVESV	left ventricular end-systolic volume
LVEDV	left ventricular end-diastolic volume
UV	unipolar voltage
LS	linear shortening
SAE	serious adverse event
ANOVA,	analysis of variance
BMI	body mass index; NYHA
ACEI	angiotensin- converting enzyme inhibitor
ARB	angiotensin receptor blocker
MRA	mineralocorticoid receptor antagonist

INTRODUCTION

Non-ischemic dilated cardiomyopathy (DCM) is now the leading cause of advanced heart failure accounting for more than 50% of all heart transplantations[1]. This suggests that there is currently an unmet need in heart failure therapeutics and that alternative treatment strategies should be explored. Recently, there is increasing evidence that cell therapy may be associated with improved ventricular remodeling, better exercise tolerance, and improved outcome in DCM[2-4]. Although the results of cell therapy in patients with DCM may be more favorable than in ischemic cardiomyopathy[5], the benefits appear be confined to the early period after application, and the long-term effects are less pronounced[3]. The response to cell therapy seems to be dose-dependent, with lower doses related to inferior clinical response[4]. Furthermore, it appears that clinical response to cell therapy is better in patients in whom cell injections are more diffuse and cover more myocardial areas than in patients where cell delivery is confined to one myocardial segment[6].

Many of these limitations could theoretically be overcome by repetitive cell therapy. Repeating cell administration at the time when the clinical effect of the first dose appears to cease may further boost the efficacy of this therapy and provide superior long-term benefits. Furthermore, repeated cell administration would allow for higher numbers of delivered cells and increase the cell distribution to different myocardial areas. In addition, the use of repetitive transendocardial cell transplantation would also allow a direct evaluation of the effects of cell therapy on electroanatomical properties of the myocardium by analysing serial electroanatomic maps performed at the time of cell delivery[7]. Based on these hypotheses, the aims of this study are (1) to evaluate the safety and efficacy of repetitive $CD34^+$ cell therapy in patients with non-ischemic DCM and (2) to better define the effects of transendocardial $CD34^+$ cell delivery on electromechanical properties of the heart.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient population.

This study consists of a prospective randomized study design conducted at the Advanced Heart Failure and Transplantation Center at University Medical Center Ljubljana between January 2014 and September 2017 in collaboration with the Stanford Cardiovascular Institute.

Patient inclusion criteria consisted of the following: age 18 to 70 years, diagnosis of DCM according to European Society of Cardiology (ESC) position statement[8], optimal medical management for ≥3 months, left ventricular ejection fraction (LVEF) <40%, and New York Heart Association (NYHA) functional class III for ≥3 months before referral. Patients with acute multi-organ failure or a history of hematologic neoplasms or inadequate response to granulocyte-colony stimulating factor (G-CSF) stimulation resulting in less than 80 million $CD34^+$ cells were excluded from the study. Informed consent was obtained in all patients prior to participation in the study and the study protocol was approved by the National Ethics Committee of the Republic of Slovenia (No: 121/01/14), Agency for Medicinal Products and Medical Devices of the Republic of Slovenia (No: 425-2007/08), and European Medical Agency (EudraCT No: 002153-38). The trial was registered with Clinicaltrials.gov (NCT02248532) in 2014.

Study design.

After enrollment, patients were randomly allocated in 1:1 ratio to receive either repetitive (Group A) or single dose (Group B) $CD34^+$ cell therapy. At baseline, patients in both groups received G-CSF (10 μg/kg, 5 days); thereafter, $CD34^+$ cells were collected via apheresis and injected transendocardially guided by electro-anatomical mapping. In Group A, G-CSF stimulation, apheresis, electroanatomical mapping, and cell injections were repeated at 6 months. Patients were followed for 1 year. The flowchart

of study design, together with timeline, is presented in Figure 1. At the time of enrollment, 6 months, and 12 months thereafter, we performed detailed clinical evaluation, echocardiography, 6-minute walk test (6MWT), and measured plasma levels of N-terminal probrain natriuretic peptide (NT-proBNP).

Echocardiography, 6-minute walk test, and NT-proBNP measurements.
The echocardiography data were recorded and analyzed at the end of the study by an independent echocardiographer (S.F.) who was blinded to the patient's treatment status and the timing of the recordings. Left ventricular end-systolic dimension (LVESD) and end-diastolic dimension (LVEDD) were measured in the parasternal long-axis view. Left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), and LVEF were estimated using the Simpson's biplane method. All echocardiographic measurements were averaged over 5 cycles. In all patients, 6MWT was performed by a blinded observer according to the standard protocol[9]. All NT-proBNP assays were performed at a central independent laboratory, blinded to the patient's clinical data using a commercially available kit (Roche Diagnostics, Mannheim, Germany).

Peripheral blood stem cell mobilization and collection.
All patients underwent stem cell mobilization and collection as described previously[10]. In short, peripheral blood stem cells were mobilized by daily subcutaneous injections of G-CSF (10 mcg/kg qd) for 5 days. Peripheral blood stem cells were then collected with Miltenyi cell separator (Miltenyi Biotech, Germany) and the magnetic cell separator Isolex 300i (Nexell Therapeutics Inc., California, USA) was used for the immunomagnetic positive selection of the $CD34^+$ cells. Of the recovered $CD34^+$ cells, a standardized dose of 80 million was used for transendocardial injection. Patients not generating 80 million cells were excluded from the study.

Transendocardial cell delivery.
Electroanatomical mapping was performed using the Biosense NOGA system (Biosense-Webster, Diamond Bar, California)[7], which allows for point-by-point analysis of left ventricular viability and local contractility. Using this technique, maps of color-coded myocardial viability (unipolar voltage; UV) and regional myocardial contraction (linear shortening; LS) and their corresponding bull's-eye maps, consisting of ≥150 sampling points were generated for each patient prior to stem cell transplantation. In accordance with previous studies in non-ischemic DCM[11], segments with electromechanical mismatch were defined as areas with average unipolar voltage ≥8.27 mV and average linear shortening <6%, scarred myocardium was defined as areas with unipolar voltage <8.27 mV and linear shortening <6%, and normal myocardium was defined as areas with unipolar voltage ≥8.27 mV and linear shortening ≥6%. Immediately after the completion of electoanatomical mapping, we injected the cells in the target zones with Myostar catheter (Biosense-Webster, Diamond Bar, CA) using the pre-specified criteria of UV ≥8.27 mV and LS <6%. Each patient received 20 injections of stem cell suspension (0.3 mL each).

Follow-up and endpoints.
Patients were followed for 12 months. The primary endpoint was the difference in change in LVEF over 12 months between the groups. Secondary endpoints included changes in LVEDD, NT-proBNP levels, and 6MWT distance. In an exploratory analysis, we also sought to analyze the impact of stem cell therapy on local and global electroanatomical properties of the myocardium, measured by changes in UV. Favorable clinical response to cell therapy was defined as the presence of increase in LVEF of at least 5% at 6 months after stem cell transplantation[12]. Serious adverse event (SAE) was defined as any serious event that may result in persistent or significant disability or incapacity and included death, heart transplantation, ventricular assist device implantation, sustained ventricular arrhythmia (ventricular tachycardia or ventricular fibrillation), and heart failure exacerbation requiring hospitalization. A data and safety monitoring committee (G.P., M.J.) was responsible for monitoring SAEs during the study and would report to the study principal investigator (B.V.).

Statistical methods and analysis.
Based on our previous findings on $CD34^+$ cell transplantation in non-ischemic DCM[3,4], the sample size for this study was chosen to detect a treatment difference in the change in ejection fraction from baseline

to 12 months between Groups A and Group B of 3.1% (assuming a standard deviation of 5.2%) with 90% power. This yielded a minimal sample size of 30 patients in each group. In our previous trials[3,4], we found that up to 20% of patients may have inadequate response to G-CSF stimulation, therefore we increased our estimated sample size accordingly, resulting in a total of 80 patients**.** This number would be readjusted according to the actual enrollment in the 2 arms.

Continuous variables were expressed as mean ± SD and categorical variables were expressed as a number and a percentage. Continuous variables were explored for normal distribution with the Shapiro-Wilk test. Differences within the groups were analyzed using a T-test for continuous variables with correction for unequal variance when appropriate, and with chi-square or Fisher exact test when appropriate. Differences between Groups A and B were analyzed with repeated measures 2-way analysis of variance (ANOVA); patients who died or underwent heart transplantation were excluded. In an exploratory analysis, we aimed to better define potential predictors of favorable response to repeated cell therapy by performing backward method multiple regression analysis with an inclusion criteria P <0.05 and exclusion criteria of P >0.10. A value of P <0.05 was considered significant. All statistical analyses were performed with SPSS software (version 20.0).

RESULTS

Patient characteristics.

To achieve the required sample size of 60 patients, we screened 89 DCM patients and enrolled a total of 66 patients, 6 of whom presented with inadequate response to G-CSF stimulation and were thus excluded and followed for clinical outcomes for the duration of study follow-up. The remaining patients were allocated into repetitive (Group A) or single-dose (Group B) cell injection. During 1-year follow-up, there was one heart transplantation in Group A versus one death and one heart transplantation in Group B. Of the 6 excluded patients, 1 patient died, and 1 underwent heart transplantation (Figure 1). At baseline, groups A and B did not differ in demographic parameters, LVEDD, LVEF, NT-proBNP, exercise capacity, heart failure medications, prevalence of diabetes mellitus or renal and liver function tests (Table 1).

Clinical outcomes.

Data on clinical parameters are presented in Figure 2. When directly comparing groups A and B at 1 year, there was no significant difference in change in LVEF (from 32.2±9.3% to 41.2±6.5% in Group A and from 30.0±7.0% to 37.9±5.3% in Group B, P=0.40). Similarly, we found no inter-group differences in the change in LVEDD (from 6.7±1.0 cm to 6.6±0.7 cm in Group A and from 6.6±0.9 cm to 6.5±0.7 cm in Group B, P=0.87), NT-proBNP (from 1525±1030 pg/mL to 732±725 pg/mL in Group A and from 1753±1008 pg/mL to 1087±978 pg/mL in Group B, P=0.33), and 6MWT (from 320±92 m to 434±71 m in Group A and from 341±87 m to 445±96 m in Group B, P=0.65). From baseline to 6 months, both groups displayed a significant improvement in LVEF (+6.9±3.3% in Group A, P=0.001 and +7.1±3.5% in Group B, P=0.001), decrease in NT-proBNP (-578±211 pg/ml, P=0.02 and -633±305 pg/ml, P=0.01), and increase in 6MWT (+87±21 m, P=0.03 and +92±25 m, P=0.02). In contrast, we observed no additional changes between 6 months and 1 year in any of the groups (LVEF: +2.1±2.3% in Group A, P=0.19 and +0.8±3.1% in Group B, P=0.56; NT-proBNP: -215±125 pg/ml, P=0.26 and -33±205 pg/ml, P=0.77; 6MWT: +27±11 m, P=0.2 and +12±18 m, P=0.42). We found no significant changes in LVEDD at any time point.

Changes in electroanatomical properties.

In patients receiving repetitive cell therapy (Group A), we were able to directly evaluate changes in myocardial viability measured by UV by comparing electroanatomical maps at baseline and 6 months. On repeated electroanatomical maps, we found a significant improvement in both global UV and local UV at the cell injection sites (Figure 3).

Response to repeated cell therapy.

Within Group A, we found a favorable response to the second cell injection (improvement in LVEF of at least 5%) in 10 of 30 patients (30%). When compared to the remaining cohort, these patients demonstrated lower LVEF and higher global UV at the time of second cell injection (Table 2). In a multivariate model that included LVEF before the second injection, change in LVEF after the first injection, and global UV before second injection, change in UV after the first injection was the only independent correlate of good response to the second dose of cell therapy (Table 3).

Adverse events.

Data on SAEs are presented in Table 4. The numbers of SAEs within the 1-year follow-up were low and did not differ between the repetitive and single-dose groups.

DISCUSSION

This is the first clinical study to date investigating the effects of repetitive transplantation of $CD34^+$ cells in patients with nonischemic DCM. Our data confirm that transendocardial cell therapy in patients with non-ischemic DCM is feasible and safe, and associated with favorable changes in LVEF, NT-proBNP, and exercise capacity when using a non-placebo controlled trial design. When compared to single-dose therapy, repetitive cell administration was not associated with better clinical response. Based on our exploratory sub-group analysis patients with increase of myocardial viability after the first dose could potentially benefit from repetitive therapy although this needs to be confirmed in a randomized controlled trial.

To date, clinical data on repetitive cell therapy are scarce and limited to patients with ischemic heart disease. In a study involving patients after anterior myocardial infarction, repeated intracoronary infusion of bone marrow mononuclear cells at 3-7 days and again 3 months after infarction was associated with superior improvement in left ventricular function and reduction of myocardial scar size when compared to single cell administration[13]. Similarly, in patients with refractory ischemic heart failure, repeated intracoronary infusion of peripheral blood mononuclear cells at 6 months resulted in a more pronounced improvement in LVEF when compared to single cell dosing[14]. In contrast with these findings, the DanCell-CHF trial failed to demonstrate significant improvement in left ventricular function after repeated intracoronary injection of bone marrow mononuclear cells administered 4 months apart in patients with ischemic heart failure[15]. However, in this patient cohort, they did find a significant correlation between long-term survival and the numbers of injected $CD34^+$ cells[16]. Consistent with the findings of these trials, we were able to demonstrate a significant improvement in LVEF after transendocardial injection of mobilized $CD34^+$ cells in patients with nonischemic DCM. However, we failed to demonstrate further improvements of heart function with repeated cell injection. Although the reasons for these results remain speculative, they may be related to differences in patient characteristics at the times of both injections, with mean LVEF before the second injection being significantly higher than before the first injection. Furthermore, we found that the response to the second dose of cell therapy was more prominent in patients with lower LVEF. Together, these data suggest that there may be a 'ceiling effect' for cell therapy in DCM, and that patients with higher LVEF may have less benefits from this therapeutic approach. Alternatively, the relatively poor response to repetitive cell therapy may be related to the timing of the second dose, which was based on our previous experience

in DCM, where the most prominent clinical effects were observed 6 months after intracoronary cell delivery[4], with little additional benefit beyond the first year after stem cell administration. In the present study we used transendocardial cell delivery, which has been associated with higher myocardial cell retention rates and possibly different cell kinetics when compared to intracoronary route[2]. Since the benefits of cell therapy appear to stabilize at 1 year, one might speculate that repeating cell injection at this time may yield different clinical effects. Finally, an alternative hypothesis could be that there is a non-negligible placebo effect to stem cell therapy that will need to be further tested in future trials.

Despite these shortcomings, our exploratory analysis suggests an incremental benefit of second cell injection in a subgroup of patients who displayed an increase in myocardial viability after the first cell dose. Similarly, in a study of patients with ischemic heart disease, repeated transendocardial application of bone marrow mononuclear cells resulted in further increase in perfusion and decrease in angina frequency only in patients who responded favorably to the first cell dose[17]. This suggests that repeated cell therapy may be more effective when applied selectively based on patient response to the first dose.

In the present study, we found no correlation between changes in LVEF and changes in myocardial viability as measured by UV. However, patients with increase of myocardial viability after the first cell dose demonstrated a significant improvement in LVEF after the second dose; in contrast to patients without improved viability, who did not respond to repeated cell therapy. This is consistent with the findings of transendocardial cell injections in ischemic heart failure, where higher myocardial viability at the time of cell injection was associated more pronounced improvement in left ventricular function[18]. Furthermore, in our previous study investigating the predictors of response to transendocardial $CD34^+$ cell transplantation in a larger cohort of patients with chronic heart failure we found that left ventricular scar burden was significantly higher in non-responders (58%) compared to responders (47%)[19]. Although the underlying mechanisms for these findings are not clearly defined, they may be related to myocardial changes after cell therapy that do not reflect as changes in LVEF. In accordance with this hypothesis, data from electrophysiological studies in nonischemic DCM suggest that the potential for reversibility of left ventricular dysfunction correlates with higher endocardial UV, but not with baseline LVEF[11]. Thus, one might speculate that by increasing myocardial viability with the first cell injection, we were able to change the myocardial properties in a way to make it more susceptible for the effects of second dose of cell therapy.

In a rat model of post-infarction heart failure, it has been demonstrated that 3 repeated doses of cardiac progenitor cells are safe and considerably more effective in improving left performance and structure than a single dose, and that these effects are mainly the result of paracrine mehanisms[20]. These findings have been confirmed using mice with 3-week-old myocardial infarction receiving one or three doses of cardiac mesenchymal cells[21]. Furthermore, intramyocardial transplantation of allogeneic cardiosphere-derived cells in a rat model of myocardial infarction was associated with improved ventricular function and reduced infarct size[22]. Taken together, the results of these pre-clinical studies suggest that repetitive cell therapy is effective and may represent an important strategy for the future development of the field of cell therapies.

In accordance with previous clinical trials[3,23], the findings of our study are confirming that transendocardial cell therapy leads to improvement in left ventricular function and exercise capacity in DCM patients. Furthermore, we have demonstrated that in this patient cohort, G-CSF stimulation, apheresis and transendocardial cell delivery can be repeated at 6-month interval without serious adverse events, and that repeated cell therapy may be effective in a subgroup of patients with an increase of myocardial viability after the first cell dose. Although the underlying mechanisms are not clearly defined, these findings may serve as a background for the design of future trials investigating the role of repetitive cell therapy in chronic heart failure patients.

Study limitations.

The results of our study are subject to several limitations. For instance, our patient population included patients with DCM, but no biopsies were performed to exclude secondary cardiomyopathies, though we obtained careful clinical history, detailed echocardiography, and coronary angiogram in all patients. Although we only included patients with NYHA class III symptoms, the baseline 6-minute walk test distance was higher than expected. The study design did not include a placebo arm, which limits the evaluation of the cell therapy effects. Our sample size was small, which makes the study underpowered to be definitive, but the groups of patients were well matched at baseline. The exploratory analysis of predictors of response to second dose of cell therapy was performed post-hoc, which diminishes the impact of its results. Based our previous results suggesting that LS represents the cumulative effects of local linear contraction, local relaxation and local dyssynchrony[24] we opted not to include it as a measure of changes in contractility in the present study. Finally, we recognize that patients with DCM are a heterogeneous patient population and dynamic changes in ventricular function may be multi-factorial. However, to minimize the potential effects of medical management, all patients were followed in our heart failure outpatient clinic for at least 3 months before inclusion, which allowed for adequate optimization of medical therapy. During the 1-year follow-up, no additional changes in heart failure therapy were performed.

Conclusions.

Repetitive transendocardial $CD34^+$ cell transplantation does not appear to be associated with incremental clinical improvement when compared to single-dose transplantation in non-ischemic DCM. Further studies are warranted to better define the patient responder profiles and to investigate whether or not such approach can be safely and effectively used in selected subgroups of chronic heart failure patients.

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DISCLOSURES
None.

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Table 1. Baseline Patient Characteristics

	All (n=60)	Group A (n=30)	Group B (n=30)	*P*
Age, y	55±10	56±9	54±11	0.42
Male gender	53 (88)	27(90)	26 (87)	0.69
BMI, kg/m^2	25.4±3.4	24.8±3.3	26.1±3.2	0.71
Duration of heart failure, mo	35.3±4.8	33.7±5.2	36.2±4.4	0.40
DCM etiology				
History of viral infection	48 (80)	25 (83)	23 (77)	0.52
Familial	1 (2)	1 (3)	0 (0)	
Idiopathic	11 (18)	4 (14)	7 (23)	
Diabetes	8 (13)	3 (10)	5 (17)	0.45
LVEF, %	31.2±8.4	32.2±9.3	30.0±7.0	0.39
LVEDD, cm	6.7±0.8	6.7±1.0	6.6±0.9	0.83
Creatinine, mg/dl	1.46±0.34	1.43±0.32	1.49±0.47	0.52
Bilirubin, µmol/L	37±14	36±12	38±15	0.59
Sodium, mmol/l	136±7	135±4	136±6	0.41
NT-proBNP, pg/ml	1690±1074	1525±1030	1753±1008	0.47
6-minute walk, m	330±90	320±92	341±87	0.72
Drug therapy				
ACEI/ARB	60 (100)	30 (100)	30 (100)	1.00
Beta blockers	59 (98)	30 (100)	29 (97)	1.00
MRA	55 (92)	28 (93)	27 (90)	1.00
Digoxin	5 (8)	2 (7)	3 (10)	0.64
Loop diuretics	58 (97)	30 (100)	28 (93)	0.49

Values are presented as mean±SD or number of patients (percent). BMI, body mass index; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; ACEI, angiotensin- converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist

Table 2. Characteristics of Good Responders and Poor Responders to the Second dose of Cell Therapy

	Good Responders (n=10)	Poor Responders (n=20)	*P*
Age, y	56±8	59±9	0.22
Male sex	8 (80)	19 (95)	0.20
Diabetes	2 (20)	1 (5)	0.25
Creatinine, mg/dl	1.50±0.32	1.35±0.50	0.35
Total bilirubin, μmol/L	37±12	32±15	0.38
LVEF before 2nd injection, %	34.9±8.9	40.0±7.8	0.02
ΔLVEF after 1st injection, %	+1.6±6.8	+7.5±7.1	0.01
LVEDD before 2nd injection, cm	6.7±0.8	6.7±1.0	0.71
ΔLVEDD after 1st injection, cm	-0.05±0.71	-0.13±0.43	0.79
NT-proBNP before 2nd injection, pg/ml	814±774	1012±1587	0.71
ΔNT-proBNP after 1st injection, pg/ml	-1066±871	-557±1665	0.19
Global UV before 2nd injection, mV	15.3±3.1	11.4±1.9	0.01
Δ Global UV after 1st injection, mV	+6.6±2.9	+1.3±2.3	0.01

Values are presented as mean±SD or number of patients (percent). LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; UV, unipolar voltage.

Table 3. Multivariate Correlates of Response to the Second Dose of Cell Therapy

	Hazard Ratio	95% Confidence Interval	*P*
LVEF before 2nd injection >30 %	1.70	0.06-47.51	0.75
ΔLVEF after 1st injection >5 %	0.93	0.10-8.68	0.95
UV before 2nd injection >12 mV	4.99	0.56-44.44	0.14
ΔUV after 1st injection >3 mV	8.60	1.25-59.12	0.02

LVEF, left ventricular ejection fraction; UV, unipolar voltage.

Table 4. Serious Adverse Events

	All (n=60)	Group A (n=30)	Group B (n=30)	*P*
Death	1 (2)	0 (0)	1 (3)	1.00
Heart transplantation	2 (3)	1 (3)	1 (3)	1.00
LVAD implantation	0 (0)	0 (0)	0 (0)	1.00
Sustained ventricular arrhythmia	3 (5)	2 (7)	1 (3)	1.00
Heart failure worsening	4 (7)	2 (7)	2 (7)	1.00

Values are presented as number of patients (percent). LVAD, left ventricular assist device.

FIGURE LEGENDS

Figure 1. Flow chart of the study design. Patients were randomly allocated in 1:1 ratio to receive either repetitive (Group A) or single dose (Group B) cell therapy. At baseline, patients in both groups received G-CSF; thereafter, $CD34^+$ cells were collected via apheresis and injected transendocardially. In Group A, G-CSF stimulation, apheresis, and cell injections were repeated at 6 months. Patients were followed for 1 year from baseline.

Figure 2. Clinical outcome after repetitive and single-dose therapy. Within 1 year**,** we found no significant differences between repetitive or single-dose injection with regards to LVEF (Panel A), LVEDD (Panel B), NT-proBNP (panel C), or 6-minute walk test distance (Panel D). Differences between repetitive and single-dose groups were analyzed with repeated measures 2-way analysis of variance (ANOVA).

Figure 3. Changes in electroanatomical properties. A representative 3D quantitative unipolar voltage map before (bottom left panel) and 6 months after cell injection (bottom right panel). High unipolar voltage is depicted as purple, blue, or green areas, and low unipolar voltage as red and yellow areas. Brown dots represent the sites of cell injections. On repeated mapping, we found a significant improvement in global unipolar voltage and local unipolar voltage at the cell injection sites (top panel).

NOVELTY AND SIGNIFICANCE

What Is Known?

- Single-dose $CD34^+$ cell therapy has been associated with improvements in heart function in non-ischemic dilated cardiomyopathy (DCM) patients.
- Data from preclinical models suggest that repetitive cell therapy may be superior to single dose cell administration.

What New Information Does This Article Contribute?

- Repetitive $CD34^+$ cell therapy did not appear to be associated with incremental clinical improvement when compared to single-dose transplantation in non-ischemic DCM patients.
- Exploratory analysis suggested an incremental benefit of second cell injection in a subgroup of patients who displayed an increase in myocardial viability after the first cell dose.

This is the first clinical study to date investigating the effects of repetitive transplantation of $CD34^+$ cells in patients with nonischemic DCM. Our data confirm that transendocardial cell therapy in patients with non-ischemic DCM is feasible and safe, and associated with favorable changes in heart function and exercise capacity. When compared to single-dose therapy, repetitive cell administration was not associated with better clinical response. However, based on our exploratory sub-group analysis, patients with increase of myocardial viability after the first dose could represent a subgroup of DCM patients who would benefit from repetitive cell therapy.

FIGURE 1

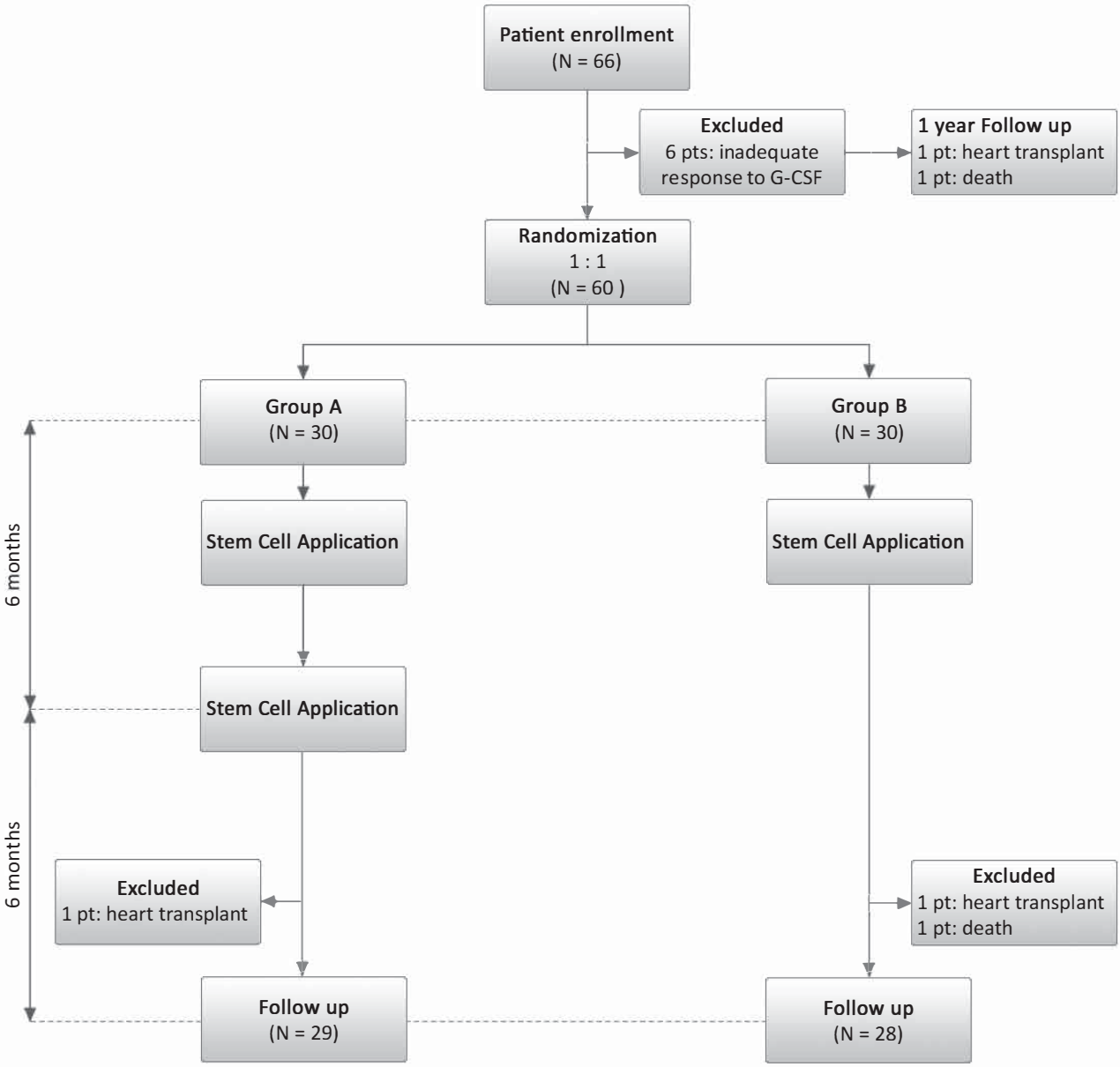


FIGURE 2

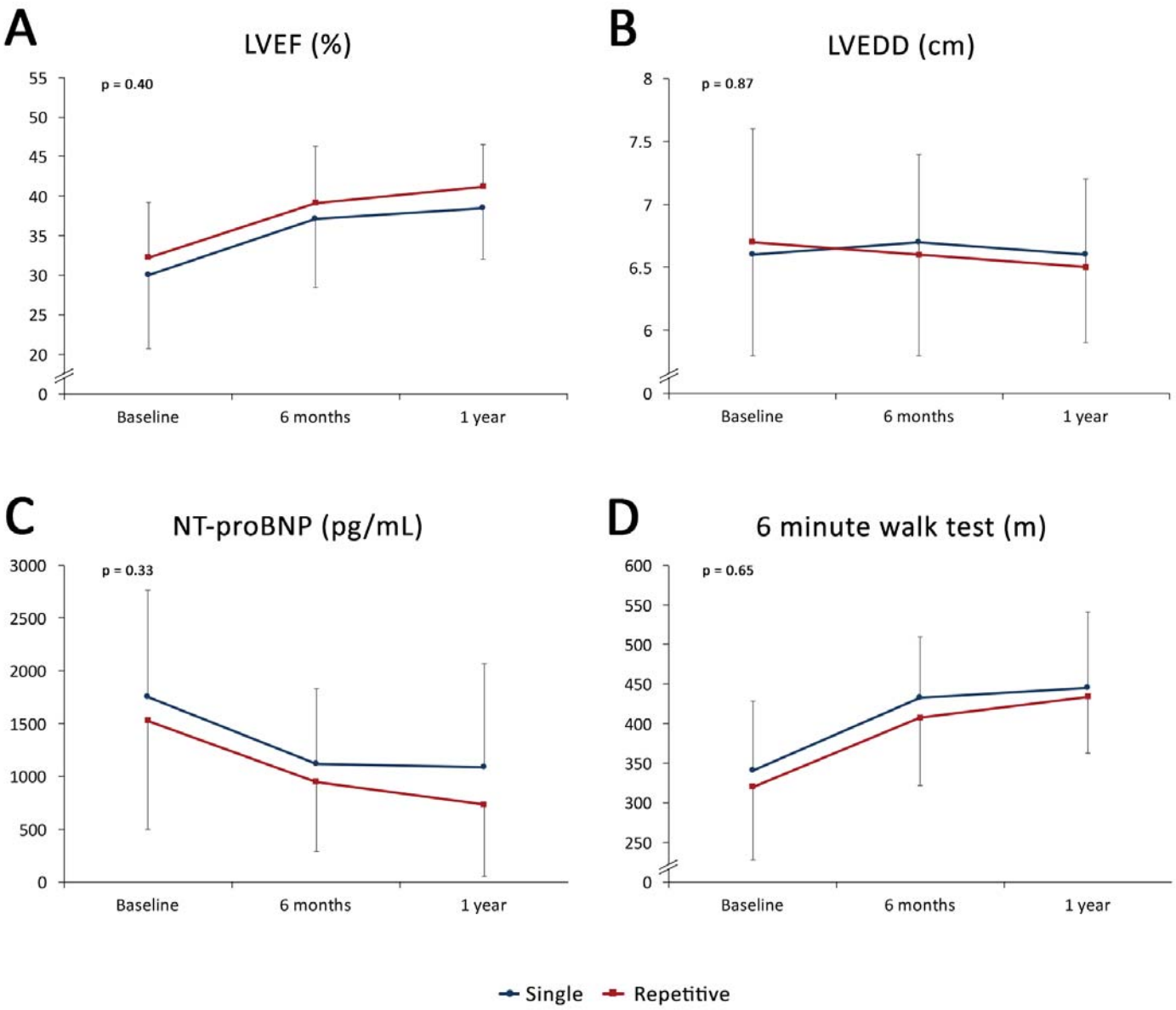
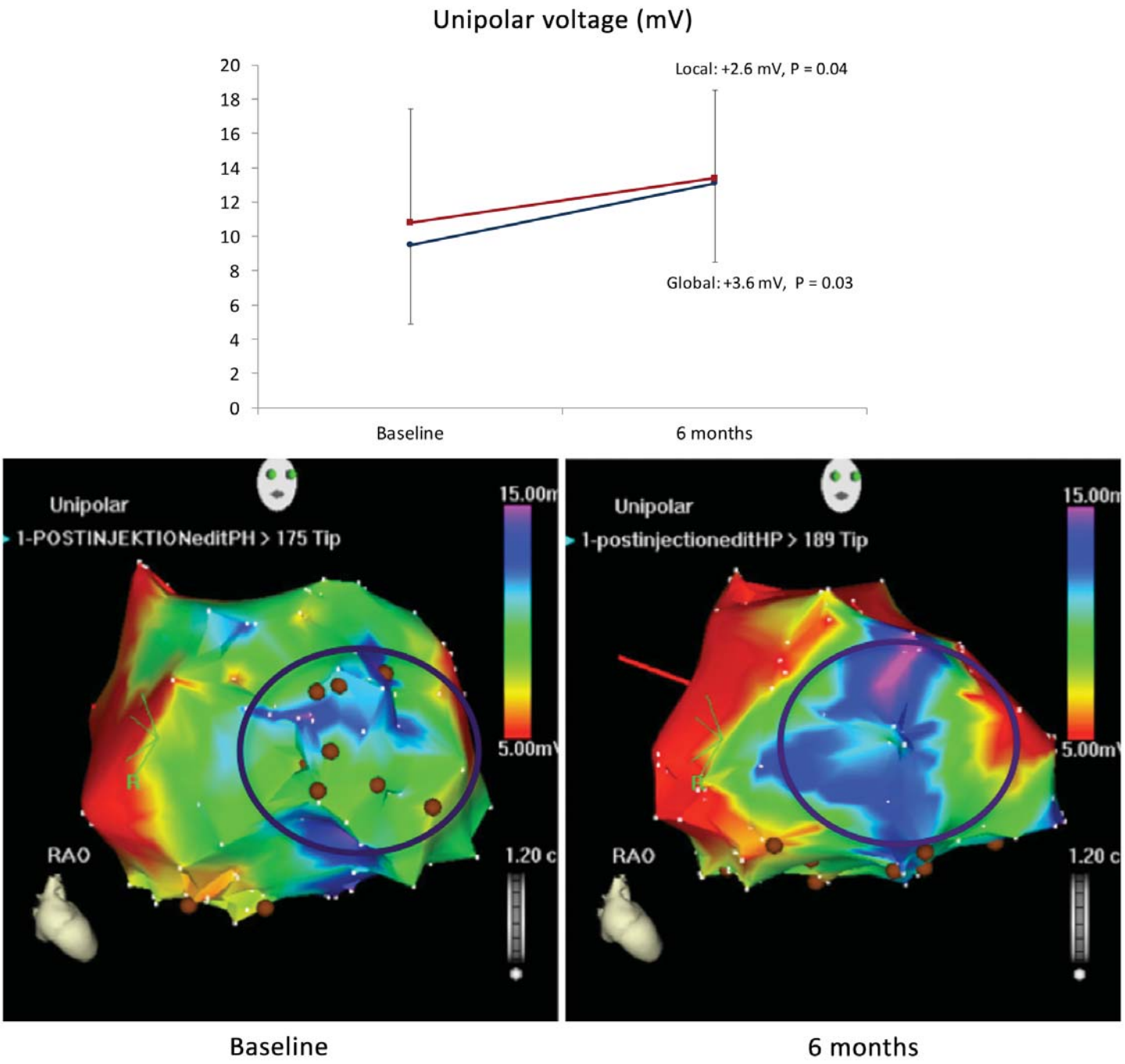


FIGURE 3



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