Human MuStem Cell Grafting into Infarcted Rat Heart Attenuates Adverse Tissue Remodeling and Preserves Cardiac Function

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Myocardial infarction is one of the leading causes of mortality and morbidity worldwide. Whereas transplantation of several cell types into the infarcted heart has produced promising preclinical results, clinical studies using analogous human cells have shown limited structural and functional benefits. In dogs and humans, we have described a type of muscle-derived stem cells termed MuStem cells that efficiently promoted repair of injured skeletal muscle. Enhanced survival rate, long-term engraftment, and participation in muscle fiber formation were reported, leading to persistent tissue remodeling and clinical benefits. With the consideration of these features that are restricted or absent in cells tested so far for myocardial infarction, we wanted to investigate the capacity of human MuStem cells to repair infarcted hearts. Their local administration in immunodeficient rats 1 week after induced infarction resulted in reduced fibrosis and increased angiogenesis 3 weeks post-transplantation. Importantly, foci of human fibers were detected in the infarct site. Treated rats also showed attenuated left-ventricle dilation and preservation of contractile function. Interestingly, no spontaneous arrhythmias were observed. Our findings support the potential of MuStem cells, which have already been proposed as therapeutic candidates for dystrophic patients, to treat myocardial infarction and position them as an attractive tool for muscle-regenerative medicine.

INTRODUCTION

The heart lacks the intrinsic ability to repair damage caused by severe injuries, such as myocardial infarction (MI).1,2 Intramyocardial transplantation of stem/progenitor cells constitutes a promising approach to restore cardiac function and prevent progression to heart failure.3 Preclinical studies conducted over the past 2 decades have evaluated the regenerative potential of multiple cell types. These include myoblasts,4–6 bone marrow (BM) mononuclear cells,7,8 mesenchymal stem cells (MSCs),9,10 and endogenous cardiac progenitors, such as c-kit+ cardiac progenitor cells (CPCs),11 sca1+ CPCs,12 and cardiosphere-derived cells,13 as well as embryonic stem cells (ESCs)14,15 and induced pluripotent stem cell (iPSC)-induced cardiomyocytes (CMs).16,17 Decreased myocardial fibrosis, neovascularization, prevention of left-ventricle (LV) dilation, and enhancement of local cardiac contractility have been successively described for stem cells of different origins,18–20 leading to rapid progression to clinical trials for a subset of these cell types. To date, however, these encouraging preclinical findings have not been replicated in a clinical setting, and randomized clinical trials for MI have shown only modest long-term efficacy,21 mainly due to poor survival and engraftment of injected cells in the harsh cardiac environment and negligible direct differentiation of stem cells into CMs and/or vascular cells.22,23

A type of murine muscle-derived stem cells (MDSCs) isolated on the basis of an initial adhesion defect has been shown to implant into the infarcted zone, induce neo-angiogenesis, and significantly improve cardiac function after transplantation into the hearts of adult immunodeficient mice with acute MI.24 These outcomes were much better than those obtained with myoblasts, which when transplanted into small and large animal models, were associated with poor retention, arrhythmias, and limited spreading from the injection site.25–27 In recent years, we have isolated and characterized a type of MDSC (MuStem cells) from healthy canine skeletal muscle and have demonstrated their therapeutic potential after intramuscular and systemic delivery in the golden retriever muscular dystrophy (GRMD) dog.28

In this clinically relevant animal model of Duchenne muscular...
dystrophy, MuStem cells show improved survival and greater potential than myoblasts for implantation into severely damaged tissue, as well as the potential to contribute to long-term myofiber regeneration, satellite cell replenishment, and restriction of fibrosis. Through signaling mediated by several biological pathways, MuStem cell implantation limits the progression of muscle damage and stabilizes the clinical status of recipient dogs. More recently, we isolated the human counterpart of MuStem cells (hMuStem cells), for which we have demonstrated comparably robust muscular-regeneration capacity after delivery into injured skeletal muscle in immunodeficient mice. These compelling findings point to hMuStem cells as attractive candidates for the treatment of skeletal muscle diseases. Given the positive results obtained following murine MDSC implantation in allogeneic MI models, as well as the limitations of other cell types tested for the treatment of MI, it is of particular interest to characterize the behavior of hMuStem cells in the context of MI.

Here, we report for the first time the transplantation of hMuStem cells in an immunodeficient rat model of MI. We provide an extensive histological and molecular analysis of their behavior 3 weeks after local administration by considering the post-transplantation survival, engraftment, and differentiation of hMuStem cells. Cardiac tissue and functional remodeling was investigated through analysis of the cardio-myogenic, fibrotic, and angiogenic compartments, as well as the electrocardiography (ECG) and echocardiography parameters. Overall, we demonstrate the potential of hMuStem cells to counteract adverse cardiac tissue remodeling and preserve cardiac function, suggesting that they could represent a valuable therapeutic tool for the treatment of cardiac diseases, in addition to their demonstrated benefits in the treatment of injured skeletal muscle.

RESULTS

hMuStem Cells Correspond to Early Myogenic Progenitors Uncommitted to the Cardiac Lineage

To characterize the hMuStem cell populations used in the transplantation protocol, 4 independent cell batches expanded at passage 5 (P5) under good manufacturing practice-like conditions were tested for a panel of lineage-specific markers. In agreement with our previous findings, flow cytometry revealed that more than 71% of in vitro-expanded cells expressed the canonical satellite cell and myoblast marker cluster of differentiation 56 (CD56), whereas all cells were robustly positive for the recently identified satellite cell markers CD29, CD82, and CD318, respectively. (A) Representative flow cytometry profiles of hMuStem cells (n = 4 independent batches) cultured in growth medium (passage 5). Expression of markers of myogenic/satellite cells (CD56, CD29, CD82, and CD318), mesenchymal stem cells (CD73, CD90, and CD105), hematopoietic stem cells (CD45), and perivascular cells (CD140b, CD146) was evaluated. Isotype controls and specific signals are shown in white and gray, respectively. (B) Expression of transcripts specific to cardiac-lineage markers (NKX2.5, TBX5, RYR2, SCN5A, TNNT2, and GJA1 encoding the Cx43 protein) in 4 MuStem cell batches. HPRT1 was used as a housekeeping gene. (C) Representative western blot showing Cx43 protein (encoded by the GJA1 gene) expression on hMuStem cells compared to myoblasts and glioblastoma cells (positive and negative control, respectively). GAPDH was used as a loading control.

Figure 1. Cell Lineage-Specific Phenotype of the hMuStem Cell Population
pressed at low levels. Only the gap junction protein Cx43 (Figure 1B). Other classical markers, including voltage-gated sodium channel α subunit 5 (SCN5A) and troponin T2 (TNNT2), were expressed at low levels. Only the gap junction protein α1 (GJA1) gene encoding connexin 43 (Cx43), which is required to establish intercellular connections between CMs and is also expressed by other progenitor cells, such as MSCs,\(^3\)–\(^5\) was detected in hMuStem cells. In addition, a large amount of the phosphorylated isoform of Cx43, which is involved in the electrical cell-to-cell coupling through its action on the gap junction function, were detected by western blotting on hMuStem cells compared to those obtained in proliferating myoblasts known to express Cx43 (Figure 1C). Taken together, these results indicate that cultured hMuStem cells are early myogenic progenitors with a perivascular/mesenchymal signature that do not exhibit any intrinsic cardiac lineage commitment in their native condition.

### Skeletal hMuStem Cells Can Engraft and Persist in Healthy Myocardium

We sought to determine whether hMuStem cells derived from skeletal muscle could be implanted into heart tissue. hMuStem cells were delivered into 6 sites within the mid-portion of the LV of 9 immunodeficient rats. Functional and histopathological studies were performed 3 weeks after stem cell administration, as shown in the schematic in Figure 2A. The extent of hMuStem cell engraftment was also analyzed by immunohistochemistry using an anti-human lamin A/C antibody (Ab) that does not crossreact with rodents. The presence of human cells was confirmed by detection of large clusters of lamin A/C\(^+\) nuclei in all injected hearts. 4′,6-diamidino-2-phenylindole (DAPI) counterstaining of the lamin A/C\(^+\) nuclei showed no change in nucleus appearance, typically corresponding to pyknosis, karyorrhexis, and chromatin condensation, indicating that the transplanted hMuStem cells correspond to intact cells. These cells, which were visualized by wheat germ agglutinin (WGA) labeling,\(^4\) were mainly randomly scattered throughout the connective tissue and rarely observed in myocardial tissue (Figure 2B). Also, they correspond to small cells with a poorly developed cytoplasm and harboring only one nucleus (Figure 2B, insets). Topographic hematoxylin-eosin-saffron (HES) staining confirmed that these cells had either very scant, barely visible cytoplasm or a moderate amount of eosinophilic cytoplasm around a large, paracentral euchromatic nucleus (Figure 2C). Activated macrophages were not detected close to the hMuStem cells, as expected in this immunodeficient rat model characterized by a drastic reduction of monocytes and macrophages.\(^2\) Picrosirius staining confirmed that these cells were primarily localized in dense, connective tissue. The number of hMuStem cells was estimated using an Alu-based technique for detecting genomic human DNA (hDNA)\(^5\). Noninfarcted hearts retained 16,600–139,390 cells, depending on the rat, corresponding to 1%–5% of the total number of cells originally transplanted. By contrast, samples from the liver, lung, spleen, brain, kidney, and skeletal muscle were all negative for hDNA (data not shown).

Analyses were performed to rule out the presence of electrical, structural, or contractile cardiac dysfunction associated with the persistence of hMuStem cells in injected hearts. ECG analysis revealed no electrical abnormalities (Figure 2D; Tables 1 and S1). Similarly, the presence of hMuStem cells had no effect on heart rate, atrial (P-wave duration), atrio-ventricular (PR interval), or ventricular (QRS complex duration) conduction or ventricular repolarization (QT interval). Measurement of the LV end-diastolic diameter (LVEDD) and end-diastolic wall thickness (LVEDWT) using time-movement mode (M-mode) echocardiography revealed no structural differences compared to Sham Group (without Inducing Infarction).

### Table 1. ECG and Echocardiographic Parameters Measured in Rats from the Sham Group (without Inducing Infarction) before (Baseline) and 3 Weeks after Sham Procedure

| Parameter                  | Baseline          | 3 Weeks Post-transplantation |
|----------------------------|-------------------|-----------------------------|
| Heart rate (bpm)           | 399.4 ± 41.3      | 382.2 ± 37.6                |
| P wave (ms)                | 19.9 ± 2.1        | 19.4 ± 1.9                  |
| PR interval (ms)           | 48.7 ± 2.8        | 47.8 ± 4.1                  |
| QRS (ms)                   | 20.0 ± 2.2        | 22.0 ± 2.3                  |
| QT interval (ms)           | 80.3 ± 5.5        | 77.2 ± 7.2                  |

Data are expressed as the mean ± SD. N = 9 animals. bpm, beats per minute.
remodeling and no alterations in LVEDD (7.22 ± 0.27 mm and 7.43 ± 0.23 mm at baseline and 3 weeks postinjection, respectively) or LVEDWT (1.63 ± 0.07 mm and 1.73 ± 0.08 mm, respectively; Figure 2D; Tables 1 and S2). Furthermore, we observed no change in systolic function, as assessed by determination of ejection fraction (EF): EF = 84% ± 3% and 87% ± 2% at baseline and 3 weeks postinjection, respectively. Similarly, we observed no change in diastolic markers (Figure S2), i.e., early-diastolic/late-diastolic (E/A) ratio (E/A = 2.3 ± 0.4 ms, respectively), or isovolumetric relaxation time (IVRT; = 27 ± 0.5 and 26 ± 0.9 ms, respectively). These data demonstrate the absence of cardiac structural remodeling or functional alterations 3 weeks after myocardial hMuStem cell transplantation and indicate that hMuStem cells are capable of engrafting in uninjured hearts without inducing structural remodeling or causing adverse effects on electrical, systolic, or diastolic function.

hMuStem Cells Attenuate the Infarction-Induced Adverse LV Remodeling

To investigate the potential of hMuStem cells to induce cardiac repair in the context of MI, we designed a protocol for cell transplantation in female-immunodecient rats that had undergone permanent occlusion of the left anterior descending (LAD) coronary artery 1 week earlier. Transplantation was followed by functional and histopathological examinations, as outlined in the schematic shown in Figure 3A. The coronary ligature caused a 19.79% ± 1.93% reduction in LV EF (LVEF) after 1 week, indicating induction of moderate MI. 1 week postinftarction, 20 rats were blindly randomized to receive injections of either 2.7 × 10^6 hMuStem cells (treated group) or vehicle (mock group) into the infarct border zone. 3 weeks postinjection, rats were sacrificed and their LV transversely sectioned from the apex to the base. Depending on the experiment planned, all or one-half of the sections were frozen and/or embedded in paraffin (Table S3). In the mock group, analysis of H&E-stained sections confirmed MI, as evidenced by extensive areas of remodeling and an irregular shape, resulting in multiple foci of varying size within the section (Figure 3B). These areas displayed a similar organization that could be divided in two parts: a central zone and a border zone. The central zone was characterized by a complete loss of CMs, which had been replaced by fibrotic tissue rich in collagen, as confirmed by saffron staining, with large fibroblasts displaying a euchromatic nucleus and infiltrated with mixed inflammatory cells. An irregular boundary-demarcated border zone, 30–70 μm thick, surrounded the central area and was mainly composed of hyper-eosinophilic ovoid CMs with marked anisocytosis, individually surrounded by thick bundles of collagen-rich connective tissue. At the periphery of the infarct, viable myocardium retained an organized structure with clearly intact cells. The central zone in all rats in the treated group was much smaller than that of the mock group. Moreover, the border zone in treated rats was thinner than that of vehicle-treated counterparts or in some cases, completely absent, with normal CMs directly adjacent to the central zone.

Fibrotic tissue accounted for 12.6% ± 2.9% and 9.4% ± 1.8% of the entire section in the mock and treated groups, respectively, indicating a trend toward a global decrease in fibrosis following hMuStem cell treatment (Figure 3C). The number of fibrotic foci within the infarct was 14.4 ± 1.2 and 7.6 ± 2.8 in the mock and treated groups, respectively (p = 0.059, Mann-Whitney test), indicating that hMuStem cell administration mitigated the development of myocardial fibrosis. Comparison of the infarct and peripheral (i.e., viable) zones revealed that fiber size was more heterogeneous and globally increased in the infarct zone. Fiber size was measured and expressed as the minimal Feret (minFeret) diameter (Figure 3D). One-way ANOVA revealed a difference in fiber size between zones (p < 0.001) but no effect of treatment (vehicle versus hMuStem cells). The size distribution of fibers in the infarct zone is depicted in Figure 3E. Marked anisocytosis was confirmed in rats in both the mock and treated groups. Strikingly, some small fibers (<5 μm in diameter) were exclusively identified in the treated group.

Revascularization of the heart by the formation of new vessels is one of the preferred therapeutic approaches for MI. Capillary density within the infarct was assessed by von Willebrand factor (vWF) labeling of endothelial cells, whereas the conjunctive frame was labeled with the WGA. In the mock group, the central MI zone was characterized by the presence of dense WGA+ areas with a low density of blood capillaries and a few larger blood vessels. Conversely, in the border zone, capillaries were more numerous and had a dilated lumen delineated by large endothelial cells (Figure 4A). The WVF+ surface accounted for 16.13% ± 3.32%, 7.46% ± 1.81%, and 5.87% ± 1.82% of the infarct border, and viable zones, respectively, in the mock group (Figure 4B) and for 32.83% ± 4.52%, 18.30% ± 3.64%, and 9.60% ± 0.56% of the corresponding zones in the treated group. There was a 2-fold and 2.5-fold increase in vWF+ structure in the infarct and border zones, respectively, in the treated versus the mock group, indicating an increased density of capillaries in both zones following hMuStem cell administration (p < 0.001). No such differences between groups were observed in the viable zone. These findings indicate that hMuStem cell transplantation induced an angiogenic effect in infarcted hearts by promoting host angiogenesis, not only in the border zone, where vascular structures are typically preserved, but also in the infarct zone, in which blood vessels are typically absent.
Figure 4. Myocardial Transplantation of hMuStem Cells Enhances Angiogenesis

(A) Representative cross-sections of recipient hearts (rats from the mock and treated groups are shown in the left and right columns, respectively) following coimmunolabeling with specific Abs against von Willebrand factor (vWF) and wheat germ agglutinin (WGA). Scale bars, 1 mm and (insets) 150 μm. (B) Quantification of the vWF+ surface in the infarct, border, and viable zones in rats from the mock and treated groups. Statistics: 2-way ANOVA with Sidak post hoc test. n = 5 animals per group; ***p < 0.001; ****p < 0.0001.
Taken together, our results show that hMuStem cell administration induces profound remodeling of the infarcted heart by acting on cardiomyogenic, fibrotic, and angiogenic compartments.

**hMuStem Cells Engraft into Infarcted Tissue**

Post-transplantation survival of stem/progenitor cells is considered a crucial determinant of the long-term efficacy of transplantation protocols. We therefore investigated whether the beneficial effects observed after hMuStem cell administration were associated with colonization of the injured heart by human cells. 3 weeks postimplantation, hMuStem cells populated the infarct of all rats from the treated group (Figure 5A). Foci composed of 15 to over 300 large lamin A/C+ nuclei per section were detected throughout the entire depth of the infarct. Topographic HES staining showed that cells containing lamin A/C+ nuclei displayed either very scant, barely visible cytoplasm or a moderate amount of eosinophilic cytoplasm around the paracentral nucleus. The presence of lamin A/C+ nuclei in the WGA+ area on confocal microscopy showed that most cells were located inside the fibrotic infarct zones (Figure 5A). Few donor cells were observed within the viable host myocardium. Quantitative analysis revealed that 98.5% of donor nuclei were located within the infarct zone, whereas 1.0% and 0.5% were located in the border and viable zones, respectively, suggesting that hMuStem cells concentrated in the acute infarct zone despite their initial injection in the border zone. To confirm further the retention of transplanted hMuStem cells, the LV of 3 rats from the treated group was analyzed to detect human Alu (hAlu) sequences on genomic DNA (gDNA) extraction. At LV of 3 rats from the treated group was analyzed to detect human con

Analysis of cell behavior showed that 1% of donor nuclei expressed proliferating cell nuclear antigen (PCNA), indicating persistence of donor cell proliferation in vivo, 3 weeks after transplantation (Figure 5B). Moreover, less than 1% of cells containing lamin A/C+ nuclei were positive for TUNEL, demonstrating that not quite all implanted donor cells are apoptotic cells and rather correspond to resident cells in the quiescent state. Next, we tracked the fate of hMuStem cells by examining their in vivo differentiation potential (Figures 5C–5F). Fluorescent coimmunolabeling for human lamin A/C and the fast skeletal myosin heavy chain (MHC) isoform, the slow skeletal troponin T1 (TNNT1) isoform, or cardiac troponin I (TNNI) showed that a proportion of the transplanted hMuStem cells contributed to the formation of fast or slow skeletal muscle fibers, as well as cardiac fibers. As observed in HES-stained sections, these human nuclei-containing fibers colonized the connective tissue with occasional clusters of skeletal muscle fibers. By contrast, human TNNT1+ fibers appeared isolated in the fibrotic zone. Fibers positive for skeletal MHC and TNNT1 isoforms accounted for 18% and 19% of fibers, respectively, whereas 5% expressed the TNNI isoform. Even though the Cx43 protein is detected in cultured hMuStem cells, none of the human TNNT1+ fibers expressed it, indicating an absence of electrical cell-to-cell coupling with host CMs (data not shown). Histomorphometry of skeletal and cardiac fibers showed minFeret diameters of 9.0 ± 5.2 µm and 13.53 ± 8.3 µm, respectively, corroborating the aforementioned detection of small fibers in the infarct zone of rats from the treated group. These data show that about 42% of the hMuStem cells that survived after myocardial administration differentiated toward a skeletal myogenic lineage or, to a lesser extent, a cardiac phenotype. Importantly, we observed no hybrid fibers (i.e., those containing both human skeletal and cardiac markers). Lamin A/C+ nuclei were observed sporadically in cells positive for alpha-smooth muscle actin (α-SMA) labeling, although not in all rats, indicating that some hMuStem cells differentiated into myofibroblasts (Figure 5G). Finally, we detected muscle fibers containing several lamin A/C+ nuclei, indicating that hMuStem cells primarily fused with one another when injected into the heart (Figure 5H). Overall, these results show that hMuStem cells have the potential to remain in the infarcted heart with a multifocal presence throughout the depth of the infarct, locating mainly in the acute fibrotic zone, where they contribute to the formation of skeletal and cardiac muscle fibers or constitute resident cells in the connective matrix.

**hMuStem Cells Preserve Cardiac Function in the Infarcted Heart**

4 weeks after infarction, ECG revealed ST segment depression in lead I in rats from both mock and treated groups (Figure 6A). As observed in the sham-operated rats, engraftment of hMuStem cells caused no alterations in heart rate (RR interval) or other ECG parameters (Figure 6A; Tables 2 and 5A) and did not induce spontaneous arrhythmias: 4 weeks postinfarction, only 1 rat in the mock group and 1 rat in the treated group showed ventricular premature beats (16 and 1
premature beats during the ECG recording period, respectively). Our data therefore demonstrate the absence of arrhythmia genesis following injection or implantation of hMuStem cells. Ultrasound studies were performed 1, 3, and 4 weeks post-infarction. As expected, MI induced an increase in LV structural remodeling characterized by an increase in LV chamber dimensions (LVEDD), leading to a decrease in systolic function, as evidenced by EF values (Figure 6B; Tables 2 and S5). Diastolic diameter was increased to 108.93% ± 3.74%, 122.13% ± 5.81%, and 115.20% ± 3.70% of control values, respectively, 1, 3, and 4 weeks post-infarction in rats from the mock group as compared with 102.23% ± 2.39%, 110.29% ± 4.59%, and 105.64% ± 4.01% of control values in rats from the treated group (Figure 6C; Videos S1, S2, S3, and S4). Although not significant, these differences suggest that the presence of hMuStem cells results in greater preservation of LVEDD as compared with the mock group. The lack of change in LV wall thickness at the end of diastole was expected at this stage of postinfarction remodeling (first phase of pathological ventricle dilation). Whereas we observed no changes in diastolic function parameters, such as the E-wave DT, there was a significant improvement in LV performance in the treated versus the mock group. Whereas EF was reduced to 80.56% ± 2.40%, 70.39% ± 4.53%, and 72.78% ± 3.56% of baseline values, respectively, 1, 3, and 4 weeks post-infarction in rats from the mock group, the corresponding values in the treated group were 79.88% ± 3.09%, 90.50% ± 3.79%, and 91.10% ± 2.64%.
in previously published studies, and expected in the clinic. Nonetheless, 9 to 15 tens of thousands of hMuStem cells were detected in each recipient animal, 3 weeks after transplantation, indicating a remarkable retention rate. These data distinguish hMuStem cells from MSCs and genetically engineered myoblasts, which are characterized by poor viability in the harsh environment of the damaged myocardium. This suggests that hMuStem cells are less sensitive to the hypoxia and oxidative stress typical of the infarct site. It should be noted that the sensitivity of these cells to the inflammatory response characteristic of MI could not be evaluated in the present study, given that the animal model used was immunodeficient.

One-half of the hMuStem cells that engrafted into the infarct area generated predominantly skeletal fibers, possibly reflecting their pretransplantation specification. Far fewer frequent fibers displayed a cardiac phenotype. This finding is consistent with the very limited contribution of BM MSCs to cardiac muscle cells following transplantation in a mouse model of acute MI, in which less than 0.1% of grafted cells expressed specific cardiac markers. Although scarce, the presence of muscle fibers expressing the human cardiac marker TNNI indicates an intrinsic potential of hMuStem cells to commit to the cardiomyogenic lineage, in line with previous studies describing the oligopotent nature of these cells. A study in which BM MSCs were stimulated with a cardiopoietic cytokine cocktail before endomyocardial delivery in MI patients reported a 7% increase in LVEF as compared with 0.2% for nonstimulated cells. In line with this observation and given the potential of hMuStem cells to differentiate into cardiac cells, it may be interesting to investigate further the consequences of in vitro preconditioning of these cells. A little over one-half of the donor nuclei were detected in tissue other than skeletal or cardiac fibers or myofibroblasts, with a location in the collagen matrix 3 weeks after transplantation. This indicates that a large proportion of the hMuStem cells that survived in infarcted tissue did not directly differentiate but instead adopted an interstitial location. Interestingly, we previously described this behavior in hMuStem cells injected into cryo-injured skeletal muscle in immunodeficient mice. These observations are also consistent with those of Quevedo et al., who found that 76% of MSCs were located within the interstitial compartment several weeks after transendocardial injection in a pig model of MI. Those MSCs were smaller than CMs and did not show a cardiogenic phenotype, evoking an immature state.

Arrhythmogenesis is a major risk associated with the use of stem cell-based therapies in heart diseases. Several serious ventricular arrhythmic events, including tachycardia, bradycardia, and bigeminy, have been described following administration of murine MDSCs in a mouse model of chronic MI. Similar results were reported after transplantation of human ESC (hESC)-derived CMs into nonhuman primate models of MI, in which significant ventricular arrhythmias were detected. Interestingly, in the present study, the implantation and subsequent persistence of hMuStem cells in the heart were not associated with any arrhythmias or cardiac electrical alterations other than those caused by the infarct. The absence of arrhythmias may be,  

### Table 2. ECG and Echocardiographic Parameters Measured before (Baseline) and 4 Weeks after Induction of Myocardial Infarction (Post-MI) in Rats Injected with Either Vehicle (Mock Group) or hMuStem Cells (Treated Group)

| Group       | Parameters | Baseline | 4 Weeks Post-MI | Baseline | 4 Weeks Post-MI |
|-------------|------------|----------|-----------------|----------|-----------------|
| **Mock Group** |            |          |                 |          |                 |
| Heart rate (bpm) | 428.9 ± 26.1 | 394.4 ± 41.4 | 396.7 ± 35.1 | 371.5 ± 28.7 |
| F wave (ms)    | 18.1 ± 2.1  | 19.4 ± 3.2  | 19.6 ± 2.9  | 20.9 ± 6.6  |
| PR interval (ms) | 44.8 ± 2.1 | 46.8 ± 2.1 | 45.1 ± 4.3 | 47.9 ± 3.4 |
| QRS (ms)       | 19.1 ± 1.1  | 20.5 ± 2.6  | 20.0 ± 1.6 | 20.9 ± 6.6  |
| QT interval (ms) | 76.0 ± 5.1 | 78.3 ± 6.8 | 78.2 ± 5.7 | 78.5 ± 10.1 |

**Echocardiography**

| Group       | Parameters | Baseline | 4 Weeks Post-MI | Baseline | 4 Weeks Post-MI |
|-------------|------------|----------|-----------------|----------|-----------------|
| Heart rate (bpm) | 381 ± 32  | 371 ± 37  | 349 ± 62 | 353 ± 37  |
| LVEDWT (mm)   | 1.62 ± 0.22 | 1.63 ± 0.22 | 1.49 ± 0.37 | 1.64 ± 0.29 |
| LVEDD (mm)    | 5.76 ± 0.24 | 6.62 ± 0.58 | 6.13 ± 0.45 | 6.51 ± 0.45 |
| Deceleration time (ms) | 39.7 ± 7.2 | 42.4 ± 7.03 | 37.4 ± 9.09 | 42 ± 4.78 |
| E/A ratio     | 1.38 ± 0.23 | 1.25 ± 0.25 | 1.55 ± 0.31 | 1.60 ± 0.14 |
| IVRT (ms)     | 24.5 ± 2.01 | 26.1 ± 1.85 | 25.8 ± 3.85 | 25 ± 2.31 |
| Ejection fraction (%) | 87.8 ± 6.16 | 64.3 ± 13.06 | 86.4 ± 6.65 | 79.9 ± 6.81 |

Data are expressed as the mean ± SD. N = 10 animals per group.

**DISCUSSION**

In this study, we examined the intramyocardial transplantation of human skeletal muscle-derived MuStem cells in a rat model of MI in order to assess the feasibility of this approach as a potential advanced therapy medicinal product. Previous preclinical studies have established the ability of MuStem cells to survive in severely damaged tissue, such as dystrophic or cryo-injured skeletal muscle. Moreover, we have previously demonstrated that MuStem cells can generate significant and persistent clinical and tissue benefits in the GRMD dog model of Duchenne muscular dystrophy. In the present study, we provide original data demonstrating the reparative potential of this adult stem cell population in the context of MI, highlighting its potential for use in regenerative medicine for muscle diseases. We successfully demonstrate that hMuStem cell administration in the border zone of the infarct area is associated with the persistence of this cell population within the injured heart, attenuation of adverse LV tissue remodeling, and an improvement in cardiac function during the next 3 weeks. Angiogenesis and neo-myo genesis were the main factors that contributed to preservation of the architecture of the LV myocardium.

The number of hMuStem cells injected into sites surrounding the infarct area was less than 3 million, a lower number than that injected...
at least partially, explained by the small number of newly formed CMs. Another potential explanation is that donor cell-derived CMs were implanted into the conjunctive matrix, preventing close contact with host CMs and consequent alterations in their electrical properties.

The infarct zone in hMuStem cell-treated rats was characterized by the presence of many human fast and slow skeletal fibers, as well as human TNNI1 fibers, which limited the formation of fibrotic scar tissue. It is tempting to speculate that the formation of these new muscle fibers, although mainly of skeletal origin, may have a more beneficial effect on LV remodeling than fibrous scar tissue, allowing mechanical stabilization of the weakened myocardium. In keeping with this idea, the use of acellular epicardial patches has shown therapeutic efficacy, increasing the mechanical integrity of damaged rat LV tissues.66,67 A key finding of the present study is the preservation of LVEF, 3 weeks after hMuStem cell transplantation into the border region of the infarct. The presence of differentiated cells could promote healing of the infarcted region by maintaining greater elasticity of the ventricular wall, thereby facilitating long-term preservation of function, which in turn, may be associated with a reduction in the LV dilation characteristic of scar formation in MI. Consistent with these findings, the functional benefit of MSC therapy appeared to be much greater than that expected based on the observed rate of engraftment.68 Given the limited number of human TNNI1 fibers detected in the host myocardium, this outcome cannot be attributed to the acquisition of a cardiac phenotype by hMuStem cells. Our findings are in agreement with those of Oshima et al.,24 who also reported a positive functional effect in the mouse model of MI, despite a low incidence of cardiac phenotype acquisition.

Intramyocardial delivery of hMuStem cells promoted marked angiogenesis by increasing capillary density in both central and border zones of the infarct, likely directly contributing to structural and functional recovery. These observations are in agreement with the increased microvascular density observed within the infarct and peri-infarct area after human pericyte transplantation in a mouse model of acute MI.18 Interestingly, microRNA-138 (miR-138) increases angiogenesis associated with infarct repair following adventitial progenitor cell transplantation.20 Moreover, MSC-derived exosomal miR-21 has been shown to increase the expression of angiogenic proteins in a rat model of MI,69 whereas delivery of adipose MSC-derived exosomes to the peri-infarct area promotes angiogenesis in a mouse model of ischemic heart disease.70 Further studies of the microRNA (miRNA) secreted by hMuStem cells will be required to better understand the underlying mechanism of action.

One approach to treat MI is to open the artery as soon as possible after the onset of symptoms. The experimental model that best replicates this approach is the ischemia-reperfusion model. However, in this scenario, angioplasty becomes impossible if the intervention is not sufficiently prompt or if coronary obstruction prevents the passage of a catheter. These clinical cases may require cell injection as soon as possible after MI to promote regeneration or at least preserve function in the injured areas of cardiac muscle. Our permanent ligation model with a short delay of 4 weeks post-MI therefore has clinical relevance. However, despite routine use of this protocol in the laboratory, the LVEF decrease has never exceeded a mean of 20%, 1 week after LAD ligation. These values are likely related to the attenuated inflammatory response in this rat model. Given that cardiac muscle and the associated vasculature receive considerable damage in MI, the LV remodeling observed in our model is particularly relevant, confirming the anti-apoptotic activity of hMuStem cells on host CMs and a proangiogenic effect. Moreover, this global beneficial impact on tissue organization was observed after administration of a very small number of hMuStem cells, suggesting that an even greater effect could be obtained by injecting a larger number of cells or by administering a concomitant injection of hMuStem cell secretome. Over the past decade, it has become increasingly apparent that paracrine factors associated and delivered by several types of stem cells are responsible for most of the beneficial effects observed after cell transplantation rather than the cell engraftment and the direct tissue repair.62–65 Thus, it may be informative to determine the tissue and functional impacts generated by the cell-free supernatant in the case of the hMuStem cells to clarify to what extent it recapitulates the therapeutic effect of the parent cells. The use of immunodeficient rats is one aspect of the present study that limits our understanding of the behavior and actions of hMuStem cells in damaged heart tissue in the context of MI. Indeed, due to the immunodeficient nature of these rats, we could not investigate the immunological component that is yet a key determinant of the post-transplantation survival, recruitment, and function of donor cells with notably a major role of inflammatory cells.66–68 Moreover, this immunological response had important consequences for cardiac remodeling and in particular, the critical supportive role of monocytes and/or macrophages in cardiogenesis and tissue repair.69–72 Another study limitation that should be borne in mind is that we were unable to evaluate the clinical relevance of the beneficial effect of hMuStem cell transplantation on long-term LV remodeling and cardiac function, since our observations were made only 4 weeks after MI induction. Further, more long-term studies will be required to evaluate post-transplantation responses after the initial compensatory phase.

MATERIALS AND METHODS

Human Skeletal Muscle Tissue

Four Paravertebral muscle biopsies were collected from patients aged 12–19 years. Patients were free of known muscle disease and underwent surgery for acute scoliosis at the Department of Pediatric
Surgery of the Centre Hospitalier Universitaire (CHU) de Nantes (France). Written, informed consent was obtained from all patients. All protocols were approved by the Clinical Research Department of the CHU (Nantes, France), according to the rules of the French Regulatory Health Authorities (approval number: MESR/DC-2010-1199). The biological sample bank was created in compliance with national guidelines regarding the use of human tissue for research (approval number: CPP/29/10).

**Animals**

Immunodecient recombination activating gene 1 (Rag1) and interleukin-2 receptor γ (Ilig2) knockout (KO) Sprague-Dawley rats (RRG rats)\(^{42}\) were obtained from the platform transgenic rats immunophenomic (TRIP; Nantes, France) and housed in a controlled environment (temperature 21 ± 1°C, 12 h light/dark cycle) in the specific pathogen-free animal facilities at the l’Institut de Recherche en Santé de l’Université de Nantes (IRS-UN; Nantes, France). All efforts were made to minimize suffering. All animals were provided with environmental enrichment: provision of rolls is reported to potentially reduce procedural dysfunction. Two groups each consisted of 10 rats in which infarction was induced by coronary ligation (see Coronary Artery Ligation below for description of the procedure) and treated without coronary ligature. Rats from this group were all injected respectively (see Table S3). The last group (sham group) consisted of 9 rats provided using the same animals. Animals of the different groups were progressively included in order to be able to anticipate any procedural dysfunction. Two groups each consisted of 10 rats in which infarction was induced by coronary ligation (see Coronary Artery Ligation below for description of the procedure) and treated with vehicle (mock group) or hMuStem cells (treated group), respectively (see Table S3). The last group (sham group) consisted of 9 rats undergoing the same surgical procedure of the previous groups but without coronary ligature. Rats from this group were all injected with hMuStem cells. All experimental procedures involving animals were carried out in strict accordance with European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and were authorized by the French Ministry of Higher Education and Research after approval by the Ethics Committee on Animal Experimentation from the Pays de la Loire region (approval number: APAFIS 9573-2017041311597719).

**Isolation and Culture of hMuStem Cells**

hMuStem cells were independently isolated from skeletal muscle from 4 patients free of known muscle disease, as previously described.\(^{33}\) Cells were expanded under standard conditions (37°C in a humidified atmosphere containing 5% CO\(_2\)) in growth medium (GM; Macopharma, Mouvax, France), supplemented with 10% human serum (EFS, Nantes, France), 1% 10,000 IU/mL penicillin, 10 mg/mL streptomycin, 25 μg/mL fungizone (PSF; Sigma-Aldrich, Saint-Quentin-Fallavier, France), 10 ng/mL human recombinant basic fibroblast growth factor (bFGF; Miltenyi, Bergisch Gladbach, Germany), and 25 ng/mL human recombinant epidermal growth factor (EGF; Miltenyi). Cells were seeded on CELLstart substrate-coated plastic flasks (Invitrogen, Cergy-Pontoise, France) at 1.0–1.5 × 10\(^4\) cells/cm\(^2\), and GM was replaced every 4 days.

**Flow Cytometry**

Cultured hMuStem cell samples (n = 4; P5 corresponding to 10.4–12.7 population doublings) were resuspended in cold phosphate-buffered saline (PBS)/2%/human serum, and 1.0 × 10\(^5\) cells were incubated (30 min, 4°C in darkness) with fluorochrome-conjugated Abs against the appropriate cell-surface marker at a saturating concentration (Table S6). Isotype-matched Abs and fluorescence minus control samples were used as negative controls for gating and analyses. Where applicable, 7-amino-actinomycin D (7-AAD; BD Biosciences, Franklin Lakes, NJ, USA) was added to evaluate cell viability. Samples were acquired using a FACS®Aria flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (FlowJo, Ashland, OR, USA). For each labeling experiment, at least 15 × 10\(^3\) viable cells were considered.

**Reverse Transcription PCR**

Total RNA was extracted from dry pellets of 10\(^5\) cells using the RNAeasy mini kit following the manufacturer’s instructions (QIAGEN, Santa Clara, CA, USA). After DNase treatment (Ambion, Austin, TX, USA), RNA was quantified using a NanoDrop spectrophotometer (Labtech, Wilmington, DE, USA) and processed for reverse transcriptase, as previously described.\(^{32}\) Quantitative real-time PCR reactions were performed with specific TaqMan primers and TaqMan universal PCR Master Mix (Life Technologies, Carlsbad, CA, USA) on the CFX96 PCR System (Bio-Rad, Marnes-la-Coquette, France). Data were normalized using HPR1T as an internal control, and differential expression was calculated using the ΔCt method. The oligonucleotide primers used are listed in Table S7.

**Western Blotting**

Proteins were extracted from 1 × 10\(^6\) cell pellets generated from the primary culture of hMuStem cells, myoblasts and glioblastoma cells that were lysed in radioimmunoprecipitation assay (RIPA) buffer (30 min, 4°C). Then, samples were centrifuged at 10,000 × g (10 min, 4°C), and protein concentration on supernatants was determined using a bicinchoninic acid (BCA) protein assay (Sigma-Aldrich, Saint-Quentin-Fallavier, France). 50 μg of proteins was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% polyacrylamide gels (Invitrogen, Carlsbad, CA, USA) and electroblotted onto nitrocellulose membranes (Protran BA 83; GE Healthcare, Chicago, IL, USA) using a Bio-Rad liquid blotting system at 300 mA for 2 h. The membranes were blocked using 50% blocking buffer (Odyssey; LI-COR Biosciences, Lincoln, NE, USA) in PBS (1 h, room temperature) and incubated (2 h, room temperature) with primary Abs against Cx43 (1:5,000; C6219; Sigma-Aldrich) and loading control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:500; sc-25778; Santa Cruz Biotechnology, Dallas, TX, USA) at 4°C overnight. Membranes were washed (5×, 10 min) with Tris-buffered saline (TBS)/0.1% Tween-20 (TBS-T) and incubated (1 h, room temperature) with the appropriate secondary antibodies labeled with fluorochromes (1:5,000; Odyssey; LI-COR Biosciences). Protein bands were visualized using Odyssey infrared imaging (LI-COR Biosciences).
3-phosphate dehydrogenase (GAPDH; 1:1,000; Sc25778; Santa Cruz, Dallas, TX, USA). After washing with Tween 0.1% in PBS, the blots were incubated with Alexa Fluor780-conjugated anti-rabbit secondary Ab (1:10,000; A11369; Invitrogen). Equal protein loading was checked through GAPDH labeling and Ponceau S staining of the membranes. The western blot bands were scanned and analyzed with Odyssey.

**Coronary Artery Ligation**

MI was induced by ligation of the LAD coronary artery. Briefly, female RRG rats were anesthetized with a mix of inhaled 3% isoflurane/97% oxygen and subcutaneously injected with 0.1 mg/kg buprenorphine (Buprecare), after which, the chest was shaved. One-half of the buprenorphine dose was injected 15 min before beginning surgery and the remainder when the animal awoke. A subcutaneous injection of 1% lidocaine (Xylocaïne) was administered at each cutaneous incision point. Postoperative analgesia (0.1 mg/mL buprenorphine, subcutaneously [s.c.]) was maintained for 3–6 days as needed. Animals were then intubated and ventilated using a Harvard Rodent Ventilator (Harvard Apparatus, Les Ulis, France). The tidal volume was 2.5 mL, and the respiratory rate was 63 breaths/min. Animals were placed in a supine position on a heating pad. A left lateral thoracotomy at the fourth intercostal space exposed the anterior surface of the heart, after which, the location of the LAD coronary artery on the surface of the LV anterior wall was identified. The proximal LAD coronary artery was ligated, and occlusion was confirmed by observation of a color change and akinesia of the affected area of LV wall. The lungs were expanded before the chest was closed to avoid pneumothorax. To locate and quantify infarction, the wall motion score index was calculated based on echocardiography measurements taken 7 days after MI. M-mode acquisitions were performed in parasternal incidence (short axis) at the base, in the middle, and at the apex of the LV. Systolic function was further assessed by calculation of LVEF using averaged measurements from 3 to 5 consecutive cardiac cycles, in accordance with the guidelines of the American Society of Echocardiography. LV end-diastolic and end-systolic volumes (LVEDV and LVESV, respectively) were calculated from bi-dimensional short-axis parasternal views, taken through the infarcted area by means of the single-plane area-length method. These indexes were calculated in accordance with the standard, widely accepted formulas.73 Transmirtal flow measurements of ventricle filling velocity were obtained with pulsed Doppler, using an apical 4-chamber orientation. Doppler-derived mitral DT, isovolumic relaxation time, E wave, A wave, and the E/A ratio were determined to assess diastolic dysfunction. To avoid bias, experiments were performed and the data analyzed by investigators blind to treatment. Echocardiography measurements were performed 1 day before induction of MI (baseline) to rule out any initial functional differences, after which, the MI was induced. 3 and 4 weeks after MI induction, ultrasound was also used to assess the consequences of hMuStem cell delivery.

**Echocardiography**

Rats underwent 2-dimensional (2D) echocardiography using a Vivid 7 Dimension ultrasound system (GE Healthcare, Chicago, IL, USA). Body temperature was maintained at 37°C with a heating pad (Harvard Apparatus), and ECG was monitored during measurements. Anesthesia was induced using 4% isoflurane (Abbott Laboratories, Chicago, IL, USA) and maintained with 2.5% isoflurane during recording. Caution was taken not to apply excessive pressure to the chest, which could cause bradycardia and deformation of the heart. To detect possible structural remodeling, LV diameter and free-wall thickness were measured from short- and long-axis images obtained by M-mode echocardiography. Systolic function was further assessed by calculation of LVEF using averaged measurements from 3 to 5 consecutive cardiac cycles, in accordance with the guidelines of the American Society of Echocardiography. LV end-diastolic and end-systolic volumes (LVEDV and LVESV, respectively) were calculated from bi-dimensional short-axis parasternal views, taken through the infarcted area by means of the single-plane area-length method. These indexes were calculated in accordance with the standard, widely accepted formulas.73 Transmirtal flow measurements of ventricle filling velocity were obtained with pulsed Doppler, using an apical 4-chamber orientation. Doppler-derived mitral DT, isovolumic relaxation time, E wave, A wave, and the E/A ratio were determined to assess diastolic dysfunction. To avoid bias, experiments were performed and the data analyzed by investigators blind to treatment. Echocardiography measurements were performed 1 day before induction of MI (baseline) to rule out any initial functional differences, after which, the MI was induced. 3 and 4 weeks after MI induction, ultrasound was also used to assess the consequences of hMuStem cell delivery.

**ECG**

For ECG, rats were anesthetized with isoflurane, as described above. Body temperature was maintained at 37°C with isoflurane during measurement (Harvard Apparatus). With the use of a computer attached to an analog-digital converter IOX 1.585, 6-lead ECG recordings were taken using 25-gauge subcutaneous electrodes (EMKA Technologies, Paris, France) and later analyzed with ECG Auto v3.2.0.2 (EMKA Technologies). ECG parameters were measured on lead II. As described above for echocardiography, ECG measurements were taken 1 day before induction of MI (baseline) to rule out any initial functional differences and again 4 weeks after the induction of MI to assess the consequences of hMuStem cell delivery.

**Histopathological Evaluation**

4 weeks after MI induction, rats were sacrificed by intravenous administration of sodium pentobarbital (300 mg Dolethal). After gross examination, the heart, lungs, liver, spleen, kidney, brain, and biceps femoris muscle were sampled to examine the fate of the
hMuStem cells in the infarcted heart. The heart was weighed and the tibial bone measured. Next, the heart was divided into 7–8 sections using a rat heart slicer matrix (Zivic Instruments, Pittsburgh, PA, USA). The odd sections were frozen, and 10 μm-thick serial sections were cut for immunolabeling and histo-enzymological assays. The even sections were fixed in 10% neutral-buffered formalin and embedded in paraffin wax and 5 μm-thick sections cut and routinely stained with HES for histopathological evaluation. Additional sections were stained with Picrosirius red stain for collagen. A skilled pathologist certified by the European College of Veterinary Pathology recorded all lesions.

**Immunohistochemistry**
The frozen sections were first permeabilized (30 min, room temperature) using 0.1% Triton X-100 (Sigma-Aldrich), incubated (30 min, room temperature) in blocking buffer (10% goat serum in 0.1 M PBS; Sigma-Aldrich), and incubated overnight at 4°C with the following primary Abs: mouse monoclonal IgG2ak anti-PCNA (1:1,000, M0879; Dako, Glostrup, Denmark); Alexa Red 555-conjugated WGA (1:500, W32464; Invitrogen); mouse monoclonal IgG2b anti-lamin A/C (1:100, MA3-1000; Invitrogen); mouse monoclonal IgG1 anti-skeletal fast myosin (1:400, M4276; Sigma-Aldrich); rabbit anti-lamin A/C (1:100, MA3-1000; Invitrogen); mouse monoclonal IgG2a anti-cardiac troponin T (1:500, HPA058448; Ozyme SAS, Saint-Quentin-en-Yvelines, France); rabbit monoclonal IgG2b anti-cardiac troponin T (1:250, Ab52852; Abcam, Cambridge, UK); and rabbit polyclonal anti-α-smooth muscle actin (A5280; Sigma-Aldrich). Biotinylated secondary Abs (1:300, E433; E432; Dako) in 1 h at room temperature, after which, the nuclei were counterstained with Alexa Red 555 or Green 488 secondary Abs (1:300, A11008; Invitrogen). Next, the appropriate primary Abs were visualized using a rat heart slicer matrix (Zivic Instruments, Pittsburgh, PA, USA) and syn-thesized by MWG Operon (Eurofins, Ebersberg, Germany). Following the manufacturer’s instructions, PCR reactions were carried out on 0.5 μg of input gDNA. hAlu-specific oligonucleotide primers were designed using Oligo Primer Analysis Software, v.7 (Molecular Biology Insights, Colorado Springs, CO, USA) and synthesized by MWG Operon (Eurofins, Ebersberg, Germany): forward primer, 5′-CATGGTGAACCCCGTCTCTA-3′; reverse primer, 5′-GCCTCAGGCCGAGTGG-3′; probe, 5′-ATTAGCGGG CGTGGTGGCG-3′. Primers targeting rat GAPDH were also prepared: forward primer, 5′-GACATCATTCCGTCCATCA-3′; reverse primer, 5′-GCCTCAGCGCCTCCGAGTGG-3′; probe, 5′-ATTAGCGGG CGTGGTGGCG-3′. PCR amplifications were performed using the following program: initial denaturation (10 min, 95°C), followed by 40 cycles (15 s, 95°C; 1 min, 60°C; and 1 min, 72°C). With the generation of a scatterplot of the standard concentrations versus the (Ct) values and the determination of the equation of the best-fit line, the number of human cell equivalents in each qPCR well was calculated.

**Statistical Analysis**
Data were reported as the mean ± standard deviation (SD). Statistical analysis was performed in GraphPad Prism software, v6.0f (GraphPad Software, La Jolla, CA, USA) using the Mann-Whitney test and 1- or 2-way ANOVA, followed by the Sidak multiple comparison post hoc test. Differences were considered significant at p < 0.05.
SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtm.2020.06.009.

AUTHOR CONTRIBUTIONS

A.R. performed the in vivo protocol; collected, assembled, and interpreted all results; and participated in the preparation of the manuscript. G.T. performed the in vivo protocol and the echocardiography analyses, collected and interpreted the results, and participated in the writing of the manuscript. T.L. sacrificed the animals, participated in the tissue-sampling process, and provided histopathology/histomorphology expertise. I.L. isolated the hMuStem cell batches, prepared the cell suspensions for transplantation, and performed some of the immunohistochemistry experiments. M.L. participated in the tissue sampling, generated the histology slides, and performed the immunohistochemistry experiments. A.H. participated in the in vivo experiments, recorded and analyzed the ECGs, and took care of the animals. C.B. performed the molecular biology experiments. R.F. provided expertise in confocal microscopy and participated in the histomorphometry analyses. L.D. provided bioimaging expertise. S.M. and I.A. generated the immunodeficient rat model and provided the immunodeficient rats required for the study. F.C., K.R., and L.G. designed the study, wrote the manuscript, and coordinated the research and strategy. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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