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Transplantation of Human Embryonic Stem Cell–Derived Cardiovascular Progenitors for Severe Ischemic Left Ventricular Dysfunction

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ABSTRACT

BACKGROUND In addition to scalability, human embryonic stem cells (hESCs) have the unique advantage of allowing their directed differentiation toward lineage-specific cells.

OBJECTIVES This study tested the feasibility of leveraging the properties of hESCs to generate clinical-grade cardiovascular progenitor cells and assessed their safety in patients with severe ischemic left ventricular dysfunction.

METHODS Six patients (median age 66.5 years [interquartile range (IQR): 60.5 to 74.7 years]; median left ventricular ejection fraction 26% [IQR: 22% to 32%]) received a median dose of 8.2 million (IQR: 5 to 10 million) hESC-derived cardiovascular progenitors embedded in a fibrin patch that was epicardially delivered during a coronary artery bypass procedure. The primary endpoint was safety at 1 year and focused on: 1) cardiac or off-target tumor, assessed by imaging (computed tomography and fluorine-18 fluorodeoxyglucose positron emission tomography scans); 2) arrhythmias, detected by serial interrogations of the cardioverter-defibrillators implanted in all patients; and 3) alloimmunization, assessed by the presence of donor-specific antibodies. Patients were followed up for a median of 18 months.

RESULTS The protocol generated a highly purified (median 97.5% [IQR: 95.5% to 98.7%]) population of cardiovascular progenitors. One patient died early post-operatively from treatment-unrelated comorbidities. All others had uneventful recoveries. No tumor was detected during follow-up, and none of the patients presented with arrhythmias. Three patients developed clinically silent alloimmunization. All patients were symptomatically improved with an increased systolic motion of the cell-treated segments. One patient died of heart failure after 22 months.

CONCLUSIONS This trial demonstrates the technical feasibility of producing clinical-grade hESC-derived cardiovascular progenitors and supports their short- and medium-term safety, thereby setting the grounds for adequately powered efficacy studies. (Transplantation of Human Embryonic Stem Cell-derived Progenitors in Severe Heart Failure [ESCORT]; NCT02057900) (J Am Coll Cardiol 2018;71:429–38) © 2018 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

CT = computed tomography

DSA = donor-specific antibody

EF = ejection fraction

ESC = embryonic stem cell

hESC = human embryonic stem cell

HLA = human leukocyte antigen

ICD = implantable cardioverter-defibrillator

LV = left ventricular

LVEF = left ventricular ejection fraction

PET = positron emission tomography

SSEA-1 = stage-specific embryonic antigen-1

The current treatment of heart failure rests on 2 major pillars: drugs; and interventional, surgical, or catheter-based procedures. These therapies are either palliative or, at the other extreme, radical (cardiac replacement). In the past 2 decades, an intermediate strategy has emerged that aims at repairing the diseased heart by transplanting cells.

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The phenotype of the "ideal" cells is still unsettled. However, there has been a recent shift toward cells committed to a cardiac lineage. In this setting, clinical trials have tested right atrial c-*Kit* cardiac stem cells (1) and right ventricle–derived cardiospheres (2) that have a derivation from adult tissues in common. We adopted a different strategy consisting of starting "upstream" from human embryonic stem cells (hESCs) and leveraging their intrinsic pluripotentiality to drive their fate toward a cardiovascular lineage. Several experimental studies have validated the concept that ESC-derived cardiomyocytes improve the function of infarcted hearts (3). Taking advantage of the knowledge that ESCs can be used at a defined stage of differentiation, we selectively generated hESC-derived cardiovascular progenitor cells, primarily defined by the coexpression of the stage-specific embryonic antigen-1 (SSEA-1) and cardiac transcription factor *Isl*-1 markers, whereas the recognition that biomaterials can efficiently promote cell engraftment (4) led us to incorporate these progenitors in a fibrin scaffold that was delivered onto the epicardium of the infarct area. The safety and efficacy outcomes of this approach, supported by almost a decade of preclinical studies in small and large animal models, have been sufficient (5) to allow regulatory approval for this safety trial (6).

METHODS

PATIENTS AND PROCEDURES. Six patients were selected on the basis of 3 major inclusion criteria: 1) left ventricular (LV) systolic dysfunction reflected by an ejection fraction (EF) ≥15% and ≤35%, as assessed by echocardiography; 2) a history of myocardial infarction that had occurred at least 6 months before screening; and 3) an indication for surgical coronary revascularization. The other inclusion and exclusion criteria are reported in the Online Appendix. The protocol was approved by the Ethics Committee of Paris Descartes University (2010-A00794-35), and written informed consent was obtained from all patients.

The technique of cellularized patch preparation (7) is detailed in the Online Appendix. Briefly, expanded pluripotent hESCs were committed to the cardiovascular lineage by a 4-day exposure to 2 cytokines, immunomagnetically sorted for a positive expression of SSEA-1 (a marker for loss of pluripotency) and finally mixed with fibrinogen and thrombin to form a gel (**Figure 1A**), which could be easily manipulated.

All surgical procedures were performed with the patient under normothermic blood antegrade or retrograde cardioplegic arrest, except for 1 patient, whose left anterior descending coronary artery was bypassed off-pump on the beating heart. On completion of the distal coronary artery anastomoses, a piece of autologous pericardium, matching approximately that of the fibrin patch (20 cm^2), was sutured around one-half the circumference of the infarct area, thereby creating a "pocket" into which the fibrin patch was slid (**Figure 1B**). The pericardial flap was then folded over and finally stitched to the remaining one-half of the infarct circumference (**Figure 1C**). The transplanted segments were marked on a 17-segment map matching the echocardiographic division of the left ventricle. Corticosteroids (methylprednisolone) were given intraoperatively at a total dose of 240 mg, split into 2 equal injections, and an

Paris, France; [k]Sorbonne Universités, Université Pierre et Marie Curie, University Paris-6, Institut de Biologie Paris-Seine, UMR CNRS 8256, Biological Adaptation and Ageing, Paris, France; [l]Assistance Publique-Hôpitaux de Paris, University Paris Sud, Histology-Embryology-Cytogenetics, Hôpitaux Universitaires Paris Sud, Clamart, France; [m]School of Pharmacy, University Paris Descartes, Paris, France; [n]Central Pharmacy, Pharmaceutical Innovation Department, Assistance Publique-Hôpitaux de Paris, Paris, France; [o]Central Pharmacy, Clinical Trials Department, Assistance Publique-Hôpitaux de Paris, Paris, France; [p]Human Leukocyte Antigen and Médecine, Hôpital Saint-Louis, INSERM U976, Paris, France; and the [q]University Paris Diderot, Sorbonne Paris Cité, Paris, France. This study was primarily funded by public grants from the French Ministry of Health (Programme Hospitalier de Recherche Clinique PCR11001, P100303) and Assistance Publique-Hôpitaux de Paris, which approved the protocol but did not participate in data collection, analysis and interpretation, and writing of the report. Additional funding was provided by charity grants from the Association Française contre les Myopathies and the Fondation Coeur et Artères, but neither of these bodies was involved in data collection or interpretation. The trial was sponsored by Assistance Publique-Hôpitaux de Paris (Direction de la Recherche Clinique et du Développement). All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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