Randomized, double-blind pilot study of transendocardial injection of autologous aldehyde dehydrogenase–bright stem cells in patients with ischemic heart failure

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Background The optimal type of stem cell for use in patients with ischemic heart disease has not been determined. A primitive population of bone marrow–derived hematopoietic cells has been isolated by the presence of the enzyme aldehyde dehydrogenase and comprises a multilineage mix of stem and progenitor cells. Aldehyde dehydrogenase–bright ($ALDH^{br}$) cells have shown promise in promoting angiogenesis and providing perfusion benefits in preclinical ischemia studies. We hypothesize that $ALDH^{br}$ cells may be beneficial in treating ischemic heart disease and thus conducted the first randomized, controlled, double-blind study to assess the safety of the transendocardial injection of autologous $ALDH^{br}$ cells isolated from the bone marrow in patients with advanced ischemic heart failure.

Methods Aldehyde dehydrogenase–bright cells were isolated from patients' bone marrow on the basis of the expression of a functional (aldehyde dehydrogenase) marker. We enrolled 20 patients (treatment, n = 10; control, n = 10). Safety (primary end point) and efficacy (secondary end point) were assessed at 6 months.

Results No major adverse cardiovascular or cerebrovascular events occurred in $ALDH^{br}$-treated patients in the periprocedural period (up to 1 month); electromechanical mapping–related ventricular tachycardia (n = 2) and fibrillation (n = 1) occurred in control patients. Aldehyde dehydrogenase–bright–treated patients showed a significant decrease in left ventricular end-systolic volume at 6 months (P = .04) and a trend toward improved maximal oxygen consumption. The single photon emission computed tomography delta analysis showed a trend toward significant improvement in reversibility in cell-treated patients (P = .053).

Conclusions We provide preliminary evidence that treatment with the novel cell population, $ALDH^{br}$ cells, is safe and may provide perfusion and functional benefits in patients with chronic myocardial ischemia. (Am Heart J 2012;163:415-421.e1.)

Ischemic heart disease remains a major cause of death and disability in the Western world despite the availability of revascularization and medical treatment strategies. Cell therapy has emerged as an innovative approach for treating advanced ischemic heart disease. The use of autologous bone marrow mononuclear cells has improved myocardial ischemia in patients with ischemic heart failure who are not eligible for revascularization procedures.[1-13] Although benefits have been seen in clinical trials of unselected autologous bone marrow mononuclear cells, the optimal type of stem cell for treating ischemic heart disease has not been determined.

In general, stem cells used in clinical trials have been isolated based on cell phenotype, such as the expression of cell surface markers. However, cell phenotype can be altered depending on the developmental stage or the cell cycle or by culturing cells ex vivo. Thus, the isolation and purification of cells based on stem/progenitor cell function may provide a more reliable way to identify the most effective cell population for angiogenesis.

A very primitive population of hematopoietic cells (Lin^{-} $CD^{34+}CD38^{-}$) has been isolated, originally from

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human cord blood, using the presence of the cytosolic enzyme aldehyde dehydrogenase (ALDH), which is considered a marker for stem and progenitor cells.[14] These cells, referred to as ALDH-bright ($ALDH^{br}$) cells, have also been isolated from human bone marrow and peripheral blood. The population of $ALDH^{br}$ cells comprises cells expressing CD34, CD117, CD105, CD133, and CD166 antigens and includes primitive $CD34^{+}CD38^{-}$ cells.[15] Thus, this suggests that these cells could have angiogenic properties. In characterizing $ALDH^{br}$ cells from human bone marrow, Gentry et al[16] reported that $ALDH^{br}$ populations showed more hematopoietic colony-forming activity and generated more endothelial colonies than did bone marrow mononuclear populations depleted of $ALDH^{br}$ cells ($ALDH^{dim}$). Furthermore, in a preclinical study in immunodeficient mice, the intravenous administration of human bone marrow–derived $ALDH^{br}$ cells was more effective at promoting angiogenesis and restoring blood flow to ischemic hindlimbs than was unfractionated bone marrow mononuclear cells or $ALDH^{dim}$ cells.[17]

We hypothesize that $ALDH^{br}$ cells may be beneficial in the treatment of ischemic heart disease. Therefore, we conducted the first randomized, controlled, double-blind pilot study to assess the safety of the transendocardial injection of autologous $ALDH^{br}$ cells isolated from the bone marrow (ALD-201 cells; Aldagen, Inc, Durham, NC) in patients with advanced ischemic heart failure. This study is the first step in determining the usefulness of $ALDH^{br}$ cells in treating patients with chronic ischemic heart disease not amenable to revascularization therapies.

Methods

Study design

This phase 1 randomized, double-blind, placebo-controlled clinical trial was designed to enroll 20 patients with advanced ischemic heart failure and no other option for revascularization. Patients who met the inclusion and exclusion criteria were randomized in a 1:1 fashion (treatment:placebo) via a computer-generated randomization sequence to receive transendocardial injections of $ALDH^{br}$ cells (n = 10) or placebo (n = 10). The study was approved by the institutional review board and as an Investigational New Drug Submission by the Food and Drug Administration (www.clinicaltrials.gov trial no. NCT00314366).

After randomization, baseline assessment of both treatment and control groups was performed by measuring maximal oxygen consumption (MVO_2) with the use of ergospirometry (ramp treadmill protocol) and by performing 2-dimensional Doppler echocardiography and adenosine single photon emission computed tomography (SPECT) perfusion scanning. Two blinded, independent echocardiologists reviewed the echocardiograms, and the average of the 2 readings was reported. Clinical status was assessed by New York Heart Association (NYHA) class and Canadian Cardiovascular Society (CCS) angina score. Before the injection procedure, patients underwent left ventricular angiography and electromechanical mapping (EMM) as previously described.[10,11] Follow-up examination at 6 months comprised assessment of clinical status by CCS and NYHA class, 24-hour Holter monitoring (also at 1 and 4 weeks), SPECT analysis (see online Appendix), 2-dimensional echocardiography (see online supplement), and MVO_2 testing. The perfusion defect was assessed by using a standard, widely accepted protocol.[18] Specifically, perfusion defect severity (stress total severity score, rest total severity score, and reversible total severity score) assessment was provided by an independent core laboratory with the use of Emory Tool Box software (MEDX, Arlington Heights, IL). The total severity score was defined as the sum of blackout pixels in the blackout polar map, each weighted by the number of SDs below the mean. At the 6-month follow-up examination, patients also underwent left ventricular angiography.

End points

The primary end point was safety as assessed by the occurrence of adverse events (major adverse cardiovascular and cerebrovascular events [MACCEs] and hospitalizations). Early safety assessments included the periprocedural period up to 2 weeks, and then safety was assessed at 6 months. Efficacy was the secondary end point. We evaluated efficacy by clinical status, left ventricular ejection fraction (LVEF), perfusion on SPECT imaging, and MVO_2.

Patient population

Inclusion criteria. To be included in the study, patients had to meet the following conditions: CCS class II to IV angina or NYHA class II or III heart failure (able to walk on a treadmill) on maximum tolerable medical therapy, ejection fraction ≤45% by echocardiography, the presence of a reversible perfusion defect on SPECT, coronary artery disease ineligible for percutaneous or surgical revascularization in the target area as assessed by an interventional cardiologist and a cardiovascular surgeon not involved in this study, and maximal medical therapy. All patients had to provide signed, informed consent.

Exclusion criteria. Patients were excluded from the study if they were <18 years or >70 years. Other key exclusion criteria were atrial fibrillation, interventricular thrombus as shown by echocardiography, anatomy that precluded obtaining vascular access for percutaneous procedures, severe valvular disease, left ventricular aneurysm (wall thickness <8 mm at the target site), international normalized ratio >2 in the absence of warfarin therapy, a history of malignancy within 5 years or other comorbidities that could affect short-term survival, acute myocardial infarction within the past month or a high risk of acute coronary syndrome, significant ventricular dysrhythmias (including sustained ventricular tachycardia or firing of an automatic implantable cardioverter defibrillator within 60 days of enrollment), or any condition that the investigator believed would place the patient at undue risk.

Bone marrow aspiration and cell processing procedures

One hundred (±20) milliliters of bone marrow was harvested from the iliac crest under local anesthesia unless institutional guidelines required general anesthesia. The bone marrow was transported via an interhospital courier to current

Good Manufacturing Practices facilities at The University of Texas MD Anderson Cancer Center (Houston, TX) or via on-board courier to the Good Manufacturing Practices Cell Manufacturing Facility at Aldagen, Inc. For the ALDH^{br} group, bone marrow cells were depleted of CD15 and glycophorin A–expressing cells by using immunomagnetic beads (EasySep; Stem Cell Technologies, Vancouver, British Columbia, Canada). The cells were reacted with ALDH substrate, and ALDH^{br} cells were isolated by using a cell sorter (MoFlo; Dako/Cytomation, Glostrup, Denmark, or FACSAria; BD Biosciences, San Jose, CA), as described elsewhere. After centrifugation, the cells were resuspended in 3.5-mL 5% pharmaceutical grade human serum albumin. The final products were transferred to a 3-mL fluorinated ethylene propylene bag (American Fluoroseal, Gaithersburg, MD) with a needleless entry port. Control patients underwent an identical bone marrow harvest procedure, including insertion of the needle, except that bone marrow was not aspirated. Personnel involved in the harvesting procedure acted independently of the study team, thus maintaining the blinding.

Cell products and injection procedure

Aldehyde dehydrogenase–bright cells were administered via a NOGA Myostar catheter (Biosense Webster, Diamond Bar, CA) to patients who were randomized to the treatment group. The products manufactured at Aldagen were administered to all patients within 50 to 55 hours of bone marrow aspiration, whereas those produced locally at the University of Texas MD Anderson Cancer Center were administered within 30 to 36 hours of aspiration. Cell numbers and viability were similar in bone marrow samples processed at the 2 centers. Aldehyde dehydrogenase–bright cells comprised a mean of 0.74% ± 0.28% of the nucleated bone marrow cells in the unprocessed aspirates from patients (median 0.73%, range 0.35%-1.16%). Cell injections were targeted to areas of the myocardium identified as ischemic on SPECT and as viable by EMM (unipolar voltage ≥6.9 mV). Coronary anatomy was also taken into account. The transendocardial injections were performed according to previously described injection and safety criteria.[10,11,19,20] Control patients underwent the same procedures but received transendocardial injections of placebo solution (5% albumin) instead of the cell preparation. All personnel involved were blinded, except for the research manager, who was responsible for assignment to the study groups.

Statistical analyses

Continuous variables were expressed as mean ± SD. Comparisons of continuous variables between groups were performed by using a 2-tailed unpaired Student *t* test for data that were normally distributed. The Wilcoxon rank sum test was used to analyze nonparametric data. Differences in follow-up assessments within the study were analyzed by paired *t* test. NCSS 2007 (2007 edition; Kaysville, UT) software was used for statistical analyses.

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Table I. Patient baseline characteristics

	Control (n = 10)	ALDH^{br} treatment (n = 10)
Age (y)	57.8 ± 5.5	58.2 ± 6.1
Female	2 (20%)	1 (10%)
CCS	2.5 ± 0.5	2.5 ± 0.5
NYHA	2.6 ± 0.5	2.5 ± 0.5
Congestive heart failure	10 (100%)	10 (100%)
Coronary interventions	9 (90%)	10 (100%)
Diabetes mellitus	5 (50%)	2 (20%)
Hypertension	9 (90%)	9 (90%)
Hyperlipidemia	10 (100%)	10 (100%)
Laboratory data		
BNP (pg/mL)	205.1 ± 239.6	131 ± 146.2
C-reactive protein (mg/dL)	0.59 ± 0.6	0.78 ± 0.9

BNP, B-type natriuretic peptide.

Results

Patients and cell injections

We screened 60 patients, and we enrolled 20 patients in the study from August 22, 2006, to June 12, 2008 (10 control patients, average age 57.8 ± 5.5 years, and 10 cell-treated patients, average age 58.2 ± 6.1 years). The 2 groups did not differ significantly in demographics or medical history (Table I). Moreover, laboratory and functional data were also similar between the control and treatment groups (Tables I and II) (LVEF 32.1% ± 10.6% vs 36.1% ± 10.9%, respectively). The low MVO_2 values indicate the severely compromised functional capacity of both groups of patients (Table II).

Aldehyde dehydrogenase–bright cells were prepared successfully from all patients in the treatment group. There were no differences in viability or in the number of ALDH^{br} cells between samples processed it the 2 centers; colony-forming unit ability and viability of ALDH^{br} cell populations after removing CD15 and glycophorin A–expressing cells were also similar. Aldehyde dehydrogenase–bright cells or placebo was delivered transendocardially via 15 injections in a volume of 0.2 mL per injection. The mean number of nucleated cells administered to the ALDH^{br} cell treatment group (n = 10) was 2.94 ± 1.58 × 10^6 cells (median 2.78 × 10^6, range 0.53-5.42 × 10^6). When the total cell doses were corrected for the proportion of ALDH^{br} cells in the cell product, the mean number of ALDH^{br} cells administered to the cell treatment group was 2.37 ± 1.31 × 10^6 cells (median 2.27 × 10^6, range 0.35-4.42 × 10^6). The difference in the highest and lowest dose indicates individual variability in ALDH^{br} cell content of the bone

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Table II. Clinical and efficacy parameters at baseline and 6-month follow-up for the treatment and control groups

	Control (n = 10)		ALDH^{br} treatment (n = 10)	
	Baseline	6 m	Baseline	6 m
NYHA	2.6 ± 0.5	2.1 ± 0.3	2.5 ± 0.5	2.3 ± 0.5
CCS	2.5 ± 0.5	2.0 ± 0.0	2.5 ± 0.5	2.0 ± 0.5
Echo EF (%)	32.1 ± 10.6	34 ± 9.3	36.1 ± 10.9	36.0 ± 11.3
LVESV (mL)	94.9 ± 59.8	94.7 ± 62.2	93.2 ± 46.1	85.9 ± 46.2*
LVEDV (mL)	132.7 ± 65.2	131.3 ± 67.4	138.3 ± 43.0	127.2 ± 51.3
Echo WMSI	2.13 ± 0.38	2.06 ± 0.41	1.92 ± 0.51	1.90 ± 0.57
MVO_2 (mL/kg per minute)	14.1 ± 4.8	14.6 ± 6.7	15.5 ± 6.3	17.7 ± 4.1†
EF (%) (LV angiogram)	41.9 ± 11.8	42.2 ± 7.6	38.0 ± 17.5	40.4 ± 15.8
Total severity score (stress)	1007.6 ± 352.4	1132 ± 588.5	798.2 ± 528.7	785.9 ± 554.6
Total severity score (rest)	812.4 ± 304.5	828 ± 396.9	542.8 ± 440.7	594.6 ± 488.9
Total severity score (reversible)	46.5 ± 48.5	87.3 ± 60.2	72.6 ± 95.2	22.9 ± 39.2

Results are presented as the mean ± SD. *Echo*, echocardiography; *EF*, Ejection fraction; *WMSI*, wall motion score index.
Note: We present nuclear, echocardiographic, and MVO_2 data for 9 control patients at baseline and at 6 months and MVO_2 data for 9 ALDH^{br}-treated patients at 6 months. Data for ejection fraction (left ventricular angiogram) are for 7 control and 8 ALDH^{br}-treated patients at baseline and at 6 months.
* P = .04 versus baseline.
† P = .05 versus baseline.

Table III. Combined early and late adverse events

Event	Control (n = 10)	ALDH^{br} treatment (n = 10)
Atrial arrhythmia	0	2 (20)
EMM-related VT*	2 (20)	0
EMM-related VF*	1 (10)	0
Cerebrovascular event	1 (10)	0
Angina exacerbation	1 (10)	5 (50)
Myocardial infarction (NSTEMI)	0	1 (10)
Intracardiac thrombus	0	1 (10)

VT, Ventricular tachycardia; *VF*, ventricular fibrillation; *NSTEMI*, non–ST-segment elevation myocardial infarction.
* Successfully cardioverted.

marrow; however, 7 of the 10 patients received doses that were closely clustered between 2×10^6 and 4.5×10^6 cells. Cell viability of the final product was 96.7% ± 2.9% before injection.

Safety

No MACCE was associated with the injection procedures in ALDH^{br} patients, including no perforations. Electromechanical mapping–related ventricular tachycardia occurred in 2 control patients, and ventricular fibrillation occurred in 1 control patient, all of whom were successfully cardioverted by external means. No deaths occurred, and heart failure was not exacerbated in any patient. All patients were discharged the day after the procedure. Holter monitoring showed no sustained ventricular arrhythmia in any patient. Table III shows all combined early (30-day) and late adverse events up to 6 months. Angina exacerbation was seen in 5 ALDH^{br}-treated patients and in 1 control patient.

Efficacy

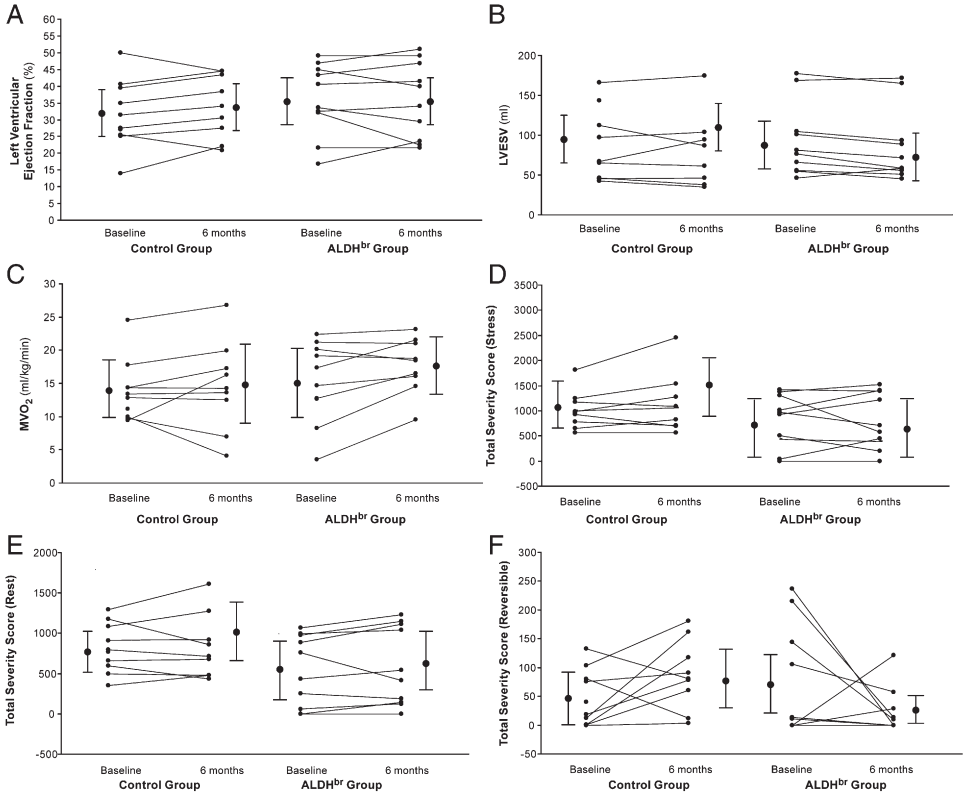
No differences were observed between groups in NYHA class and CCS classification (Table II).

On echocardiography, there were no differences over time in LVEF or wall motion score in either cell-treated or control patients (Table II). Similarly, we found no effect of cell therapy on LVEF by angiography. However, left ventricular end-systolic volume (LVESV) decreased significantly from baseline to 6 months in ALDH^{br}–treated patients (93.2 ± 46.1 to 85.9 ± 46.2 mL, P = .04). Left ventricular end-diastolic volume (LVEDV) decreased over time in treated patients, but the change did not reach statistical significance (Table II). No differences in LVESV or LVEDV were seen in control patients.

Maximal oxygen consumption increased in patients treated with ALDH^{br} cells, showing a trend toward statistical significance from baseline to 6 months (15.5 ± 6.3 to 17.7 ± 4.1 mL/kg per minute, P = .05), whereas control patients showed no differences during the same period (14.1 ± 4.8 to 14.6 ± 6.7 mL/kg per minute). Figure 1A to C shows the individual patient data for LVEF, LVESV, and MVO_2.

On SPECT analysis, the perfusion defect severity (total severity score) did not change significantly in either group. However, the SPECT findings showed a small increase in both stress total severity score and reversible total severity score at 6 months in the control group and a small decrease in both stress total severity score and reversible total severity score in the cell-treated group (Table II) (individual patient data are shown in Figure 1D-F). The comparison of the change from baseline to 6 months showed a trend toward significant improvement in reversible total severity score in the treatment group compared with the

Figure 1



Individual patient data showing left ventricular ejection fraction (**A**), LVESV (**B**), MVO_2 (**C**), and the total severity scores from SPECT analysis (**D**-**F**).

control group (−49.7 ± 109.5 vs 40.1 ± 72.8, respectively, $P = .053$).

Discussion

We present here the results of the first randomized, controlled, double-blind pilot study to assess the safety of the transendocardial injection of autologous $ALDH^{br}$ cells isolated from the bone marrow in patients with advanced ischemic heart failure. We provide preliminary evidence suggesting improved perfusion and a trend toward improved functional capacity in no-option heart failure patients treated with $ALDH^{br}$ cells. In the cell therapy group, there were no periprocedural (from enrollment up to 1 month of follow-up) complications or adverse events such as ventricular arrhythmias or MACCE. Our findings provide a sound basis for proceeding with the study of this cell type in a larger population of patients.

Clinical trials have shown that cell therapy is associated with modest functional or clinical improvements in patients with acute and chronic ischemic cardiomyopathy.[11,21,22] In most of these trials, unselected mononuclear cell populations from autologous bone marrow have been used. The equivocal results of cell therapy trials to date have brought into question the "shotgun" approach of using a cellular amalgamation of all mononuclear cells in the bone marrow. In the present study, we have examined a new cell type obtained by isolating a cell population based on the presence of a cytosolic enzyme (ALDH) rather than by using density gradient separation alone or the expression of cell surface markers. Thus, in our study, we have used the unique approach of selecting cells by function rather than phenotype.

Aldehyde dehydrogenase–bright cell populations constitute approximately 1% of the mononuclear cells in human bone marrow but include most of the hematopoietic, endothelial, and mesenchymal progenitor cells in the marrow. Each of these cell types has been proposed to play a role in repairing heart tissue damaged by ischemia. Bone marrow $ALDH^{br}$ cell populations are also

highly enriched in cells expressing surface markers that have been used by others to select therapeutic cell populations for cardiovascular diseases, including CD34, CD133, and CD117.[16,17] Because of this diversity, $ALDH^{br}$ bone marrow cells may be particularly well suited for providing positive benefits in an ischemic setting in that the progenitor and stem cell types believed to be necessary for tissue repair and angiogenesis are contained within this population.

The effects of bone marrow–derived ALDH cells ($ALDH^{hi}$) on perfusion and revascularization have been studied in mice with hindlimb ischemia by Capoccia et al[17] who used a sorting method similar to the one used in our study to obtain a bone marrow–derived, ALDH-enriched population. After ligation of the femoral artery, nonobese diabetic/severe combined immunodeficient mice were given one of the following via intravenous tail vein infusion: $ALDH^{hi}$ cells ($ALDH^{br}$ cells, 1×10^5 cells), unsorted bone marrow cells (50×10^6 cells), $ALDH^{lo}$ cells (the mononuclear fraction of bone marrow cells without high ALDH activity, 5×10^5 cells), or phosphate-buffered saline (control). The $ALDH^{hi}$ cells homed specifically to the ligated cells and could be detected at the ischemic site for up to 2 weeks. Treatment with $ALDH^{hi}$ cells significantly restored perfusion and increased capillary density in the ischemic limb by 7 days after therapy. No other treatment regimen had a significant effect, suggesting that the bone marrow cells responsible for angiogenesis and perfusion-restoring activity were contained with the $ALDH^{hi}$ population. Moreover, in the study of Capoccia et al,[17] the unsorted bone marrow cells were administered at a higher dose and contained 4-fold more $ALDH^{hi}$ cells than the isolated $ALDH^{hi}$ population. This finding may indicate the presence of $ALDH^{br}$ cell inhibitory activity in the unsorted mononuclear bone marrow population and suggests that the enriched ALDH population may have increased therapeutic potency.

In another acute cardiovascular injury model,[22] $ALDH^{br}$ cells isolated from umbilical cord blood were used to treat mice in which cardiac ischemia was induced by ligating the left descending cardiac artery. The $ALDH^{br}$ cells homed to the ischemic anterior surface of the heart and contributed to the formation of collateral arteries in the ischemic tissue.[23]

The clinical data on cell therapy in patients with chronic ischemic heart disease are relatively sparse.[1] In the present study, we have shown a trend of improved perfusion and exercise capacity in $ALDH^{br}$-treated patients. Our results support those of van Ramshorst et al[24] who reported improved perfusion in patients with refractory angina treated with autologous bone marrow cells. Moreover, in their study, LVEF was significantly improved. In our study, end-systolic volume decreased without an improvement in LVEF. This discrepancy may be explained by the difference in target patient populations. Although our patients did not have extensive angina, ischemia was present on SPECT at baseline. Our SPECT findings are interesting regarding the trend in decrease in reversible total severity score, but these results should be interpreted cautiously. Furthermore, we found no overall significant changes in the stress total severity score. In another study of patients with refractory angina, Losordo et al[25] reported that low-dose autologous $CD34^+$ cells improved angina and exercise tolerance; they also reported significant improvements in perfusion in cell-treated patients at 6 months. Larger studies are necessary to assess SPECT analysis more closely in this population.

In large cohort studies of patients with chronic ischemic heart failure, MVO_2 has been shown to be a valuable predictor of survival.[26] Thus, modest, directional changes in MVO_2 might represent a significant mortality benefit in this population.[27] Patients treated with $ALDH^{br}$ cells in our study showed a trend toward improvement in MVO_2. If similar findings are confirmed in larger studies, cell therapy could be viewed in the context of possibly affecting prognosis in patients with chronic ischemic heart disease by improving MVO_2.

The main limitation of the present study is the small number of patients. Given this limitation, the improvements that we have reported could have occurred by chance alone. In addition, the variability in $ALDH^{br}$ cell dose administered to patients may have affected the results.

In conclusion, we provide preliminary evidence that the transendocardial delivery of $ALDH^{br}$ cells is safe and may provide perfusion and functional benefits in the setting of chronic myocardial ischemia. Importantly, we present a unique approach for selecting a diverse population of active cells for cell therapy by using a physiologic rather than a single phenotypic marker, which may result in the isolation of a more efficacious population comprising the multiple cell types required for ischemic repair. We believe that our study provides a strong rationale for examining this innovative approach in a larger population.

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Appendix. Online Supplement

Online Supplemental Methods

Echocardiography protocol. We used 2-dimensional Doppler echocardiography. Images were obtained in the parasternal, apical, subcostal, and suprasternal views, using both short- and long-axis projections. Left ventricular systolic and diastolic volumes and diameter were measured. Left ventricular ejection fraction and volumes were calculated according to Simpson's method.

SPECT imaging protocol. Each patient received 370 MBq (±10%) of technetium Tc 99m sestamibi injected intravenously at rest. Single photon emission computed tomography was performed beginning 45 to 75 minutes after injection. If initial images were suboptimal due to persistent gastrointestinal system activity or due to patient motion, images were repeated, and the best image set was used for interpretation. Supine SPECT images were performed using a dual-headed scintillation camera (General Electric Millenium series) with low-energy high-resolution collimators in a cardiac (approximately 100°) configuration. Acquisition parameters included a 15% window centered at the 140-keV photopeak. Images were obtained for 72 stops at 25 seconds per stop in a step and shoot mode with circular orbit. No attenuation or scatter correction was applied. Image data were processed using filtered back projection as per the manufacturer's standard protocols. After rest imaging, the patient underwent pharmacologic stress using intravenous adenosine. One thousand one hundred ten megabecquerels (±10%) of technetium Tc 99m sestamibi was injected intravenously at 3 minutes into a 4-minute adenosine infusion (140 <mu>gm/kg per minute). The SPECT was also performed after stress injection, using the same imaging protocol, except that poststress images were gated with a 20% window and 8 frames per cycle. The same camera was used for resting and poststress images. Images were displayed and qualitative and semiquantitative analysis was performed using Emory Tool Box (MEDX, Arlington Heights, IL).

Technical data such as injection-to-image time and all raw and processed (multiplanar tomographic images and quantitative/semiquantitative analysis) data were provided and interpreted by a single experienced nuclear medicine physician who was blinded to all clinical data, except patient weight and gender. Each study was assessed for study quality as excellent, good (minor artifact such as hepatic or bowel activity or patient motion noted but not interfering with interpretation), adequate (significant artifact present but study interpretable), or uninterpretable (due to artifact or other condition). Myocardial perfusion (tracer uptake) at rest and after stress, potential myocardial viability was assessed by using the Emory Tool Box. The total severity score is defined as the sum of blackout pixels in the blackout polar map, each weighted by the number of SDs below the mean as previously described (Lin GS, Hines HH, Grant G, Taylor K, Ryals C. Automated quantification of myocardial ischemia and wall motion defects by use of cardiac SPECT polar mapping and 4-dimensional surface rendering. J Nucl Med Technol 2006;34:3-17).