

## Cardiopoietic index predicts heart repair fitness of patient-derived stem cells

**Background:** Stem cell therapy shows promise for regeneration in heart disease, yet interpatient variability challenges implementation into practice. **Aim:** To establish a biomarker profile, predictive of reparative potential in patient-derived progenitors, human mesenchymal stem cells were isolated from patients undergoing coronary artery bypass grafting. **Materials & methods:** Stem cell delivery postinfarction translated into divergent benefit, distinguishing reparative from nonreparative populations. **Results:** While the nonreparative subtype was characterized by low expression of cardiac transcription factors, reparative human mesenchymal stem cells demonstrated high expression of cardiac transcription factors. **Conclusion:** This index of factors (cardiopoietic index) was found sensitive and specific in predicting impact of stem cell benefit on left ventricular function. The cardiopoietic index thus offers a tool to screen stem cell fitness for heart repair prior to intervention.

**Keywords:** cardiopoiesis • cardiopoietic stem cells • heart failure • mesenchymal • regenerative medicine • stem cells

The advent of coronary revascularization has reduced mortality rates in acute myocardial infarction [1]. This enhanced survival following myocardial infarction has however precipitated an epidemic of patients suffering from chronic ischemic cardiomyopathy [2]. Despite aggressive medical management, heart failure prognosis is poor mandating a growing need for novel therapies to rescue the failing myocardium [3].

Regenerative medicine provides an opportunity to supplement current pharmacologic and hemodynamic therapies through restoration of myocardial function and structure. Recent clinical trials provide evidence for feasibility and safety of adult stem cells as a promising therapeutic modality [4–7]. Although distinct individuals demonstrate benefit from stem cell therapy, treated cohorts display significant variability in outcome [8]. Patient-related modifiers, including age, gender, co-morbidities and concomitant pharmacotherapy, are thought to alter stem cell-based repair proficiency [9,10].

A biomarker-based quality control assay capable of assessing stem cell repair aptitude prior to transplantation is needed to inform clinical use.

### Materials & methods

All procedures were performed in compliance with institutional guidelines for patient sample acquisition and preclinical experimentation.

### Bone marrow-derived mesenchymal stem cells

Derivation of the cardiopoietic phenotype was performed as previously described [11]. Briefly, bone marrow aspirates were obtained from patients with ischemic heart disease undergoing coronary artery bypass surgery (11 males, one female; age: 65–85 years old) (Table 1). Plastic adherent cells were evaluated by fluorescence activated cell sorting to obtain the human mesenchymal stem cells (hMSCs) phenotype characterized by cell surface profile CD34<sup>-</sup>/CD45<sup>-</sup>

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Table 1. Patient demographics and medication regimens.

Patients	Age (years)	Sex	Left main	High LDLs	HTN	Smk	β-b	ACE	ASA	STAT	AMIO
Patient 1	75	Male	Yes	Yes	Yes	No	Yes	No	Yes	No	No
Patient 2	65	Male	No	No	Yes	No	Yes	No	Yes	Yes	No
Patient 3	57	Male	No	Yes	No	Yes	No	No	Yes	Yes	No
Patient 4	66	Male	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Patient 5	66	Male	Yes	Yes	Yes	No	No	Yes	Yes	Yes	No
Patient 6	71	Male	No	Yes	No	No	No	No	No	No	No
Patient 7	69	Male	No	Yes	No	No	Yes	No	Yes	No	No
Patient 8	52	Male	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Patient 9	51	Female	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Patient 10	52	Male	No	No	No	No	Yes	No	Yes	Yes	No
Patient 11 <sup>†</sup>	69	Male	No	Yes	No	No	Yes	No	Yes	Yes	Yes
Patient 12 <sup>†</sup>	77	Male	No	Yes	No	No	Yes	No	Yes	Yes	No

Patients 1–10 harbored nonregenerative human mesenchymal stem cells derived from bone marrow.

<sup>†</sup>Patients 11 and 12 were rare individuals with regenerative human mesenchymal stem cells derived from bone marrow.

ACE: Angiotensin converting enzyme inhibitor; AMIO: Amiodarone; ASA: Aspirin; βb: Beta-adrenergic receptor blocker; HTN: Hypertension; LDL: Low density lipoprotein; Smk: Smoking; Stat: Statin.

CD14<sup>-</sup>/CD105<sup>+</sup>/CD90<sup>+</sup>. Derived cells were cultured in advanced-MEM medium with 10% fetal bovine serum at 37°C until 95% confluence.

### Gene-expression profiling

Real-time and quantitative PCR was evaluated using a TaqMan PCR kit (Applied Biosystems, CT, USA) in triplicate. Threshold cycle ( $C_T$ ) values were determined using the  $2^{-\Delta\Delta C_T}$  method, normalized to human specific *GAPDH* (P/N 4352662–0506003). The cardiopoietic index (CP index) was calculated by averaging the mRNA fold expression levels of the following cardiac transcription factors: *MESPI* (Hs00251489\_m1), *Nkx2.5* (Hs00231763\_m1), *GATA4* (Hs00171403\_m1), *GATA6* (Hs00232018\_m1), *Tbx5* (Hs00361155\_m1), *MEF2C* (Hs00231149\_m1) and *FOG1* (Hs00542350\_m1) in cells harvested from all patients [12]. Cell engraftment was quantified with human-specific *GAPDH* standard curve as previously shown [4].

### Immunoprobing & microscopy

Staining for CP index factors was performed using Mef2C (1:400, Cell Signaling Technologies, TX, USA), Nkx2.5 (1:150, Santa Cruz Biotechnology), Gata-4 (1:150, Santa Cruz Biotechnology), Tbx5 (1:5000, Abcam, MA, USA), Mesp-1 (1:250, Novus Bio, CO, USA), Fog-1 (1:100, Santa Cruz Biotechnology) alpha-smooth muscle actin (1:500, Sigma-Aldrich, MO, USA), human lamin A/C (1:50, Novocastra Microsystems, Newcastle upon Tyne, UK), human-specific troponin I (1:100, Abcam), MLC-2a (1:200, Synaptic Systems, BC, Canada) and MLC-

2v (1:200, Synaptic Systems) antibodies. Human Alu probes (BioGenex, CA, USA) were hybridized required hybridization at 85°C for 10 min to detect human cell, followed by overnight incubation at 37°C and fluorescent labeled detection. Images were acquired using the LSM 510 confocal and apotome structured illumination microscopes (Carl Zeiss, CA, USA).

### Electron microscopy

Cultured cells were fixed with 1% glutaraldehyde and 4% formaldehyde in phosphate buffered saline. Nucleus was exposed with sarcolemmal stripping using 1% Triton X-100 solution. Cells were rinsed in phosphate-buffered saline solution and 1% osmium. This was followed by dehydration with ethanol and dried in a critical point dryer. Samples were coated with platinum and examined on a 4700 field-emission scanning microscope (Hitachi, Tokyo, Japan).

### CP index induction

Following expansion hMSCs were treated with a cocktail of growth factors consisting of TGF-β1 (2.5 ng/ml), BMP-4 (5 ng/ml), Activin-A (5 ng/ml), FGF-2 (10 g/ml), IL-6 (100 ng/ml), factor IIa (α-thrombin, 1 U/ml), IGF-1 (50 ng/ml) and retinoic acid (1 μM) supplementation for 5 days [11,13]. Cells were then analyzed for expression of cardiogenic transcription factors at the mRNA and protein levels.

### Cell therapy & analysis of cardiac function

Immunocompromised athymic nude mice (8–12 weeks old, male, Harlan) underwent myocardial infarction with permanent ligation of the proximal left anterior

descending artery. Induced ST-changes on electrocardiography (MP150, Biopac; CA, USA) and akinetic regions on echocardiography (Sequoia 512, Siemens; Vevo2100, VisualSonics, ONT, Canada) documented injury. In the initial experiment, 600,000 of patient-derived hMSCs were injected in five peri-infarct epicardial sites, one-month postinfarction to assess endogenous regenerative potency. Follow-up experiments used the same injection approach, and compared hMSCs displaying various degrees of CP index induction. Left ventricular (LV) ejection fraction was serially monitored, in blinded fashion, by echocardiography at preinfarction, 1 month after infarction (precell therapy) and 1, 2, 6 and 12 months postcell therapy. LV ejection fraction (%) was calculated as:

$$[(EDV-ESV)/EDV] \times 100$$

where EDV is left ventricular end-diastolic volume ( $\mu$ l), and ESV is left ventricular end-systolic volume ( $\mu$ l) [14].

### Statistics

Data is presented as mean  $\pm$  SD or SEM (JMP 8; SAS Institute; NC, USA). Paired group analysis was performed using Student's *t*-test for each pair of samples. CP index was constructed by averaging the fold expression of each cardiac transcription factor gene probed relative to nonreparative hMSCs. Bivariate analysis comparing ejection fraction change as a function of CP index was performed with analysis of variance for continuous variables and chi-square test for categorical variables. Linear regression probed the relationship between CP index as the primary predictor variable and the ejection fraction change as the outcome variable. All analyses were conducted with JMP 8.0 software (SAS) and a *p*-value <0.05 was considered significant.

## Results

### CP biomarker profiling

Bone marrow hMSCs were harvested from the sternum of patients undergoing coronary artery bypass grafting (Table 1) and injected into an immunodeficient murine model of chronic myocardial infarction. While no statistical difference in ejection fraction (EF), before and 1 month after treatment with hMSCs, was observed ( $29 \pm 6\%$  and  $29 \pm 10\%$ , respectively,  $n = 12$  patient cell lines), individual EF evaluation revealed rare instances of significant repair (Figure 1A). Immunoprobings of rare reparative cells documented an increased expression and nuclear translocation of cardiac transcription factors Nkx2.5, Tbx-5, Gata4 and MEF2C compared with nonreparative cells (Figure 1B). mRNA analysis

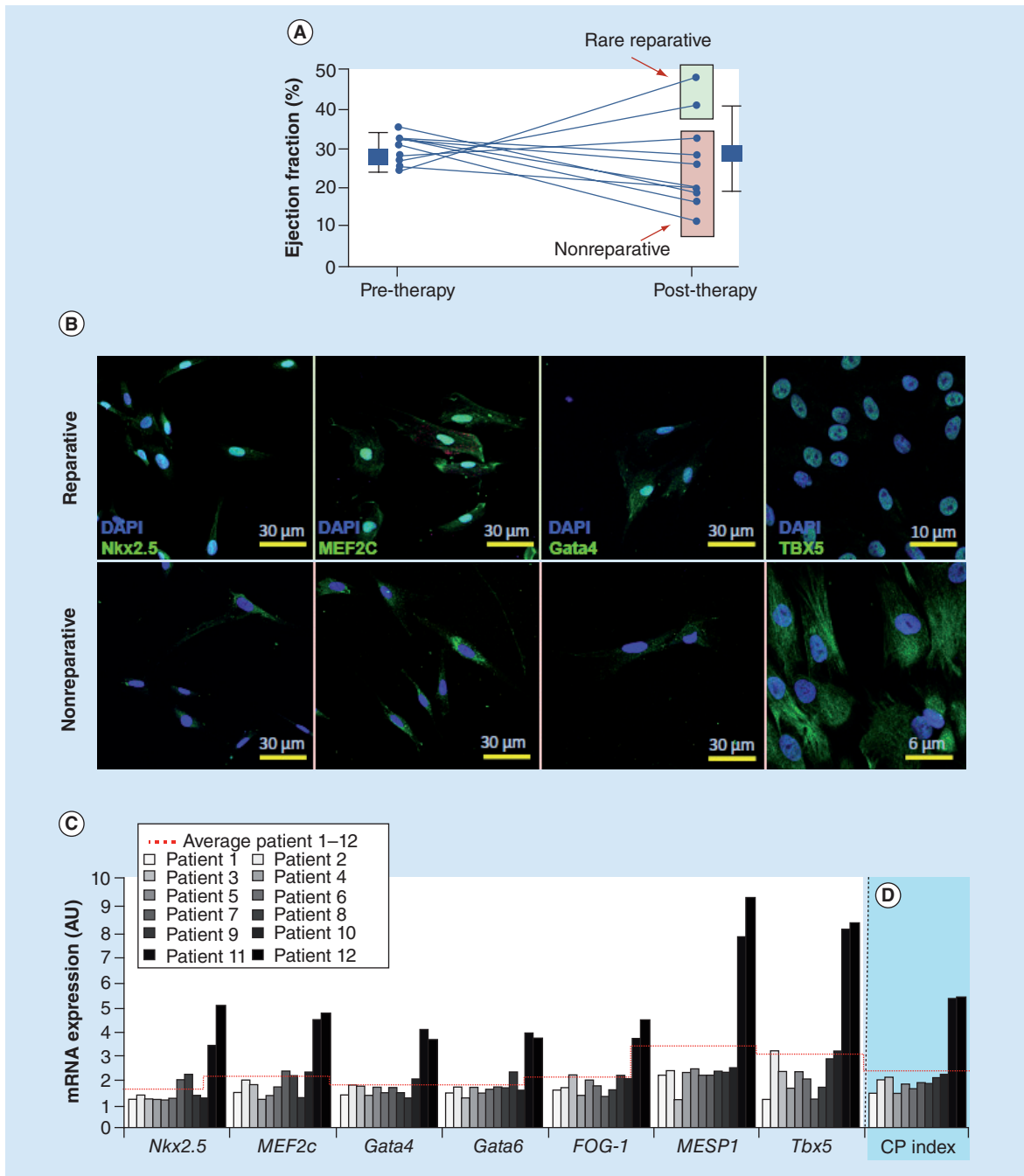
confirmed upregulation of Nkx2.5, MEF2c, Gata4 and Tbx5, as well as additional cardiac transcription factors Gata6, Fog-1, MESP1 in the rare reparative cellular subtype in comparison to nonreparative hMSCs (Figure 1C). To forecast repair efficiency, mRNA expression of transcription factors was integrated into a CP index. The average index value was  $2.24 \pm 1.43$  for all 12 patients (red dashed lines) and ranged from 1.31 to 5.14, 95% CI (Figure 1D). Based on the index, hMSCs could be categorized as high-reparative (CP index >5) or low-reparative (CP index <2) (Figure 1D).

### Induction of CP index

To prospectively establish the association between CP index and repair aptitude, a guiding microenvironment was utilized to induce cardiopoiesis in all patient-derived hMSCs [15]. Cardiogenic guidance resulted in mRNA CP index induction from  $1.6 \pm 1.0$  pre- to  $6.8 \pm 0.50$  postinduction ( $p < 0.001$ ) (Figure 2A) and was associated with an increase in expression and nuclear translocation of Nkx2.5, MEF2C, GATA4 and Tbx5 (Figure 2B). Ultrastructural imaging of high-index hMSCs revealed myotube and sarcomere formation in tandem with mitochondrial maturation (Figure 2C) suggesting enhanced proclivity toward a cardiomyogenic fate.

### CP index forecasts stem cell repair efficacy

High and low-CP index hMSCs ( $6.0 \times 10^5$ ) were injected into mice suffering from chronic ischemic cardiomyopathy. Echocardiographic follow-up studies were performed to measure end diastolic volume (EDV), end systolic volume (ESV) and EF difference between high CP index and low CP index treated mice. No statistically significant difference in EDV was observed at baseline and 1 month after infarction in high CP index mice compared with low CP index (Figure 3A). At 1 month and 2 months postcell therapy (Figure 3A), high CP index treated mice had an EDV of  $51.9 \pm 3.2$  ml and  $61.7 \pm 3.4$  ml, respectively ( $p > 0.05$ ), whereas low CP index treated mice reported an EDV of  $61.7 \pm 9.6$  ml and  $62.5 \pm 6.1$  ml, respectively ( $p > 0.05$ ). Similarly, EDV change at 1 and 2 months postcell therapy (Figure 3B) relative to precell therapy was not statistically significant between these two groups. EDV change at 1-month postcell (Figure 3B) therapy in high and low CP index cohorts were  $2.1 \pm 3.3$  ml and  $6.4 \pm 6.2$  ml, respectively ( $p > 0.05$ ). EDV change at 2-months following cell therapy (Figure 3B) in high CP index cohorts was  $8.0 \pm 4.7$  ml, whereas the low CP index was  $7.2 \pm 4.6$  ml ( $p > 0.05$ ). Pre-cell therapy measurements of ESV were  $31.6 \pm 2.6$  ml and  $30.7 \pm 5.0$  ml in the high and low CP index, respectively ( $p > 0.05$ ). At 1 month after cell therapy (Figure 3A) ESV record-



**Figure 1. Interpatient variability reveals rare stem cells expressing an index enriched with cardiogenic genes.**

(A) A rare reparative population was identified following transplantation of patient-derived human mesenchymal stem cell in a model of ischemic cardiomyopathy. (B) Immunofluorescence identification of cells with low or high regenerative impact was associated with a change in cardiac transcription factor expression. (C) mRNA-expression profiling for cardiac transcription factors in 12 distinct patient-derived bone marrow mesenchymal stem cell lines and (D) identifies seven upregulated genes to formulate a cardiopoietic index. CP: Cardiopoietic.

ings were  $23.8 \pm 2.8$  ml and  $35.9 \pm 9.8$  ml in the high and low CP index, respectively ( $p > 0.05$ ). Two months following cell injection (Figure 3A) the ESV in high CP index group was  $28.8 \pm 2.5$  ml and  $38.8 \pm 6.0$  ml in the low CP index cohort ( $p > 0.05$ ). However, ESV

change (Figure 3C) at 1 month and 2 months following cell injection exhibited statistically significant volume reduction in the high CP index treated cohort compared with the low CP index group. ESV change at 1 month following stem cell treatment was  $-6.1 \pm$

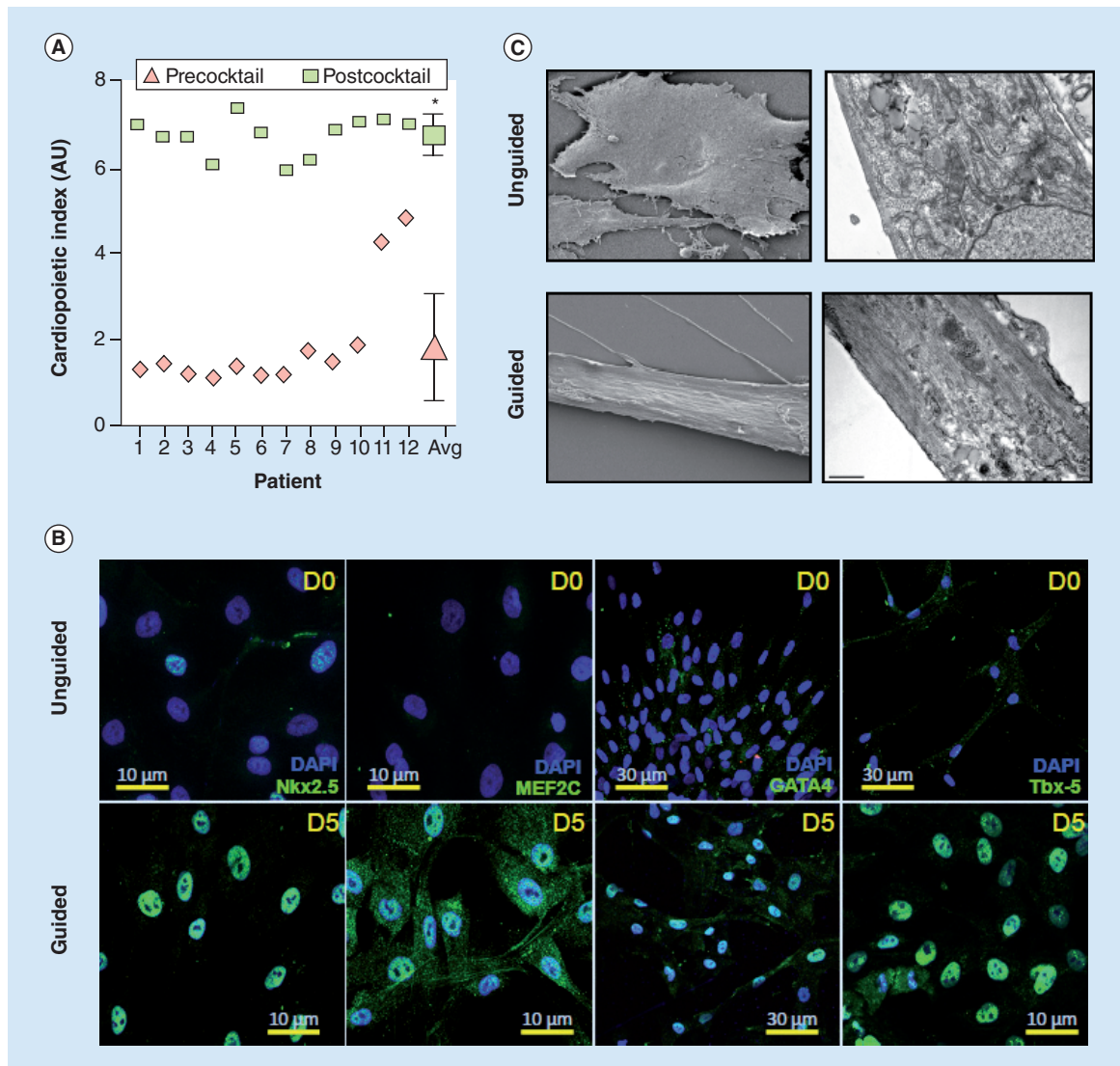


1.9 ml in the high CP index and  $5.2 \pm 5.3$  ml in the low CP index mice ( $p < 0.05$ ). Two months following cell therapy demonstrated sustained ESV reduction of  $-6.23 \pm 3.3$  ml in high CP index treated mice compared with  $8.0 \pm 4.5$  ml in low CP index treated ( $p < 0.05$ ) (Figure 3C). Furthermore, ejection fraction improved from  $39.5 \pm 3.3\%$  before cell therapy to  $53.5 \pm 3.5\%$  at 2 months following cell therapy in high CP index treated mice ( $p < 0.05$ ) versus no benefit in the low CP index group ( $46.6 \pm 3.3\%$  and  $47.1 \pm 4.1\%$ ;  $p > 0.05$ ) (Figure 3D). An average CP index increase of  $5.2 \pm 0.5$  corresponded to an ejection fraction increase

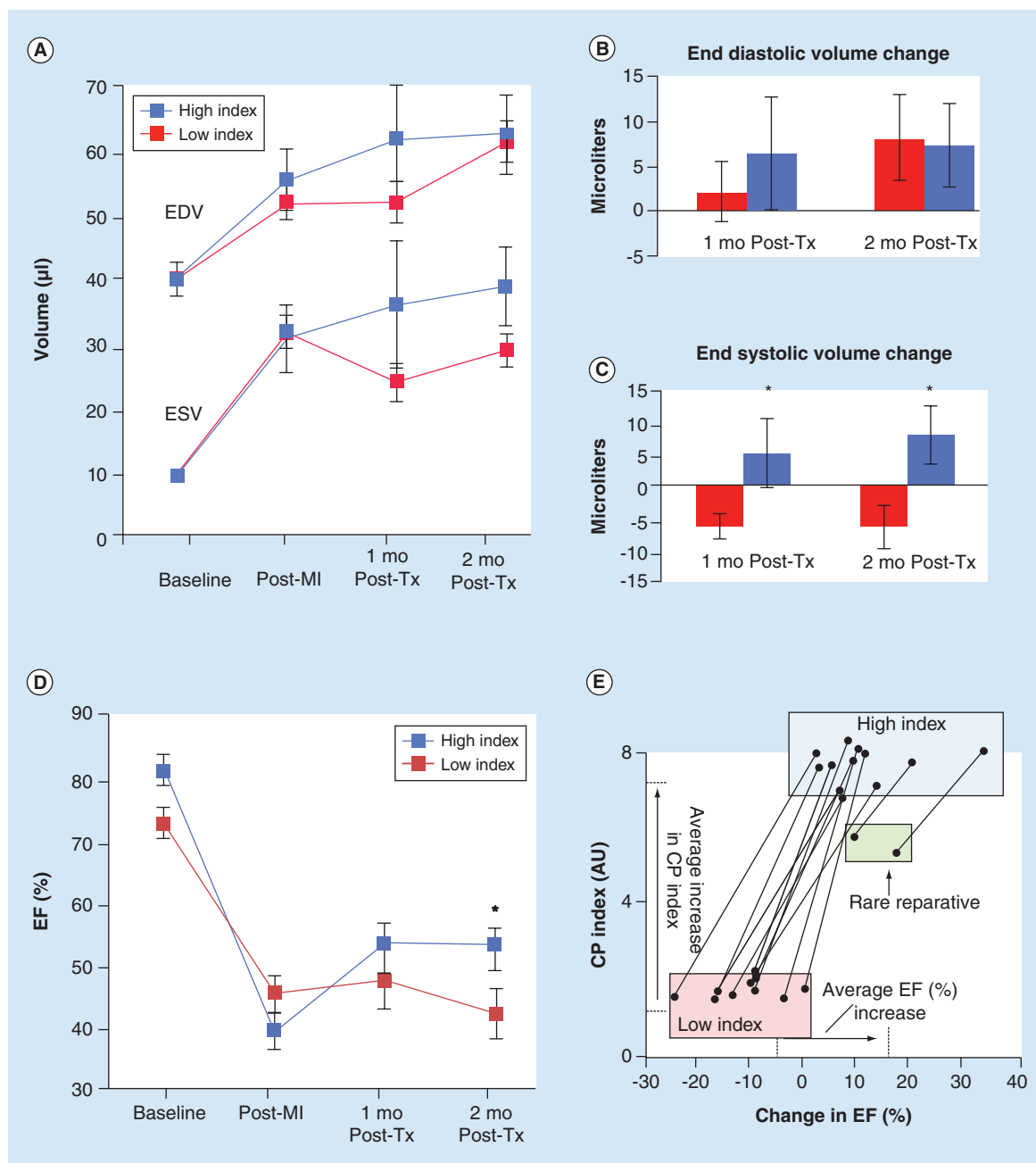
of  $14.2 \pm 4.1\%$ , 2 months following stem cell therapy (Figure 3E). Thus, induction of the CP index resulted in cardiac function improvement.

**Regenerative threshold determined as a function of CP index**

High CP index treated animals demonstrated a sustained functional benefit over a 12 month observation period with a 95% CI of ejection fraction change: 8.8–19.8% at 1 month, 9.0–22.2% at 2 months, 0.1–7.3% at 6 months and 0.8–10.6% at 1 year (Figure 4A). Low CP index treated cohort had a pro-



**Figure 2. Cardiopoietic guidance of human mesenchymal stem cells. (A)** Cardiopoietic index, reflecting mRNA-expression profile, prior to (diamond) and following (square) exposure to a guiding environment. **(B)** Immunoprobng reveals induction and nuclear translocation of cardiopoietic index factors following cardiogenic cocktail-based induction. **(C)** Ultra-structural imaging revealed capacity for myotube and sarcomere formation as well as organelle maturation 20 days following cardiopoietic index induction. \* $p < 0.05$ . AU: Arbitrary units; Avg: Average value of cardiopoietic index.



**Figure 3. Induction of cardiopoietic index augments human mesenchymal stem cell-based cardiac repair.** (A) End diastolic and systolic volume at baseline, post-myocardial infarction, 1 month and 2 months following cell therapy. (B–C) Change in end diastolic and systolic volume relative to pre-cell injection. (D) Ejection fraction at baseline, post-myocardial infarction, 1 month and 2 months following cell therapy. (E) Patient matched comparison of repair efficacy in low- vs high-index hMSCs revealed that an increase in CP index is associated with ejection fraction improvement. CP: Cardiopoietic; EDV: End diastolic volume; EF: Ejection fraction; ESV: End systolic volume; MI: Myocardial infarction; mo: Months; Tx: Stem cell therapy.

gressive decline in ejection fraction change with a 95% CI of -3.2 to -4.3% at 1 month, -11.4 to -2.9% at 2 months, -30.2 to -6.0% at 6 months and -26.2 to -6.0% at 1 year (Figure 4A). These findings formulated linear regression models with CP index as the primary predictor variable, and ejection fraction change as the

outcome variable. Spearman correlation between these two variables at 1 month, 2 months, 6 months and 1 year following stem cell treatment was 0.66 (red line;  $p = 0.005$ ), 0.78 (purple line;  $p = 0.001$ ), 0.68 (blue line;  $p = 0.018$ ) and 0.67 (black line;  $p = 0.009$ ), respectively (Figure 4C). Sensitivity and specificity were calculated to

test the prognostic value of the CP index with threshold values set at zero for ejection fraction and a 5 AU threshold for CP index corresponding to the upper 95% CI of high-index hMSCs (Figure 3C). CP index sensitivity and specificity at selected thresholds were calculated as 91 and 95%, respectively, producing an area of 0.91 under the Receiver-operating-characteristic curve. The 96% positive predictive value and 89% negative predictive value, indicate a high probability for the CP index to accurately forecast stem cell repair fitness.

### Functional validation of the high CP index cell

Cardiovascular lineage proclivity was probed via differentiation of high index CP cells to evaluate capacity for myofibrillogenesis and vasculogenesis (Figure 5). Sarcomere formation was absent in low-CP index hMSCs subjected to a 20-day differentiation environment (Figure 4A) but was documented in high CP index cells by expression of human Troponin-I, MLC2v and MLC2a (Figure 5A–E) after 15 days of differentiation (Figure 4B) and persisting at day 21 (Figure 5C–E). Potential for vasculogenesis was noted with *in vitro* differentiation into smooth muscle actin positive cells (Figure 5F). *In vivo* evaluation of high CP index cells within the myocardium further validated system biology overrepresentation of vascular contribution with identification of collateral blood vessel networks subtending the infarcted area in CP treated mice (Figure 5G) demonstrating evidence for human cell integration in the vessel wall as evidenced by human lamin co-localization with smooth muscle actin staining (Figure 5H). Furthermore, despite low initial myocardial retention for both high guided and low unguided CP index cells, 3 months after therapy the number of human cells detected in the murine heart increased to  $8.3 \times 10^4 \pm 1.4 \times 10^4$  in high index guided versus  $2.0 \times 10^4 \pm 2.0 \times 10^3$  in unguided cells ( $p < 0.05$ ). Beyond 12 months guided high index and unguided low-index hMSCs were quantified as  $5.8 \times 10^4 \pm 7.0 \times 10^3$  and  $2.0 \times 10^4 \pm 1.0 \times 10^3$ , respectively ( $p < 0.05$ ), with histological evidence for myocardial integration as documented by human-ALU probing (Figure 5J).

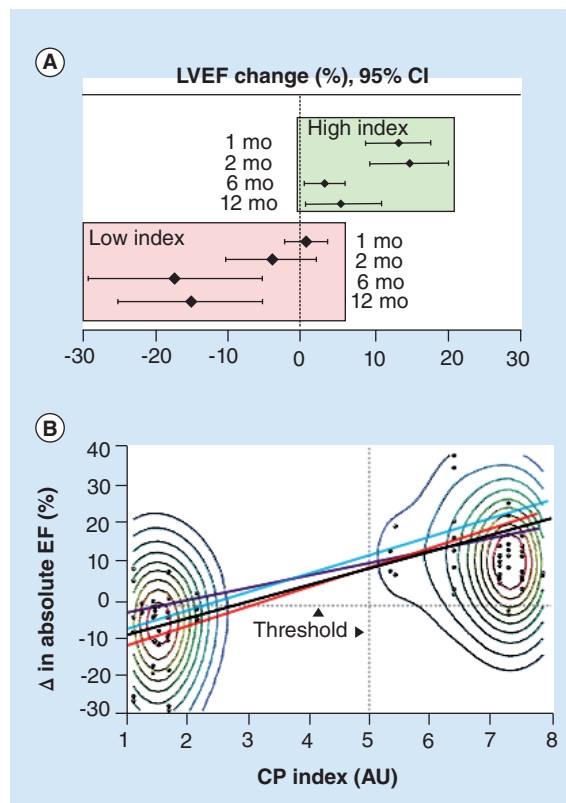
### Discussion

This work establishes an index that predicts the cardiac repair aptitude of patient-derived stem cells. The CP index was formulated as an integrated readout, based on mRNA expression of seven canonical cardiogenic transcription factors, Nkx2.5, MEF2c, Gata4, Gata6, Fog-1, MESP1 and Tbx5. hMSCs featuring high CP index levels prior to myocardial transplantation demonstrated superior repair capacity on long-term follow-up. Through cardiogenic guidance, CP index titrated above threshold levels were a reliable determinant of

positive ejection fraction change. With a sensitivity and specificity of 91 and 95%, respectively, the CP index provides a biomarker-based measure to anticipate therapeutic efficacy of hMSCs prior to transplantation.

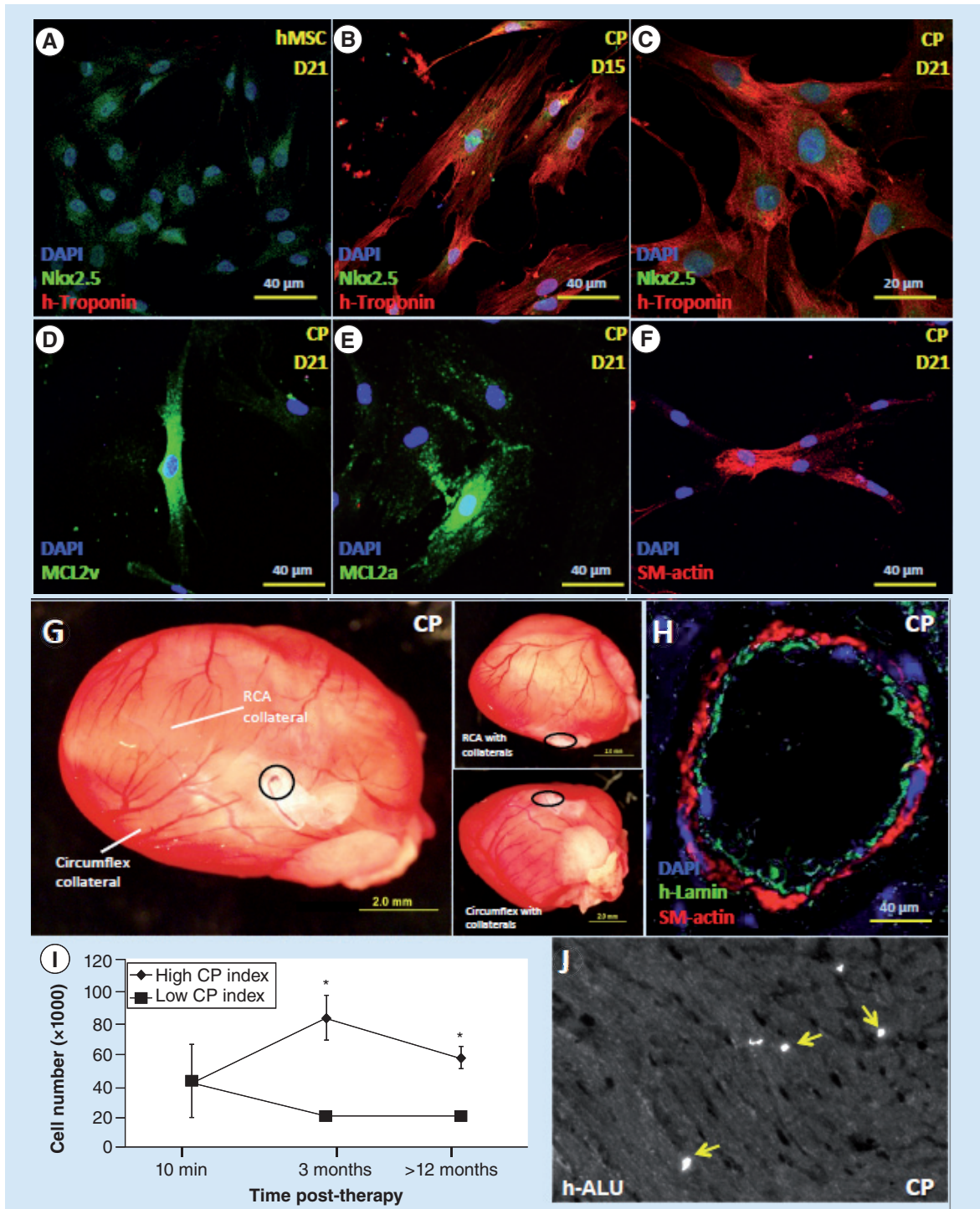
Trials assessing cell-based treatment for ischemic cardiomyopathy demonstrate significant interpatient variability, in part, due to divergent cell derivation methodologies and patient senescence and morbidity [3,10,16]. In fact, the incidence of patients harboring bone marrow derived stem cells demonstrating, in their native state, a repair quotient is rare and estimated to be in the order of 5% [17,18].

Typically, patient-derived stem cells are distinguished and separated from one another based on specific surface marker profiles [19]. Although this approach provides phenotypic characterization, it does not offer sufficient resolution to predict cellular repair aptitude [20]. At the time of isolation, adult stem cells are typically confined to a state of



**Figure 4. Cardiopoietic index correlates with human mesenchymal stem cell repair aptitude.** (A) 95% CI for overall therapeutic efficiency following transplantation of low index vs high- CP index human mesenchymal stem cells. (B) Prognostic aptitude of CP index as determined with thresholds of zero for ejection fraction and 5 AU for CP index corresponding to the upper 95% CI.

AU: Arbitrary units; CP: Cardiopoietic; EF: Ejection fraction; LVEF: Left ventricular ejection fraction; mo: Months.





perpetual stemness limiting the plasticity required for regeneration. When placed in a growth factor-rich environment, either *in vivo* or *ex vivo*, some stem cells recover plasticity while others remain confined [21,22]. A heterogeneous capacity of patient stem cells diminishes however the ultimate therapeutic reliability upon transplantation. Such phenotypic variability has resulted in unpredictable benefit following transplantation of adult-derived stem cells in patients with acute or chronic heart disease [8]. Meta-analysis of cell-based regenerative approach highlights rare individuals as benefiting, while the majority show limited improvement [23].

*In vivo* stem cells are mobilized through activation of specific signals within the microenvironment of injured tissue in order to restore tissue or organ homeostasis [24,25]. By providing mitogenic stimulus to derived stem cells, adjuvants within the artificial culture environment can mimic this process to drive cellular propagation [26]. The present study highlights that in rare instances, cell cycle reactivation in mesenchymal stem cells also results in resuscitation of plasticity as tracked by the CP biomarker index. Use of a preclinical model of chronic myocardial infarction provides a validated *in vivo* potency assay assessing repair capacity. This model, established with rare reparative hMSCs, was utilized to decipher the regenerative potency of mesenchymal stem prior to and after cardiogenic cocktail stimulation. To this end, an mRNA-expression profile consisting of biomarkers indexed to predict heightened cellular capacity for myocardial revitalization is here provided.

## Conclusion

The CP index provides a platform by which to evaluate the regenerative aptitude of adult stem cells prior to transplantation. Application of this standard allows assessment of repair potential at the time of stem cell harvest. Implementation of this approach identifies individuals harboring cells with an innate capacity for repair and provides a framework by which to switch-on regenerative signaling within stem cells predicted to be nonreparative.

## Future perspective

The biomarker-based index, described herein, provides the first quality control standard to forecast stem cell repair potency prior to myocardial transplantation. The CP index employs gene-expression profiling as a means to assess the regenerative quotient of patient derived cells. As such, this tool aims to reduce heterogeneity inherent to cell-based therapeutics in cardiovascular medicine. Inherent to this approach is the reliance on a targeted snapshot of cardiac transcription factors, profiling of the full transcriptome, miRNome and secretome will be needed to deconstruct the complexity of the biological system regulating regenerative potency. Beyond cell phenotyping, additional benchmarks – including stratification of patient vulnerability to ischemic cardiomyopathy – are required to secure definitive therapeutic outcome.

## Financial & competing interests disclosure

The study was supported by the NIH, Marriott Foundation, Russ and Kathy Van Cleve Foundation, Michael S and Mary Sue Shannon Family, and Center for Regenerative Medicine, Mayo Clinic; Cardio3 BioSciences, Walloon Region General Di-

## Executive summary

### Background

- Stem cell therapy has shown promise for regeneration in heart disease, yet observed interpatient variability in clinical outcomes challenges implementation into practice. This work aims to establish a biomarker profile, predictive of reparative potential in patient-derived stem cells.

### Materials & methods

- Bone marrow-derived human mesenchymal stem cells (hMSCs) were isolated from 12 patients undergoing coronary artery bypass grafting. The expression profile of canonical cardiogenic factors (Nkx2.5, Mef2C, Gata4, Gata6, FOG1, Tbx5 and Mesp1) in hMSCs was characterized and averaged into a cardiopoietic index. Cardiac function was prospectively monitored over a 12-month period following hMSC transplantation in chronically infarcted immunodeficient murine model.

### Results

- Stem cell delivery postinfarction translated into a divergent long-term outcome in myocardial performance, distinguishing reparative from nonreparative hMSC populations. While the nonreparative subtype was characterized by a consistently low expression of cardiac transcription factors, reparative counterparts harbored a robust cardiopoietic profile defined by high expression of cardiac transcription factors. Cardiogenic cocktail-based treatment of hMSCs induced a high mRNA cardiopoietic index profile (>5 arbitrary units threshold) associated with consistent repair proficiency. Accordingly, the cardiopoietic index was found sensitive (91%) and specific (95%) in predicting impact of stem cell benefit on left ventricular function.

### Conclusion

- The cardiopoietic index may thus serve to stratify patient-derived stem cells, offering a biomarker capable of assessing fitness for heart repair prior to intervention.

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No writing assistance was utilized in the production of this manuscript.

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## Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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