Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2761807 C 2018/01/09

(11)(21) **2 761 807**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) **Date de dépôt PCT/PCT Filing Date:** 2010/05/20
(87) **Date publication PCT/PCT Publication Date:** 2010/11/25
(45) **Date de délivrance/Issue Date:** 2018/01/09
(85) **Entrée phase nationale/National Entry:** 2011/11/10
(86) **N° demande PCT/PCT Application No.:** US 2010/035616
(87) **N° publication PCT/PCT Publication No.:** 2010/135555
(30) **Priorité/Priority:** 2009/05/20 (US PCT/US2009/044751)

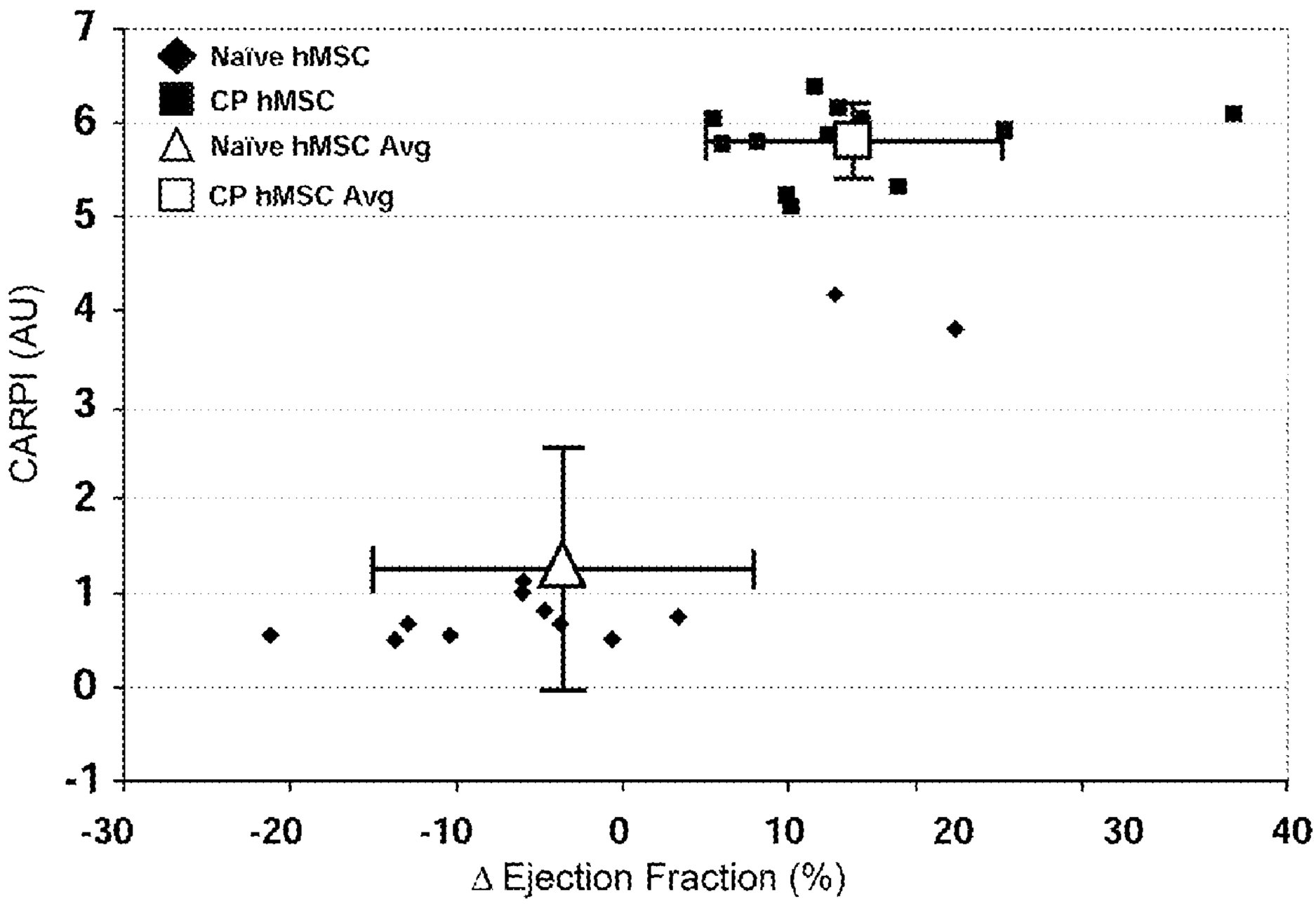
(51) **Cl.Int./Int.Cl.** ***G01N 33/50*** (2006.01)

(72) **Inventeurs/Inventors:**
TERZIC, ANDRE, US;
BEHFAR, ATTA, US;
GORDON-BERESFORD, ROLAND, BE;
GAUSSIN, VINCIANE, BE;
HOMSY, CHRISTIAN, BE

(73) **Propriétaires/Owners:**
MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH, US;
CELYAD S.A., BE

(74) **Agent:** BORDEN LADNER GERVAIS LLP

(54) **Titre : PROCEDE DE DETERMINATION DU POTENTIEL CARDIO-GENERATEUR DE CELLULES DE MAMMIFERES**
(54) **Title: METHOD FOR DETERMINING THE CARDIO-GENERATIVE POTENTIAL OF MAMMALIAN CELLS**



(57) **Abrégé/Abstract:**
This document is related to a method for determining the cardio generative potential of mammalian cells which comprises the assessment of a CARdiac generation Potential Index (CARPI) as a function of the quantification of the expression of genes of said cells. It also relates to a method for quantitatively assessing the modification of this cardio-generative potential and the cardiogenic potential of a treatment aiming at cellular differentiation.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 November 2010 (25.11.2010)

PCT

(10) International Publication Number
WO 2010/135555 A1

(51) **International Patent Classification**:
G01N 33/50 (2006.01)

(21) **International Application Number**:
PCT/US2010/035616

(22) **International Filing Date**:
20 May 2010 (20.05.2010)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
PCT/US2009/044751 20 May 2009 (20.05.2009) US

(71) **Applicant** *(for all designated States except US)*: **MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH** [US/US]; 200 First Street S.W., Rochester, Minnesota 55905 (US).

(72) **Inventors; and**
(71) **Applicants** : **GORDON-BERESFORD, Roland** [BE/BE]; Avenue Antoinette Herlin 11, B-1310 La Hulpe (BE). **GAUSSIN, Vinciane** [BE/BE]; Avenue de l'Esplanade 6, B-1970 Wezembeek-Oppem (BE). **HOMSY, Christian** [BE/BE]; Avenue des Sittelles 99, B-1150 Bruxelles (BE).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **TERZIC, Andre** [FR/US]; 803 8th Street S.W., Rochester, Minnesota 55902 (US). **BEHFAR, Atta** [US/US]; 1121 Plummer Circle S.W., Rochester, Minnesota 55902 (US).

(74) **Agent**: **FINN III, J. Patrick**; Fish & Richardson P.C., P.O. Box 1022, Minneapolis, Minnesota 55440-1022 (US).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

[Continued on next page]

(54) Title: METHOD FOR DETERMINING THE CARDIO-GENERATIVE POTENTIAL OF MAMMALIAN CELLS

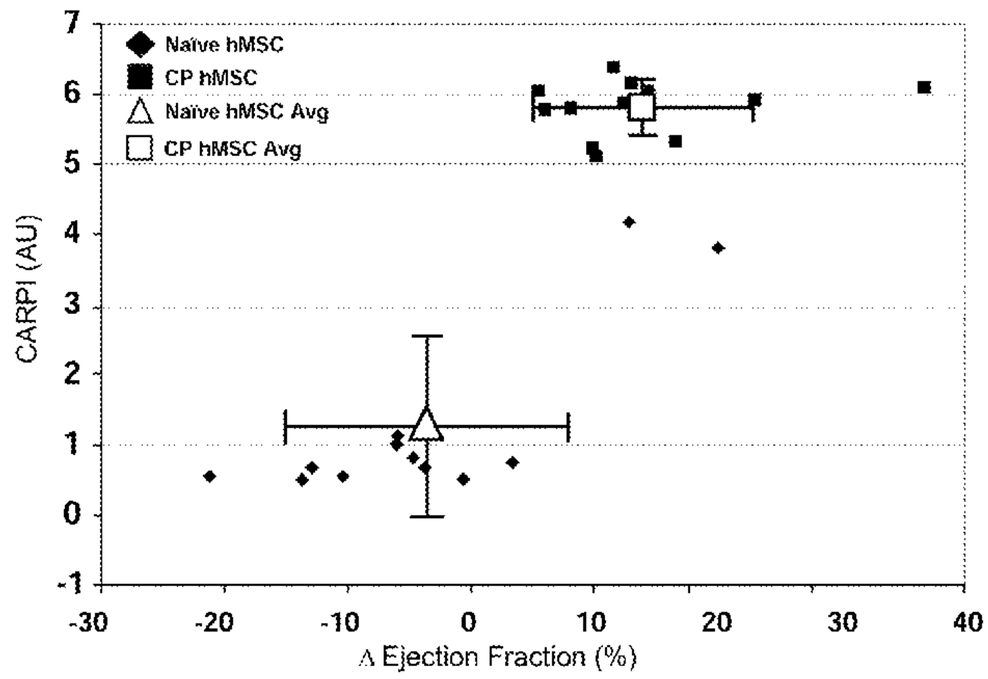


Fig. 1

(57) Abstract: This document is related to a method for determining the cardio generative potential of mammalian cells which comprises the assessment of a CARdiac generation Potential Index (CARPI) as a function of the quantification of the expression of genes of said cells. It also relates to a method for quantitatively assessing the modification of this cardio-generative potential and the cardiogenic potential of a treatment aiming at cellular differentiation.

WO 2010/135555 A1

WO 2010/135555 A1

Published:

— *with international search report (Art. 21(3))*

METHOD FOR DETERMINING THE CARDIO-GENERATIVE POTENTIAL OF MAMMALIAN CELLS

Field of the invention

The present invention relates to the treatment of heart disease disorders through injection of mammalian cells. In particular, it relates to a method for quantitatively assessing the cardio-generative potential of mammalian cells, thereby allowing a good predictability of the success of repairing a heart in need. It also relates to a method for quantitatively assessing the modification of this cardio-generative potential and the cardiogenic potential of a treatment aiming at cellular differentiation, and a computer device comprising a processor, and a memory encoding one or more non-neural network programs coupled to the processor, wherein said programs cause the processor to perform a method, said method comprising calculating a CARPI.

State of the art

Cardiovascular diseases are leading cause of morbidity and mortality worldwide, despite advances in patient management. In contrast to tissues with high reparative capacity, heart tissue is vulnerable to irreparable damages. Cell-based regenerative cardiovascular medicine is now being pursued in the clinical setting to address heart disease disorders.

Recent advent of stem cell biology extends the scope of current models of practice from traditional palliation towards curative repair. Typically, clinical experience has been based on adult stem cells delivered in an unaltered state. First generation biologics are naive human stem cells, identified as readily accessible cytotypes. It has been shown that a few individuals improve on delivery of naive human stem cells. The state of the art in the field of naive cell transplantation in the heart of humans was described inter alia in the review carried by Abdel-Latif A. et al. '*Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis.*' Arch Intern Med. (2007) 167:989-997, and citations therein.

To improve clinical outcome, second-generation stem cell therapies were developed to guide naive human stem cells towards the cardiac lineage prior to injection into the patient. In the review by Behfar et al. '*Guided stem cell cardiopoietic: Discovery and translation*' J. Mol. and Cell. Cardiology (2008) 45: 523–529, the concept of using cardiac precursor cells, such as cardiopoietic cells, for heart regeneration was discussed.

Cardiopoietic cells have a unique phenotype: they are characterized by nuclear translocation of Nkx2.5 and MEF2C polypeptides, combined to the absence of detectable sarcomeric proteins. This cardiopoietic status corresponds to an intermediate cell phenotype, i.e. committed to the cardiac lineage but not yet fully differentiated. Non-detectable level of sarcomeric protein expression is a unique feature of cardiopoietic cells which distinguishes them from contractile and sarcomeric-containing cardiomyocyte-like cells derived from stem cells and described in other applications such as by Chunhui Xu (US 2005/0164382) and Lough et al (US 2002/0061837).

Increased protein content of a transcription factor may not imply its subcellular localization, which could be either cytoplasmic or nuclear. Nuclear translocation of Nkx2.5 and MEF2C polypeptides is necessary for definitive cardiac lineage commitment. This is further explained in Behfar A. et al, (*Derivation of a cardiopoietic population from human mesenchymal stem cells yields cardiac progeny, Nature Clinical Practice, 2006, 3:S78-S82)*. Although nuclear translocation may be qualitatively observed by immunocytochemistry or immunohistochemistry, techniques such as western blotting or Fluorescence Activated Cell Sorting (FACS) that look at total protein content are not suitable for quantitative assessment of the subcellular distribution of a polypeptide. The observation of subcellular distribution of a polypeptide, as described in US 2008/0019944, is not only qualitative but also time-consuming in the industrial perspective and operator-dependent. Thus clinical outcome, i.e. the cardio-generative potential of these "first-generation" naive stem cells and "second-generation" guided stem cells could not be readily predicted prior to injection.

A method to quantitatively assess the cardio-generative potential of mammalian cells remained to be proposed.

The present invention now provides such a predictive method for determining the cardio generative potential of mammalian cells which comprises the quantitative assessment of a CARdiac generation Potential Index (CARPI) as a function of the quantification of the expression of genes of said cells. It also addresses the quantitative assessment of the modification of the cardio generative potential of

mammalian cells and the cardiogenic potential of a treatment aiming at cellular differentiation.

Definitions

Within the frame of the present document, and unless indicated to the contrary, the terms designated below between quotes have the following definitions.

The '*cardio-generative potential*' of a cell designates the ability of this cell to succeed to generate heart cells, for instance cardiac myocytes.

'*Cardiopoietic cells*' are an intermediate cell phenotype, i.e. committed to the cardiac lineage but not yet fully differentiated. Cardiopoietic cells are characterized by nuclear translocation of Nkx2.5 and MEF2C, combined to the absence of detectable sarcomeric proteins (*Behfar et al. 'Derivation of a cardiopoietic population from human mesenchymal stem yields progeny', Nature Clin. Pract., Cardiovasc. Med. (2006) 3: S78–S82*). Cardiopoietic cells retain a proliferative capacity. Cardiopoietic cells can be derived from stems cells including for example, human adult mesenchymal stem cells (*Terzic et al. US 2008/0019944)*, mouse embryonic stem cells (*Behfar et al, 'Cardiopoietic programming of embryonic stem cells for tumour-free heart repair' J Exp Med 2007 204: 405-420*), embryonic-like stem cells, inducible pluripotent stem cells, umbilical cord blood cells, resident cardiac stem cells and the like, or any other adapted source (provided their production implies no human embryo destruction).

A '*cocktail*' or '*cardiogenic cocktail*' designates a composition containing at least two cardiogenic substances.

A '*cardiogenic treatment*' is a treatment which improves the cardio-generative potential of a cell. Example of such treatment consists in putting said cell in contact with a cocktail. Examples of such cocktails comprise at least two substances selected in the group consisting of growth factors, cytokines, hormones and combinations thereof. Said at least two substances may be selected in the group consisting of bone morphogenetic proteins (BMP) such as BMP-1, BMP-2, BMP-5, BMP-6; epidermal growth factor (EGF); erythropoietin (EPO); fibroblast growth factors (FGF) such as FGF-1, FGF-4, FGF-5, FGF-12, FGF-13, FGF-15, FGF-20; granulocyte-colony stimulating factor (G-CSF); granulocyte-macrophage colony stimulating factor (GM-CSF); growth differentiation factor-9 (GDF-9); hepatocyte growth factor (HGF); insuline-like growth factor (IGF) such as IGF-2; myostatin (GDF-8); neurotrophins such as NT-3, NT-4, NT-1 and nerve growth factor (NGF); platelet-derived growth factor (PDGF) such as PDGF-beta, PDGF-AA, PDGF-BB; thrombopoietin (TPO); transforming growth factor alpha (TGF-α); transforming growth factors β (TGF- β) such as TGF-β1, TGF-β2, TGF-β3; vascular

endothelial growth factor (VEGF) such as VEGF-A, VEGF-C; TNF-α; leukemia inhibitory factor (LIF); interleukin 6 (IL-6); retinoic acid; stromal cell-derived factor-1 (C SDF-1); brain-derived neurotrophic factor (BDNF); periostin; angiotensin II; Flt3 ligand; glial-derived neurotrophic factor; heparin; insulin-like growth factor binding protein-3; insulin-like growth factor binding protein-5; interleukin-3; interleukin-8; midkine; progesterone; putrescine; stem cell factor; Wnt1; Wnt3a; Wnt5a; caspase-4; chemokine ligand 1; chemokine ligand 2; chemokine ligand 5; chemokine ligand 7; chemokine ligand 11; chemokine ligand 20; haptoglobin; lectin; cholesterol 25-hydroxylase; syntaxin-8; syntaxin-11; ceruloplasmin; complement component 1; complement component 3; integrin alpha 6; lysosomal acid lipase 1; β-2 microglobulin; ubiquitin; macrophage migration inhibitory factor; cofilin; cyclophillin A; FKBP12; NDPK; profilin 1; cystatin C; calcyclin; stanniocalcin-1; PGE-2; mpCCL2; IDO; iNOS; HLA-G5; M-CSF; angiopoietin; PIGF; MCP-1; extracellular matrix molecules; CCL2 (MCP-1); CCL3 (MIP-1α); CCL4 (MIP-1β); CCL5 (RANTES); CCL7 (MCP-3); CCL20 (MIP-3α); CCL26 (eotaxin-3); CX3CL1 (fractalkine); CXCL5 (ENA-78); CXCL11 (i-TAC); CXCL1 (GROα); CXCL2 (GROβ); CXCL8 (IL-8); CCL10 (IP-10); and combinations thereof.

A '*cocktail-guided cell*' or a '*cell guided towards cardiac differentiation*' is a cell which has been put into contact with a cocktail.

'*Differentiation*' is the process by which a less specialized cell becomes a more specialized cell.

'*Ejection fraction*' means the fraction of blood pumped out during a heartbeat. Without a qualifier, the term ejection fraction refers specifically to that of the left ventricle (left ventricular ejection fraction or LVEF).

As used in the subject specification, the singular forms '*a*', '*an*' and '*the*' include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to '*a stem cell*' includes a single cell, as well as two or more cells; reference to '*an agent*' or '*a reagent*' includes a single agent or reagent, as well as two or more agents or reagents; reference to 'the invention' or 'an invention' includes single or multiple aspects of an invention; and so forth.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below.

In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Summary of the invention

The invention provides a method for determining the cardio-generative potential of mammalian cells or cardiogenic potential of a treatment which comprises the assessment of a CARdiac generation Potential Index (CARPI) as a function of the quantification of the expression of genes of said cells.

Preferably, the CARPI is a function of the quantification of messenger RNA (mRNA) levels of specific genes of said cells.

Preferably, at least one gene is chosen from the group consisting of Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1, FOG2, Flk1, homologues thereof in mammals and combinations of these genes. The cells may be cardiac progenitor cells. They may also be somatic, germ, umbilical cord blood, cardiac progenitor, embryonic, and/or genetically modified cells.

In some cases, the cells can belong to one individual, and a CARPI can be assessed for those cells before and after exposing the cells to any cardiogenic treatment.

In another embodiment, a CARPI is assessed for cells of an individual or group of individuals *versus* another individual or group of individuals.

In a method particularly preferred, the CARPI is a multivariate equation where the expression of genes at the mRNA level is quantified as variables.

The equation is preferably chosen from the group consisting of polynomials functions, transcendental functions, and combinations thereof.

In a particular embodiment of a method provided herein a CARPI is measured to quantitatively assess the cardiogenic potential of a treatment.

According to one embodiment of a method provided herein, the CARPI may be put into correlation with a parameter of cardiac function.

The invention also relates to a computer device comprising a processor, and a memory encoding one or more programs coupled to the processor, wherein the one or more programs cause the processor to perform a method, said method comprising calculating a CARPI.

Brief Description of the Drawing

Fig. 1 shows in Y ordinate the CARPI, in arbitrary units (AU), calculated for both naive human MSC (hMSC) and cocktail guided-hMSC (CP-hMSC) on the basis of quantification of the expression of genes at the mRNA level and in X ordinate the change of LVEF (ΔEF) in percent prior and after injection in mouse infarcted hearts. Black symbols represent individual data; open symbols represent averaged data (Avg).

Detailed description of the invention

Example 1

Bone marrow samples were harvested from patients undergoing coronary artery bypass for ischemic heart disease. Patients provided informed consent, as approved by competent Institutional Ethics Committees.

Mesenchymal stem cells were recruited by plating of raw bone marrow on plastic dishes, with a wash at 12h, selecting adhesive cells with identity confirmed by Fluorescence-Activated Cell Sorting (FACS) analysis using the $CD34^-/CD45^-/CD133^+$ marker panel. Cells were further cultured and expanded at 37° C in DMEM supplemented with 5% human platelet lysate (Mayo Clinic Blood Bank, Rochester, MN).

Naive human bone marrow-derived mesenchymal stem cells were cultured in either platelet lysate or serum supplemented with a cardiogenic cocktail consisting in TGFβ-1 (2.5 ng/ml), BMP4 (5 ng/ml), FGF2 (5 ng/ml), IGF-1 (50 ng/ml), Activin-A (10 ng/ml), Cardiotrophin (1 ng/ml), α-thrombin (1 U/ml), and Cardiogenol C (100 nM) in order to derive a cardiopoietic population.

The present invention allows the quantitative assessment of the cardio-generative potential of said cardiopoietic population, by quantifying the expression of two or more genes at the RNA level. This invention obviates the problems of qualitative observations, issue of time, and operator-dependence, inherent to the observation of subcellular location of transcription factor polypeptides. One method of choice is real-time quantitative reverse transcription polymerase chain reaction (qPCR). This method gives faster results (within one day) that are operator-independent and quantified relative to a reference standard. In addition, while immunostained samples require

one-by-one fluorescent microscopy evaluation, up to 48 different samples (or conditions) can be tested in duplicate by qPCR using 96-well plates.

In order to identify suitable markers for qPCR, mRNA was extracted from cardiopoietic cells that were evaluated by immunofluorescence staining.

The reference standard consisted of cells from the same batch cultured in the absence of the cardiogenic cocktail.

Genes listed in Table 1, which are representative of cardiac transcriptional activity were evaluated.

qPCR was performed using a TaqMan PCR kit with an Applied Biosystems 7,900HT Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan Gene Expression reactions were incubated in a 96-well plate and run in triplicate. The threshold cycle (C_T) was defined as the fractional cycle number at which fluorescence passes a fixed threshold. TaqMan C_T values were converted into relative fold changes determined using the $2^{-\Delta\Delta C}{}_T$ method, normalized to a housekeeping gene expression, i.e. *GAPDH* (P/N 435,2662-0506003).

Results for treated cells were normalized to results obtained for the corresponding reference standard.

A CARPI, which is a function of the quantification of the expression of two or more genes of said cells, was calculated as a linear average of the expression at the RNA level of Nkx2.5, Tbx-5, MEF2C, GATA-4, GATA-6, MESP-1 and FOG-1 using a calculation spreadsheet (Microsoft Excel 2007®, Microsoft Corporation). The following formula was used:

$$CARPI = \frac{1}{n}\sum_{i=1}^{n} RNA\ level_i$$

where '*i*' represents the selected gene and 'n' represents the total number of genes selected, with a minimum of 2. In this particular example, n=7.

The cardio-generative potential of hMSC-derived cardiopoietic cells was evaluated in nude, immunocompromised mice (Harlan, Indianapolis, IN). The protocol was approved by the competent Institutional Animal Care and Use Committee.

Myocardial infarction was performed. Following a blinded design, one month post-infarction a total of 600,000 total viable naive hMSC or 600,000 total viable hMSC-derived cardiopoietic cells, suspended in 12.5 µl of platelet lysate-free propagation medium, were injected under microscopic visualization in five epicardial sites on the anterior wall of the left ventricle (2.5 µl per injection site).

Left ventricular function and structure were serially followed by transthoracic echocardiography (Sequoia 512; Siemens, Malvern, PA and VisualSonics Inc, Toronto, Canada). Left ventricular ejection fraction (LVEF, %) was calculated as [(LVVd - LVVs)/LVVd] × 100, where LVVd is left ventricular end-diastolic volume (μl), and LVVs is left ventricular end-systolic volume (μl).

A change of LVEF (ΔEF) was calculated as the difference between LVEF measured one month after cell injection and LVEF measured prior to cell injection.

Fig. 1 is a graph plotting the CARPI for each individual cell culture against the corresponding ΔEF for the mouse injected with the respective said individual cell culture. Naive hMSC (small black diamonds) typically demonstrated a low CARPI associated with no significant improvement in myocardial function (negative ΔEF) one month post-cell injection. It is worth noting rare batches of naive hMSCs innately possessing high CARPI value together with an innate regenerative potential. The average for all batches of naive hMSCs is shown by a large white triangle. hMSC-derived cardiopoietic cells (small black squares) typically demonstrated an elevated CARPI associated with robust increase in myocardial function (positive ΔEF). The average for all batches of hMSC-derived cardiopoietic cells is shown by a large white square. Averages are represented together with the corresponding 95% confidence interval.

Thus, the inventors demonstrate that there is a positive correlation between an elevated CARPI of the cells to be injected and the change in ejection fraction after injection in the infarcted heart. Thus, the CARPI is a predictive index of cardio-generative potential.

Table 1

Applied Biosystems Assay ID	Gene name	Gene symbol
Hs00231763_m1	-Homeobox transcription factor or -NK2 transcription factor related, locus 5	Nkx2.5 or NKX2-5 or NKX2.5
Hs00171403_m1	-Zinc finger cardiac transcription factor or -GATA binding protein 4	GATA-4 or, GATA4 (AB)
Hs00231149_m1	-Myocyte enhancer factor 2C	MEF2c or MEF2C
Hs00361155_m1	-T-box transcription factor or -T-box 5	Tbx5 or TBX5
Hs00542350_m1	-GATA co-factor (“Friend of GATA”) or -zinc finger protein, multitype 1	FOG 1 of FOG-1 or FOG1
Hs00251489_m1	-Helix-loop-helix transcription factor -Mesoderm posterior 1 homolog (mouse) (AB)	Mesp1 or MESP1
Hs00232018_m1	-GATA binding protein 6 (AB)	GATA-6 or GATA6
Hs00911699_m1	-Kinase insert domain receptor (a type III receptor tyrosine kinase)	Flk-1, or FLK1 or KDR

Example 2

Similar results have been observed by treating stem cells with a cocktail containing recombinant TGFβ-1(2.5 ng/ml), BMP4 (5 ng/ml), Activin-A (5 ng/ml), FGF-2 (10 ng/ml), α-thrombin (1 U/ml), IGF-1 (50 ng/ml), Cardiotrophin (1 ng/ml) and Cardiogenol C (100 nM) used in a combinatorial fashion.

Example 3

Similar results have been observed by treating stem cells with a cocktail containing recombinant TGF-β1 (2.5 ng/ml), BMP-4 (5 ng/ml), Activin-A (5 ng/ml), FGF-2 (10 ng/ml), α-thrombin (1 U/ml), IGF-1 (50 ng/ml), IL-6 (100 ng/ml) and retinoic acid (1 μM) used in a combinatorial fashion.

Other embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS:

1. A method for determining the cardio-generative potential of cardiopoietic cells, said method comprising the steps of:

a) collecting the expression level in cardiopoietic cells of the following genes: Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1 or homologues thereof;

b) determining a CARdiac generation Potential Index (CARPI), the CARPI being calculated as an average of the quantified expression levels of said genes of said cells;

wherein said expression of genes is quantified at the level of messenger RNAs (mRNAs), functional RNAs, or a combination thereof.

2. The method according to claim 1, wherein the functional RNAs are microRNAs.

3. The method according to claim 1 or claim 2, wherein said cells are selected from the group consisting of somatic, germinal, umbilical cord blood, cardiac progenitors, embryonic cells, and combinations thereof.

4. The method according to any one of claims 1 to 3, wherein said cells are genetically modified.

5. The method according to any one of claims 1 to 4, wherein said cells contain no detectable sarcomeric proteins.

6. The method according to any one of claims 1 to 5, wherein the CARPI is assessed for said cells before and after any cardiogenic treatment.

7. The method according to claim 6, wherein the cardiogenic treatment comprises exposing said cells to a cocktail containing cardiogenic substances.

8. The method according to any one of claims 1 to 7, wherein said cells belong to one individual mammal.

9. The method according to any one of claims 1 to 8, wherein said cells are from an individual mammal or group of individual mammals and the CARPI is assessed and

compared to the CARPI of cells belonging to another induvial mammal or group of individual mammals.

10. The method according to any one of claims 1 to 9, wherein the CARPI is calculated as a linear average of the expression levels of said two or more genes using the formula:

$$CARPI = \frac{1}{n}\sum_{i=1}^{i=n} RNAlevel_i$$

, where i represents the selected gene and n represents the total number of genes selected.

11. The method according to any one of claims 1 to 10, wherein the CARPI is measured to quantitatively assess the cardiogenic potential of a treatment.

12. The method according to any one of claims 1 to 11, wherein the CARPI is put into correlation with a parameter of cardiac function.

13. A computer device comprising a processor, and a memory encoding one or more non-neural network programs coupled to the processor, wherein said programs cause the processor to perform a method, comprising the steps of:

a) collecting the expression level in cardiopoietic cells, of the following genes: Nkx2.5, Tbx5, MEF2C, GATA4, GATA6, Mesp1, FOG1 or homologues thereof;

b) determining a CARdiac generation Potential Index (CARPI), the CARPI being calculated as an average of the quantified expression levels of said genes; and

c) displaying the CARPI;

wherein said expression of genes is quantified at the level of messenger RNAs (mRNAs), functional RNAs, or a combination thereof.

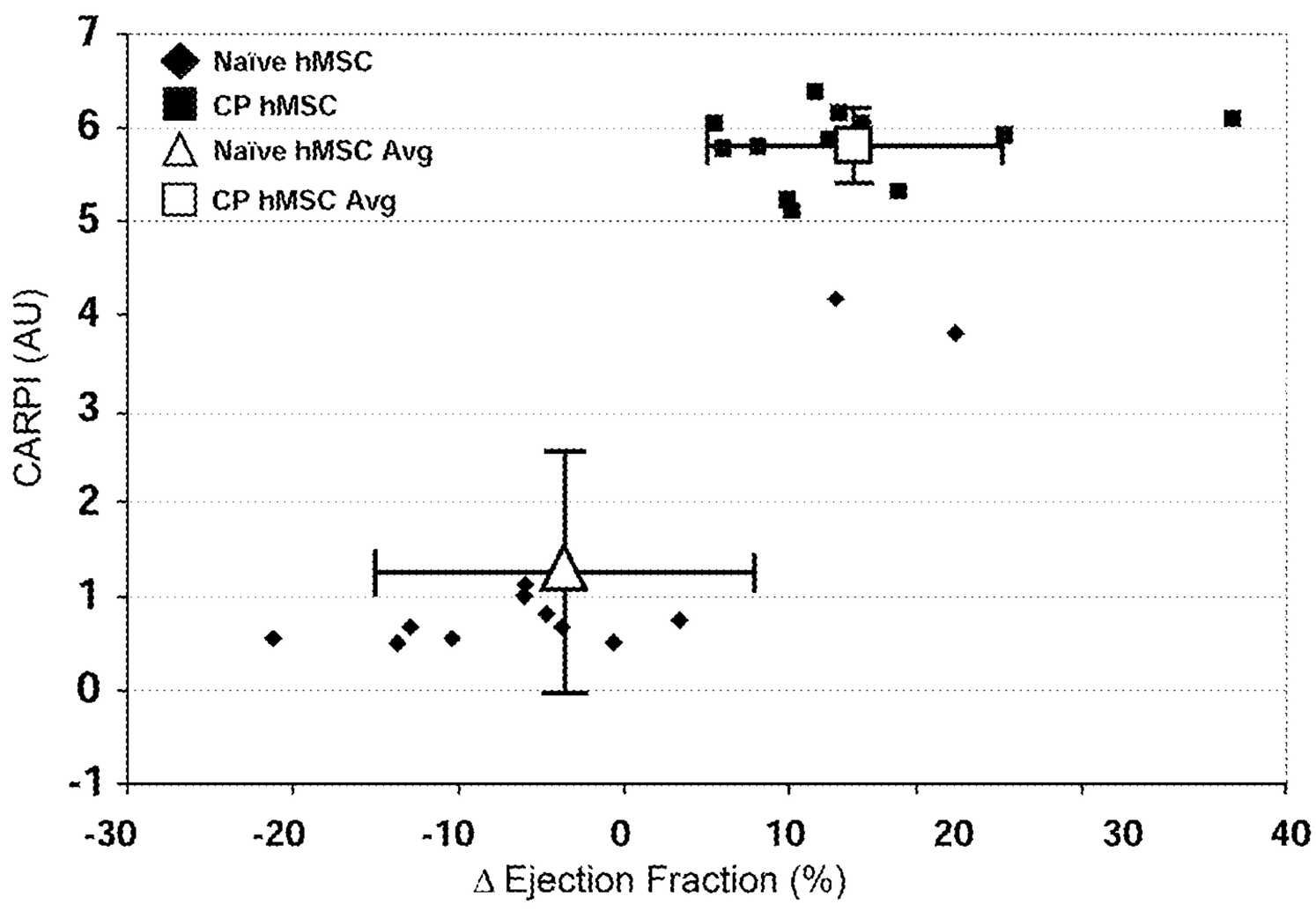
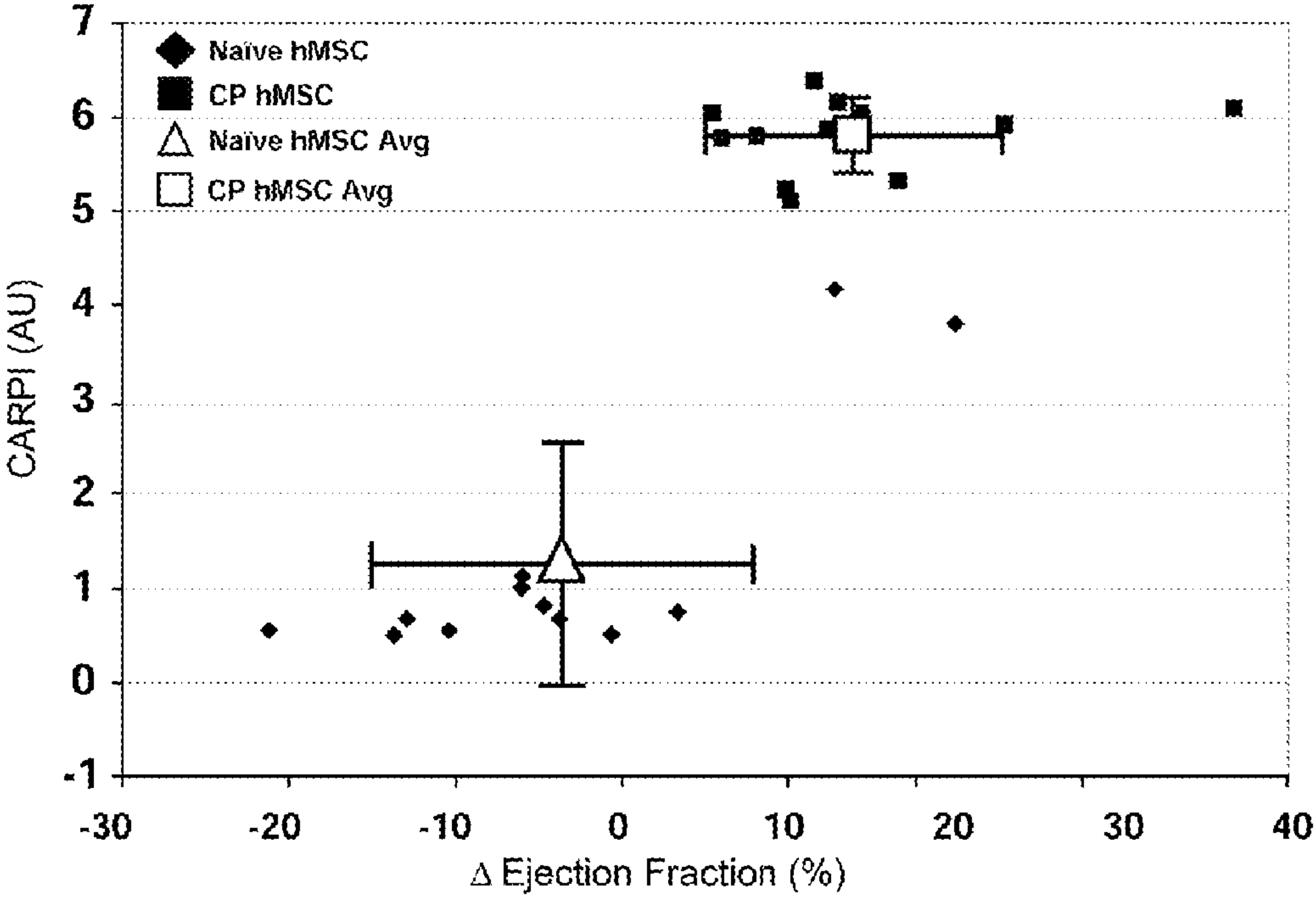

Naïve hMSC
CP hMSC
Naïve hMSC Avg
CP hMSC Avg
CARPI (AU)
Δ Ejection Fraction (%)
7
6
5
4
3
2
1
0
-1
-30
-20
-10
0
10
20
30
40

Fig. 1


Naïve hMSC
CP hMSC
Naïve hMSC Avg
CP hMSC Avg
CARPI (AU)
7
6
5
4
3
2
1
0
-1
-30
-20
-10
0
10
20
30
40
Δ Ejection Fraction (%)