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ENDOTHELIAL PROGENITOR CELLS IN CELL-BASED THERAPY FOR CARDIOVASCULAR DISEASE

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Abstract – Coronary Artery Diseases (CAD) is the first mortality cause in industrialized countries. The possibility of regenerating myocardium injured tissue using the cell therapy is a promising option to regenerate cardiac tissue. Currently, a variety of adult stem/ progenitor cells are undergoing clinical evaluation, but it is very important to study and characterize the bone marrow-derived progenitor/ stem cells, the main source of cells used for human cardiac repair, before their clinical use. Bone marrow-derived endothelial progenitor cells (EPC) home sites of ischemia and differentiate into endothelial cells, increase the neovascularization of ischemic tissue. Moreover recently, it has been observed that EPC can be able to differentiate or transdifferentiate to like-adult cells resident in cardiac tissues. The characterization of phenotype EPC is complex, because express hematopoietic stem cells (CD133 and/ or CD34) and endothelial markers such as vascular endothelial growth factor receptor 2 (KDR). Several studies described subpopulation of EPC expressing *CD34+D133+KDR+* phenotype in literature, but some other authors suggest other phenotype. The EPC capacity of mobilization or recruitment/ homing to ischemic tissue areas by cytokines are reviewed. Finally are described clinical studies in CAD using bone marrow-derived progenitor cells permitting human cardiac tissue repair.

Key words: EPC: Endothelial progenitor cells; CD: cardiovascular disease; RM: regenerative medicine; AGN: angiogenesis.

INTRODUCTION

Vessel proliferation is under stringent control in healthy people and only occurs during: embryonic development, endometrial regulation, the reproductive cycle and lesion repair. Vasculogenesis was originally defined as the process of vascularization during embryogenesis based on bone marrow-derived stem or progenitor cells, including endothelial progenitor cells (EPC) or angioblasts.

Abbreviations: AMI: Acute Myocardial Infarction; **CAD**: Coronary Artery Diseases; **CFU-EC**: Colony Forming Unit Endothelial Cells; **EPC**: Endothelial Progenitor Cells; **HF**: Heart Failure; **HGF**: Hepatocyte Growth Factor; **VEGF**: Vascular Endothelial Growth Factor.

Angiogenesis is the generation of new capillaries from pre-existing microvessels and occurs during postnatal life. Postnatal neovascularization was believed to result from the proliferation, migration, and remodeling of fully differentiated endothelial cells derived from preexisting blood vessels. However, EPC have been described as being involved in angiogenesis (6, 46) .Vasculogenesis involves EPC that differentiate into mature endothelial cells, whereas angiogenesis involves mature endothelial cells and EPC. Neovascularization, which is vital to the healing of damaged tissue, including cardiac tissue after myocardial infarction, involves both angiogenesis and vasculogenesis (62, 68, 91-95).

Circulating bone marrow-derived stem/progenitor cells have an important role in the normal physiological maintenance of the

body's vasculature. However, bone marrow-derived EPC can also participate in pathological processes contributing to the formation of new vessels in ischemic tissue in the context of several pathologies, such as tumor neoangiogenesis, retinopathies or postnatal liver regeneration after hepatic injury (20, 48,43, 86). Several angiogenic factors have been identified as being involved in vasculogenesis and angiogenesis. Physiological angiogenesis is regulated by the balance between proangiogenic/activators like VEGF (vascular endothelial growth factor), bFGF (basic Fibroblastic Growth Factor), HGF (Hepatocyte Growth Factor), etc… and antiangiogenic/inhibitors growth factors such as endostatin, angiostatin, TSP-1 (thrombospondin-1), etc... (Table I). This balance is shifted under pathological conditions, such as cancer, towards a proangiogenic phenotype that promotes angiogenesis and the increase in tumor neovascularization associated with progression and worse prognosis in cancer patients (28,34,88).

Table I Endogen molecules implicated in the physiopathological angiogenesis process

Activators	Inhibitors
VEGF	
Angiopoietin-1 y 2	
Fibroblastic Growth Factor (bFGF y a FGF)	
PD-ECGF	Trombospondin-1
TGF α and β	Angiostatin
HGF	Endostatin
EGF	Angiopoietin-2
TNFα	Interferons α, β and γ
Angiogenin	PF-4
Interleucin-8	Interleucin-12
Vascular Integrin αvβ3	Vasostatin
Matrix Metallopeptidase	TIMP
Platelet-activating Factor	
PIGF	
G-CSF	
Prostaglandins (E1 and E2)	

VEGF, Vascular Endothelial Growth Factor; HGF, Hepatocyte Growth Factor; PIGF, Placental Growth Factor; TGF, Transforming Growth Factor; PD-ECGF, Platelet Derived-Endothelial Cell Growth Factor; EGF, Epidermic Growth Factor; TNFα, Tumor Necrosis Factor alpha; PIGF, Placental Growth Factor; G-CSF, Granulocyte-Colony Stimulating Factor; PF-4, Platelet Factor 4; TIMP, Tissue Inhibitor of Metallopeptidase.

Although several growth factors have been identified as influencing angiogenesis, VEGF is the main activator of angiogenesis known to date. EPC mobilization from bone marrow and recruitment by ischemic tissues have been positively correlated with an increase in circulating VEGF levels, but other cytokines, such as stromal cell-derived factor 1 (SDF-1) and angiopoietin-1, can also be involved (6,60,84).

Endothelial Progenitor Cell Phenotype

The definition of EPC is complex due to the lack of a single specific marker and, thus, different cell populations are termed EPC. The term EPC may define a group of cells existing in a variety of stages ranging from hemangioblasts to fully defined adult endothelial cells (41,75). The hemangioblast is a common precursor for both hematopoietic stem cells (HSC) and EPC. Although the existence of hemangioblasts was hypothesized as a transient subpopulation in the hematopoietic system, restricted to embryonic development, it was later identified in postnatal contexts (50, 70). Li et al, have recently described the generation of large numbers of hemangioblasts from human embryonic stem cells using an ***in vitro*** differentiation system based on a serum-free media culture system supplemented by a combination of several morphogens and hematopoietic cytokines and growth factors, including VEGF (49). The generated hemangioblasts can differentiate into multiple hematopoietic lineages and into endothelial cells, and these hemangioblasts can participate in ischemia tissue repair and reduce the mortality rate after myocardial infarction in animal models.

The immunocytochemical and Genechip studies of hemangioblasts generated ***in vitro*** from human embryonic stem cells demonstrate the expression of hematopoietic lineage markers (CD45+, CD13+ and CD235a+), but not the expression of typical endothelial markers such as KDR or CD31. However, when these cells were induced to differentiate ***in vitro***, they expressed endothelial markers (KDR, VE-Cadherin and CD31) (45, 69, 89, 92) (Figure 1).

Two different EPC subpopulations have also been described in relation to primitive hematopoietic progenitor marker CD133/AC133 expression, denoted as early and late EPC (Figure 1). Early EPC originates from hemangioblast acquired hematopoietic cell marker CD133/AC133. It is considered as an immature marker from early endothelial progenitor cells. When the early EPC is involved in ischemic tissues, it lacks several immature hematological antibodies (CD133) but it acquires molecular specific markers from endothelial cell as the VE-Cadherin and E-selectin markers (Table II) (15, 45, 71). Early EPC reach peak growth in culture after 2-3 weeks and secrete a number of angiogenic activators, growth factor inhibitors and neuroregulatory cytokines, expressing the surface markers CD34+ CD133+/- and KDR+. However, late EPC appear in culture after 2-3 weeks and can be maintained in culture up to 12 weeks and on surface expressing CD34+/-

CD133- KDR+ and typical markers of EC, such as VE cadherin, vWF or E-selectin (14, 75).

Endothelial progenitor cells in human adults are a rare cell subpopulation in CD34+ cells whereas hematopoietic stem cells are found in rich sources like bone marrow, umbilical cord blood and through growth factor mobilized peripheral blood. Normal peripheral blood is receptor), vascular endothelial cadherin (VE cadherin), platelet endothelial cell adhesion molecule 1 (CD31), CD146, von Willebrand factor (vWF) and Tie-1 and Tie-2, have been used to define the EPC phenotype by flow cytometry and/or immunocytochemical techniques.

In most studies described to date, the number of EPC has been counted by flow cytometry in human bone marrow, peripheral blood and human umbilical cord blood and the number of circulating EPC measured by the same technique has been used as a biomarker of cardiovascular disease. Although most studies on rather a poor source of hematopoietic stem cells. In order to identify the EPC subpopulation, a series of surface receptors of hematopoietic stem/progenitor cells and EC cells that appear early in the development of EC from EPC, such as CD34, CD133, CD45, VEGF-receptor 1 (VEGF-R1, fms-like kinase, flt-1) and 2 (VEGF-R2, KDR, kinase insert domain EPC from 1997 onwards, when these cells have been described by Asahara et al (6) and identified as an human EPC subpopulation of cells co-expressing the CD34+CD133+KDR+ phenotype, some authors have suggested that human CD34+CD133+KDR+ cells are hematopoietic progenitor cells that do not differentiate into adult endothelial cells and do not form capillary-like structures in Matrigel assays (71, 79, 80, 101). However, a human umbilical cord blood-derived subpopulation showed that the CD34+CD45- phenotype had higher endothelial colony-forming cell activity than the CD34+CD45+ subpopulation (15).

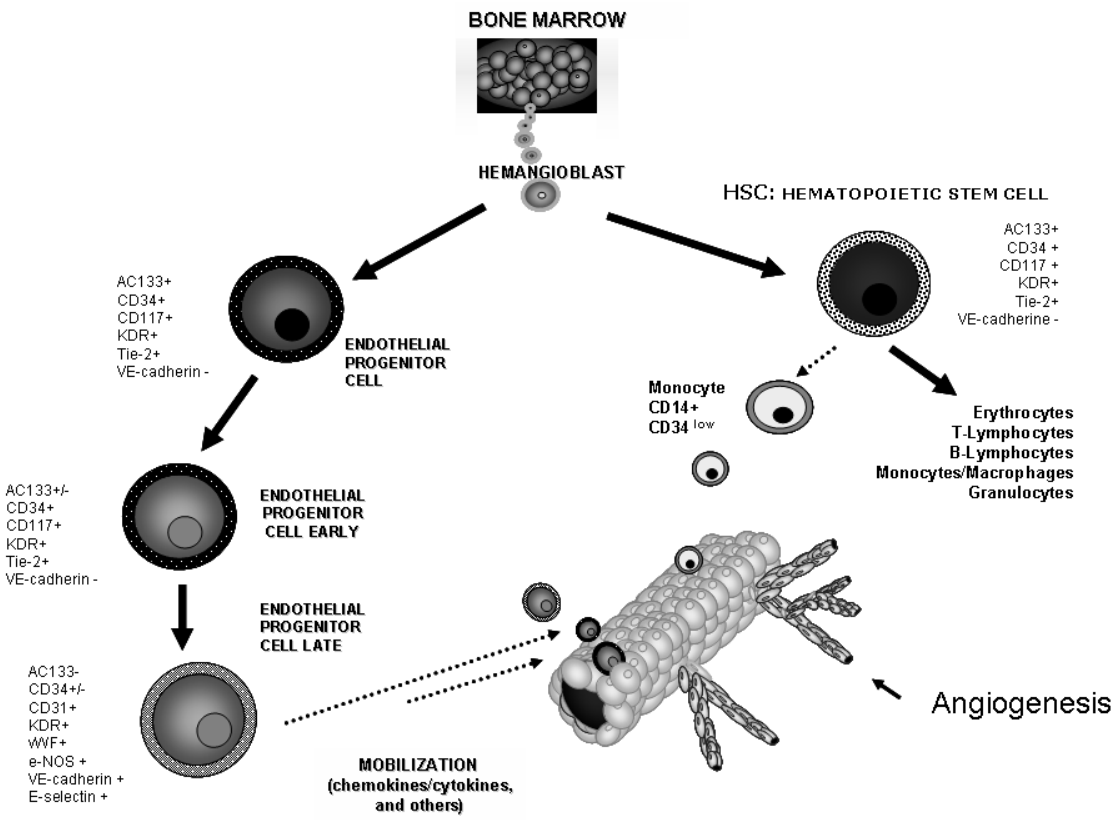

Figure 1. Expression profile and mobilization of human bone marrow-derived EPC

Table II Immunophenotype and identification of human EPC

Early circulating EPC	Late circulating EPC	EPC tissues	Reference
CD133+/- CD34+ KDR+ CD177- VE-cadherin+ vWF+ CD31+	CD133+/- CD34+ KDR+ VE-cadherin- vWF+ P1H12- CD31+ CD14+/-		(3,36)
CD133++ CD34++ KDR+	CD133+ CD34+ KDR+	CD133- CD34low KDR- CD31+ VE-cadherin+ vWF+ ("mature" EPC)	(69, 76, 82, 93, 102)
CD133+/- CD34+ KDR+ CD31+ CD14+/- vWF+ VE-cadherin- E-selectin- eNOS-	CD133 - CD34+/- KDR+ CD31+ CD14 - vWF+ VE-cadherin+ E-selectin+ eNOS+		(14)
	CD133- CD34- CD14+ CD16+ CD31+ CD105+ CD86+ CD11c+ (culture day 5)		(77)

HSC, Hematopoietic Stem Cell

Characterization of EPC in Culture

Endothelial progenitor cells have been identified by their high clonogenic and proliferative potential in appropriate cell culture. Bone marrow-derived cells or peripheral blood mononuclear cells have been isolated ***in vitro*** and can be differentiated into an endothelial cell-like phenotype when they are grown in EC culture medium, supplemented with growth factors such as VEGF, bFGF or IGF (insulin growth factor) which induce the differentiation of EPC into an EC-like phenotype.

Counting the number of EPC by measuring colony forming units (CFU-EC) has emerged as a useful technique in recent years (33,101).

The EPC ***in vitro*** acquired the ability to take up acetylated low-density lipoprotein (Dil-acLDL) and bind to *lectin Ulex europeanus agglutinin* (UEA), so that these cells are defined as double positive cells for both markers (Dil-acLDL and UEA). Endothelial progenitor cells in culture expressed the mRNA of their typical surface markers (CD133, CD34, KDR, etc) and of cytokines such as VEGF, HGF or TGF-β, and released in culture media several proangiogenic cytokines, such as VEGF, IGF-1 and SDF-1, (39, 91).

EPC in culture are identified by flow cytometry or immunocytochemical techniques using specific monoclonal antibodies to surface markers (19, 90-94). Although bone marrow and

growth factor-mobilized peripheral blood have more EPC than normal peripheral blood, normal peripheral blood is a better source for obtaining CFU-EC derived from EPC than bone-marrow-derived EPC or mobilized peripheral blood-derived EPC, suggesting that different blood sources have different CFU-EC formation potential for EPC (89).

Moreover, Romagnani et al described a circulating $CD34^{low}CD14+$ subpopulation (typical monocyte phenotype) that can be a new source of EPC, because these cells proliferate in response to stem cell factor and can differentiate ***in vitro*** into endothelial cells, but can also differentiate in culture into other adult cells, such as osteoblasts, adipocytes or neural cells (77). It has recently been observed that isolated human peripheral blood mononuclear cells cultured in an EPC culture medium can produce colonies similar to EPC colonies, and display a typical monocyte/macrophage phenotype (CD45+CD14+ and lysozyme). Moreover, when cultured in EPC culture media supplemented with IGF (insulin growth factor), VEGF and bFGF, isolated human monocytes can produce "typical EPC colonies", showing that EPC can be derived from a monocyte-macrophage cell lineage (51,52).

Endothelial Progenitor Cells and Cardiovascular Disease

Vascular trauma and tissue ischemia induce the production and release of several cytokines, including several proangiogenic growth factors, such as VEGF and bFGF, that potentiate mobilization from bone marrow progenitor cells, such as EPC (27), and regulate the recruitment and homing of several circulating inflammatory cells and of EPC to the site of damaged vascular tissue or ischemic tissue areas, promote angiogenesis and vasculogenesis and accelerate vascular or tissue wound healing. A decrease in the number and the functionality of circulating EPC has been associated with cardiovascular disease and several cardiovascular risk factors, such as circulating lipid levels, hypertension, diabetes mellitus or smoking habit (82). The same pronostic of population is connected with the free survival of events (102). The mobilization of EPC to the periphery has been observed in patients after acute myocardial infarction (82), instable angina (25), stable angina (21) and almost all processes related to chronic systemic process and chronic heart failure (93).

In animal models, circulating human EPC isolated from peripheral blood and injected intravenously into athymic nude rat after experimental myocardial infarction, predominantly home to the infarction border, although EPC can also be localized in other organs, such as the spleen or liver (2).The mobilization of EPC from bone marrow by growth factors and/or cytokines produced by ischemic tissues (as occurs in the infarcted area of myocardial tissues) or by the exogenous administration of growth factor (G-CSF, granulocyte colony-stimulating factor; erythropoietin, etc..) or cytokines (VEGF, SDF-1, etc..) that can mobilize progenitor cells from bone marrow to peripheral blood, may contribute to the repair of damaged vessels or tissues mainly via vasculogenesis and/or angiogenesis mechanisms (60-63). Several clinical trials using granulocyte monocyte colony-stimulating factor (GM-CSF) and results there obtaining was different. (35).

On the other hand, ***in vitro*** models have demonstrated that progenitor cells of human origin cocultivated with rat cardiomyocytes can transdifferentiate into cells expressing cardiac genotypes and phenotypes (73). It is well known that isolated human EPC differentiate into endothelial cells when cultured in a medium supplemented with the appropriate cytokines. It has recently been reported that human CD133+ cells isolated by a clinical-grade immunoselection method from umbilical cord blood can differentiate in vitro into EC and cardiomyocyte-like cells (13).

The mechanisms by which adult EPC acquire the cardiomyocyte phenotype are not well determined. Several mechanisms including differentiation, transdifferentiation and fusion have been proposed as possible mechanisms (30). Human EPC isolated as well from healthy subjects as from coronary artery disease patients can also transdifferentiate ***in vitro*** into functionally active cardiomyocytes (9). The coculture of human EPC isolated from peripheral blood with neonatal or adult rat cardiomyocytes, with the aim of mimicking the cardiac microenvironment, triggers the expression of cardiac genes and cardiac marker proteins, such as myosin heavy chain (MHC) or troponin-I, within human EPC. The expression of specific cardiac proteins has also been observed, when rat cardiomyocytes were fixed with

paraformaldehyde, indicating the presence of some proteins and/or membrane glycoproteins necessary for human EPC to acquire the cardiomyogenic phenotype.

In coculture studies, Koyanagi et al have observed the participation of E-cadherin and the formation of intercellular nanotubular structures that allow the transport of proteins and/or organelles from the rat cardiomyocyte to the human EPC (44, 49); this communication between human EPC and rat cardiomyocyte may contribute to the acquisition of the cardiomyogenic phenotype by human EPC isolated from healthy subjects. This has been explained as the process of cell fusion thereby originating hybrid cells co-expressing cardiomyocytes and EPC markers. However, other authors have not observed evidence of human EPC transdifferentiation into cardiomyocytes using coculture methods (30).

In animal models of myocardial infarction, Murosawa et al, have demonstrated the transdifferentation "***in vivo***" of human EPC isolated from the peripheral blood of healthy subjects into cardiomyocytes (44, 45). However, these authors have observed that human EPC injected into the tail of rat, first home to the ischemic infarcted myocardial tissue, and then transdifferentiate into cardiomyocytes or endothelial or smooth muscle cells depending on the microenvironment or the niche within the myocardial tissues where EPC are localized. Although clinical studies using EPC have demonstrated moderate improvements in cardiac function (82), clinical applications employing EPC may be of promise for the future treatment of cardiovascular disease due to their potential for growth and differentiation, and vasculogenesis and angiogenesis (7,11,16,21).

Bone marrow derived progenitor cell-based therapy for cardiovascular disease patients

Coronary Artery Diseases (CAD) is the first mortality cause in industrialized countries, heart ischemia and brain vascular events being the main components of CAD. Heart failure (HF) constitutes the third mortality cause due to the prevalence of heart failure. The prevalence of heart failure grows parallelly to the increase of the life expectancy of the population. Inspite the effectiveness of the therapeutic treatments for ischemic heart diseases and of blood hypertension, there may be observed an increased population of patients with those chronic diseases. The recent improvements on the prognostic of CAD by the use of new treatments such as primary angioplasty, use of pharmacologic drugs (β-blockers, angiotensin II antagonists, anti-aldosterone drugs) or by the use of recent devices such as resynchronization therapy for the treatment of myocardium infarction or HF, respectively, have enlarged the prevalence of these CAD.

These facts lead to a concomitant rising in the sanitary economic expenses based on the high cost of these new therapies and the re-hospitalization of patients enduring a chronic disease (74). However, until now, it has been impossible to obtain a complete recuperation of the damaged myocardium tissue induced by an infarction, so that the majority of the experimented survivals of patients that have suffered it based only on so-called "palliative measures", maintaining several physiopathologic mechanisms and transforming them into chronic diseases.

In the last decenny, several works and studies (Table III) have been developed with the aim to regenerate these tissues when cell death has occurred. This approach has been named "regenerative therapy" or "regenerative medicine". The cardiology could not remain indifferent, and really, if this new growing field would not have existed, several studies would not have been developed using basic and clinical experimental designs. Most of these studies have yielded optimist results, but, nowadays there is still a long way left (19).

Several techniques have been employed to regenerate an infarcted myocardium tissue, firstly using the onset of cell transplantation and the mobilization of autologic progenitor cells, intra-coronarily via the most used approach (36, 1).

These studies (security clinical assays) showed a fair tendency to improve the ejection fraction and the myocardic perfusion, together with a lack of significant adverse events and inspite the absence of an important clinical improvement of the patients. But these results show the limitations of the phase II clinical trials (security) as well as the small sample of cases studied. In addition, the different studies found in the literature show a great heterogeneity in relationship to the studied experimental designs (chronic or acute infarction phase; presence or not of an important left ventricular dysfunction, diagnostic methods used: ventriculography,

echocardiography and resonance methods, intracoronary injection of several cell lines, inconsistent cell doses also with different inoculation moments in the acute myocardium infarction).

These incongruencies have not only been found in one assay, but have also been assumed to be generalized in several studies about cell transplantation and mobilization induced by growth factors. Although most of these hopeful results (1) show a wide security spectrum, the heterogeneity of the methods, the sample size that has been employed, the almost absence of clinically significant results and the low magnitude of the results, seem to be almost identical to the controls during the assessment

These limitations in addition to the unknown processes that mediate during the stimulation, migration and chemotaxis of autologic cells have led back from the initial euphoria to reach a reflexive and autocritic state of the concept, developing deeper basic studies and generating common criteria (66). It is advisable to base clinical trials on solid results obtained from animal research studies with relevant human applications (1, 11).

Several cell lines have been used in clinical research previously described on cell therapy. Although the plasticity of adult human stem cells still remains unclear, data proceeding from animal research show that those cells are able to differentiate into vascular or cardiac cells (31, 67). Human mesenchymal stem cells isolated from bone marrow, mononuclear cells and EPC can differentiate to cardimyocytes both ***in vivo*** and ***in vivo***.

However, since the following of the transplanted cell is difficult, their role in the improvement of the ventricular function and its remodelling is on controversy, mainly because it is still unknown it these cells remain at the same place where they have been injected, if they promote transdifferentiation and migration of stem cells to myocardium injured areas. In addition, another topics such as the optimum number of cells to inject, the exact moment and the via to do it, as well as the most effective cell line in terms of prognostic improvement of ventricle should be clarified with the development of new experimental and clinical studies. This idea remains more consistent due to the high variability observed in the studies previously described in the corresponding literature (17).

Few works have been described in literature employing EPC in cell therapy. EPC were the first stem cells injected intracoronarily in the acute phase of the infarction (TOP-CARE AMI). The patients injected with EPC showed a significant improvement of ventricle function that was of the same magnitude than latter results obtained with the injection of stem cell isolated from bone narrow (8, 80). Conversely, it has been recently described the absence of an improvement of the injection of bone narrow stem cells on ventricle function in chronic phases of the infarction, mainly due to the absence of chemokines from heart (7).

Most of the clinical trials published on cell therapy at this moment, are focused on the acute myocardium infarction. The usefulness of the cell therapy to be used in different pathologies to ischemic diseases will require the realization of experimental and clinical studies in other pathologies, a fact that nowadays they have not been investigated yet from as well a basic as from a clinical point of view.

CONCLUSIONS

Several bone–marrow-derived stem/progenitor cells, such as CD34+ stem cells, mesenchymal stem cells and EPCs, have been extensively studied as biomarkers of cardiovascular disease, and several clinical trials are currently underway to study the usefulness of cell-based therapy for repairing damaged blood vessels or cardiac tissue after myocardial infarction. To improve the possible success of EPC-based therapy for cardiovascular disease, EPC biology has to be well characterized, including a well-defined panel of surface markers for flow cytometry techniques to define the EPC subpopulation with regenerative capacity, to discover whether their mechanism of action for myocardial repair is due to putative EPC and/or if it depends from a paracrine mechanism in relation with cytokines/growth factors released in the area of myocardial infarction, including several pro- and anti-angiogenic growth factors. It is also necessary to define at what extend the cellular and molecular processes involved in EPC homing may damage myocardial tissues, including the myocardial-specific cytokines and/or chemokines, which can facilitate the selective recruitment of circulating EPC to the myocardial infarction border as well as endogenous regeneration and repair of damaged myocardial tissue.

Table III Main studies with cellular therapy in humans

N	Cell type	Numbers of cells	Clinical follow-up (months)	Way of administration	Parameters	Days of event To cellular therapy	Ref
20	BMMNC	$28 \pm 22 X\ 10^6$	3	IC	AMI	8 ± 2	(80)
69	MSC	$48\text{-}60\ X\ 10^6$	6	IC	AMI	18.4 ± 0.5	(15)
35	BMMNC (CD133)	$12.6 \pm 2.2 \times 10^6$	4	IC	AMI	11.6 ± 1.4	(10)
26	CPC	$69 \pm 14\ X\ 10^9$	3	IC	ICM	225 ± 87	(21)
22	MSC/EPC	$2\text{-}4\ X\ 10^6$	4	IC	AMI/ICM	224 ± 470	(38)
20	BMC	NR	6	IC	AM I	1	(75)
36	BMMNC	$90\ X\ 10^6$	3	IC	ICM	823.5 ± 945.5	(80)
92	BMMNC CPC	$22 \pm 11\ X\ 10^6$ (CPC), $205 \pm 110 \times 10^6$ (BMMNC)	3	IC	ICM	2348 ± 2318 (CPC) 2470 ± 2196 (BMMNC)	(7)
20	BMMNC	$40\ X\ 10^6$	6	IC	AMI	1	(23)
20	BMMNC	$60.25 \pm 31 X\ 10^6$	4	IM	ICM	217 ± 162	(31)
67	BMMNC	$172 \pm 72\ X\ 10^6$	4	IC	AMI	1-2	(36)
82	CPC	$14 \pm 5 X\ 10^8$	6	IC	AMI/ICM	7 ± 1 (AMI) 517 ± 525 (OMI)	(37)
100	BMMNC	$87 \pm 47.7\ X\ 10^6$	6	IC	AMI	6 ± 1.3	(50)
36	BMMNC	$292 \pm 232\ X\ 10^6$	3	IM	ICM	NR	(54,55)
20	BMMNC	$25.5 \pm 6.3\ X\ 10^6$	12	IM	ICM	NR	(68)
204	BMMNC	$236 \pm 174 X\ 10^6$	4	IC	AMI	4.3 ± 1.3	(76)
70	CPC	$72.5 \pm 73.3 X\ 10^6$	6	IC	AMI	7 ± 5	(47)

AMI, Acute Myocardial Infarction; BMC, Bone Marrow Cell; BMMNC, Bone marrow Mononuclear Cell; CPC, Circulating Progenitor/ Stem Cell; EPC, Endothelial Progenitor Cell; IC, Intracoronary Injection; ICM, Ischemic Cardiomyopathy; IM, Intramyocardial Injection; MI, Myocardial Infarction

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