Myocardial regeneration protocols towards the routine clinical scenario: An unseemly path from bench to bedside

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Summary

Heart failure secondary to cardiomyocyte loss and/or dysfunction is the number one killer worldwide. The field of myocardial regeneration with its far-reaching primary goal of cardiac remuscularization and its hard-to-accomplish translation from bench to bedside, has been filled with ups and downs, steps forward and steps backward, controversies galore and, unfortunately, scientific scandals. Despite the present morass in which cardiac remuscularization is stuck in, the search for clinically effective regenerative approaches remains keenly active. Starting with a concise overview of the still highly debated regenerative capacity of the adult mammalian heart, we focus on the main interventions, that have reached or are close to clinical use, critically discussing key findings, successes, and failures. Finally, some promising and innovative approaches for myocardial repair/regeneration still at the pre-clinical stage are discussed to offer a holistic view on the future of myocardial repair/regeneration for the prevention/management of heart failure in the clinical scenario.

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An introduction to regenerative medicine for cardiovascular diseases

Cardiovascular diseases (CVD), accounting for 32% of all deaths,[1] are the leading cause of death worldwide. Alongside the increasing prevalence of risk factors, morbidity and mortality for CVD have progressively increased over the last twenty years.[1] The continuous improvement in primary and secondary prevention, as well as the introduction in clinical practice of new and more efficient therapeutic strategies, have led to a significantly better prognosis for patients affected by acute CVD, with the direct consequence of steadily increasing the population at high risk to develop chronic heart failure (HF).[1]

From an etiopathogenic point of view, HF is most often the final stage of a process triggered by heart injury. Once acute or chronic myocyte injury is established, current guideline-recommended therapies can only reduce the pathologic remodelling process, slowing but not arresting the inevitable progression toward overt cardiac failure.[2] Stem cell therapy, with its potential to generate new parenchymal cells of any tissue,[3] including the cardiomyocytes (CMs), has become an attractive and highly promising treatment for heart disease and failure. Currently, research into its design and application remains at the cutting edge of biomedical research.

Historically, stem cell-based regenerative cardiology has developed mainly in two directions: **one**, based on the concept of the myocardium lacking myogenic stem cells, has focused on the transplantation of either autologous or allogeneic but cardiac exogenous stem cells (Figure 1 and Box 1); **the other**, based on the evidence that the adult myocardium harbours an endogenous regenerative potential constituted by a population of mainly dormant multipotent cardiac stem cells, has been focused on methods to harness this endogenous

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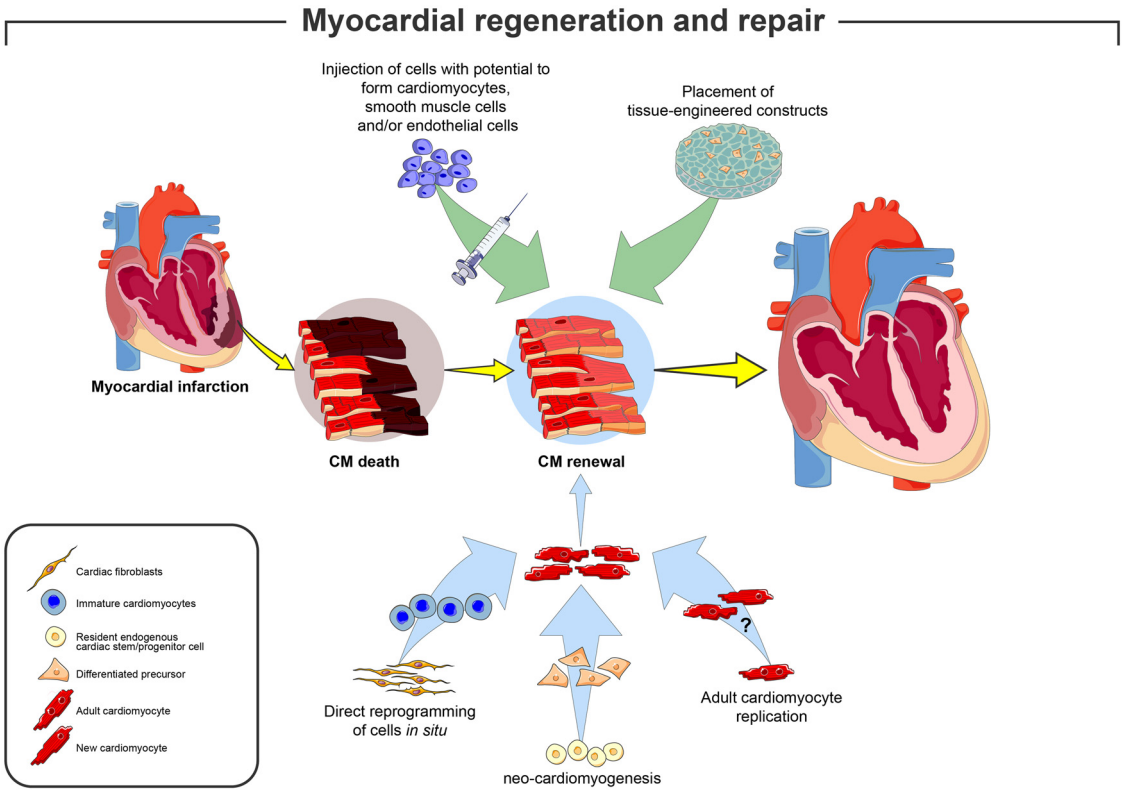

Figure 1. Potential approaches for successful myocardial remuscularization. The cartoon depicts the main approaches tested pre-clinically and clinically to obtain cardiac remuscularization. Two main approaches have been pursued: (1, top) the exogenous approach has been based on the injection of pluripotent/multipotent ex vivo expanded stem cells (ESCs, iPSCs, CSCs and their derivatives) capable to form new cardiac tissue (including new cardiomyocytes), either directly administered to the injured myocardium or through the use of engineered materials; (2, bottom) the endogenous approach has been based on the injection of cells or factors able to 'boost' the endogenous regeneration potential of the adult heart to form new cardiac muscle: the latter has been tested by the paracrine potential of allogenic/autologous stem cells or directly by regenerating factors able to activate (i) the endogenous cardiac stem progenitor cells (CSCs), (ii) the claimed proliferative potential of adult cardiomyocytes or (iii) to reprogram somatic cells like fibroblasts to acquire cardiomyocyte identity.

regenerative potential of the adult myocardium (Figure 1 and Box 1). On this premise, this review starts with the known and some controversial aspects of cardiac regenerative biology to introduce the up-to-date attempts of their clinical application through cell therapy.

The endogenous regenerative capacity of the adult heart

The long-standing paradigm of the heart as a non-regenerative organ because the cardiomyocyte, the main parenchymal cell type of the heart, is a terminally differentiated cell with no replication competence, has been dismantled by a wealth of data showing that new CMs are indeed formed throughout life in the adult mammalian heart.[4,5] It is also clear, however, that this regenerative phenomenon on its own is not robust enough to prevent post-myocardial infarction (MI) as well as non-ischemic pathologic ventricular remodelling leading to HF. Thus, augmentation of this endogenous regenerative activity has become a compelling strategy for cardiac repair and regeneration.[6] Such amplification has been pursued with the two main approaches described in Box 1.

We, together with many others, have documented that the adult heart contains a pool of resident tissue-specific endogenous multipotent cardiac stem/progenitor cells (eCSCs).[7–11] These cells have all the characteristics expected from a tissue-specific adult stem cell: self-renewal, clonogenicity and multipotency *in vitro* and *in vivo*.[10,12] *Ex-vivo* amplified eCSCs share the potential to differentiate in bona fide CMs *in vitro* and *in vivo*.[11,13] Using cellular, genetic, cell transplantation and molecular means, we established that the eCSCs are necessary and sufficient to support myocardial cell homeostasis, repair and regeneration.[8] However, using a genetic strategy with site specific recombinases (SSRs, i.e. systems that, when properly used, allow to label these stem/progenitor cells *in vivo*, and determine their

The exogenous and endogenous approaches to obtain myocardial regeneration are reflective of two very different views about the biology of the adult myocardium. The first ("*the exogenous*"), is grounded on the view that the adult myocardium is an exception among all other tissues and, in contrast to them, it lacks a physiologically relevant tissue-specific stem/progenitor cells with cardiomyogenic potential and new cardiomyocytes formation after the early post-natal period can be obtained only by transplanted exogenous sources (see Figure 1). The other ("*the endogenous*") views the heart as a low regenerative organ harboring tissue specific stem/progenitor cells, which are mostly dormant and need to be properly activated to effectively generate a significant number of cardiomyocytes needed for the repair/regeneration of the damaged myocardium (see Figure 1). The endogenous approach has been recently expanded by the claim that adult terminally differentiated cardiomyocytes unexpectedly maintain a low but still targetable proliferative capacity; the latter, however, despite having an endogenous cell target for regeneration (the cardiomyocytes) is based on the 'exogenous' biology view of the heart lacking a tissue specific stem/progenitor cell pool. These two different approaches, while based on mutually exclusive concepts of myocardial cell biology, are not mutually exclusive in practical terms and could be complementary: myocardial repair/regeneration protocols based on the stimulation of the endogenous cardiac stem/progenitor cells complemented by the transplantation of exogenous myocardial stem/precursor cells or vice versa. Unfortunately, these two different concepts of the adult myocardium and the derived respective approaches to myocardial repair have evolved throughout an unseemly and competitive path that has contraposed their respective promise while simultaneously muddling the scientific underpinnings of adult cardiac cell biology.

***Box 1*: The apparently contrasting views to design protocols of myocardial regeneration.**

developmental plasticity by permanently marking their cell progeny) to fate-map eCSCs *in vivo*, several studies have claimed that the "c-kit+/Sca-1+ (i.e., the main two markers used to detect cardiac cells enriched with *bona fide* multipotent CSCs) eCSCs" minimally contribute CMs or, more assertively, that the adult heart lacks an endogenous functional pool of myogenic precursor cell to effectively replenish CMs in the adult life.[14–18] Nevertheless, using these same genetic animal models,[14–18] we demonstrated that, as used, these specific SSRs recombine resident eCSCs very poorly (<10%), while severely affecting their myogenic and regenerative potential *in vivo* and *in vitro*.[19,20] Therefore, contrary to their claims, these cell-fate reports have failed to test the CSCs cardiomyogenic potential.

Because the generation of new CMs in adulthood had been shown by many authors and in different species, including the human, the failure of the cell-fate mapping studies to track the source of these new CMs back to CSC activation and differentiation, was swiftly followed by reports claiming that the main/sole source of adult neo-cardiomyogenesis was indeed the replication of adult and terminally differentiated CMs.[21] The latter was mainly based on a deductive approach more than on scientific experimental evidence. Indeed, because it was and continue to be claimed that no adult CSCs (more precisely, no adult cardiomyocyte progenitors) exist, new CM formation has to be the product of pre-existing CM division.[22] Yet, no data so far reported has clearly proven that adult terminally differentiated CMs can actually divide *in vivo* or *in vitro* unless specific genetic modifications allowing for cell cycle competence are introduced.[23,24] The latter have been plagued by the detrimental consequences of forcing the re-activation of the cell cycle in terminally differentiated cells, owing to cardiac dysfunction, heart failure and generation of tumors.[22] Nevertheless, two intriguing approaches targeting adult CMs with overexpression of specific microRNAs[25] or the four pluripotency transcription factors[26] appear to reverse the terminal differentiation allowing for CM re-entry into the cell cycle followed by their duplication. Cell transformation and tumour formation remains a high risk for such strategies, but the findings are biologically important because, if correct and reproduced, they would demonstrate that terminal differentiation is functionally reversible (Box 2).

Main cell types used in clinical trial tests

In last two decades clinical studies demonstrated the safety and feasibility of cell therapy both for ischemic heart disease and heart failure using a wide range of cell types such as bone marrow mononuclear cells, bone marrow derived mesenchymal cells, adult tissue stem/progenitor cells including endothelial progenitor cells and CSCs.[27,28] Several mechanisms of action have been proposed (Figure 2), however because the endogenous regenerative biology of the adult is still controversial (see above), no mechanism can be considered fully underlying the effects of cell therapy for CVD. While the first claimed mechanism was that the transplanted cells would differentiate in cardiac muscle and vascular cells, thereby contributing to balanced "remuscularization" of the heart and increasing its contractile function, a second, alternative and currently prevailing, mechanism of action is that the transplanted cells release a blend of factors/biomolecules, which harness endogenous repair pathways, leading to stimulation of angiogenesis and reduction of inflammation, fibrosis and cell death, while inducing some yet debated new cardiomyocyte formation[29] (Figure 2). The main clinical cell therapy studies for HF and acute MI are listed in Tables 1 and 2, respectively. The characteristics of the main cell types used are discussed below.

Bone marrow mononuclear cells

The majority of clinical cell-therapy studies for cardiac repair have been based on the use of bone-marrow-

Heart regeneration is nowadays one of the most active and contentious field of biomedical research, while being a relatively new branch of cardiac biology. Given the epidemic size and poor prognosis of heart failure, the potential significance of successful human heart regeneration strategies cannot be understated. The biology underlying the myocardial regenerative process, however, is extremely complicated, and several data of effective heart regeneration have sparked both intrigued interest and nasty controversy. Although myocardial regeneration necessitates the replenishment of a variety of cell types, including cardiomyocytes, vasculature, lymphatics, conduction system cells, and the interstitium, the real focus is on cardiomyocyte replenishment/refreshment/renewal. For a long time, the mammalian heart was thought to be a postmitotic organ incapable of self-renewal because of terminal differentiation of its main parenchymal cell type, the cardiomyocyte, which is permanently withdrawn from the cell cycle and unable to efficiently re-enter it under physiological and pathological stimuli. This old paradigm supported the idea that the heart is made up of a fixed number of cardiomyocytes, which is decided at birth and maintained until the organ's death. However, the findings that new cardiomyocytes are formed throughout life as shown by the evidence of small mononucleated cardiomyocytes undergoing division and that tissue-specific multipotent adult cardiac stem/progenitor cells (CSCs) with a robust potential to differentiate into cardiac muscle and vascular cells exist in the heart, have revolutionized cardiac biology. The above findings seemed at first to go hand in hand as it was logically to envision that as for all the other body tissue, also for the heart, the resident tissue specific stem cells (the CSCs) get activated in response to wear and tear or tissue damage to differentiate into immature small mononucleated cardiomyocytes, which are still capable of few rounds of division similarly to neonatal cardiomyocytes before terminal differentiation. Nevertheless, a few studies recently claimed that CSCs have low if not negligible 'remuscularization' capability and that new cardiomyocytes are instead the product of pre-existing terminally differentiated cardiomyocytes' duplication. The latter view challenges the undisputable evidence that adult mammalian cardiomyocytes as opposed to contractile cardiac cells in lower vertebrates stop dividing relatively early after birth. This has postulated the existence of a yet undefined very rare population of hypoxic cardiomyocytes able of a continuous slow turnover. The resolution of this biology conundrum is clearly necessary to design proper myocardial regeneration protocols in the clinical setting.

***Box 2*: The biology of endogenous myocardial regeneration.**

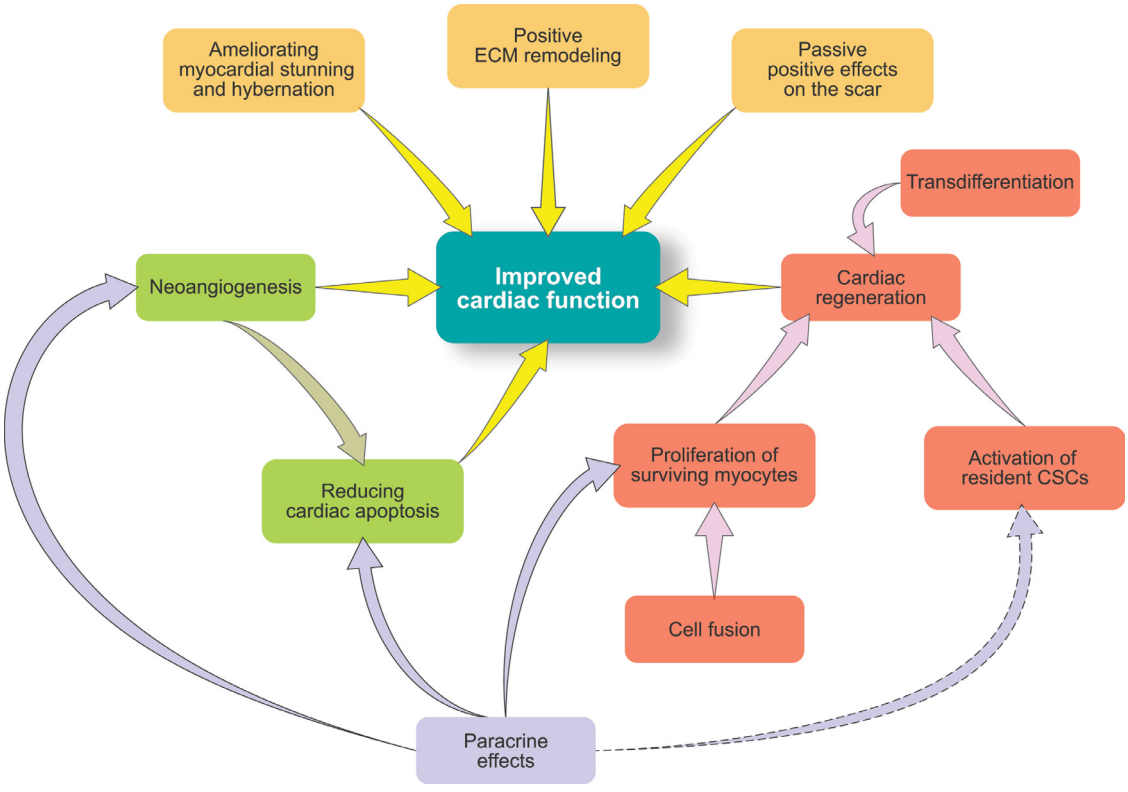

Figure 2. Proposed mechanisms of action of (stem) cell therapy. The cartoon summarizes the main mechanisms claimed to underline the potential benefits of (stem) cell therapy. While cardiac regeneration was the stated goal of (stem) cell therapy, the realization that many clinical attempts of so-called stem cell therapy did not contain actual stem cells with cardiac remuscularization potential shifted the interest on the paracrine ability of the injected cells. This paracrine potential includes the ability of the injected cells to stimulate the repair of the endogenous myocardium through (i) boosting new cardiomyocytes formation either from the endogenous CSCs or from the unexpected division of pre-existing cardiomyocytes, (ii) fostering cardiac protection (reducing hypertrophy, cell death and fibrosis), (iii) improving new vessel formation (angiogenesis) and (iv) overall ameliorating pathologic cardiac remodeling.

Study name	Year	Study design	N	Cell type	Setting	Primary outcome
TOPCARE-CHD (Assmus et al.)[1]	2006	RCT	75	Autologous CPC vs BMPC	Ischaemic heart failure	Improvement in LVEF in BMPC group
MAGIC (Menasché et al.)[2]	2008	RCT	97	Autologous SM	Ischaemic heart failure	No effect on LVEF and incidence of arrhythmia
Ang et al.[3]	2008	RCT	63	Autologous BMC	Ischaemic heart failure	No additional benefit
SEISMIC (Duckers et al.)[4]	2011	RCT	40	Autologous SM	Ischaemic heart failure	No change in LVEF
FOCUS HF (Perin et al.)[5]	2011	RCT	30	Autologous BM MNC	Ischaemic heart failure	No functional improvement, improved symptoms and QoL
MARVEL-1 (Povsic et al.)[6]	2011	RCT	23	Autologous SM	Ischaemic heart failure	No functional improvement, higher incidence of ventricular arrhythmias
FOCUS CCTRN[7]	2012	RCT	92	Autologous BM MNC	Ischaemic heart failure	No improvement in LVEF, infarct size, wall motion
POSEIDON[8]	2012	RT	30	Allogenic BM MSC vs autologous BM MSC	Ischaemic heart failure	Improved LVEF, QoL and ventricular remodeling
TOPCARE-G-CSF (Honold et al.)[9]	2012	RCT	32	Autologous CPC + G-CSF	Ischaemic heart failure	Safe, no effect on cardiac function and NYHA
C-CURE (Bartunek et al.)[10]	2013	RCT	36	Autologous BM MSC	Ischaemic heart failure	Improved LVEF and symptoms
Lu et al.[11]	2013	RCT	50	Autologous BM MNC	Ischaemic heart failure	Improved LVEF, reversed ventricular remodeling, scar reduction
CELLWAVE (Assmus et al.)[12]	2013	RCT	103	Autologous BM MNC	Ischaemic heart failure	Improved LVEF, regional wall thickness, MACE
Pätilä et al.[13]	2014	RCT	39	Autologous BM MNC	Ischaemic heart failure	Reduced scar size, no improvement in systolic function or viability
PRECISE (Perin et al.)[14]	2014	RCT	27	Autologous ADRC	Ischaemic heart failure	Improved ventricular function, myocardial perfusion, exercise capacity
TAC-HFT (Heldman et al.)[15]	2014	RCT	59	Autologous BM MNC vs MSC	Ischaemic heart failure	No improvement in LVEF and improved QoL in cell treated group, improved infarct size, exercise and functional capacity in MSC group
Ascheim et al.[16]	2014	RCT	30	Allogenic MPC	Ischaemic heart failure	Increased but not significant possibility of LVAD weaning
Cardio133 (Nasseri et al.)[17]	2014	RCT	60	Autologous BM CD133+	Ischaemic heart failure	No effect on LV function or symptoms with some improvement in scar size and regional perfusion
Perin et al.[18]	2015	RCT	60	Allogenic MPC	Ischaemic heart failure	Safe, no improvement in LVEF
MSC HF (Mathiasen et al.)[19]	2015	RCT	60	Autologous BM mesenchymal stromal cells	Ischaemic heart failure	Improved LEVF, stroke volume and myocardial mass
Zhao et al.[20]	2015	RCT	59	Allogenic hUC-MSC	Ischaemic heart failure	Improved LVEF, NT-proBNP and functional tests
IMPACT-CABG[21]	2016	RCT	40	Autologous BM CD133+, CD34+, CD45+	Ischaemic heart failure	No improvement in LVEF
xiCELL-DCM (Patel et al.)[22]	2016	RCT	126	Autologous CD90+MSC+CD45 +CD14+auto-fluorescent+activated macrophages	Ischaemic heart failure	Reduction in clinical cardiac events
CHART-1 (Teerlink et al.)[23]	2017	RCT	351	Autologous BM cardiopoietic MSC	Ischaemic heart failure	Decreased LV volumes
PERFECT (Steinhoff et al.)[24]	2017	RCT	82	Autologous BM CD133+	Ischaemic heart failure	Safe, no significant improvement in LVEF
REGENERATE-IHD (Choudhury et al.)[25]	2017	RCT	90	Autologous BMPC+G-CSF	Ischaemic heart failure	Improved LVEF, NYHA and NT-proBNP in IM group
Gwizdala et al.[26]	2017	RCT	13	Connexin 43 muscle-derived progenitor cells	Ischaemic heart failure	Improvement in exercise capacity and myocardial viability
TRIDENT (Florea et al.)[27]	2017	RCT	30	Allogenic MSC	Ischaemic heart failure	Reduced scar size, improved NYHA
RIMECARD (Bartolucci et al.)[28]	2017	RCT	30	Allogenic hUC-MSC	Ischaemic heart failure	Increased LVEF, improved symptoms and QoL
HUC-HEART (Ulus et al.)[29]	2020	RCT	54	Allogenic hUC-MSC or autologous BM MNC	Ischaemic heart failure	Cell treated group: reduced NT-proBNP and necrotic myocardium. hUC-MSC: increased LVEF, stroke volume, exercise capacity
He et al.[30]	2020	RCT	50	Allogenic hUC-MSC	Ischaemic heart failure	Safe, improved cardiac function, infarct size and QoL
CONCERT HF (Bolli et al.)[31]	2021	RCT	125	Autologous MSC&c-kit+ CSC	Ischaemic heart failure	Improvement in MACE and QoL
ABCD Study (Seth et al. 2006)[32]	2006	RCT	44	Autologous BM MNC	Dilated cardiomyopathy	Improvement in LV function and NYHA class
Vrtovec et al.[33]	2011	RCT	55	Autologous PB CD34+G-CSF	Dilated cardiomyopathy	Improvement in LVEF, exercise tolerance and NT-proBNP
Perin et al.[7]	2012	RCT	20	Autologous ALDH	Dilated cardiomyopathy	No MACE; decreased LVESV, improved maximal oxygen consumption
Vrtovec et al[34]	2013	RCT	40	Autologous PB CD34+filgrastim	Dilated cardiomyopathy	Improved LVEF, NT-proBNP, exercise capacity
Vrtovec et al[35]	2013	RCT	110	Autologous PB CD34+G-CSF	Dilated cardiomyopathy	Improved LVEF, exercise tolerance, long term survival
IMPACT-DCL, CATHETER-DCM (Henry et al.)[36]	2014	RCT	61	Autologous lxmyelocel-T	Dilated cardiomyopathy	Reduction in MACE and improved symptoms in ischemic DCM population
INTRACELL (Sant'Anna et al.)[37]	2014	RCT	30	Autologous BM MNC	Dilated cardiomyopathy	No improvement in LVEF
MiHeart (Martino et al.)[38]	2015	RCT	160	Autologous BM MNC	Dilated cardiomyopathy	No improvement in LVEF
REGENERATE-DCM (Hamshere et al.)[39]	2015	RCT	60	Autologous BM MNC+G-CSF	Dilated cardiomyopathy	Improved LVEF, exercise capacity, QoL, NT-proBNP

Table 1 *(Continued)*

Study name	Year	Study design	*N*	Cell type	Setting	Primary outcome
Butler et al.[40]	2017	RCT	22	Allogenic MSC	Dilated cardiomyopathy	Improvement in functional status
Xiao et al.[41]	2017	RCT	53	Autologous BM MNC or BM MSC	Dilated cardiomyopathy	Similar effectiveness on LVEF and NYHA class
POSEIDON DCM (Hare et al.)[42]	2017	RCT	37	Allogenic vs autologous BM MSC	Dilated cardiomyopathy	Less adverse events, improved LVEF, increased exercise capacity and QoL in allogenic group
REMEDIUM (Vrtovec et al.)[43]	2018	RCT	60	Autologous PB CD34+G-CSF	Dilated cardiomyopathy	Improvement in LVEF, NT.proBNP, 6 minute walking test
CCTRN SENECA (Bolli et al.)[44]	2020	RCT	37	Allogenic BM mesenchymal stromal cells	Dilated cardiomyopathy	Safe, no difference in clinical outcomes

***Table 1*: Clinical trial of cell therapy for heart failure.**

Abbreviation: ADRC, adipose tissue-derived regenerative cell; ALDH, aldehyde dehydrogenase; BMC, bone-marrow cell; BMMNC, bone-marrow-derived mononuclear cell; BMPC, bone-marrow-derived progenitor cell; CPC, circulating progenitor cell; CSC, cardiac stem cell; G-CSF, granulocyte-colony stimulating factor; EPC, endothelial progenitor cell; ESV, end systolic volume; hUC-MSC, human umbilical cord-derived mesenchymal stem cell; LV, left ventricle; LVAD, left ventricular assist device; LVEF, left ventricle ejection fraction; IM, intramyocardial; MACE, major adverse cardiac events; MNC, mononuclear cell; MPC, mesenchymal precursor cells; MSC, mesenchymal stem cell; NT-proBNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association; PB, peripheral blood; PBSC; peripheral blood stem cell; QoL, quality of life; RCT, randomised controlled trial; RT, randomised trial; SM, skeletal myoblast; UC-MSC, umbilical cord-derived mesenchymal stem cell.

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Study name	Year	Study design	N	Cell type	Primary outcome
BOOST (Wollert et al.)[1]	2004	RCT	60	Autologous BMPC	Improved LV function
TOPCARE-AMI (Schächinger et al.)[2]	2004	RT	59	Autologous BMPC or CPC	Safe, improved LVEF, decreased ESV, reduced infarct size
Chen et al.[3]	2004	RCT	69	Autologous BM MSC	Improved LV function
Bartunek et al.[4]	2005	RCT	35	Autologous BM CD133+	Improved LV performance, myocardial perfusion and viability
Meluzin et al.[5]	2006	RCT	66	Autologous BM MNC	Improvement in myocardial function
LEUVEN-AMI (Janssens et al.)[6]	2006	RCT	67	Autologous BMPC	Reduction in infarct size, no effect on LVEF
ASTAMI (Lunde et al.)[7]	2006	RCT	97	Autologous BM MNC	No effect on LV function
REPAIR-AMI (Schächinger et al.)[8]	2006	RCT	204	Autologous BMPC	Improvement in LVEF
TCT-STAMI (Ge et al.)[9]	2006	RCT	20	Autologous BM MNC	Improved LV performance, myocardial perfusion, prevented myocardial remodeling
Penicka et al.[10]	2007	RCT	27	Autologous BM MNC	No improvement of LVEF
FINCELL (Huikuri et al.)[11]	2008	RCT	80	Autologous BM MNC	Improvement in LVEF
Lipiec et al.[12]	2009	RCT	39	Autologous BM MNC	Improvement in myocardial perfusion, no effect on LVEF
BALANCE (Yousef et al.)[13]	2009	RCT	62	Autologous BM MNC	Improvement in LV function, mortality and QoL
MYSTAR (Gyöngyösi et al.)[14]	2009	RCT	60	Autologous BM MNC	Improvement in infarct size and LV function
REGENT (Tendera et al.)[15]	2009	RCT	200	Autologous BM MNC vs selected BM CD34+ CXCR4+	No improvement in LVEF
Hare et al.[16]	2009	RCT	53	Autologous BM MNC	Improvement in symptoms
Cao et al.[17]	2009	RCT	86	Autologous BM MNC	Long term improvement in myocardial function
Quyyumi et al.[18]	2011	RCT	31	Autologous BM CD34+	Dose-dependent perfusion improvement
COMPARE-AMI (Mansour et al.)[19]	2011	RCT	20	Autologous BM CD133+	Safe, improvement in LVEF
Colombo et al.[20]	2011	RCT	15	Autologous BM CD133+ vs PB CD133 +	Increased myocardial flow in BM group
HEBE (Hirsch et al.)[21]	2011	RCT	200	BM MNC vs PB MNC	No effect on LV function
LATE-TIME (Traverse et al.)[22]	2011	RCT	87	Autologous BM MNC	No effect on LVEF or infarct size
BONAMI (Roncalli et al.)[23]	2011	RCT	101	Autologous BM MNC	Improved myocardial viability
TIME (Traverse et al.)[24]	2012	RCT	120	Autologous BM MNC	No effect on LVEF
APOLLO (Houtgraaf et al.)[25]	2012	RCT	14	Autologous ADRC	Improved LVEF and perfusion
SWISS-AMI (Sürder et al.)[26]	2013	RCT	200	Autologous BM MNC	No effect on LVEF
CADUCEUS (Malliaras et al.)[27]	2014	RCT	25	Autologous CDC	No effect on LVEF, reduction in scar size, increased viability and contractility
Lee et al.[28]	2014	RCT	80	Autologous BMMSC	Improvement in LVEF
Gao et al.[29]	2015	RCT	116	Allogenic Wharton's Jelly-derived MSC	Safe, improvement in LVEF, myocardial viability and perfusion
CHINA-AMI (Hu et al.)[30]	2015	RCT	22	Autologous hypoxia preconditioned BMMNC	No effect on LVEF, improved myocardial perfusion and wall motion score
Chullikana et al.[31]	2015	RCT	20	Allogenic BM mesenchymal stromal cells	Safe, no effect on LVEF, perfusion and infarct size
REGENERATE-AMI (Choudry et al.)[32]	2016	RCT	100	Autologous BMC	No effect on LVEF

Table 2 *(Continued)*

Study name	Year	Study design	*N*	Cell type	Primary outcome
Zhu et al.[33]	2016	RCT	10	Autologous T04 pre-treated EPC	Improved cardiac function and exercise capacity
BOOST (Wollert et al.)[34]	2017	RCT	153	Autologous BMC vs irradiated BMC	No improvement in LVEF
PreSERVE-AMI (Quyyumi et al.)[35]	2017	RCT	161	Autologous BM CD34+	No improvement in myocardial perfusion
CAREMI (Fernandez-Aviles et al.)[36]	2018	RCT	49	Allogenic CSC	Safe
ADVANCE (Duckers et al.)[37]	2018	RCT	23	Autologous ADRC	Safe
ALLSTAR (Makkar et al.)[38]	2020	RCT	134	Allogenic CDC	Safe, reduced LV volumes and NT-proBNP
BAMI (Mathur et al.)[39]	2020	RCT	375	Autologous BMMNC	No significant improvement in mortality
Zhang et al.[40]	2021	RCT	43	Autologous BMMSC	No significant effect on cardiac function

***Table 2*: Clinical trial of cell therapy in acute myocardial infarction.**

Abbreviation: ADRC, adipose tissue-derived regenerative cell; BM, bone-marrow-derived; BMMNC, bone-marrow-derived mononuclear cell; BMPC, bone-marrow-derived progenitor cell; CDC, cardiosphere-derived cell; CPC, circulating progenitor cell; CSC, cardiac stem cell; EPC, endothelial progenitor cell; ESV, end systolic volume; hMSC, human mesenchymal stem cell; LV, left ventricle; LVEF, left ventricle ejection fraction; MNC, mononuclear cell; MSC, mesenchymal stem cell; NT-proBNP, N-terminal pro B-type natriuretic peptide; QoL, quality of life; RCT, randomised controlled trial; RT, randomised trial.

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derived mononuclear cells (a.k.a. BM MNCs or BMNCs). BMNCs are a heterogeneous population that includes hematopoietic lineage-committed cells such as lymphocytes and monocytes together with hematopoietic stem cells (HSCs), side population cells (defined by their ability to exclude the Hoechst 33342 dye) and endothelial progenitor cells as well as mesenchymal stromal cells together with mesenchymal stem cells. Stem cells within the BMNCs have an extensive capability to generate many non-haematopoietic cells, such as skeletal myoblasts, endothelium, epithelium, hepatocytes, neuroectodermal cells and, finally, CMs.[30] However, the fraction of stem cells with multipotent differentiation plasticity within BMNCs is different in each preparation but always minimal (below 1%).[30] Therefore, to label BMNC administration as "stem cell therapy" is a misnomer and it should be correctly defined as a cell therapy. This therapy arose very shortly after the first documentation of HSCs transplantation for cardiac regeneration in a small animal model of MI.[31] In a race to the clinic, in the next six months several small non-randomised clinical trials, using autologous BMNCs, were published reporting moderately positive outcomes for the treatment of acute MI and HF.[32] These publications led to larger randomised controlled clinical trials. Several meta-analyses of these controlled, randomised trials[27,28] concluded that BMNC therapy is safe, suggesting that BMNC transplantation is associated with modest improvements in physiologic and anatomic parameters in patients with both acute MI and chronic ischemic heart disease, above and beyond conventional therapy. In turn, the findings of the meta-analyses supported conducting larger, multicentre, randomised trials to evaluate the impact of BMC therapy on overall and event-free long-term survival (see Tables 1 and 2).

Following up, in 2012 the FOCUS trial[33] showed no improvement in left ventricle (LV) volume or ejection fraction (EF) at 6 months in patients with ischemic HF after BMNC injection. The TAC-HFT trial, in 2014, demonstrated similar findings.[34] In 2015, the REGENERATE-DCM study showed a significant increase in LVEF from baseline at 3 and 12 months in patients treated with G-CSF and BMNC.[35] Finally, in 2020 the BAMI trial, the largest phase III study with autologous BMNC in the treatment of acute MI, demonstrated that coronary injection failed to improve all-cause mortality, or death/HF hospitalization.[36]

Bone marrow derived mesenchymal cells

Bone marrow derived mesenchymal cells (BM-MSCs) are a rare population of fibroblast-like cells in the bone marrow stroma[37] that can differentiate into important cell lineages under defined conditions *in vitro* and in limited situations after implantation *in vivo*.[37] BM-MSCs also secrete a range of proangiogenic factors, matrix metalloproteinase and factors involved in tissue specific stem/progenitor cells mobilization.[37] The MSCs have broad anti-inflammatory and immune-modulatory properties.[37] Due to their significant expansion ability, paracrine effects and immunomodulatory properties, MSCs have been the focus of several clinical trials in cardiovascular diseases.

The POSEIDON trial in 2012 demonstrated a reduction in scar size at 12 months in patients with ischemic HF treated with autologous or allogenic BM-MSC, with a reduction in LV end-diastolic volume in the allogenic group, generating the surprising hypothesis of allogenic superiority over autologous cells.[38] In 2014, the TAC-HFT trial showed a reduction in scar size and an increase in regional myocardial function in the autologous BM-MSC-treated vs. the BM-MNC-treated group.[34] In 2015, and then in 2020 with the 4-year follow up, the MCS-HF study showed a significant reduction in LV end-systolic volume and a significant improvement in LVEF, myocardial mass and quality of life at 12 months after autologous BM-MSCs injection.[39] The TRIDENT study in 2017 compared two different doses of allogenic BM-MSCs in patients with ischemic HF, proving that the higher dose improved LVEF.[40] The recent CONCERT-HF trial, comparing transendocardial injection of MSCs combined with CPCs, MSCs alone, or CPCs alone, did not show improvement in LV function or structure at 12 months after transendocardial injections of autologous BM-MSCs, whereas a significant reduction of HF-related major adverse cardiac events (HF-MACE) was observed in the CPCs group.[41]

Finally, the recently results of the largest clinical trial conducted so far using BM-MSCs in patients with ischemic and non-ischemic HF, the DREAM-HF study showed that although the study missed the primary endpoint (reduction in recurrent HF-related hospitalizations) and key secondary endpoint, the risk of nonfatal MI or nonfatal stroke was lower in the group treated with allogenic BM-MSCs.[42]

Overall, clinical trials show that MSCs paracrine cardioprotective and vasculo-regenerative effects along with their immunomodulatory properties produce benefits in the setting of HF patients on top of current recommended optimal management. Importantly, no safety concerns emerged.

Endothelial progenitor cells

Since the discovery of endothelial progenitor cells (EPC) in the landmark study by Asahara et al. in 1997,[43] an increasing number of basic science and pre-clinical studies have shown that EPC-based therapy is feasible, safe, and efficacious in multiple disease states.[44,45] Consequently, several, mainly early-phase, clinical trials demonstrating the feasibility and safety profile of EPC therapy have been conducted, with the suggestion of efficacy in several conditions, including ischemic heart disease.[45]

While clinical testing of EPCs started with patients with acute ischemic heart disease, the most relevant findings have come from treatment of refractory angina (RA).[46] Cell therapy utilizing autologous CD34+ (auto-CD34+) EPCs is a promising therapy for RA patients, as shown by two early phase clinical trials, which established the feasibility[47] and dose-response[48] for intramyocardial (IM) delivered auto-CD34+ cells to improve exercise capacity. The RENEW, a phase III pivotal trial, terminated prematurely by the sponsor solely for financial reasons.[49] Nevertheless, a recent patient-level pooled analysis of randomised double-blinded trials show that autologous CD34+ cell therapy improves exercise capacity, angina frequency and reduces mortality in no-option RA.[50] Furthermore, a pilot study in patients with ischemia with non-obstructive coronary arteries (INOCA) and endothelial-independent coronary microvascular dysfunction (CMD) and persistent angina, treated with autologous intracoronary CD34+ stem cells, demonstrated a significant improvement in coronary flow reserve, angina frequency, Canadian Cardiovascular Society class, and quality of life (ESCaPE-CMD, NCT03508609).[51] This work is being further evaluated in the ongoing FREEDOM (NCT04614467) placebo-controlled trial.

Overall data from clinical studies using intramyocardial injections of CD34+ cells in patients with refractory angina show safety and efficacy with respect to pain relief and improvements of mortality, making this cell therapy the one closer to become part of broad clinical scenario while waiting for the results of the larger clinical trials.

Cardiac stem/progenitor cells

The characteristics and regenerative potential of CSCs (also called CPCs) have been described above. Only one

trial, the SCIPIO, tested their potential as autologous cell source in HF patients.[52] However, the study has been retracted.[53] Despite the high scepticism and the moratorium proposed on the entire field of myocardial cell therapy because of the misconduct of a single investigator, more than 50 independent studies from 26 independent research groups have established the benefit of c-kit positive CPCs on LV function in animal models (e.g., mice, rats, pigs, and cats) of ischemic heart disease.[54] Additionally, when properly identified and expanded, endogenous CSCs, as well as transplanted exogenous CSCs, robustly differentiate into CMs both *in vitro* and *in vivo*. Yet, *in vitro* the CSC-derived CMs remain immature contractile cells that resemble foetal/neonatal CMs.[13] Nevertheless, their maturation progresses and reaches the adult terminally differentiated CMs when the CSCs are injected in the injured myocardium.[8,10] Furthermore, similar to MSCs, CSC (CPCs), either as autologous or allogenic cell products, through paracrine mechanisms have potent immunomodulatory actions reducing inflammation, fibrosis, and apoptosis while promoting angiogenesis and by stimulating the endogenous CSCs increase the regenerative potential of the adult heart.[55]

The feasibility and safety of allogenic CSCs has been tested in the CAREMI trial, which administered allogeneic CSCs in patients with large STEMI. Even though no differences in cardiac magnetic resonance imaging–based efficacy parameters were observed at ≤12 months,[56,57] CAREMI shows that AlloCSCs can be safely administered in STEMI patients and their low immunogenicity and absence of immune-mediated events should facilitate adequately powered studies to test their clinical efficacy in this or other clinical settings.

Cardiosphere-derived cells (CDCs), a heterogenous type of cardiac mesenchymal progenitor/precursor cells have potent immunomodulatory, antifibrotic, and cardiomyogenic regenerative activity.[58–60] On this basis, autologous CDCs were initially tested in patients with left ventricular dysfunction in the CADUCEUS trial, appearing to be safe and effective in decreasing scar size and increasing viable myocardium. In the longer follow-up study, autologous CDC infusion proved to have ameliorated the regional function of the infarcted myocardium.[61]

Allogenic CDCs have been tested in two clinical studies: the ALLSTAR trial and the DYNAMIC trial.[59,60] AlloCDCs treatment proved to be safe but clearly their efficacy in HF needs to be tested in larger randomised trials.

Finally, two Duchenne muscular dystrophy (DMD) clinical trials, HOPE and HOPE-2 (Halt cardiOmyoPathy progression) (NCT02485938 and NCT03406780, respectively) have been performed with CDCs[62,63] with promising preliminary results in terms of improvement in LVEF and LV chamber dimensions reduction.[64]

Overall, despite the robust *in vitro* and *in vivo* evidence showing that clonogenic CSCs are cardiomyogenic and a flurry of preclinical data showing from mouse to pigs the beneficial effects of CSCs and CPCs in ischemic cardiomyopathy,[65] the clinical translation of these cells has been severely downplayed by the scandal surrounding one laboratory and the consequent retraction of the SCIPIO trial even though the positive clinical findings of that trial have not been specifically questioned.[53] On the other hand, the data from the CONCERT-HF (see above) show that a single administration of allogenic CPCs in patients with chronic ischaemic HF on maximal guideline-driven therapy has measurable beneficial effects over the ensuing 12 months, namely, a reduction in hospitalization for HF.[41] Whether these beneficial effects are related to anti-inflammatory, immunomodulatory, antifibrotic, proangiogenic, endothelial protective or endogenous CSC-activating actions by the injected cells on the host myocardium of the transplanted cells remains to be elucidated. Furthermore, it should also be pointed out that both CONCERT-HF and CARE-MI injected an heterogenous c-kit^{pos} CSC/CPC population, which not uniformly have myogenic capacity. Indeed, only 10% of this population is clonogenic and multipotent.[19,20] Therefore, if remuscularization of damaged myocardium is the clinical endpoint, then future trials using autologous CSCs should be designed entailing CSCs expanded from single cell-derived clones, which are robustly myogenic.[10]

Pluripotent/embryonic stem cells

For many years, embryonic stem cells (ESCs) have been considered the only source of truly pluripotent stem cells (PSCs), or rather, stem cells with the potential to differentiate into all cell types of the organism, except for a viable embryo. ESCs are however limited for clinical application because of ethical concerns, potential genetic instability, and requisite immunosuppression therapy. Yet, a clinical-grade approach of hESC-derived CMs xeno-transplantation has been evaluated in a large animal model of myocardial infarction, showing remuscularization of the infarcted macaque heart with human myocardium and a durable improvement in left ventricular function.[66,67] Nevertheless, a subset of the hESC-CM transplanted animals experienced graft-associated ventricular arrhythmias.[66,67]

On the other hand, the generation of human induced PSCs (hiPSCs) was met by a widespread enthusiasm for its potential clinical application, which was set to overcome many of the limitations of ESCs.[68] hiPSCs were originally generated from fibroblasts through co-expression of four pluripotent transcription factors (c-Myc, Oct3/4, Sox2, Klf-4)[69] and like ESCs, they have an indefinite proliferative potential and can be differentiate into any cell type of the three germ layers. In contrast to ESCs, the use of iPSCs does not raise ethical issues and can be derived from the patient to be treated. CMs can then be produced from iPSCs *in vitro* with a similar efficiency and functionality of ESCs.[70] iPSC technique

makes possible autologous cell transplantation, with a theoretical reduction in risk of immune rejection.[68] Nevertheless, the use of patient-specific iPSCs or iPSC-derived CMs to generate autologous muscular grafts to circumvent immune rejection has been largely debated due to the time and cost of producing clinical grade autologous cells for each patient, which remains a realistic option only for a selected number of patients.[71] An alternative and more cost-effective approach would be the use of allogeneic iPSCs that allow for the development of a cryopreserved, ''off-the-shelf'', widely available product for transplantation. To this end, hiPSCs have been engineered to remove human leukocyte antigens (HLAs), to generate a "universal donor".[72–74] Of note, in a real allogenic setting (i.e., xenotransplantation of human cell in animal recipients), primate iPSC-derived CMs were transplanted into allogeneic and immune-suppressed primate recipients, demonstrating engraftment, electrical integration in the host myocardium, but modest improvements in global contractile function in infarcted recipients. Yet, the incidence of ventricular tachycardia was transiently, but significantly, increased when compared to vehicle-treated controls.[75]

The possibility to remuscularize the heart by PSC-derived cardiac cells paved the way to the first clinical trials of open-chest epicardial delivery of cell-laden patches in patients with advanced HF. One of them has used ESC-derived cardiac progenitor cells embedded in a fibrin patch (ESCORT Trial) (NCT02057900)[76] with a concomitant coronary artery bypass grafting and has successfully met its primary safety end point. The other 2 trials will deliver iPSC-derived CMs under the form of a cell sheet (jRCT2053190081)[77] or a collagen-based construct (BioVAT-HF Trial; NCT04396899).[78]

So far, none of the study procedures have been associated with adverse events. However, due to the small number of patients enrolled in these first-in-man studies, solid conclusions are not yet available. It will be key to show true and sustained heart remuscularization by PSC-derived CMs, electromechanically coupled with host pre-existing CMs that do not cause life-threatening cardiac arrythmias. Additionally, it will be important to verify that the genetic modifications used to obtain the iPSCs and the culture protocols to derive cardiac progenitors or CMs do not increased cell transformation capability of teratoma formation of the injected cells within the damaged myocardium. Furthermore, the PSC-derived cells used in the above clinical trials are allogeneic, including the iPSCs. This is so because despite being postulated as a pluripotent stem cell to be used as an autologous source for all patients in need, their use as autologous cells is economically not affordable even for the wealthiest health system in the world when considering the large patient populations to be treated for cardiac repair. Consequently, immunosuppression would be required if long-term remuscularization is the intended mechanism of action, and this immunosuppression would be lifelong, which raises the issue of the long-term adverse effects of immunosuppressive drugs. On the other hand, if reliance is on paracrine mechanisms, but still remuscularization is the goal then this could be accomplished only if the injected cells would target the endogenous regenerative capacity of the human heart. Yet the latter remains unknown.

Gaps in evidence

Twenty years ago, satellite cells, the resident tissue specific stem cells of the skeletal muscle, were intramyocardially injected into a patient with severe left ventricular decompensation undergoing coronary artery bypass.[79] This attempt started the era of cell-based human cardiac regeneration. Since then, multiple experimental and clinical studies have been performed. Unfortunately, despite the goal of "remuscularizing" the failing heart, the outcomes of the many clinical trials have been either neutral or marginally positive at best. Indeed, despite the many types of cells and methods of administration used, the possible mechanisms of action of these therapies have not been established. This is not surprising because there is still no agreement on whether the myocardium, like the other tissues, has a population of stem cells to replace the myocytes lost by wear and tear throughout life, or whether it is lacking them.

Is the goal of myocardial cell therapy to replace some of the CMs lost, to improve the performance of the surviving ones or a combination of both? If the goal is to replace lost myocytes, is the target the endogenous CSCs/CPCs or, if they do not exist, the de-differentiation, re-entry in the cell cycle and division of the surviving myocytes? Unlikely we will make significant progress until answer to these fundamental questions becomes available.

Additionally, there remain important issues related to this potential therapeutic approach that need to be defined before its implementation in the clinical routine. The clinical trials performed have shown that cell therapy for cardiovascular disease is safe and that allogeneic cells have had low immunogenicity. However, neither the effective cell dose nor the best route and timing of administration have been firmly defined.[80] Furthermore, while the best approach in terms of engraftment and efficacy appears to be the transendocardial injection route, intracoronary injection remains the easiest applicable choice. The latter is further reinforced considering that recently the intravenous injection route has been shown to be very promising in preliminary clinical applications.[28]

A paracrine mechanism to explain the improvement in cardiac function after cell transplantation has been widely investigated (Figure 2).[81] The latter has been followed up by recent data showing that non-myogenic cardiac cells, like cardiac fibroblasts[82] and endothelial

cells[83,84] play paracrine roles that may significantly affect cardiac repair and regeneration. This mechanism has been deemed responsible for the restorative processes associated with cell therapy, including positive myocardial remodelling, cardioprotection, neovascularization, and neo-myogenesis[85] (Figure 2). The now emerging paradigm is that exogenous cells may exert most of their beneficial actions via an immune-modulation, in particular recent studies have highlighted the emerging role of macrophages in triggering cell regeneration[28,86] The above considerations bring into question the contraposition of autologous vs allogenic stem cell therapy. Most of the studies seem to agree that if the paracrine effect is what a cell therapy approach has to achieve, then allogenic cells are the strategy to prefer. The latter should include the view of a heart as a regenerative organ whereby allogenic cells through their paracrine milieu can boost the intrinsic regenerative potential of the damaged cardiac tissue. Deciphering the real regenerative molecules within the paracrine secretome of the allogenic cells is at the forefront of the new frontiers of the cell-free myocardial regeneration approaches (such as exosomes, microRNAs, RNA therapeutics and nanotechnologies). Clearly if this view is denied, then allogenic cell therapy would exert paracrine effects that are mainly cardioprotective. On the other hand, if the aim of cell therapy is remuscularization by the injected cells then allogenic cell therapy would not be the preferred approach for the need of long-lasting immunosuppression that for the number of patients in need would run the risk of generating a very large number of immunosuppressed people. To the aim of functional CM regeneration by the injected cells, autologous stem cells with true myogenic potential should be the correct approach. However, there is no agreement as to which stem cell type with myogenic potential should be preferred. It is also unclear whether uncommitted stem cells or instead their CM progeny should be used for effective anatomical and functional myocardial regeneration. The answer to the above questions will point to the type of cell to be used and the parameter that best evaluate their potential effects (Figure 2). Finally, it would not be surprising if it turns that the allogenic cell therapy approach to modify the damage cardiac tissue from an hostile to a receptive microenvironment would be indeed needed to allow for an efficient remuscularization by the autologous cell strategy.

Additionally, it is still very uncertain what are the CVD pathologies best suited for cell therapy and the proper stage for these interventions. Among ischemic cardiomyopathies, the STEMI clinical trial has shown that cell therapy, as tested, is hardly going to make an impact over the standard therapy, including early reperfusion strategy.[27,28] On the other hand, meta-analyses of cell therapy trials for refractory angina and heart failure suggest clinical benefit.[27,28] Yet no data exist for HF with preserved ejection fraction HF (HFpEF), an increasingly prevalent clinical condition.[87]

Conclusion

As it stands now the myocardial cell therapies used are a black box within a black box. We are ignorant about the true reparative agent used (the cells or their paracrine emissions), the target of the therapy (stimulate myocyte regeneration or improve the function of the surviving cohort), the real administered dose or the appropriate one, the idoneous CVD to be treated, the optimal time and route to administer the cell therapy. It stands to reason that until most of these questions are answered, pre- and clinical repair/regenerative tests will fall short of providing convincing and conclusive answers about their clinical potential. On one hand, basic research is needed to provide the needed answers. On the other hand, basic research continues to provide exciting new findings that are never followed up with robust reproducible scientific experiments to justify clinical tests. Unless this approach changes, regenerative biology medicine in cardiovascular diseases will always remain the "best next future therapy" while in the present, save heart transplantation for the lucky few, the millions of patients in need of an effective therapy will be treated with palliative drugs or devices with the only possible goal of slowing down the progression of chronic disease towards terminal HF. In the meanwhile, a large fraction of biomedical investment will be used to foster the "biomechanical era" (left ventricular assist device, and artificial hearts) and learning how to prevent/eliminate/reduce biomechanic-induced adverse effects on the human body instead of better understanding the human body itself. In this dreary panorama, the recent "successful" transplantation of a genetically modified swine heart in a human[88] has provided a ray of light on the future. Yet, even if proven long term successful, the very high costs of this therapy will only expand the small cohort of the "lucky few" and leave the millions behind. Cardiovascular research should have the ambition to get out of the "on-treadmill" effort on cardiac regenerative biology by pursuing the realistic and timely goal to settle the question of whether the myocardium has or lacks regenerative potential and advancing our understanding of its biology in order to prevent its progressive deterioration. The goal should be to make the need for a human or porcine heart transplantation a rarity that can be met by many health care systems.

Search strategy and selection criteria

Search strategy and selection criteria data for this Review were identified by searches of MEDLINE, Current Contents, PubMed, and references from relevant articles using the search terms "stem cells and myocardial regeneration", "cardiac cell therapy and clinical trials", 'bone marrow and cardiac cell therapy", "pluripotent stem cells and cardiac regeneration", "cardiac progenitors", and "heart stem cells". Abstracts

and reports from meetings were excluded. Only articles published in English between 2000 and 2022 were included.

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Contributors

Conceptualization: N.S. and D.T.; Design of the work: N.S., L.S. and D.T. Provided critical feedback and helped shape the manuscript: E.C. and A.DeA; Writing original draft preparation and editing N.S., L.S., F.M., M.S., A. C., G.P., E.C., K.U. and D.T. All of the authors approved the final version of this manuscript.

Declaration of interests

The authors declare no conflict of interest.

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