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Cell therapy for the treatment of heart disease: renovation work on the broken heart is still in progress

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ABBREVIATIONS

- autologous Bone marrow cell therapy in Acute Myocardial Infarction (BAMI)
- adult CHD (ACHD)
- ALLogeneic heart STem cells to Achieve myocardial Regeneration (ALLSTAR)
- Allogeneic hMSC Injection in Patients With Hypoplastic Left Heart Syndrome (ELPIS)
- bone marrow mononuclear cells (BM-MNCs)
- Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease (PERSEUS)
- Cardiac Stem/progenitor Cell Infusion in Univentricular Physiology (APOLLON)
- Cardiology Stem Cell Centre Adipose Stem Cell (CSCC_ASC)
- cardiosphere-derived cells (CDCs)
- CARDiosphere-Derived aUTologous stem CELls to reverse ventricUlar dysfunction (CADUCEUS)
- cardiovascular disease (CVD)
- Carvedilol Or Metoprolol European Trial (COMET)
- Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS)
- Combination of Mesenchymal and c-kit+ Cardiac Stem Cells as Regenerative Therapy for Heart Failure (CONCERT-HF)
- congenital heart disease (CHD)
- Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1)
- disability-adjusted life years (DALYs)
- Double-Blind Randomized Assessment of Clinical Events With Allogeneic Mesenchymal Precursor Cells in Advanced Heart Failure (DREAM-HF)
- embryonic stem cells (ESCs)
- Efficacy and Safety of Targeted Intramyocardial Delivery of Auto CD34+ Stem Cells for Improving Exercise Capacity in Subjects With Refractory Angina (RENEW)
- European Union (EU)
- Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI)
- heart failure (HF)
- hypoplastic left heart syndrome (HLHS)
- induced pluripotent stem cells (iPSCs)
- ischaemic heart disease (IHD)
- left ventricular (LV)
- left ventricular ejection fraction (LVEF)
- magnetic resonance imaging (MRI)
- major adverse cardiac events (MACE)
- mesenchymal stromal cells (MSCs)
- myocardial infarction (MI)
- PercutaneOus StEm Cell Injection Delivery Effects On Neomyogenesis in Dilated CardioMyopathy (POSEIDON-DCM)
- Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)
- Relative Risk (RR)
- Reactive oxygen species (ROS)
- right ventricle ejection fraction (RVEF)
- right ventricle outflow tract (RVOT)
- Seniors With Heart Failure (SENIORS)
- Stem Cell therapy in IschEmic Non-treatable Cardiac disease (SCIENCE)
- ST-segment elevation myocardial infarction (STEMI)
- Transcoronary Infusion of CPCs in patients with single ventricle Physiology (TICAP)
- umbilical cord blood (UCB)

ABSTRACT

Cardiovascular disease (CVD) continues to be the number one killer in the aging population. Heart failure (HF) is also an important cause of morbidity and mortality in patients with congenital heart disease (CHD). Novel therapeutic approaches that could restore stable heart function are much needed in both paediatric and adult patients. Regenerative medicine holds promises to provide definitive solutions for correction of congenital and acquired cardiac defects. In this review article, we recap some important aspects of cardiovascular cell therapy. First, we report quantifiable data regarding the scientific advancements in the field and how this has been translated into tangible outcomes according clinical studies and related meta-analyses. We then comment on emerging trends and technologies, such as the use of second-generation cell products, including pericyte-like vascular progenitors, and reprogramming of cells by different approaches including modulation of oxidative stress. The more affordable and feasible strategy of repurposing clinically available drugs to awaken the intrinsic healing potential of the heart will be discussed in the light of current social, financial, and ethical context. Cell therapy remains a work in progress field. Uncertainty in the ability of the experts and policy makers to solve urgent medical problems is growing in a world that is significantly influenced by them. This is particularly true in the field of regenerative medicine, due to great public expectations, polarization of leadership and funding, and insufficient translational vision. Cardiovascular regenerative medicine should be contextualized in a holistic program with defined priorities to allow a complete realization. Reshaping the notion of medical expertise is fundamental to fill the current gap in translation.

1. BACKGROUND

1.1. The burden of cardiovascular disease in adult people

The burden of cardiovascular disease (CVD) is greater than that of any other disease. The 2017 statistics document from the European Heart Network indicates that CVD causes 3.9 million deaths in Europe and over 1.8 million deaths in the European Union (EU), meaning 45% of all deaths in Europe and 37% of all deaths in the EU (<http://www.ehnheart.org/images/CVD-statistics-report-August-2017.pdf>). Death rates from both ischaemic heart disease (IHD) and stroke showed geographically heterogeneity, being higher in Central and Eastern Europe than in Northern, Southern and Western Europe.

Recent data from the Global Burden of Disease database estimate that, in the EU, more than 60 million people live with CVD, and that about 13 million new cases of CVD occur every year. Despite the absolute number of cases has steadily increased over the past 25 years, the age-standardized prevalence rate of CVD has fallen in most European countries, with disability-adjusted life years (DALYs) following the same trend except for two middle-income countries recording an increase.¹ IHD and stroke accounted for 82% of DALYs associated to CVD in EU countries. In the European Heart Network study, CVD was estimated to cost the EU economy €210 billion a year in 2015, of which 53% (€111 billion) was due to healthcare costs, 26% (€54 billion) to productivity losses and 21% (€45 billion) to the informal care of people with CVD.¹

1.2. A new epidemic of cardiovascular disease in children

Heart failure (HF) is also an important cause of morbidity and mortality in patients with congenital heart disease (CHD).²⁻⁴ It can manifest early after birth or later in life through progressive deterioration of contractile function. It is estimated that about 25% of adult CHD (ACHD) patients manifest overt clinical signs by the age of 30. Patients with severe malformations and repeated interventions are more susceptible to complications.⁵⁻⁸ Patients with Tetralogy of Fallot having their first operation within the first 3-6 months of life have a 10-20% chances of requiring another intervention (either surgical or catheter intervention) within their first 10 years of life, with the majority of these patients requiring a pulmonary valve replacement within their second decade of life. For patients with pulmonary atresia requiring a valved conduit in their first few years of life, the chances of undergoing a reintervention for conduit failure within their first decade of life is almost 100%.

The individuals with lower-complexity defects have a higher chance to grow into the adulthood and thus represent the majority of the ACHD population. Adverse cardiovascular events are frequent in these patients, with hazard ratios ranging from 2 for IHD to 13 for HF with respect to the general population.⁹ This increased risk may reflect some primitive frailty of the heart, yet the underpinning molecular determinants remain unknown.¹⁰ Therefore, conventional therapy of HF is currently unsupported by clinical evidence.¹¹ In the US, hospitalization for ACHD-related HF has increased by 91% from 1998 to 2011 and charges have augmented by 258%, more than twice that for non-ACHD HF. The NHS England estimated that, by 2021, costs for CHD will surpass budget availability by £30 billion, primarily due to the increased expenditure on adult patient care.

1.3. The aid from regenerative medicine

Novel therapeutic approaches that could restore stable heart function are much needed in both paediatric and adult patients. Implementing treatments and repurposing available drugs represents a priority within a general program to combat health inequalities. Investing in new reparative approaches is also of vital importance; this strategy may be deployed in special categories of patients before becoming a standard medical treatment.

Cell therapy is a paradigmatic example of modern regenerative therapeutics. While numerous encouraging preclinical data have facilitated rapid translation to clinical studies, current evidence of efficacy remains questionable. Very few Phase III clinical trials have been conducted so far, with results that did not provide definitive answers regarding hard endpoints such as reduction of mortality. However, there are lessons we have learnt from initial investigation which can be useful for the future.

2. SCOPE OF THE REVIEW

In this review article, we summarize some important aspects of cardiovascular cell therapy. First, we report quantifiable data on PubMed-referenced publications. We then comment the results of recent trials in IHD and HF. The experience of our preclinical research is described as an exemplar narrative illustration of academic contribution to the field. The more affordable and feasible strategy of repurposing clinically available drugs to awaken the intrinsic healing potential of the heart is discussed in the light of current social, financial, and ethical context. Finally, we pinpoint medical priorities where cardiovascular regenerative medicine could favourably impact to support health.

3. QUANTIFIABLE EVIDENCE SUPPORTING CARDIOVASCULAR CELL THERAPY

3.1. PubMed analysis

An analysis of articles reported in PubMed from 2000 to 2020 using stem cell and heart as key words indicates a steadily increasing interest with a peak of 1982 publications in 2017 and maintenance of similar publication rate in the following 2 years (**Figure 1A**). The first meta-analysis dates 2007, and 2015 was the year with the highest number of meta-analyses published so far (**Figure 1B**). In general, there is a temporal gap between the publication of experimental results and clinical studies. In the case of stem cell therapy, cardiac trials started soon and peaked in 2014 but declined remarkably in the last 3 years, with randomized studies following the same trend (**Figure 1C**). These data suggest that the enthusiasm about this new approach has been declining in the last few years.

4. RECENT CLINICAL TRIALS IN ADULT CARDIOVASCULAR PATIENTS

4.1. Myocardial infarction

A list of main trials conducted with bone marrow derived cells is reported in **Table 1**. The Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial represents a milestone in this field, influencing and conditioning all the subsequent clinical investigation of cardiac cell therapy. It was a randomized, placebo-controlled trial aimed to determine the efficacy of the intracoronary delivery of bone marrow mononuclear cells (BM-MNCs) versus placebo in patients with acute ST-segment elevation myocardial infarction (STEMI) following successful percutaneous coronary intervention.

At 4-month follow-up, patients treated with BM-MNCs showed a significant improvement in left ventricular ejection fraction compared with placebo (+5.5 vs +3.0%, absolute difference +2.5%). Cell therapy with BM-MNCs was associated with a significant reduction in adverse clinical events at 1-year follow-up. At 2 years, the cumulative end point of death, myocardial infarction, or need for revascularization was reduced in the BM-MNCs group compared with placebo (hazard ratio, 0.58). Likewise, the combined end point death and recurrence of myocardial infarction (MI) and rehospitalization for HF was reduced in the active treatment group (hazard ratio, 0.26). In addition, magnetic resonance imaging (MRI) indexes of left ventricular (LV) contractility were improved in the treated arm. The conclusions of REPAIR-AMI were that the cell therapy product was safe and effective in improving cardiac function and reducing major adverse cardiac events (MACE) in patients with STEMI.¹² This success fuelled a number of subsequent Phase I–II trials, which consistently failed to demonstrate therapeutic benefit.^{13–20} This failure was attributed to the small group size, which did not allow sufficient power for definitive conclusions.

The need for a definitive study of BM-MNCs encouraged the enterprise of the autologous Bone marrow cell therapy in Acute Myocardial Infarction (BAMI) trial with support from the European Commission.²¹ Initially, this was designed as an open-label, multicentre Phase III trial to test the safety and efficacy of intracoronary infusion of BM-MNCs in reducing the time to all-cause mortality in patients with reduced left ventricular ejection fraction (LVEF, ≤45%) after primary angioplasty for STEMI. In total, only 375 of 3000 planned patients were eventually recruited: 185 patients were randomized to the treatment arm (intracoronary infusion of BM-MNCs 2–8 days after angioplasty) and 190 patients to the control arm (optimal

medical therapy). BM-MNCs (range 25–500 × 10⁶ as per protocol) were infused into the culprit coronary artery, that was treated with primary angioplasty at admission, using an over-the-wire balloon.

Because of low recruitment negating definitive conclusions, the trial was converted into an observational study. Noteworthy, mortality was much lower than anticipated. The group size was calculated considering a 25% effect size on mortality. However, instead of the anticipated all-cause mortality of 12%, this was only 3.8% in control group at the 2-year follow-up. It was calculated that more than 15,000 patients should have been enrolled to have a 90% probability of demonstrating a 25% reduction in mortality (from 3.82% to 2.87%).

The results of this trial have consequences that go beyond cell therapy. The 12% mortality rate reflected the clinical scenario at the stage of the initial trial design. In the last years, the outcome of STEMI patients has improved substantially, yet the study shows that 4 out of 100 die within 2 years from the acute event. New life-saving treatments of STEMI are still required but their verification through clinical trials will be increasingly difficult. This particularly applies to cell therapy with autologous BM cells, which, despite extensive demonstration of safety, still require an invasive harvesting procedure.²² A recent commentary highlighted the BAM1 trial provided invaluable information to the broad cardiovascular community for designing future studies of other therapies.²³ In addition, the commentary endorses the case of cell therapy for treatment of chronic HF and refractory angina, two settings where Phase I or II trials showed encouraging results.²³

Apart from BM-MNCs, other cell products have been trialled in the context of acute MI. Preclinical and clinical evidence suggests that mesenchymal stromal cells (MSCs) may be beneficial in treating acute myocardial MI. One of the following sections reports on meta-analyses on MSC therapy. Here, we expand some interesting data from studies that examined heart-derived stromal cell products.

4.1.1. Cardiosphere-derived cells

Cardiosphere-derived cells (CDCs) are heart-derived progenitor stromal cells that have been tested pre-clinically before undergoing a systematic clinical assessment. Initially, autologous CDCs were employed to avoid potential immune rejection.^{24, 25} In the randomized, controlled CARDiosphere-Derived aUtologous stem CELls to reverse ventricUlar dysfunction (CADUCEUS) trial (NCT00893360), autologous CDCs (12.5 to 25 × 10⁶) grown from endomyocardial biopsy specimens were infused via the intracoronary route in 17 patients with left ventricular dysfunction 1.5 to 3 months after an acute MI. An additional patient was treated off-protocol 14 months post-MI. Eight patients were followed as routine-care control patients.

Cell treatment was safe and was associated with improvements regarding scar size, myocardial viability, and regional function at 1-year post-treatment, supporting the case for larger trials. In designing such an endeavour, investigators considered the limitations of the autologous approach, including the ethical, logistic, and economic constraints introduced by the need for tissue harvesting through a myocardial biopsy, cell processing and quality control, and the influence of donor age, risk factors and comorbidities on bioactivity. Moreover, pre-clinical studies had already shown that allogeneic CDCs, administered without immunosuppression, were safe and had efficacy and mechanisms of action similar to that of autologous CDCs.²⁶⁻²⁸

Based on these grounds, Makkar et al performed the ALLogeneic heart STEm cells to Achieve myocardial Regeneration (ALLSTAR, NCT01458405) trial, a multicentre, randomized, double-blinded, placebo-controlled study evaluating the safety and efficacy of intracoronary administration of allogeneic CDCs in patients with post-MI with LV dysfunction.²⁹ They enrolled patients 4 weeks to 12 months after MI, with LVEF <45% and LV scar size >15% of LV mass as assessed by MRI. Patients were randomly allocated in a 2:1 ratio to receive CDCs or placebo in the infarct-related artery by stop-flow technique. The primary efficacy endpoint was the relative percentage change in infarct size at 12 months post-infusion as assessed by contrast-enhanced cardiac MRI. Of 142 eligible patients, 134 were eventually randomized (90 to the CDC group and 44 to the placebo group). Treatment was safe but did

not reduce scar size relative to placebo at 6 months. The investigators reported reductions in end-diastolic LV volume and N-terminal pro b-type natriuretic peptide in the cell treated group.

4.2. *Chronic heart failure*

Available evidence suggests that cell therapy can improve the outcome of chronic HF. In particular, Phase II trials of MSCs have yielded encouraging results. For instance, ixCELL-DCM (NCT01670981) was a double-blind, placebo-controlled phase 2B trial that recruited patients with New York Heart Association class III or IV symptomatic HF due to ischaemic dilated cardiomyopathy from 31 sites in North America.³⁰ Recruitment criteria were LVEF 35% or less, use of an automatic implantable cardioverter defibrillator, and ineligibility for revascularization procedures. Patients were randomly allocated to receive ixmyelocel-T or placebo at the time of bone marrow aspiration and followed for 12 months. A total 126 participants were eventually recruited and assigned to ixmyelocel-T (n=66) or placebo (n=60). The primary endpoint was a composite of all-cause death, hospitalization due to cardiovascular symptoms, and unplanned clinic visits to treat acute HF. Primary efficacy endpoint analyses and safety analyses were done by modified intention to treat. The trans-endocardial delivery of ixmyelocel-T resulted in a significant reduction in clinical cardiac events compared with placebo leading to improved patient outcomes.

The MSC-HF trial was a double-blind, placebo-controlled trial, where patients were randomized 2:1 to intramyocardial injections of MSCs or placebo.³¹ The primary endpoint was LV end-systolic volume, assessed by MRI or computed tomography. A total 60 patients with ischaemic HF, New York Heart Association class II-III, LVEF <45% and no further treatment options were recruited. After 12 months, volumetric indexes were reduced in the MSC group only, with a reduction of 17.0 mL compared with placebo. There were also improvements in LVEF of 6.2% and myocardial mass between groups. Furthermore, the improvements were dose related. After 4 years, the MSC group had experienced fewer hospitalizations for angina whereas no differences in hospitalizations or survival were reported.

The PercutaneOus StEm Cell Injection Delivery Effects On Neomyogenesis in Dilated CardioMyopathy (POSEIDON-DCM) trial in patients with non-ischemic cardiomyopathy demonstrated that allogeneic MSCs significantly increased LVEF and 6-minute walk test distance in comparison with the autologous group.³²

The Congestive Heart Failure Cardiopoietic Regenerative Therapy (CHART-1, NCT01768702) trial aimed to validate cardiopoiesis-based biotherapy in a larger HF cohort.³³ This multinational, randomized, double-blind, sham-controlled study was conducted in 39 hospitals, with a pre-screening of 484 patients with symptomatic ischemic HF who were on guideline-directed therapy. Of eligible subjects, 348 underwent bone marrow harvest for MSC expansion, which achieved the goal of 24 million cells in 315. This cohort was randomized to cardiopoietic cells delivered endomyocardially with a retention-enhanced catheter (n = 157) or sham procedure (n = 158). Procedures were eventually performed in 271 patients (n = 120 cardiopoietic cells, n = 151 sham). The primary efficacy endpoint was a Finkelstein-Schoenfeld hierarchical composite (all-cause mortality, worsening heart failure, Minnesota Living with Heart Failure Questionnaire score, 6-min walk distance, LV end-systolic volume, and LVEF) at 39 weeks. The primary endpoint was neutral, with safety demonstrated across the cohort. The authors performed an exploratory analysis according to the baseline LV volumes, which showed a benefit of cell treatment on the primary composite in patients with baseline LV end-diastolic volume 200–370 mL (corresponding to 60% of patients). They concluded that further evaluation of cardiopoietic cell therapy in patients with elevated end-diastolic volume is warranted.³³

Four additional trials are ongoing in HF [Combination of Mesenchymal and c-kit+ Cardiac Stem Cells As Regenerative Therapy for Heart Failure (CONCERT-HF),³⁴ Double-Blind Randomized Assessment of Clinical Events With Allogeneic Mesenchymal Precursor Cells in Advanced Heart Failure (DREAM-HF),³⁵ Stem Cell therapy in IschEmic Non-treatable Cardiac disease (SCIENCE, a Horizon 2020 funded multicentre phase II study using allogeneic Cardiology Stem Cell Centre Adipose-derived Stromal Cells),³⁶ and Cardiology Stem Cell Centre Adipose Stem Cell (CSCC_ASC) in patients with LVEF ≤45% and HF].

4.3. *Refractory angina*

A significant number of patients are not amenable to or have residual myocardial ischemia after revascularization.³⁷ The majority of these patients suffer from refractory angina (100,000 new cases per year in Europe), which is associated with a markedly impaired quality of life. Furthermore, nearly a quarter of STEMI patients in the UK are not revascularized within the recommended time targets, resulting in larger infarcts and increased risk of HF and death.³⁸ New treatment principles, such as therapeutic angiogenesis, metabolic modulation, and novel interventional techniques, have been proposed to improve the quality of life and prevent the development of ischemic HF in these critical patients.^{39, 40} Proangiogenic therapies may help stabilize the infarct scar and reduce its extension, by modulating the extracellular matrix remodelling, promoting cardiomyocyte salvage in the area at risk, and awakening the hibernated myocardium. Furthermore, the improvement in myocardial perfusion is expected to treat angina and improve exercise tolerance.

Strong preclinical data have led to the development and implementation of three double-blind, placebo-controlled trials of CD34+ cells with similar designs.⁴¹⁻⁴³ A meta-analysis of these trials demonstrated a significant improvement in exercise time, a reduction in angina and in major adverse cardiac events, and an improvement in exercise capacity compared with subjects randomized to placebo.⁴⁴ The results of the Efficacy and Safety of Targeted Intramyocardial Delivery of Auto CD34+ Stem Cells for Improving Exercise Capacity in Subjects With Refractory Angina (RENEW) were also critically analyzed in an editorial article.⁴⁵ This was a multicenter, double-blind randomized controlled trial testing the intramyocardial administration of purified autologous CD34+ cells in patients with refractory angina with no coronary revascularization options.⁴³ The study was sponsored by Baxter Healthcare, Deerfield, Illinois and was designed to assess the benefit in total exercise time, which was measured using a modified Bruce protocol at 12 months. Key secondary endpoints were: 1) change in angina frequency at 3, 6, and 12 months; and 2) change in exercise time at 3 and 6 months. The safety endpoint was the incidence of MACE through 24 months. Only 112 of the planned 444 patients were enrolled, thus precluding the assessment of the primary efficacy endpoint. Nonetheless, patients treated with cell therapy displayed better exercise capacity at each time point, longer mean and median exercise time at 6 months, and fewer angina episodes at 3 and 6 months. There were no safety concerns for major MACE, cardiac perforation, or ventricular arrhythmia.

An appraisal of CD34+ cell therapy impact on health care expenditures before and after treatment was published in 2020.⁴⁶ The study assessed the effect of CD34+ cell therapy on cardiac-related hospital visits and costs during the 12 months follow-up for subjects with refractory angina. Patients randomized to cell therapy had a 50% reduction in cardiac-related hospital visits 12 months post-injection, which was associated with a 62% cost reduction corresponding to an average savings of \$5500 per cell therapy patient. Interestingly, the placebo group also demonstrated a reduction in care costs, although to a lesser degree than the CD34+ group. In addition, CD34+ cell therapy was associated with improved survival.⁴⁶

5. *SYSTEMATIC REVIEW AND META-ANALYSIS OF MESENCHYMAL STEM CELL THERAPY IN ADULT PATIENTS WITH ISCHEMIC HEART DISEASE*

As an increasing number of patients are being asked to participate in MSC therapy studies, the safety issue becomes extremely relevant, with any risk of adverse events representing a significant barrier to successful translation into clinical practice.

A meta-analysis of MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials and Web of Science searched randomized controlled trials (RCTs) from 2012–2019 that compared intravascular delivery of MSCs to controls in adult populations.⁴⁷ Pre-specified adverse events were grouped according to: (1) immediate, (2) infection, (3) thrombotic/embolic, and (4) longer-term events (mortality, malignancy). Adverse events were pooled and meta-analyzed by fitting inverse-variance binary random effects models. Primary and secondary clinical efficacy endpoints were summarized descriptively. Fifty-five studies met inclusion criteria (n = 2696 patients). MSCs as compared to controls were associated with

an increased risk of fever (Relative Risk (RR) = 2.48), but not non-fever acute infusional toxicity, infection, thrombotic/embolic events, death, or malignancy. No included trials were ended prematurely due to safety concerns. The results were reassuring and confirmed the safety profile, but also were delivered with the authors' recommendation that future trials should continue to strengthen study rigor, reporting of MSC characterization, and adverse events.

A CVD-focused systematic review evaluated the safety and efficacy of MSC therapies in patients with acute MI and ischemic HF.⁴⁸ The protocol was prospectively registered with the PROSPERO database of systematic reviews (CRD42016043540) and prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol checklist. The Cochrane risk of bias tool was used to assess bias of included studies. The primary outcome was safety (incidence of adverse events) and the secondary outcome was efficacy, mortality, and changes in LVEF. Of 23 studies eligible, 11 were on MI and 12 evaluated ischemic HF. Results showed no association between MSCs and acute adverse events. Moreover, a significant improvement in overall ventricular function was observed in patients treated with MSCs, but not on mortality.

A systematic search and critical review of MEDLINE, EMBASE, and Cochrane database literature published from inception through December 2017 was performed regarding the efficacy of randomized controlled trials of MSCs in patients with MI.⁴⁹ A total of 950 patients from 14 randomized, placebo-controlled trials were included. MSC treatment showed benefits for functional and clinical outcomes. Regarding hemodynamic parameters, LVEF was increased by 3.84% in the MSC-treated group (95% CI: 2.32~5.35, I²=43) and the effect was maintained for up to 2 years. Scar mass was reduced by -1.13 (95% CI: -1.80 to -0.46, I²=71) at 6 months after MSC treatment. Mortality rate and incidence of re-hospitalization for HF in MSC group patients was a trend to reduced incidence compared to the control group.

Another meta-analysis focusing on therapeutic effect on HF examined nine studies involving 612 patients who underwent MSCs or placebo treatment.⁵⁰ The overall rate of death showed a trend of reduction of 36% (RR [CI] = 0.64 [0.35, 1.16], p = 0.143) in the MSC treatment group. The incidence of readmission was reduced by 34% (RR [CI] = 0.66 [0.51, 0.85], p = 0.001). The patients in the MSC treatment group realised improvement exercise tests in association with a reduction in NYHA class levels.⁵⁰

A general appraisal of meta-analyses of cell therapy studies in acute MI and HF has been published by Gyöngyösi and colleagues in 2018.⁵¹ The same author had already published the ACCRUE meta-analysis (analysis of cell-based cardiac studies; NCT01098591) comprising 1252 individual patient data-sets from 12 randomized trials of intracoronary cell therapy, which showed no therapeutic advantage of cell therapy when compared to controls.⁵² It should be noted that Fisher and colleagues detected a therapeutic-effect in regard to exercise capacity, LVEF and quality of life.⁵³ According to these authors, most meta-analyses agree that the potential benefit of cell therapies were still inconclusive and statistically underpowered. More recent large trials enrolling patients with ischemic HF and MI published neutral results regarding changes in LVEF.^{30, 33, 54} In addition to the concerns regarding statistical power, meta-analyses may be confounded by 2 major sources of variation: (1) pitfalls in trial design and (2) inconsistencies in reporting and interpreting trial results. Therefore, ACCRUE consortium and guidelines published by the International Committee of Medical Journal Editors recommend data sharing on publication of trial results in order to resolve controversies in data interpretation and direct future clinical trials.^{55, 56} These concepts are reinforced by the DAMASCENE study, which has highlighted the flaws of numerous trials in reporting the outcomes. Interestingly, the more discrepancies were detected in a trial the better was the reported outcome. In contrast, trials that were seemingly flawless did not detect any effect of cell therapy.⁵⁷

6. SURROGATE ENDPOINTS AND NEW PREDICTIVE MARKERS OF MORTALITY TO DESIGN WELL POWERED CLINICAL TRIALS

As mentioned above, mortality and MACE rates have substantially improved in STEMI patients, making almost impossible to design an efficacy trial using clinical outcome as the primary endpoint.²³ Surrogate endpoints are often used to evaluate the efficacy of a drug instead of waiting to evaluate its effects on mortality. Selecting the right surrogate endpoint and proving that it is a good predictor of clinical outcome, however, is not always simple, because many background factors can act as confounders. Several surrogate endpoints have been evaluated in stem cell clinical trials, such as LV function or scar size, with results not been encouraging (reviewed in ²³). Likewise, recent meta-analyses do not support beneficial effects on scar size or LV function, as assessed by cardiac MRI.⁵⁸

Biomarkers may represent an attractive option for prediction of clinical outcome but also to identify the high-risk patients who could mostly benefit from a new treatment. In the latter case, the biomarker may help designing sufficiently powered trials without involving thousand patients. A study published in *Circulation Research* a few years ago reported that low levels of circulating CD34+ cells predict all-cause and cardiovascular mortality in patients undergoing coronary angiography over 2 years.⁵⁹ Other four longitudinal studies had previously assessed the prognostic role of peripheral blood stem/progenitor cell phenotypes.⁶⁰⁻⁶³ Of these, 2 reported an association between reduced CD34+ cells and all-cause mortality.^{60, 61} In an editorial article commenting the results from a total of 1335 patients, Fadini has calculated that the pooled hazard ratio of death from any cause was 2.78 (95% CI, 1.29–7.37) for low versus high CD34+ cell count.⁶⁴ Patients with low circulating levels of CD34+ cells may represent a preferred target population for allogeneic cell therapy aiming to overcome the lack of endogenous reparative mechanisms.

In addition, our team has proposed that the functional quality of CD34+ cells could be a predictor of cardiovascular mortality. In a proof-of-concept study, we performed a 18-month follow-up in a consecutive series of 119 patients with type 2 diabetes, who underwent revascularization for critical limb ischemia.⁶⁵ CD45dim CD34+ CXCR4+ KDR+ cells were assessed by flow cytometry upon isolation and also after spontaneous or stromal cell-derived factor 1 α -directed migration in an *in vitro* assay. The association between basal cell counts and migratory activity and the risk of an event at 18-month follow-up was evaluated in a multivariable regression analysis. Results from this analysis showed that cell migration forecasts cardiovascular mortality independently of other validated predictors, such as age, coronary artery disease, serum C-reactive protein, and estimated glomerular filtration rate. In this model, doubling of migrated cell counts increased the cardiovascular death hazard by 100% ($p < 0.0001$). Recently, we validated these findings in a new study, where the association between CD34+ cell migration and cardiovascular mortality was reassessed at 6 years after revascularization.⁶⁶ The study also comprised a new series of patients with diabetes and critical limb ischemia and control subjects, whose bone marrow CD34+ cells were profiled for miRNA expression and assessed for apoptosis and angiogenesis activity. Multivariable regression analysis confirmed that CD34+ cell migration forecasts long-term cardiovascular mortality. Moreover, we provided a mechanistic interpretation for the adverse outcome predictor. In this high-risk population, CD34+ cells become antiangiogenic and pro-apoptotic due to the adverse metabolic environment they are exposed to. Once they enter the circulation, these cells may deliver pathogenic signals to the vascular endothelium, thereby accelerating ischemic complications. The study has important clinical implications. Autologous bone marrow derived CD34+ cells are currently used not only in patients with heart disease but also in clinical trials of critical limb ischemia. Our findings calls for caution in using CD34+ cells that carry a pro-apoptotic and anti-angiogenic molecular signature that we identified as low miR-21/high PDCD4. New investigation is warranted to determine if this signature can be exploited to select patients which would benefit from an approach using healthy allogeneic cells instead of autologous cells.

7. CONGENITAL HEART DISEASE

Cell therapy and tissue engineering hold promises for correction of CHD. Application of cell-based technology is supported by the peculiar microenvironment of the youngest heart, which theoretically should permit proliferation of immature cardiac cells for functional remuscularization in association with minimal fibrotic scarring.

Preclinical studies have demonstrated the benefit of delivering different cell types in animal models, including skeletal myoblasts, UCB stem cells, and MSCs, either intramyocardially or epicardially to treat right ventricle dysfunction and pulmonary artery hypertension induced by pressure or volume overload.⁶⁷⁻⁶⁹ This evidence has supported initial clinical trials, some already concluded and others still ongoing, in patients with HLHS (reviewed in⁷⁰). This condition is one of the most complex congenital defects, with a prevalence of 2 to 3 per 10 000 live births. Anatomically, HLHS is characterized by underdeveloped LV, which cannot provide adequate systemic perfusion (**Figure 2**). The choice of HLHS as the target disease for regenerative therapies in CHD management is multi-factorial and includes the following considerations: 1) severity of the disease, 2) palliative nature and burden of long-term outcomes, 3) need for multi-stage surgical procedures that provide time points to adjunctively intervene, and 4) prenatal diagnosis enabling planned collection of cells such as those contained in UCB.

The Transcoronary Infusion of CPCs in patients with single ventricle Physiology (TICAP, NCT01273857) trial was the first study of cell therapy for congenital heart defects. This phase I nonrandomized, controlled study aimed at confirming the feasibility of intracoronary delivery of autologous cardiac progenitor cell transfer.⁷¹ The approach was safe and resulted in an improvement in right ventricle ejection fraction (RVEF) 7 percent units (from 46.9±4.6% to 54.0±2.8%) together with a significant reduction in tricuspid valve annulus diameter. Treated patients also showed significant reductions in RV free wall mass and indexed end-systolic and end-diastolic volumes at 18 months. Finally, cell therapy improved somatic growth of from baseline to 18 months, as indicated by an increase in z scores for height and weight, whereas there was no change in the control group.

The PERSEUS (Cardiac Progenitor Cell Infusion to Treat Univentricular Heart Disease) randomized Phase 2 trial was a follow up of the TICAP phase 1 study, designed to provide a rigorous efficacy assessment with 2 treatment protocols, primary and late transplantations.⁷² Results indicate clinically significant responses in terms of improved cardiac function (reduced ventricular volumes and fibrosis), HF symptoms, somatic growth, and health-related quality of life.

The APOLLON (Cardiac Stem/progenitor Cell Infusion in Univentricular Physiology: NCT02781922) was a Phase 3 trial, which was implemented by a sponsor-initiated multicentre collaboration between 3 children's hospitals. The aim was to compare the therapeutic efficacy of cardiac progenitor cells versus placebo. The study followed up 40 participants with single ventricle physiology for 12 months. No results have been posted on ClinicalTrials.gov for this study.

Several additional studies are ongoing. The allogeneic hMSC Injection in Patients With Hypoplastic Left Heart Syndrome (ELPIS, NCT02398604) is an Open Label Pilot Study. A total of 30 patients with HLHS were planned to be enrolled in a staged process. Twenty patients were attributed to receive intramyocardial injection of the allogeneic MSCs while 10 patients with no cell injection served as control. Study was completed in March 2019.⁷³

The NCT02256501 trial is a study investigating the feasibility Clinical Trial Phase I/II of Intracoronary Transplantation of Autologous BM-MNCs in idiopathic dilated cardiomyopathy in paediatric patients. According to the inclusion and exclusion criteria of the trial, 32 patients with LVEF <45% not responsive to the standard medical therapy were randomly allocated in 2 groups including BM-MNCs (n=16) or vehicle (n=16). Only the MNC group underwent the bone marrow aspiration and intracoronary injection. The investigators followed the patients at 2 weeks, 1, 2, 4 and 6 months after transplantation for cell therapy group or registration for placebo by physical examination, laboratory tests and imaging such as echocardiography, and cardiac MRI. No results are available on ClinicalTrials.gov for this Study.

Safety study of autologous UCB cells for Treatment of HLHS (NCT01883076) was a Phase I study to determine the safety and feasibility of injections of autologous umbilical cord blood (UCB) cells in children undergoing a scheduled Glenn surgical procedure. This study will determine the feasibility of collection, processing, and delivery of autologous cells as used in adult cardiac regenerative protocols. The estimated study completion date is May 2021.

The HOPE trial (NCT02485938) was aimed to assess the feasibility, safety, and efficacy of intracoronary allogeneic cardiosphere-derived cells (CAP-1002) in patients with Duchenne muscular dystrophy. It recruited 25 subjects and showed safety and signals of efficacy on both cardiac and upper limb function for up to 12 months.⁷⁴

8. SECOND GENERATION CELL THERAPY

8.1. Embryonic stem cells and induced pluripotent stem cells

Several candidate cell products have been proposed in the last five years, as revised in.^{75, 76} Regulatory agencies recommend that research should not exclude any cell source a priori in the fight against cardiac disease. The ones with the highest therapeutic potential but also associated with risk for adverse effects are derived from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Menasché et al. reported the first clinical application of cardiac patches made using human ESC-derived cardiac progenitor cells in a patient suffering from severe ischemic left ventricle dysfunction.⁷⁷ Despite the well-documented capacity of ESCs to differentiate into cardiomyocytes, a critical appraisal of the potential for clinical application identified daunting limitations, including graft rejection, arrhythmias, and potential risk of teratomas.^{revised in 78}

iPSCs share the same characteristic of ESCs, but being generated from patient-specific somatic cells, they may surmount the ethical limitations and provide an autologous source of reparative cells. The reprogramming efficiency has been improved and it is now possible to generate iPSCs without genomic insertion of transgenes. Transplantation of sheets containing allogeneic iPSC-derived cardiomyocytes reportedly regenerated the infarcted non-human primate heart.^{79, 80} However, iPSC-derived cells are not completely mature and their major application remains currently limited to drug discovery and toxicology testing.⁸¹

Extracellular vesicles secreted by iPSC may offer a safer scope in the treatment of CHF, through their specific miRNA signature and the associated stimulation of distinct cardioprotective pathways. The processing and regulatory advantages of extracellular vesicles and exosomes from iPSC and adult allogeneic stem cells could make them effective substitutes for cell transplantation.⁸²⁻⁸⁴ Moreover, extracellular vesicles can be bioengineered to increase their stability, bioactivity, presentation to target cells and capacity for on-target binding at both cell-type-specific and tissue-specific levels.⁸⁵ This potential remains almost completely unexplored in the cardiovascular field.

8.2. Our experience with pericytes

Pericytes have been isolated from several human tissues. One major achievement from our laboratory was the deployment of a reliable and reproducible method for isolation and expansion of pericytes from small tissue biopsies or leftovers of cardiac surgery. The method was then enhanced with use of clinical grade reagents.^{86, 87} Transplantation of pericytes from the human saphenous vein showed promising results in murine models of non reperfused MI, with synergistic activity when combined with cardiac stromal cells.^{88, 89} However, pericyte therapy was less effective in a swine model of reperfused MI, improving angiogenesis and reducing interstitial fibrosis, but failing to supporting LV function as assessed by cardiac MRI.⁹⁰ These discrepancies could be attributed to the inadequate upscaling of cell dosage from the mouse to the pig or to the stronger immune response of the swine to xenogeneic cells. Nonetheless, results obtained using human pericytes were replicated when allogeneic pericytes were transplanted in the infarcted swine heart.⁹⁰ These findings have discouraged further preclinical experimentation and convinced us to redirect our line of research.

Cardiac pericytes may represent a tissue specific solution for targeted repair of the ischemic heart. Distinct pericyte populations have been isolated from cardiac tissue, via selection of a spectrum of markers (CD146+/CD34-/CD45-/CD56-/CD117-11, CD31-

/CD34+12), using fluorescent activated cell sorting or magnetic activated cell sorting. We showed that from just 100 mg of human heart tissue, approximately 20 million cells can be generated within 6 weeks.⁹¹ Using flow cytometry and immunocytochemistry, we demonstrate the purity of isolated cardiac pericytes and the identity of cells expanded from the bulk population or single cells.^{91, 92} Furthermore, cardiac pericytes demonstrated a potential for differentiation into a contractile smooth muscle cells and were also able to enhance network formation with endothelial cells on a Matrigel substrate.⁹¹ Although phenotypical and functional assays indicate cardiac pericytes to be a reliable candidate for regenerative medicine, the invasive nature of acquiring a cardiac biopsy represents a relevant limitation. We privilege therefore the strategy of reprogramming endogenous pericytes using clinically available drugs, guided in this endeavour by the increasing knowledge of the molecular signalling and interactive relations between pericytes, endothelial cells, myocytes, and the heart extracellular matrix. Moreover, unbiased approaches using high-throughput small molecule screening may help to deliver clinically valuable drugs to modulate specific pericyte functions and improve their regenerative potential in the ischemic heart.

Pericytes can also offer a scope for bioengineering of prosthetic valves and conduits employed in corrective cardiac surgery. In Bristol, we combine experience in cell biology, translational medicine, and innovative cardiac surgery to reduce the burden of congenital and acquired cardiac defects. Caputo has already reported three successful randomized preclinical trials in swine.⁹³⁻⁹⁵ Two consisted in the reconstruction of the left pulmonary artery in piglets using grafts seeded with cord blood or Wharton jelly derived mesenchymal stromal cells.^{94, 95} A third study successfully demonstrated the ability of thymus derived stromal cells to confer patches with antifibrotic properties when applied onto the right ventricle outflow tract (RVOT) of piglet hearts.⁹³ Cellularized grafts showed improved RVOT strain, reduced fibrosis and increased cardiac remodelling and endothelialization when compared to unseeded grafts. An additional pilot study demonstrated the feasibility of using neonatal cardiac pericytes from littermate for reconstruction of small-size branch pulmonary arteries in piglets.⁹² This initial proof-of-concept study has been followed by a randomized, controlled trial on a total of 12 piglets, results showing cardiac pericyte-seeded grafts improved endothelialization of the graft and remodelling of the pulmonary artery stump. Nonetheless, the approach did not resolve the growth mismatch between the grafted and the untouched contralateral artery

9. DIRECT REPROGRAMMING

In the last years, the innovative idea of cell reprogramming was transferred from cell culture system to resident cardiac cells. The potentially revolutionary concept is to differentiate terminally differentiated myofibroblasts into cardiomyocyte-like cells directly, without an intermediate induction of pluripotency. This molecular intervention should simultaneously reduce the scar tissue and increase the pool of functional cardiomyocytes. Transcription factors were first used to this goal, followed by microRNAs and/or small molecules.

Srivastava et al. pioneered the field by using a combination of three developmental transcription factors (Gata4, Mef2c, and Tbx5).⁹⁶ The same group demonstrated that retroviral delivery of the same factors *in vivo* could reprogram murine cardiac fibroblasts into cardiomyocytes, with decreased infarct size and attenuated cardiac dysfunction.⁹⁷ Adding Hand2 to the above combination resulted in more relevant outcomes in the same model, according to studies from Olson's laboratory.⁹⁸ Genome-wide analyses of cardiogenic transcription factors during cardiac reprogramming showed that transcription can synergistically activate gene enhancers, thereby recruiting other transcription factors to the reprogramming process.⁹⁹ This mechanism mirrors the enhancer activation that occurs during embryonic cardiogenesis. The recognition of gene regulatory networks could ultimately lead to improved efficiency of the cardiac reprogramming strategy.

As tissue-specific miRNAs play pivotal role in cardiac development and function, the regulation of their expression would aid in generating functional cardiomyocytes starting from human fibroblasts.¹⁰⁰ Endogenous miRNAs can be easily overexpressed or downregulated through the administration of miRNA mimics or miRNA inhibitors, respectively.¹⁰¹ MiRNA

mimics and inhibitors hold a great potential as therapeutic agents because of their cytoplasmic activity, relatively small size and ability to be administered systemically or locally by nanoparticle-based delivery systems, avoiding the use of viral vectors.¹⁰²

A number of miRNAs were discovered through systematic screenings (e.g. miR-199a-3p and miR-590-3p) reviewed in ¹⁰³. Of these, several can suppress cardiomyocyte proliferation being involved in the withdrawal of cardiomyocytes from the cell cycle after birth (e.g. the let-7 and miR-15 families). Overlapping regulatory roles on cardiomyocytes proliferation are exerted by long ncRNAs. The possibility of reprogramming endogenous cardiac cells into a regenerative state through miRNA and ncRNA therapeutics is extremely exciting. Nonetheless, however extensive experimentation in large mammals and meticulous assessment of safety are required to advance this field towards clinical application. Moreover, the efficiency of the reprogramming process needs to be enhanced to provide significant clinical outcomes.

10. MODULATION OF OXIDATIVE STRESS

Reactive oxygen species (ROS) are implicated in important cellular processes including signalling, regulation of homeostasis, or induction of death. Low levels of ROS are necessary for quiescent stem cells to maintain their self-renewal. Mild increase of ROS in stem cells causes lineage differentiation, whereas excessive ROS cause stem cell senescence and death. In addition, ROS were shown to facilitate reprogramming adult lineage committed cells into iPSCs.¹⁰⁴ Mitochondrial functionality and efficient ROS scavenging mechanisms are therefore key in stem cells to maintain low oxidative stress and preserve cell viability.

In line with the purpose of this article, we underscore the importance of ROS as an invariable element that stem cells sense upon the transplantation in an ischemic tissue. ROS levels during ischemia are dictated by changes in the mitochondrial respiratory chain and oxidation of ferrous heme (Fe²⁺) in the oxymyoglobin complex.¹⁰⁵ Specifically, Fe²⁺ is converted into ferric heme (Fe³⁺) during ischemia, which is paired to the formation of O₂^{•-}.¹⁰⁶ After reperfusion, an additional burst of ROS causes incremental damage via a reaction catalyzed by xanthine oxidase and the recruitment of leukocytes, which release toxic oxidants triggering the mitochondrial permeability transition-dependent death.^{107, 108}

10.1. Oxidative stress during cell delivery

Therapeutic protocols of stem cell therapy include the delivery via different routes: intracoronary, intramyocardial, and intravenous. There are advantages and disadvantages of each route and variable retention rate of cells in the myocardium. The intracoronary delivery represents the most clinically practiced form. It is generally performed with a balloon catheter, which is inflated during a percutaneous coronary intervention for treating stenotic coronary arteries. Stem cells are infused through the catheter in one of two manners: (1) nonocclusive angioplasty at slow or high flow rates while maintaining coronary flow or (2) stop-flow method by interrupting it with balloon occlusion. The main advantages are its direct infusion into the target area and the resulting homogenous cell engraftment. In both cases, injected cells are exposed to the oxidative stress typical of ischemia; with additional ROS burden from stop-flow reperfusion. This combined stress causes selective pressure of engrafted cells having to survive less-than-ideal nutrient-deprived and hypoxic conditions. In fact, the low engraftment represents a persistent limitation of stem cell therapy.¹⁰⁹

10.2. Strategies to protect cell product from ROS-induced damage

Several strategies have been proposed to address the low engraftment in ischemic tissues, including preconditioning and genetic manipulations prior to cell transplantation. Preconditioning refers to a procedure where the cell product is exposed to various physical and chemical factors in vitro. The different approaches used for this effort have been recently reviewed.¹¹⁰ Different preconditioning protocols with hypoxia, starvation, or chemicals have been employed in preclinical studies.

Maintaining cardiac stem cells under an inductive environment, not only during expansion but also particularly during isolation, may provide the most therapeutic cells possible for transplantation into patients. A better understanding of the metabolic and oxidative

adaptation of transplanted cells is crucial to refine the preconditioning methods. Non-invasive metabolic tracing, based on nuclear magnetic resonance spectroscopy, already allows for in vivo assessment of fuel pathways adopted by the injured heart. Improvement in sensitivity at single cell level, something has not been achieved yet, may allow capturing changes in the respiratory and metabolic pathways of engrafted stem cells. In a prototype application, Zhao and colleagues developed a protocol to image and monitor NAD(+)/NADH redox state in living cells and in vivo using a highly responsive, genetically encoded fluorescent sensor known as SoNar (sensor of NAD(H) redox).¹¹¹ SoNar was applied to high-throughput chemical screening of candidate compounds targeting cell metabolism in a microplate-reader-based assay, and next to in vivo fluorescence imaging of tumor xenografts in mice.¹¹¹

Another complementary approach is to use stem cells empowered with potent detoxifier and antioxidant mechanisms. Studies in human pericytes showed SOD3 plays an important role in their therapeutic activity in ischemia.¹¹² After exposure to hydrogen peroxide, human pericytes showed mild increases in apoptosis, protein carbonylation, and γ H2AX phosphorylation.¹¹² Furthermore, ROS-stressed pericytes retained intact pro-survival and pro-angiogenic paracrine activities.^{112, 113} Conversely, pharmacological inhibition of SOD3 increased ROS levels in pericytes and impaired their survival. Likewise, pericyte differentiation was associated with SOD3 down-regulation, suggesting ROS is essential for the lineage commitment, though the increase in ROS eventually induced apoptosis.¹¹² In a mouse limb ischemia model, an injection of naïve pericytes, but not SOD3-silenced pericytes, improved perfusion recovery and neovascularization.¹¹²

10.3. Targeting transcription factors at the intersection of oxidative stress and angiogenesis

Adaptive response to increased oxidative stress involves the induction of antioxidant response element ARE genes that are controlled by the transcriptional activator Nrf2 and the transcriptional repressor Bach1. Under steady state conditions, Nrf2 levels in the cytoplasm are maintained at low levels by binding to Keap1 and Cullin 3, which leads to the degradation of Nrf2 by ubiquitination.^{114, 115} Oxidative stress inhibits ubiquitination thereby increasing Nrf2 protein levels and its transport into the nucleus, which results in transcription of antioxidant and cytoprotective genes, including SOD3, catalase, and heme oxygenase-1 (*HMOX-1*).^{116, 117} Bach1 opposes this function by binding to the small Maf proteins and ARE promoter sequences and competing with Nrf2-ARE promoter interaction, thereby resulting in suppression of Nrf2 activity.¹¹⁸ Bach1 repression is necessary for ARE genes, such as *HMOX-1*, to be fully expressed.^{119, 120}

The Nrf2-Bach1 pathway is now regarded as a valuable therapeutic target for the treatment of age-related degenerative diseases,¹²¹ in which this antioxidant mechanism is reportedly depressed.^{122, 123} There is also initial evidence supporting modulation of the Nrf2-Bach1 pathway can induce cardiovascular benefits. *BACH1* gene deletion protects from a variety of pathologies in mice, including myocardial ischemia-reperfusion, pressure overload, and limb ischemia.¹²⁴⁻¹²⁶ Conversely, global *BACH1* overexpression impairs developmental and postnatal reparative angiogenesis through inhibition of Wnt/ β -catenin signalling and decreased VEGF expression.^{127, 128} Data on Nrf2 are more controversial, as its abrogation reduced angiogenesis *in vitro*, but unexpectedly increased muscle blood flow recovery in mice with limb ischemia.¹²⁹ We discovered that targeting a new signalling pathway involving microRNA-532-5p and its inhibitory target *BACH1* results in angiopoietin-1 transcription and associated induction of in vitro angiogenesis.¹³⁰ Thus, Bach1 could represent a molecular crossroad orchestrator of oxidative stress and angiogenesis (**Figure 3**).

10.4. Metabolic reprogramming

Critical limb ischemia represents a challenging test bench for cell therapy. We have shown that transplantation of human pericytes can improve the recovery of ischemic limbs in mice.^{86, 87, 131} In the attempt to improve the potential of an autologous approach, we pursued the metabolic conditioning of pericytes that were isolated from limb muscles of vascular patients with diabetes mellitus.¹³² These cells display elevated levels of oxidative stress causing a dysfunctional phenotype, which we tried to rescue with dimethyl-2-oxoglutarate (DM-2OG), a tricarboxylic acid cycle metabolite with antioxidant properties.¹³² Using an extracellular flux

analyser and anion-exchange chromatography-mass spectrometry and redox tracers, we demonstrated that the muscular pericytes from patients with diabetes and limb ischemia had suppressed glycolytic activity and reduced ROS-buffering capacity, but no reduction of antioxidant systems when compared with controls. DM-2OG supplementation improved redox balance and mitochondrial function but failed to affecting glycolysis or antioxidant systems.¹³² Although treated pericytes were not able to regain myogenesis features, which are typical of these progenitor cells, they could be restored by DM-2OG to form vascular network in a 3D co-culture angiogenesis assay with ECs.¹³² We concluded that metabolic reprogramming improves pericyte redox balance and mitochondrial function, while concurrently allowing for enhanced pericyte–endothelial crosstalk.¹³² These preclinical data open new avenues for targeted metabolic interventions in critical limb ischemia, either through direct reprogramming of resident cells and/or transplantation of redox restored, autologous cells. A graphical abstract accompanying our previous article illustrates the metabolic mechanism relevant to pericyte restoration.¹³²

10.5. Subcellular substitutes harnessing oxidative stress and regeneration

Another fascinating method to harnessing tissue regeneration encompasses the delivery of subcellular fractions instead of entire cells. Microvesicles contain a pro-oxidant or antioxidant machinery that may produce or scavenge ROS, but also molecules that can modify ROS metabolism in target cells. Exosomes containing functional NADPH oxidase 2 complexes are reportedly released from macrophages and incorporated into injured neurons via endocytosis.¹³³ This stimulated PI3K-phosphorylated Akt signalling and importantly aided regenerative nerve outgrowth.¹³³ Stress or pathological conditions, as well as aging and diabetes, can modify the effect of microvesicles on ROS metabolism. However, the defect is not irreversible according to a study showing that infant MSC-derived microvesicles rejuvenated elderly MSCs by inhibiting ROS production and cellular senescence.¹³⁴

Moreover, artificial mitochondrial transfer has emerged as an additional subcellular option for tissue repair. The strategy has been intensively investigated in brain and cardiac disease models.^{135, 136} The administration of MSC-derived mitochondria reduced ROS production while increasing mitochondrial superoxide dismutase 2.¹³⁷ There are five trials testing the therapeutic applications of MSC derived vesicles and two proposing the use of mitochondria. Commercially valuable therapeutic products of this kind should be manufactured according to the same GMP procedures used for cell therapy. Different companies are focusing on optimizing the manufacturing platform for the transition from bench-scale to clinical production of exosomes.¹³⁸

11. REPURPOSING CLINICALLY AVAILABLE DRUGS: NEW LIFE FOR BETA-BLOCKERS

Beta-blockers are a pillar in the treatment of IHD and HF. Initially, contraindicated in this condition up to the late 1990s, this class of drugs continues to surprise scientists for their pleiotropic action on the heart. The group of Professor Harding and Professor Poole-Wilson at the Imperial College London contributed to understanding the reduced stimulatory contractile response to catecholamines in individual cardiac cells from failing heart.¹³⁹ They showed that each cell could respond to catecholamines through both stimulatory β 1-adrenoceptors (β 1ARs) and the protective β 2ARs. Pioneer trials, such as the Carvedilol Or Metoprolol European Trial (COMET, 2003),¹⁴⁰ the Seniors With Heart Failure (SENIORS, 2005)¹⁴¹ the Carvedilol Prospective Randomized Cumulative Survival (COPERNICUS, 2002) trial,¹⁴² demonstrated that an initial depression of cardiac function in the HF patients treated with beta-blockers was reversed to a clear improvement with continued use.

Interestingly, beta-blockers can also impact healing processes and regenerative mechanisms. Researchers at York University reported that beta-blockers may prevent further cell death following a heart attack and that could lead to long-lasting benefit on patient outcome.¹⁴³ They discovered that cardiac cell death is increased when MEF2, a transcription factor that acts as a hub in the network that controls cell differentiation, organogenesis, and cardiovascular development, was suppressed. However, when beta-blockers, such as Atenolol, were given to animal models, MEF2 activity was enhanced and promoted cell

survival. Another interesting study from Chimenti and colleagues showed that the functional phenotype of cardiospheres and cardiosphere-derived cells was improved in patients undergoing cardiac surgery who were treated with beta-blockers.¹⁴⁴ Remarkably, a higher number of cardiac stromal cells could be isolated from patients on beta blocker therapy.¹⁴⁵ Moreover, the β 1AR blocker Nebivolol reportedly increased the number of circulating endothelial progenitor cells,¹⁴⁶ whereas the β 2AR agonist Clenbuterol enhanced the stimulatory effect of the chemokine GCSF on CD34+ cell mobilization after MI.¹⁴⁷ It was also suggested that β 1AR can improve the cardiac differentiation of ESCs *via* mitogen-activated protein kinase pathways.¹⁴⁸ MEK inhibition, which is feasible through clinically available drugs, was shown to promote the differentiation of clonal mesenchymoangioblasts into mural cells.¹⁴⁹

There are also indications that beta-blockers can promote cardiac regeneration in patients with CHD. Liu and colleagues reported a reduction in cardiomyocyte cell division in paediatric patients, which may account for the progressive mismatch between heart growth and performance and the increasing hemodynamic needs of the organism.¹⁵⁰ The team collected heart tissue from 12 infants who underwent corrective surgery for Tetralogy of Fallot, and found that more than half of the cardiomyocytes had entered the cell cycle but then remained stalled halfway through the process. The ultimate consequence was the studied subjects had 30% fewer cardiomyocytes than a normal infant of the same age, a reduction comparable to the cardiomyocyte loss occurring after a heart attack. The underlying mechanism consisted of repression of the cytokinesis gene ECT2, which is set downstream of beta-adrenergic receptors. Importantly, Propranolol treatment increased cardiomyocyte proliferation in patient cells and in neonatal mice, resulting in improved cardiac function and remodeling after MI in adult mice. The authors concluded that early-life beta-blocker treatment could rescue cell division defects, with potential benefit to long-term cardiac health.¹⁵⁰

12. THE LANDSCAPE OF CELL THERAPY IN THE ERA OF COVID-19

12.1. Perception of success of cell therapy – based regenerative medicine

The engagement of both the clinical community and lay persons is a key contributor to the successful deployment of cell therapy products. A survey of clinicians from five specialties investigated what they believe to be the most significant barriers to cellular therapy clinical development and adoption. The study showed that the main concerns among this group are cost-effectiveness, efficacy, reimbursement, and regulation. The authors concluded that these concerns can be addressed by ensuring that future clinical trials are conducted to adequately answer the questions of both regulators and the broader clinical community.¹⁵¹

Surveys on patients' opinions about cell and gene therapy indicate the interest focused on efficacy and long-term survival; little discussion centered on trial experience and side effects DOI: 10.1016/j.jval.2018.09.444. Web-based big data digital network analytics represents an alternative to opinion polls in deciphering the distributed meanings and sense making related to controversial biotechnology applications. This approach is thought to eliminate the sampling bias that is typical of opinion polls. Large-scale surveys, such as the Eurobarometer of Biotechnology, revealed that European citizens only consent research on stem cells if they are highly regulated. Conversely, the analysis of stem cell digital network suggested promise centeredness as a key ingredient of public perception, thus making with therapeutic tourism that remains as an important aspect of the stem cell landscape.¹⁵²

13. THERAPEUTIC PRIORITIES AND COMPETING NEEDS

In the conclusive section of the review article, we would like to identify some therapeutic priorities where regenerative medicine can have a voice.

13.1. Therapeutic priority 1: Combating frailty and disability.

Coronary revascularization has remarkably modified the epidemiology of IHD. Interestingly, the benefit is escalating over time due to successful implementation, extension, and timely application of primary angioplasty, as well as the effectiveness of concomitant medical therapy.¹⁵³ Nonetheless, a medicalization of CVD has increased the burden of chronically ill patients. Interventions aimed at improving lifestyle behaviours are fundamental for reducing

cardiovascular risk in the general population. Non-pharmacological measures include promoting exercise and reducing stress to combat subclinical chronic inflammation, cardiovascular frailty, while aiding proper response of the immune system.¹⁵⁴ Secondary prevention through comprehensive cardiac rehabilitation represents the most cost-effective intervention to ensure favourable outcomes across a wide spectrum of cardiovascular disease, reducing cardiovascular mortality, morbidity and disability, and to increase quality of life.¹⁵⁵ Exercise can also have important positive action in preserving and restoring endogenous reparative mechanisms.¹⁵⁶

Likewise, advances in the surgical correction of CHD have revolutionized the outcome by reducing the postoperative mortality rate but have concomitantly expanded the population of ACHD requiring chronic treatment and reintervention.¹⁵⁷ The pathogenesis of ACHD-related HF differs in many aspects from that occurring in patients with acquired cardiac disease. Peculiar features comprise inherited architectural disorganization of myocytes and vascular cells, cardiac damage due to the surgical trauma and insufficient protection during cardiopulmonary bypass, and the hemodynamic load to the heart from residual defects and failing grafts.¹⁵⁸ Moreover, genetic and epigenetic factors play additive roles in maladaptive myocardial remodelling.⁶ Investigation of mechanisms has generally focused on canonical pathways, such as the renin-angiotensin-aldosterone system (RAS) and adrenergic system.^{159, 160} Nonetheless, trials using RAS inhibitors in ACHD-related HF failed to show any benefit on ventricular function.^{161, 162} Tissue engineering and deployment of drugs halting precocious cardiac aging may represent breakthrough solutions in this category of patients.

12.2. Therapeutic priority 2: Developing cost effective solutions.

Novel therapeutic products are increasingly requested to address the needs of specific classes of patients, which entails significant costs that may not be affordable under current circumstances.¹⁶³ The National Health Service has been already struggling to pay for cutting-edge technologies and treatments to the extent that it has over the past couple of decades – even when those new treatments have been deemed to be cost effective. The situation has become dramatic now, with emerging transmissible diseases harshly competing for financial resources and medical services. Estimates indicate that Covid-19 could cost the world more than \$10 trillion. This new crisis is expected to widen disparities between and within countries and threaten health and social insecurity for decades to come. doi.org/10.1016/S2468-2667(20)30085-2

This new scenario is remarkably reshaping how medical research will be funded in the next decade. The devastating impact of the Covid-19 pandemic means that net income, and resulting investment in new research, has dropped significantly this year, leading to reduction or cancellation of regular grant calls. The British Heart Foundation has reacted to this potentially catastrophic situation by launching a new alliance of Association of Medical Research Charities and 151 of its members, including Cancer Research UK and Parkinson's UK, to call on the UK Government to match charity funded research for the next three years. Concurrently, the Wellcome Trust has published their new vision and strategy, along with headline indications on how their funding schemes will be changing in support of this refreshed vision/strategy to solve the urgent health challenges facing everyone. They will focus on four areas: (1) a broad programme of discovery research across a wide range of disciplines with the potential to make important and unanticipated discoveries about life, health and wellbeing; (2) infectious diseases; (3) mental health; and (4) climate and health. Most importantly, the Wellcome Trust program has the ambition of improving research culture in support of integrity, diversity, inclusion, and career opportunities. It is in fact clear that the current scientific landscape is dominated by prominent figures at the expense of innovation. Today's extremely competitive funding environment can make the safe approach appear the cleverer one, at least from a career standpoint. Therefore, adjusting funding policies could make science, including cardiovascular regenerative medicine, more responsive to the novelty it needs to move forward.^{164, 165}

The new crisis will indeed impose pressing questions on the feasibility of complex therapeutic methodologies, like cell therapy and tissue engineering. Affordable access to

proven regenerative therapies will be limited to off-the-shelf and point-of-care technologies, which provide potential for scale-up in biomanufacturing and high-quality standards and mitigate variability in function and assure quality. Cellular substitutes may also offer a means to reduce the burden of costs associated with cell manufacturing, while posing less issues related to safety.

It is also evident that new professional figures are urgently needed to facilitate the translation of new ideas into medical practice (**Figure 4**). Conventional benchtop-to-bedside medical research starts in the laboratory and ends in a clinical trial. The process has been criticized and associated with a high probability of failure. Reverse translation, also called bedside-to-benchtop research, begins with real-life patient experience, and goes backward to reveal the mechanistic basis for these experiences and clinical observations. This approach has several advantages but still requires that basic scientists and clinicians use a proper communicative system.¹⁶⁶ Unfortunately, the back-and-forward information flow is often too precipitous due to persistent gap of knowledge combined with pressure to deliver tangible results. Clinical professionals devoting themselves to a career shift into experimental medicine could help bridging the translational gap.

12.3. Therapeutic priority 3: New settlement for health and wellbeing.

International organizations and population health scientists concur in the importance of a fair access and allocation of resources but also of proper contingency planning and use of alternatives.¹⁶³ Likewise, translational scientists and doctors must design alternative models to the current research development and clinical translation pipeline. This includes the adoption of holistic collaborative approaches, a strong commitment to abating barriers between large academic centres with adequate funding and insufficiently funded centres and learning from simplified solutions from poor countries. In a number of inspiring publications, Nigel Crisp pinpointed that rich countries can learn from poorer ones, as well as the other way round, and this can help us understand what is happening and what means for us all as well as stimulate new ideas and innovations around the world.¹⁶⁷

In this respect, the implication for an equitable regenerative medicine consists of repurposing clinically available drugs that can aid the endogenous reparative potential of resident cells at a relatively low cost for the benefit of million cardiovascular patients.

Figure 1: (A) Number of articles retrieved from PubMed using stem cell and heart as key words. (B) Number of meta-analyses retrieved from PubMed using stem cell and heart as key words. (C) Number of trials conducted in the same period, according to PubMed search using stem cell and heart as key words.

Figure 2: Schematics of the HLHS defective heart. Credit to Centers for Disease Control and Prevention, National Center on Birth Defects and Developmental Disabilities for usage of this image.

Figure 3. Schematic representation of the mechanism coupling disposal of ROS and promotion of vascular stabilisation. (A) In physiologic conditions, ROS acts as a signalling for pericytes to remove the inhibitory mechanism that leads to Nrf2 degradation. Nrf2 is imported to the nucleus where it induce the expression of ARE genes. In addition, Bach1 is exported to the cytosol and degraded thus removing its inhibitory effect on Nrf2 binding to ARE gene promoter and on *ANGPT-1* and *COL1A* transcription. This leads to stabilisation of the provisional neovascularisation. **(B)** Disruption of the mechanism in cardiovascular disease and diabetes leads to unbalanced Bach1 activity, impeding both the ROS scavenging activity and angiogenic signalling by pericytes.

Figure 4: (A) Cell therapy needs to be considered as a part of a holistic approach, which combines technological, clinical, cultural, policy and economic changes. Future approaches to improvement must recognize these complementary factors together with better designed trials in specific populations to make sustained change happen. (B) It is imperative to include new ideas from emerging groups in a field that has seen so far polarization, division, and inequality in funding distribution. (C) Clinicians repurposing themselves to bridge the translational gap are urgently needed. (D) Covid-19 crisis highlighted racial disparity in healthcare and economy. Novel technological advancements need to consider deploying affordable pathways to remove inequality in accessing resources.

Table 1: List of main clinical trials using bone marrow derived cells

trial	Description	N. patients	Dosage	Outcome
NCT00268307	A phase 1, randomized, placebo-controlled, double-blind trial to investigate the effects of BMC administration in patients following STEMI on recovery of LV function using cardiac magnetic resonance imaging (cMRI)	40	100 million intracoronary BMCs versus placebo	The improvement in LV ejection fraction at 6 months by cMRI in the cell therapy group was not different than the placebo group. However, BMC administration had a favorable effect on LV remodeling at 6 months.
NCT00684021	Intracoronary infusion of BMCs within 12 hours of aspiration and cell processing administered at day 3 or day 7 after treatment with PCI	120	150 × 10 ⁶ BMCs or placebo (randomized 2:1)	Among patients with STEMI treated with primary PCI, the administration of intracoronary BMCs at either 3 days or 7 days after the event had no significant effect on recovery of global or regional left ventricular function compared with placebo.
NCT00765453	A multi-centre phase II randomized, double-blind, and placebo-controlled trial. Patients with anterior AMI and significant regional wall motion abnormality were randomized to receive either intracoronary infusion of BMC or placebo (1:1) within 24 h of successful primary PCI.	100	59.8 × 10 ⁶ BMCs or placebo	At 1 year, LVEF increased compared with baseline in both groups, the between-group difference was not significant. However, there was a significantly greater myocardial salvage index in the BMC-treated group compared with placebo.
NCT00355186	A multicenter randomized study on patients with large AMI in a 1:1:1 pattern into an open-labeled control and 2 BM-MNC treatment groups. In the BM-MNC groups, cells were either administered 5 to 7 days (early) or 3 to 4 weeks (late) after AMI.	200	Between 5 × 10 ⁷ and 5 × 10 ⁸ BMCs or placebo	Among patients with AMI and LV dysfunction, treatment with BM-MNC either 5 to 7 days or 3 to 4 weeks after AMI did not improve LV function at 12 months, compared with control. The results are limited by an important dropout rate.
NCT01495364	Patients who underwent successful stenting for STEMI and had left ventricular dysfunction (ejection fractions ≤48%) were eligible for enrollment. Subjects (N=161) underwent mini bone marrow harvest and were randomized 1:1 to receive (1) autologous CD34+ cells (minimum 10 mol/L ±20% cells; N=78) or (2) diluent alone (N=83), via intracoronary infusion.	161	Autologous CD34+ cells (minimum 10 mol/L ±20% cells; N=78) or diluent alone (N=83), via intracoronary infusion	The primary efficacy end point was change in resting myocardial perfusion over 6 months. No differences in myocardial perfusion or adverse events were observed between the control and treatment groups.
Boost-2	STEMI were randomly assigned to receive a single intracoronary infusion of placebo (control group), high-dose (hi)BMCs, low-dose (lo)BMCs, irradiated hiBMCs, or irradiated loBMCs 8.1 ± 2.6 days after percutaneous coronary intervention (PCI) in addition to guideline-recommended medical treatment.	153	Autologous 20.6 × 10 ⁸ BMCs vs. vehicle.	The BOOST-2 trial does not support the use of nucleated BMCs in patients with STEMI and moderately reduced LVEF treated according to current standards of early PCI and drug therapy.
CT02439398	Double-blind, randomized, multicenter study in patients with LVEF ≤50%, successful angioplasty of infarct-related artery, and regional dysfunction in the infarct-related area analyzed before cell injection.	120	Autologous 100 × 10 ⁶ BMCs vs. saline vehicle	BMC intracoronary infusion did not improve left ventricular remodeling or decrease infarct size.

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