Assessing the therapeutic readiness of stem cells for cardiovascular repair

Edit Gara¹, Andrea Ágnes Molnár¹, Béla Merkely¹, Gábor Földes¹²
¹ Heart and Vascular Centre, Semmelweis University
² National Heart and Lung Institute, Imperial College, London

Edit Gara, MD PhD
Heart and Vascular Centre, Semmelweis University
68 Varosmajor street, HU-1122 Budapest, Hungary
Tel:+36205805256
Email: gara.editgara@gmail.com
ORCID: 0000-0003-0074-6788
LinkedIn: https://www.linkedin.com/in/edit-gara-md-phd-42238572/

Andrea Ágnes Molnár, MD PhD
Heart and Vascular Centre, Semmelweis University
68 Varosmajor street, HU-1122 Budapest
Tel:+36 308285078
Email: molnandi@gmail.com
ORCID: 0000-0003-3310-217X

Prof. Béla Merkely, PhD DSc FESC FACC
Heart and Vascular Centre, Semmelweis University
68 Varosmajor street, HU-1122 Budapest, Hungary
Tel: +36-1-458-6840
Email: merkely.bela@kardio.sote.hu
ORCID: 0000-0001-6514-0723

Correspondence:
Gábor Földes, MD PhD
National Heart and Lung Institute, Imperial College London
Imperial Centre for Experimental and Translational Medicine
Hammersmith Campus, Du Cane Road
London W12 0NN
United Kingdom
Tel: +44 020 7589 5111
Email: g.foldes@imperial.ac.uk
Heart and Vascular Centre, Semmelweis University
68 Varosmajor street, HU-1122 Budapest, Hungary
Tel: +36 20 8250558
Email: foldes.gabor@med.semmelweis-univ.hu
ORCID: 0000-0003-4415-352X
LinkedIn: https://www.linkedin.com/in/gabor-foldes-84815272/
Keywords: cell therapy, cardiovascular repair, exogenous cell support, standardized manufacturing

1. Introduction.

Ischaemic heart disease is among the most frequent causes of death and morbidity worldwide [1]. Despite advances in available cardiovascular therapies, a significant percentage of patients still die due to subsequent heart failure [2]. Regenerative medicine strategies to reduce myocardial injury and prevent left ventricular dysfunction are among the new advances in cardiovascular therapy. A quick internet search on “cardiac stem cell therapy” may suggest that these approaches are currently part of routine treatment and are available for a wider patient population. However, our enthusiasm must be tempered given that whilst bone marrow transplantation is part of clinical regime in haematology, targeting solid organs with new cell therapy products is actually still in its early stage. Amongst others, guidelines such as the one by the International Society of Stem Cell Research in 2016 discussed current limitations of these applications and collected relevant recommendations [3]. The main hurdles to overcome before standard clinical application of stem cells are the immune rejection, risk for malignant transformation and ethical concerns. On the technical side, detailed analyses of stem cells and standardization of cell sourcing, manufacturing, storage, delivery and clinical intervention protocols are needed to validate stem cell therapy for routine clinical use (Figure 1).

2. Stem cells and sourcing.

Commonly, stem cells can be categorised based on their origin, i.e. embryonic (-like) / pluripotent cells and somatic / adult ones from most adult organs. Although adult stem cells can be derived from various tissues, bone marrow and umbilical cord-derived cells still remain the primary sources for stem cell therapy. The main role of adult stem cells is assumed to be replacement of damaged and injured tissue. Cells can be grown and manipulated in vitro to obtain various mature and functional cell types. Of these, mesenchymal stem cells (MSC) show multilineage potential, a clinically substantial immunomodulatory ability and secretion of anti-inflammatory molecules which make these cells effective clinical products in autoimmune and degenerative diseases. MSC have also been investigated in a number of ongoing clinical trials for cardiac regeneration. Because of their low immunogenicity, there were no adverse events reported related to immune-responses during allogeneic transplantations. However, their main limitation remains that MSC show large diversity in origin from different tissues; characterisation and cell surface markers are controversial and nomenclature is not standardised either [4]. Ethical and regulatory concerns may be related to the invasive nature of bone marrow harvest. Their mechanisms of action are not entirely clarified either (such as a limited direct myogenic differentiation), which overall makes specification, analysis of the product comparability, stability as well as compatibility tests difficult. The attractiveness of using MSC populations alone or in combination with other cell types for regenerative therapies relies not only on their capacity to differentiate into various lineages, but also on their paracrine mediated repair mechanism via immunomodulatory, angiogenic, and pro-survival effects [5]. Another promising cell product for patient-specific therapy are the human induced pluripotent stem cells (hiPSCs) from cell banks for allogeneic uses or by reprogramming the patient’s own somatic cells. Human iPSCs have the potential to differentiate into any desired cell types and may represent an ethically acceptable alternative to the use of hESC counterparts from human embryos. An obvious advantage of iPSC technology is the recent possibility of combined repair of disease-causing mutations by homologous recombination, particularly with precise CRISPR/Cas9 genome editing. The hiPSC technology is however not completely finalised; the generation of hiPSC (and hESC) derivatives as safe and functional cell-based medicinal products requires further optimisation of reprogramming, differentiation and mutation-free maintenance of differentiated cells. Human iPSCs, even with abundant expression of pluripotency markers, may retain a unique gene and epigenetic signature of the original somatic cells. This may be linked with the observations that many hiPSC derivatives remain partly immature or have genomic and phenotypic differences when compared with adult cell types [6,7]. The genetic stability of hiPSC-derivatives is questionable (particularly karyotypic abnormalities and copy number variants) and possible tumorous malformation cannot be excluded.
Similarly, functional consequences of partially mature PSC-derivatives must be assessed *in vitro* and *in vivo* [8] before clinical translation. Assessment in small animals may be suitable to rule out acute safety issues in vivo, i.e. toxicity of the delivered product, immunological compatibility or some unknown aspects of mechanism of action. Immuno-compromised small animals (e.g. athymic nude rats) offer first models for testing human stem cell products. However, their proof-of-concept use is clearly limited by their smaller body size, shorter lifespan, and substantially different physiology. Larger animal species (large domestic species or primates) could have better ability to predict clinical efficacy in heart disease; yet recent publications emphasize great challenges such as reaching appropriate number of implanted cells, different metabolism and impaired electro-anatomical coupling of grafted hPSC-derivatives [8]. Describing the chronic situation in patients, we have currently no really good large animal models yet. This is mainly due to xenogeneic problems and insufficient immunological regime. This could be obviated in part by generation of iPSC from recipient species by reprogramming or by genetic modification in order to generate humanised animals.

3. Manufacturing, storage and delivery.

One challenge for cardiovascular cell manufacturing is that a wide range of cell types are available for an even broader array of clinical indications; each requiring a separate production process. The Guideline on Human Cell-Based Medicinal Products by the European Medicines Agency [9] discusses common quality standards to control the qualitative and quantitative composition of various cell-based products; the appropriate tests to be carried out and on ingredients and methods used for production. It sends a clear message to us that characterising complex pharmaceutical properties of cellular medicinal products is technically demanding and has major challenges in qualifications. Based on lessons learnt from current cell therapy initiatives, it is now obvious that in order to generate clinically significant benefits with cell therapy, manufacturing processes for cell therapies must be precisely designed, quality-controlled and monitored [10]. On one hand, low variability and retained stability in genotype and phenotype are critical for standardised utilisation of stem cell sources and their differentiated derivatives. To ensure their meaningful clinical use, cell products must be strictly characterised in terms of quality, culture stability, safety and efficacy both in early and late cultures, with standardised *in vitro* analytics throughout the production process [11]. Choosing the right potency assay is necessary for full characterisation of each manufactured products. Indeed, it became clear that compared to other pharmaceutical products (small molecules and proteins), cell-based ones can need more characterisation. Of note, although much effort has been put into attempts worldwide to establish such potency assays, to date there are no documented tests to characterize cardiovascular derivatives of stem cells from different sources. Yet, suitable potency assays for MSC collection and expansion are available already [11]. Carefully selected multiple parameters from clinical experience, such as patient responsiveness and adverse events should be included in design. To judge the success of the product, checklist for critical parameters should therefore include sterility, purity, plasticity as well as special (cardiovascular) adverse events, i.e. thrombogenic, arrhythmogenic, vasoactive adverse effects, immunogenicity, allergic reactions, or tumour formation. Risks may include further issues with e.g. scaffold material, genetic stability, culture conditions; transmission of infections; usual risk factors are the variability in manufacturing, donor changes, scale-up process or introducing new logistics [5,12]. All these results must be validated also at its commercial state and finally included in the Marketing Authorisation Application. For the preparation of the MAA dossier the risk-based approach is essential in the identification of various risks associated with quality, nonclinical and clinical use of the cell product.

4. Cardiovascular clinical application.

Cell sourcing should be chosen according to final aim of regenerative therapy. As the architecture of the myocardium is complex, regeneration or replacement of cardiomyocytes and other cells alone may not be efficient. Clinical and molecular biology data from these first studies may help finding those patients who can benefit the most from regenerative treatments. In addition to
identifying the optimal combination of cell population and paracrine factors, design should usually involve an optimized tissue engineering approach for cell differentiation, maturation and engraftment. It was shown that variability in engraftment partly stems from routes for cell therapy products delivery, i.e. intracoronary (e.g. RELIEF trial), intramyocardial (e.g. C-Cure trial) or systemic infusions (e.g. SCIPIO trial). Amongst these intramyocardial delivery has been shown as the most effective [13,14]; cell survival and engraftment rate in the myocardium remain low with the other routes. Of note, efficacy is not independent from the identification of right target patient population who would benefit from cell therapy and the timing of a safe cell delivery [13] (see also Table 1). Previous clinical trials using autologous non-cardiac progenitors (e.g. unfractionated bone marrow derived cells) showed inconsistent but relatively modest efficacy because of limited cell survival, maturation of cells in situ and delivery to the target area (TOPCARE-AMI, BALANCE, BOOST, ASTAMI, MYSTAR trials). The recently launched SCIENCE trial is recruiting patients with reduced left ventricular systolic function to evaluate efficacy of adipose tissue-derived stem cells to treat ischemic heart failure. The ongoing BAMI trial and the POSEIDON-DCM trial are running for results with the potential to answer whether autologous unfractionated bone marrow cells can play a role in the treatment of ischemic and dilated cardiomyopathy [15]. Autologous cardiac progenitor cells with a greater potential for cardiomyocyte regeneration may be a more relevant strategy. Nevertheless, the small number of these cells detectable in vivo represents a notable limitation for clinical use. The effect on left ventricular function appears to be similar to the unfractionated bone marrow data. Analogous to bone marrow-derived cell treatment, MSC therapy also displayed a good safety profile for patients with chronic ischemic cardiomyopathy and LV dysfunction. However, functional improvement and reduction of infarct size was proved only in MSC treated group (TAC-HFT trial) [16]. Recent publication of transcendocardial delivered allogeneic MSC in patients with heart failure and reduced ejection fraction revealed a significant reduction in clinical cardiac events leading to improved patient outcomes [17]. Allogeneic MSC have proven to be safe and may provide easier logistics with the potential availability as an off-the-shelf product. To compare the safety and efficacy between autologous and allogeneic MSC therapy in ischaemic cardiomyopathy, the POSEIDON-pilot study revealed equal clinical improvement from both therapies. Furthermore, this study demonstrated the potential of inverse dose response, as greater clinical improvement was observed with 20 million cells’ injectant than those with 200 million cells [18]. Therefore, TRIDENT study is in progress to further estimate the importance of stem cell dosage (NCT02013674). CHART-1 trial is currently the largest cardiovascular regenerative medicine study to date identifying a potential target patient population characterized by large left ventricular end-diastolic volume that might benefit from otherwise neutral cardiopoietic cell treatment.

Embryonic stem cells may not be ideal for many clinical applications because of the ethical concerns and the risk of immune rejection. Thirty-five years after the derivation of the first mouse ESC and almost 20 years after the first human ESC line, the ESCORT study is still the only clinical trial so far which actively recruited patients to test the use of human embryonic stem cell-derived CD15+/Isl-1 progenitor cells in severe heart failure [19]. Human iPSC can solve the ethical issue of ESC and may reduce the risk of immune rejection; however, the risk of malformation from residual stem cells is not negligible. Attempts to manufacture clinical grade or GMP certified iPSCs products from blood and skin samples are in progress (NCT02413450; NCT02056613). Further research should develop new methods to improve currently high cost and time-consuming iPSC manufacturing (or by using direct reprogramming of somatic cells to functional cardiomyocytes without a transit through pluripotent state).

5. Conclusions.

The increasing prevalence of cardiovascular disease worldwide and the lack of available causative therapies to reverse the loss of myocardium warrant new strategies. Advances in regenerative stem cell therapy may at least in part address this problem. However, an integrated science is needed to use stem cell-based technologies as clinical applications. Standardised, detailed designed potency and characteristics assays, furthermore pre-clinical safety and efficacy data are key for translation. Results of stem cell-based cardiovascular trials show the current lack of consensus for the optimal protocols (e.g. delivery root, cell number, timing, clinical scenario, patient population) in
these therapies. Clinically meaningful and efficient design of regenerative therapies for cardiovascular disease encompasses the integration of new knowledge in stem cell biology, cell sourcing, manufacturing, storage, delivery and clinical protocols.


Advances in regenerative medicine may hold the potential as become a causative therapy to reverse the loss of myocardium in different cardiac diseases, however further evidence is needed to achieve clinical application of stem cell based technologies. No consensus for the optimum protocol of stem cell based therapies exists yet regarding cell sourcing, manufacturing, storage and delivery and target clinical scenario. The identification of the best suited stem cell type and the right clinical setting is one of the biggest challenge before translation of stem cell therapy from preclinical studies to the clinical use. Preclinical studies showed good regenerative potential for both MSC and hPSC-derivatives in damaged or dysfunctional tissues (e.g. myocardial infarction, spinal cord injury, amyotrophic lateral sclerosis, multiple sclerosis or type 1 diabetes). Moving our scientific knowledge from bench to bedside is however still slow due to heterogeneous study designs (e.g. lack of tailored patient population, optimal raw material and cell sourcing, implantation strategy, dose and timing, repeated implantation, primary/ secondary/ composite study end-points). To achieve net clinical benefit, GMP approved standard operating protocols and study designs must be developed for each starting cell type and clinical scenario. Challenges for cardiac cell therapy are not profoundly different from those reported in other organs. Cardiovascular therapy with large amount, stable and surviving cell products requires complex modifications in vitro such as optimal cardiopoietic differentiation. To reach this, cells during differentiation steps must be characterised by array of assays and reliable and multiple biochemical, genetic or image-based markers. With regard to transplantation, cell injection alone may not be effective and an improved implantation strategy is warranted. Preclinical large-animal studies must prove safety and efficacy before any clinical use. Above safety and technical issues of stem cell therapy, it is important to obtain clear evidence on the mechanism underlying the functional improvement of the heart after administration of stem cells. Recent studies suggest that paracrine factors secreted by the stem cells may play a more significant role in the improvement of cardiac function than the direct regeneration potential of stem cells. This new field has precipitated the need for integrated science, requiring collaborative efforts across cell therapy regulatory organisations, academic institutions, manufacturing and clinical sites. This very complex process also requires well-trained clinical academics with regenerative medicine background. Increasing number of multi-disciplinary trainings in the scientific principles and clinical applications of regenerative medicine would facilitate the development of novel regenerative technologies.

Funding: This work was supported by the Hungarian National Research, Development and Innovation Fund (NVKP16). Authors declare no conflict of interest.

Annotated bibliography (* highlighted ones)


4. Ackema KB, Charite J. Mesenchymal stem cells from different organs are characterized by distinct topographic Hox codes. Stem Cells Dev 2008;17(5):979-991.


A helpful and detailed guideline covering most aspects of cardiovascular cell therapy


Important article on defining the quality of cellular therapy products


Table 1. Priorities for clinical operators in stem cell therapy

<table>
<thead>
<tr>
<th><strong>Patient screening</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Timing of enrolment in acute vs. chronic conditions</td>
<td></td>
</tr>
<tr>
<td>Concomitant diseases and medications</td>
<td></td>
</tr>
<tr>
<td>Immunology aspects</td>
<td></td>
</tr>
<tr>
<td><strong>Delivery</strong></td>
<td></td>
</tr>
<tr>
<td>Myocardial viability test before implantation</td>
<td></td>
</tr>
<tr>
<td>Guided implantation, electro-anatomical mapping</td>
<td></td>
</tr>
<tr>
<td>Standardized procedure with guaranteed safety and feasibility</td>
<td></td>
</tr>
<tr>
<td>Estimating effective dose</td>
<td></td>
</tr>
<tr>
<td>Organised logistics</td>
<td></td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
<td></td>
</tr>
<tr>
<td>State-of-the-art imaging (cMRI and nuclear imaging)</td>
<td></td>
</tr>
<tr>
<td>Responsiveness</td>
<td></td>
</tr>
<tr>
<td>Low intra and inter-observer variability (echocardiography)</td>
<td></td>
</tr>
<tr>
<td>Suitable biomarkers</td>
<td></td>
</tr>
<tr>
<td>Standardized functional tests (6-minute walk test)</td>
<td></td>
</tr>
<tr>
<td>Accurate reporting of SAEs and MACEs</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1