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Safety and efficacy of cardiopoietic stem cells in the treatment of post-infarction left-ventricular dysfunction – From cardioprotection to functional repair in a translational pig infarction model

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ABSTRACT

To date, clinical success of cardiac cell-therapies remains limited. To enhance the cardioreparative properties of stem cells, the concept of lineage-specification through cardiopoietic-guidance has been recently suggested. However, so far, only results from murine studies and from a clinical pilot-trial in chronic heart-failure (CHF) are available, while systematic evidence of its therapeutic-efficacy is still lacking. Importantly, also no data from large animals or for other indications are available. Therefore, we here investigate the therapeutic-efficacy of human cardiopoietic stem cells in the treatment of post-infarction LV-dysfunction using a translational pig-model. Using growth-factor priming, lineage-specification of human bone-marrow derived MSCs was achieved to generate cardiopoietic stem cells according to GMP-compliant protocols. Thereafter, pigs with post-infarction LV-dysfunction (sub-acute phase;1-month) were randomized to either receive transcatheter NOGA 3D electromechanical-mapping guided intramyocardial transplantation of cardiopoietic cells or saline (control). After 30days, cardiac MRI (cMRI) was performed for functional evaluation and in-vivo cell-tracking. This approach was coupled with a comprehensive post-mortem cell-fate and *mode-of-repair* analysis. Cardiopoietic cell therapy was safe and ejection-fraction was significantly higher when compared to controls ($p = 0.012$). It further prevented maladaptive LV-remodeling and revealed a significantly lower relative and total infarct-size ($p = 0.043$ and $p = 0.012$). As in-vivo tracking and post-mortem analysis displayed only limited intramyocardial cardiopoietic cell-integration, the significant induction of neo-angiogenesis (~40% higher; $p = 0.003$) and recruitment of endogenous progenitors (~2.5x higher; $p = 0.008$) to the infarct border-zone appeared to be the major *modes-of-repair*. This is the first report using a pre-clinical large animal-model to demonstrate the safety and efficacy of cardiopoietic stem cells for the treatment of post-infarction LV-dysfunction to prevent negative LV-remodeling and subsequent CHF. It further

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provides insight into post-delivery cardiopoietic cell-fate and suggests the mechanisms of cardiopoietic cell-induced cardiac-repair. The adoption of GMP-/GLP-compliant methodologies may accelerate the translation into a phase-I clinical-trial in patients with post-ischemic LV-dysfunction broadening the current indication of this interesting cell-type.

Abbreviation

MSCs	Mesenchymal stem cells
LV	Left-ventricle
MI	Myocardial-infarction
CHF	Chronic Heart-failure
cMRI	Cardiac magnetic-resonance imaging
GMP	Good Manufacturing Practice
SOP	Standard operating procedures
EF	Ejection-fraction
CO	Cardiac-output
SV	Stroke-volume
EDV	End-diastolic volume
ESV	End-systolic volume
LVMV	Left-ventricular mass volume
MPIO	Micron-sized iron-oxide particles
vWF	von Willebrand factor
NOGA	3D electromechanical mapping guided intramyocardial stem cell delivery system

1. Introduction

Stem cell therapy has been repeatedly proposed as a promising strategy to treat myocardial-infarction (MI) and chronic heart-failure (CHF) [1–3]. Based on numerous promising preclinical studies [4–6], the feasibility and safety of cell-therapies were confirmed in clinical pilot-trials [7–11]. However, with regards to efficacy, the currently available data display only heterogeneous outcomes and limited improvement of cardiac-performance [1,2,12,13]. Importantly, most of these initial trials have employed unselected, first-generation cell-types with limited cardioreparative properties. An additional element that further complicates the assessment of cell-therapies is the heterogeneity in the design of preclinical studies (i.e. methodologies and endpoints) and inconsistencies between pre-clinical and clinical study approaches [14]. Moreover, randomization, blinding, and Good Manufacturing Practice (GMP)/Good Laboratory Practice (GLP) compliant methodologies are infrequently used. Finally, the selection of a single primary outcome, while important for statistical considerations, limits the appreciation of the multi-faceted nature of heart disease and its therapy [14,15]. Therefore, there is a need for a paradigm shift to develop standardized next-generation cell therapy protocols for targeted heart-repair [1].

As one example, to enhance the therapeutic-efficacy of current cell-therapy strategies, the concept of cell lineage-specification through cardiopoietic-guidance has been reported [16–18]. Following small-animal studies [19], mesenchymal stem cells primed for cardiopoiesis (cardiopoietic stem cells) were shown to be safe in humans [7] (C-CURE trial; NCT00810238) and are currently being tested for efficacy in the larger CHART-1 trial (NCT01768702) enrolling 240 patients with CHF. However, while all previous applications of this strategy have focused primarily on CHF, little is known about its regenerative potential in the treatment of left ventricle (LV)-dysfunction in the sub-acute phase after MI to prevent negative LV remodeling and subsequent development of CHF. Moreover, to date, no preclinical large-animal data of this next-generation cell-therapy concept do exist.

Therefore, in this translational study, we investigated the safety and efficacy of cardiopoietic stem cells in the treatment of post-MI LV-dysfunction. We hypothesized that if administered in the sub-acute phase after MI, cardiopoietic cell therapy may preserve cardiac-performance, and thus prevent the potential progression from post-MI LV-dysfunction to negative LV remodeling and subsequent CHF. Importantly, we employed a fully translational pipeline including i) the choice of a relevant large-animal model; ii) GMP-compliant cell-handling; iii) transcatheter 3D-NOGA-assisted transcatheter intramyocardial delivery; iv) clinical-grade randomization, blinding, and endpoint-assessments; v) and state-of-the-art cMRI-based cell-tracking methods linked to a comprehensive post-mortem cardiopoietic cell-fate evaluation.

2. Materials & methods

For detailed and extended methods please see Supplementary file.

2.1. Production of GMP-grade human cardiopoietic stem cells

Production of human cardiopoietic stem cells was performed as previously described [7,19] using GMP protocols and standard-operation procedures (SOPs). After written informed consent and ethics approval bone marrow was aspirated from hip of six chronic heart-failure patients aged from 36 to 72 years to produce the cells (see Supplementary file for patient characteristics).

2.2. Quality-control, release-criteria, angiogenic potential and cell-labeling

A quality-control was carried out under SOPs to ensure purity, identity and homogeneity and sterility. To test the angiogenic potential of cardiopoietic stem cells CellPlayer™ Angiogenesis 96-well PrimeKit (Essen Bioscience Ltd, United Kingdom) were used to monitor the angiogenic potential of cardiopoietic stem cells on in-vitro endothelial tube-formation using conditioned medium. Next, for in-vivo and post-mortem cell tracking purpose, cardiopoietic stem cells for four animals (n = 4) were labeled with super-paramagnetic microspheres, co-labeled with Dragongreen fluorochromes (MPIOs; Bangs Laboratories; USA) allowing for additional post-mortem analysis (i.e. flow-cytometry and immunohistochemistry, (IHC)). In addition, cells underwent labeling with CellTracker CM-DiI (Invitrogen; Switzerland).

2.3. Translational post-infarction LV dysfunction pig model

Twenty-two adult landrace pigs underwent induction of MI at day0 (two pigs died peri-procedural) using a standardized *"closed-chest occlusion-reperfusion protocol"* as previously described [20–22]. At day3 post-MI all surviving animals (n = 20) underwent