Long-term results of intramyocardial CD133+ bone marrow stem cell therapy for myocardial ischemia: experience with stem cell register

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Abstract
Standardization of stem cell therapy requires application of appropriate methods to evaluate safety and efficacy, including long-term pharmacovigilance. To accomplish this objective, a long-term registry program was installed in the Regeneration and Translation Center for cardiac stem cell therapy in Rostock, Germany 14 years ago. The registry program in RTC contains parameters for the evaluation of functional outcomes and safety including major adverse cardiovascular and cerebral events (MACCEs). The register follows 223 patients with a total of 1152 patient years, who are observed yearly lifelong after stem cell application or as a control groups. The present study was done in 96 patients (n=73 cell therapy, n=23 control group) with coronary arterial disease and heart failure, treated with coronary artery bypass operation with or without stem cells injections. The analysis revealed, that owing to yearly MACCE registrations, register allow that late probable complications are carefully followed as well as unexpected complication reported, which is required to ensure patient’s safety as the main aspect of Good Clinical Practice. Nevertheless, the registry by itself cannot substitute randomized clinical trials, as patient’s cohort is heterogeneous. However, registry data can be used for long term efficiency and safety evaluation in standardized patient groups. Furthermore, registry program could help to improve patient selection revealing predictors of good response and define patients who did not respond to the stem cell therapy. It is expedient to establish an obligatory common registry program for all centers, carrying stem cell studies.

Keywords
CD133 cells, intramyocardial injection, stem cell register, responder
**Introduction**

Stem cell therapy is an approach for treatment of heart diseases since the first intracoronary implantation of autologous bone marrow mononuclear cells (Strauer, Düsseldorf) and the first intramyocardial implantation of purified CD133+ bone marrow stem cells (Steinhoff, Rostock) in 2001 in patients with ischemic heart failure [6, 8]. Nevertheless, the standard criteria for evaluation of safety and efficacy of stem cell therapy were not elaborated. According to Good Clinical Practice an insurance of safety of participants is required during and after studies. Short (1-2 years) medical observation after procedure, which is usually planned in majority studies, could unlikely satisfy the requirements of follow-up vigilance analysis. Moreover, parameters for efficacy evaluation of stem cell treatment are broad and include functional parameters as well as data of radioisotope imaging technique, quality of life and ect. The common registry program embracing all stem cell centers could help to solve named problems. In particular, it would be possible to follow probable late complication and determine the most relevant outcome parameters after stem cell therapy in the heart. Moreover, this program might be used for a standardized pharmacovigilance reporting to regulatory authorities. In addition, common stem cell register could be helpful in evaluation of the most relevant outcome parameters in patients with heart disease. Unfortunately, it is not yet exist. Meanwhile, in the Regeneration and Translation Center for cardiac stem cell therapy in Rostock, Germany (RTC) the registry program following yearly lifelong condition of patients after stem cells procedure was established 14 years ago. We would like to present our experience with stem cell register in patients with ischemic cardiomyopathy and describe the revealed relevant parameters for evaluation of safety and efficacy after procedure.

**Materials and Methods**

In the RTC practice adult bone marrow stem cell population with surface antigen CD133+ is applied for the treatment. The description of the stem cell generation is presented below. Bone marrow is aspirated primarily from the iliac crest (93,9% of cases) with preheparinized syringes. CD133+ bone marrow hematopoietic stem cells (BMSC) are isolated by magnetic separation with ferrite-conjugated antibody (Miltenyi CliniMacs System; Miltenyi Biotec, Bergisch Gladbach, Germany). Flow cytometry is performed to evaluate the purity and quality of the stem cell product. The median CD133+ BMSC dose was over 3,9×10^6 cells in 1 ml (95% CI 4,0-5,3). The stem cells are injected transepicardial during coronary-artery bypass surgery (CABG) or mitral valve surgery, before the aortic clamp is removed, or stand-alone application via mini-thoracotomy. During last 14 years, after the first intramyocardial stem cell application in 2001 [6, 7], 223 patients have been treated in RTC. Currently the Multicenter Phase III clinical randomized placebo-control study is carried out [2], following terminated clinical studies Phase I and Phase II [10]. After studies closure all participants of mentioned trials are observed yearly lifelong within the registry program established in our center. In addition, the register contains information about patients with stem cells application after mitral valve replacement or reconstruction with or without CABG, patients with stand-along stem cells application and control groups treated with standard operations without stem cells injection. The registry program in RTC includes parameters for the evaluation of functional outcomes, such as left ventricle ejection fraction (LVEF), left ventricle end diastolic diameter (LVEDD) and left ventricle end systolic diameter (LVESD), measured by ultrasound system, 6-minute walk test, NT-proBNP, heart failure class (NYHA) and angina pectoris class (CSS). Moreover, registry contains such safety parameters as results of laboratory tests (Troponin, CK-MB, CK, CRP, Leucocytes), ECG, mortality rate and major adverse cardiovascular and cerebral events (MACCEs). MACCE is defined as an incidence of cardiac death, myocardial infarction, rehospitalization and intensive care stays due to cardiac events and percutaneous or surgical revascularization, acute heart failure, ventricular arrhythmias, postoperative implantation of defibrillators or resynchronization therapy and apoplexies. In addition, we study the events of new tumor formation, immune diseases and infections after stem cell transplantation procedure.

From 2001 to 2015 two hundred twenty three patients were followed with a total of 1152 patient years. General survival rate was about 67% up to 14 years. In the present paper we report an analysis of MACCEs and trans thoracic echocardiographic (TTE) data of 96 patients (n=73 cell therapy, n=23 control group) with coronary arterial disease and heart failure, treated with CABG with or without stem cells injections. Moreover, we stratified patient population into responders and non-responders based on change in global LVEF more than 5% at 12 month follow-up, reduction of LVEDD of more than –5 mm. “Absolute” responders were defined as patients who had an improvement of both parameters.

**Results**

Two different tendencies for the short-term and long-term outcomes were revealed after analysis of heart functional parameters collected in the register before the procedure, 12 months follow-up and further yearly up to 14 years. After 12 months follow-up no significant difference in the efficiency of treatment was demonstrated in stem cell (n=73) versus control groups (n=23). The results listed that LVEF was increased by +5,3% in the stem cells group (35,7% ± 10,0 to 41,0±9,0%; P<0,001), and by +4,7% in the matched control group (37,2±2,0 to 41,9±2,0; P=0,05), LVEDD decreased by –2,3 mm in the stem cell group (57,6±6,1 mm to 55,5±6,2 mm; P<0,001) and by -2,5 mm in the control group (59,4±1,2 mm to 56,9±1,1 mm; P=0,022) 12 months after procedure. At the same time, the positive functional effect after stem cells injection was continuing during 5 years, whereas in the control group (CABG-only) – only during 2 years. Particularly, ejection fraction in patients of stem cell group was 45,3±3,2% 5 years follow up versus 35,7%±10,0 before operation (P=0,05). In the control group the LVEF, on the contrary, returned back to baseline numbers after moderate peak at 2 years follow-up. These tendencies are demonstrated in the Fig 1. Such parame-
ters as LVEDD and LVESD measured by TTE showed no significant changes after 1 year follow-up in both, stem cell and control groups (data not shown).

Mortality and MACCEs were analyzed up to 14 years. During this follow-up period 36 patients died: 28 from 114 (25%) patients in the CD133+ BMSC plus CABG group, and 8 from 36 (22%) in the CABG-only group (P=0.827). No significant difference in MACCEs between the treatment groups was observed: 45 (39%) events in the stem cell group versus 17 (47%) recorded events in the control group (P=0.442). The detailed analysis listed that cases of postoperative implantation of defibrillators or resynchronization therapy did not differ significant between stem cell and control group (17% versus 14%, P=0.799). New episodes of ventricular arrhythmias occurred in 13% and 11% cases in stem cell group versus control group (P=1.000). Moreover, the percentages of apoplexies were almost equal in stem cell and control groups (8.8% and 8.3% respectively, P=1.000) during 14 year follow-up. Another MACCEs including rehospitalization and intensive care stays due to cardiac events and percutaneous or surgical revascularization took place in 6.1% and 8% in the stem cell group and in 5.5% and 5.5% in control group, respectively. There were no cases of immune diseases in both groups. At the same time two patients (1.8%) have died from lung and bronchial cancer in stem cell group after 50 and 86 months follow up.

### Table 1. Mortality and MACCEs in responder and non-responder patient groups

<table>
<thead>
<tr>
<th>Characteristics at baseline</th>
<th>Responder (n=15)</th>
<th>Non-responder (n=58)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%) (mean±SD)</td>
<td>27.6±9.2</td>
<td>38.4±9.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LVEDD (mm) (mean±SD)</td>
<td>63.1±5.7</td>
<td>56.4±5.6</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LVESD (mm) (mean±SD)</td>
<td>49.0±8.3</td>
<td>42.3±7.7</td>
<td>0.017*</td>
</tr>
<tr>
<td>NT-proBNP (pg/ml) Median (25%-75%)</td>
<td>2558 (1756-5180)</td>
<td>762 (453-2456)</td>
<td>0.102**</td>
</tr>
<tr>
<td>Number of stem cells (mean±SD)</td>
<td>3.8±2.3</td>
<td>4.7±3.2</td>
<td>0.311*</td>
</tr>
<tr>
<td>Diabetes N (%)</td>
<td>7 (47%)</td>
<td>24 (41%)</td>
<td>0.774*</td>
</tr>
<tr>
<td>Hypertonia N (%)</td>
<td>15 (100%)</td>
<td>54 (93%)</td>
<td>0.575*</td>
</tr>
<tr>
<td>Smoking N (%)</td>
<td>6 (40%)</td>
<td>15 (26%)</td>
<td>0.341*</td>
</tr>
<tr>
<td>Dyslipidemia N (%)</td>
<td>13 (87%)</td>
<td>49 (85%)</td>
<td>1.000*</td>
</tr>
<tr>
<td>Anti coagulantia N (%)</td>
<td>5 (33%)</td>
<td>23 (40%)</td>
<td>0.770*</td>
</tr>
<tr>
<td>Aspirin N (%)</td>
<td>4 (27%)</td>
<td>8 (14%)</td>
<td>0.253*</td>
</tr>
<tr>
<td>Beta blocker N (%)</td>
<td>11 (73%)</td>
<td>48 (83%)</td>
<td>0.467*</td>
</tr>
<tr>
<td>Statin N (%)</td>
<td>14 (93%)</td>
<td>47 (81%)</td>
<td>0.438*</td>
</tr>
<tr>
<td>Diuretic N (%)</td>
<td>11 (73%)</td>
<td>29 (50%)</td>
<td>0.148*</td>
</tr>
<tr>
<td>ACE inhibitor N (%)</td>
<td>10 (66%)</td>
<td>44 (76%)</td>
<td>0.516*</td>
</tr>
</tbody>
</table>

Legend Table 2: # Fisher’s exact test, * Two-sample t-test, ** Mann-Whitney U test.
In addition, mortality rate and MACCE were used to found the most reliable outcome parameter in patient's long term prognosis. We compared rate of mortality and MACCE with changes in short-term functional outcomes: The Table 1 suggests that responders, who had an improvement of both functional parameters (LVEF and LVEDD) after 12 months follow up, had 0% mortality up to 14 years in comparison with patients, who improved only LVEF or LVEDD. In addition, 24% of deaths were noted in "absolute" non-responder group (P=0,020). Further, we compared the characteristics at a baseline between responders and non-responders to identify predictors of good response to stem cell therapy. The analysis revealed that patients who responded to CD133+ cell therapy had an average pre-operative lower LVEF (27 % versus 38%, P<0,001) and lager LVEDD and LVESD (63 mm and 49 mm versus 56 mm and 42 mm, P<0,001, P=0,017) compared to control group. In addition, NT-proBNP levels were higher in patients that responded to stem cell therapy (P=0,102). However, the numbers of CD133+ stem cells, concomitant diseases or given medication were not associated with the responsiveness of patients. This comparison of different baseline criteria for responders and non-responders is given in the Table 2.

Another sub –study was performed on registry database: the correlation between objective dates (changes in LVEF) and subjective dates (changes in Quality of life) was evaluated at 6 months follow up after stem cell injection. The study listed that 13% of patients had false-positive result or placebo effect as they reported an improvement in their physical condition, although LVEF did not increase after procedure. More interestingly, that patients, who reported negative changes (14%) after procedure, indeed had an impairment according to date of echo test.

![Figure 1. Changes in LVEF during 5 years follow up in stem cell and control groups.](image)

Legend Figure 1: *Paired t-test. The figure shows the ratio between LVEF before and after treatment with combination of CABG and stem cells injection or CABG-only. The results are represented with 1 year interval after therapy.

Discussion

The analysis of results of patients with ischemic disease and heart failure, who were observed in the frame of stem cell register in RTC, did not reveal significant difference in changes of functional outcomes between stem cell and control group 12 months follow up. In addition, we compared these data with results obtained in previous Phase II trial. On the contrary to the registry, Phase II study revealed a significant improvement in stem cells group compared to control group: LVEF increased from 37,4% ± 8,4% to 47,1% ± 8,3% in stem cell group compared to 37,9% ± 10,3% to 41,3% ± 9,1% in the CABG-only group at 6 months after the treatment (P=0,03) [Stamm et al. 2007]. We proposed that heterogeneity of patient's population between Phase II study and the register is a basis of showed disparity. We came to the conclusion that registry data cannot substitute the results of randomized placebo-control clinical trials. Nevertheless, the evaluation of functional parameters during the time in one group or long-term analysis between treated and control groups recruited for one study can be provided.

Moreover, we have concluded that MACCEs are considerably important data in the register. The late probable complications including arrhythmias, infarctions, apoplexies, calcifications, tumors etc. can be monitored closely after stem cell application and their comparison with the control group can be carried out. Patient's safety is a prerogative for any clinical trial in all the aspects of Good Clinical Practice. In addition, these data may be used for a standardized pharmacovigilence reporting to regulatory authorities. Therefore, MACCEs, which are representing main parts of safety evaluation process, could be selected as the most relevant parameter of the registry.

Another function of registry program can be applied for selecting patients with good response to stem cell therapy. It has been known for a while that the response to BMSC therapy varies with different subsets of patients [4, 5]. We stratified patient population into responders and non-responders based on change in local LVEF more than 5% at 12 month follow-up, reduction of LVEDD of more than -3 mm. "Absolute" responders were defined as patients who had an improvement of both parameters. Our study confirmed validity of this proposed criteria, since patients who were "absolute" responders after CD133+ BMSC injection showed no mortality during up to 14 years follow up (P=0,02). Moreover, our analysis revealed predictors for good response after stem cell therapy in patients with ischemic disease and heart failure. There are: LVEF below 30% and severe heart dilatation with a LVEDD over 60 mm. This observation was earlier confirmed by Wen Y. et al., [9] who demonstrated an enhanced improvement of ejection fraction after bone marrow-derived mononuclear cell therapy in patients with ischemic heart failure compared to patients with ischemic heart disease. In addition, the meta-analysis by Jeevanantham et al. [3] showed that stem cell treatment in patients with low baseline LVEF (less than 40%) resulted in greater improvement in LVESV and LVEDV (P=0,0004, P=0,01, respectively). We observed no influence of number of transplanted CD133+ BMSC (in the range of 0,5-20×10^6) as well as concomitant disease or given medication on the responsiveness of the patients. These findings are partly supported by work of Bai et al., who found no clear correlation between the number of intracoronary delivered BMSC and changes in LVEF [1].

To summarize, these results including pre-operative ejection
fraction and heart dimension, can be applied as criteria for the selection of suitable for stem cell therapy candidates.

**Conclusion**

The necessity of common stem cell registry program is increasing with expansion of stem cell therapy. New tasks including observance of Good Clinical Practice and pharmacovigilance issue the new challenges to stem cell treatment. The analysis of the registry program in the Regeneration and Translation Center for cardiac stem cell therapy showed, that the register can answer to many introduced questions. Particularly, yearly MACCE registrations allow following late probable complications after therapy and comparing them to control group. Moreover, MACCE can be a basis for standardized pharmacovigilance reporting to regulatory authorities. In addition, registry program could help to improve patient selection to stem cell therapy and define patients who did not respond to it. Nevertheless, the registry by itself cannot replace randomized clinical trials. However, registry data can be used for long term efficiency and safety evaluation in standardized patient groups. To complement these data a Phase III randomized double-blinded PERFECT trial (NCT00950274) was installed in 2009 and will be finished 2016.

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Conflict of interest: none declared.

**References**


Долгосрочные результаты внутримиокардиальной терапии CD133+ клетками костного мозга при ишемии миокарда: опыт с Регистром стволовых клеток

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Резюме
Стандартизация терапии стволовыми клетками требует методологии при оценке безопасности и эффективности лечения, а также длительное наблюдение за пациентами. Для выполнения перечисленного, в центре Регенеративной и Трансляционной медицины города Ростока, Германия, 14 лет назад была организована программа по долгосрочному наблюдению за больными после терапии стволовыми клетками и соответствующий регистр. Этот регистр содержит данные параметров для оценки эффективности и безопасности метода, в том числе основные церебральные и кардиальные события (MACCEs). Регистр включает 223 пациента (1152 пациент/год), которые наблюдаются каждый год пожизненно после имплантации стволовых клеток в контрольной группе. Настоящее исследование выполнено на 96 больных ишемической болезнью сердца с сердечной недостаточностью: 73 больных после введения стволовых клеток во время операции аорто-коронарного шунтирования (АКШ) и 23 больных контрольной группы (только АКШ без стволовых клеток). Проведенный анализ выявил, что, по средствам регистрации основных церебральных и кардиальных событий (MACCE), достигается оценка возможных поздних и неожидаемых осложнений после терапии, что является необходимым при соблюдении принципов хорошей клинической практики (Good clinical practice). Тем не менее, регистр не может заменить собой разнообразные клинические исследования, так как популяция больных в нем является гетерогенной. Однако данные регистров могут быть использованы для долгосрочной оценки безопасности и эффективности в стандартизованных группах больных. Кроме того, регистр помогает улучшить отбор больных для лечения стволовыми клетками и выявить пациентов, которые не отвечают на терапию. Введение единого обязательного регистра для всех центров, занимающихся терапией стволовыми клетками, является оправданным.

Ключевые слова
CD 133, стволовые клетки, интрамиокардиальное введение, регистр стволовых клеток, респондерная группа