In cardiology trials, defining responders to cell treatment based on functional measurements has been troublesome, and it may be considered as the Holy Grail. The functional recovery after myocardial infarction (MI) can range from only mild impairments and recovery to progression into heart failure at the next clinical visit regardless of the therapy given. In a clinical trial with adequate randomization, this will not pose an issue on the overall outcome of the trial. However, subgroup analyses become difficult, as the whimsical course of the disease influences the end result on top of the effect of the cell treatment. In other words, even patients who have suffered significant loss of functional cardiac capacity may still have benefited from cell therapy compared with the potential reference point of the same patient in the placebo group. Among other reasons, this can make subgroup analyses hard to interpret and potentially less informative. Proper subgroup analyses are ideally addressing true response, based on (pre)clinical hints, and are prospectively declared. Furthermore, the power needed to show specific responding groups might be beyond the number of participants included in hitherto conducted cell therapy trials.

Meta-analyses including all randomized controlled studies have consistently shown significant positive effects of treatment with bone marrow mononuclear cells (BMMNCs) after MI. Stratified subgroup meta-analyses hint toward different effects with increasing age and specific functional parameters. Researchers have questioned the availability and quality of autologous cells harvested from patients with multiple risk factors. The negative effects of endogenous risk factors on bone marrow and circulating progenitor cells have been confirmed with regard to age, smoking, heart failure, diabetes mellitus, and general risk factor profiles. To date, it is not known if the negative effect of clinical risk factors on BMMNC function and the recipient heart is also reflected in the outcomes of clinical studies. Furthermore, the invasiveness and cost of BMMNC therapy call for better prediction of treatment response after MI. In the present analysis, we demonstrate a method based on multivariable statistical interactions, which is able to identify potential treatment responders, while simultaneously correcting for relevant factors that affect general disease outcome. With the identification of components that positively influence the (probability of a) functional gain after cell therapy, it might be possible to predict who the real responders are.

**Methods and Results**

As a proof of concept, we used the data from the REPAIR-AMI trial (Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction), a multicenter, randomized, controlled trial, conducted from April 2004 till October 2005. In the REPAIR-AMI trial, difference in ejection fraction (EF) after 4 months compared with baseline was used as the initial primary outcome. Patient characteristics, baseline imaging, and cell characteristics were recorded. Two hundred and four patients were randomized, of which 186 had complete data after 4 months for functional outcome and characteristics.

This post hoc analysis was not prospectively declared but initiated and executed by independent researchers not affiliated with the primary study team. On the basis of an a priori power analysis, we defined 18 variables as possible predictors, based on previous literature and clinical expertise, having 1 variable per 10 outcome measures as is generally accepted. We applied linear regression analyses with difference in EF between baseline and 4-month follow-up as outcome and the statistical interaction of cell therapy with the single possible predictors as variable of interest. These interaction terms resemble the difference between the cell-treated and placebo groups, regardless of the effect of the variable on the functional outcome itself. A significant interaction therefore identifies predictors in which the effect of cell therapy compared with the placebo is different within groups.

Next, we performed multivariable linear regression for these interactions with subsequent stepwise backward selection (cutoff value used is the AIC [Akaike’s information criterion]) and estimated by the primary study team. On the basis of an a priori power analysis, we defined 18 variables as possible predictors, based on previous literature and clinical expertise, having 1 variable per 10 outcome measures as is generally accepted. We applied linear regression analyses with difference in EF between baseline and 4-month follow-up as outcome and the statistical interaction of cell therapy with the single possible predictors as variable of interest. These interaction terms resemble the difference between the cell-treated and placebo groups, regardless of the effect of the variable on the functional outcome itself. A significant interaction therefore identifies predictors in which the effect of cell therapy compared with the placebo is different within groups.

Next, we performed multivariable linear regression for these interactions with subsequent stepwise backward selection (cutoff value used is the AIC [Akaike’s information criterion]).
criterion; \( P = 0.157 \) ) to identify a combination of independent factors that most accurately predict the outcome in this data set (Table). The analysis was performed using R version 3.1.2\(^{5}\) with the additional rms package.

The randomization of the REPAIR-AMI study generated comparable groups for our analysis with minor baseline differences (Table). The combination of independent predictors for treatment response to cell therapy through interaction was patient age (\(-0.18\% / y; P = 0.05\) ), weight (\(+0.17\% / kg; P = 0.02\) ), \( \text{EF}_{\text{baseline}} \) (\(0.42\% / \%; P = 0.002\) ), and baseline end systolic volume (\(-0.09\% / \text{mL}; P = 0.08\); Table). \( \beta \) Values are expressed as \( \text{EF} \) change per unit of assessment. These outcomes suggest that advanced age is associated with poor response to BMMNC therapy, whereas higher weight and high initial functional loss are associated with greater treatment benefit in this data set (Figure [A] through [D]).

**Discussion**

Here, we show the concept of multivariably assessing the benefit of cell therapy by comparing outcomes to a patient’s reference point instead of the patient’s baseline measurement. Distinguishing responders from nonresponders could be a next step for clinical cell therapy, ultimately tailoring cell therapy to patients who will most likely benefit. Statistically correcting for the whimsical nature of the disease is an insightful step in this process. When doing so, it seems that in the REPAIR-AMI trial, younger patients with larger infarcts and risk factors such as smoking and obesity derive more benefit from BMMNC therapy compared with the patients with a negligible risk factor profile. Our findings are partially in line with results from previous meta-analyses, showing more effects of cell therapy in patients with lower baseline \( \text{EF} \) and age.\(^1\) For the effect of baseline cardiac function and cell therapy, results have been conflicting in both single studies and meta-analyses, of which a comprehensive overview was recently published.\(^6\) In these meta-analyses, the imaging method is also discussed, in which magnetic resonance imaging showed less effects compared with the left ventricular angiography used in, for example, the REPAIR-AMI trial. A recent individual patient meta-analysis could not confirm the findings with regard to

Table. Baseline Characteristics of the REPAIR-AMI Trial and Interaction Modeling of \( \Delta \text{EF} \) With Univariable Regression Analysis and Multivariable Regression Analysis With Backward Selection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n=91)</th>
<th>BMC (n=95)</th>
<th>Main Effect</th>
<th>Univariable Interaction</th>
<th>Multivariable Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta \text{EF}, 4 \text{ mo} )</td>
<td>3.2</td>
<td>5.5</td>
<td>0.02</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Age, y</td>
<td>56.6</td>
<td>55.4</td>
<td>0.55</td>
<td>0.04*</td>
<td>−0.18</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>84.6</td>
<td>81.2</td>
<td>0.52</td>
<td>0.52</td>
<td>1.69</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>84.3</td>
<td>80.4</td>
<td>0.07</td>
<td>0.07*</td>
<td>0.12</td>
</tr>
<tr>
<td>( \beta )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>27.6</td>
<td>26.8</td>
<td>0.13</td>
<td>0.26</td>
<td>0.29</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>59.3</td>
<td>52.6</td>
<td>0.36</td>
<td>0.51</td>
<td>1.32</td>
</tr>
<tr>
<td>Hyperlipidemia, %</td>
<td>60.4</td>
<td>51.6</td>
<td>0.23</td>
<td>0.79</td>
<td>−0.53</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>23.1</td>
<td>10.5</td>
<td>0.02</td>
<td>0.64</td>
<td>−1.33</td>
</tr>
<tr>
<td>Family history of CAD, %</td>
<td>36.3</td>
<td>34.7</td>
<td>0.83</td>
<td>0.08</td>
<td>3.6</td>
</tr>
<tr>
<td>Previous MI, %</td>
<td>6.5</td>
<td>5.2</td>
<td>0.95</td>
<td>0.29</td>
<td>4.7</td>
</tr>
<tr>
<td>Smoking history, %</td>
<td>68.1</td>
<td>74.7</td>
<td>0.32</td>
<td>0.06</td>
<td>4.1</td>
</tr>
<tr>
<td>Active smoker, %</td>
<td>42.9</td>
<td>47.4</td>
<td>0.54</td>
<td>0.11</td>
<td>3.2</td>
</tr>
<tr>
<td>Baseline imaging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{EF}, % )</td>
<td>47</td>
<td>48.3</td>
<td>0.36</td>
<td>0.02*</td>
<td>−0.25</td>
</tr>
<tr>
<td>( \text{ESV}, \text{mL} )</td>
<td>74</td>
<td>67.4</td>
<td>0.12</td>
<td>0.11*</td>
<td>0.06</td>
</tr>
<tr>
<td>( \text{EDV}, \text{mL} )</td>
<td>138.2</td>
<td>128.5</td>
<td>0.12</td>
<td>0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Intervention</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days of MI therapy</td>
<td>4.3</td>
<td>4.3</td>
<td>0.66</td>
<td>0.05*</td>
<td>1.4</td>
</tr>
<tr>
<td>Days MI-BM asp</td>
<td>3.9</td>
<td>3.8</td>
<td>0.6</td>
<td>0.09</td>
<td>1.6</td>
</tr>
<tr>
<td>Basal migration</td>
<td>91.5</td>
<td>103.7</td>
<td>0.16</td>
<td>−0.02</td>
<td></td>
</tr>
<tr>
<td>( \text{SDF} ) stim migr</td>
<td>161.8</td>
<td>170.9</td>
<td>0.49</td>
<td>0.46</td>
<td>−0.008</td>
</tr>
</tbody>
</table>

\( \text{BMI} \) indicates body mass index; CAD, coronary artery disease; days MI-BM asp, days between MI and bone marrow aspiration; EDV, end diastolic volume; \( \text{EF} \), ejection fraction; \( \text{ESV} \), end systolic volume; MI, myocardial infarction; and \( \text{SDF} \) stim migr, stromal-derived factor stimulated migratory capacity.

\*Significant or remaining in the multivariable model.
stratified variables for age and functional parameters, nor find other associated risk factors with any outcome. Weight as an effect modifier on functional response after cell therapy has never been described before to our knowledge.

Our first multivariable results might imply that cell therapy predominantly affects adverse remodeling after MI. An increased effect of cell therapy with increasing weight is in line with this hypothesis because waist circumference and body mass index are associated with increased incidence of heart failure after MI, and increase in body weight/body mass index is associated with an increased risk of developing heart failure in general. The same holds true for lower baseline EF and developing heart failure after MI. BMMNC therapy might be predominantly counteracting this process through its paracrine mechanisms.

Decreased numbers of circulating progenitor cells have been observed in, for example, smoking, diabetes mellitus, and obesity, and this decrease in circulating cells ultimately leads to worse cardiovascular prognosis. Interestingly, the acute increase in circulating progenitor cells after MI is also diminished with risk factors like diabetes mellitus and history of MI. In BMMNC therapy after MI, this defect in progenitor cell mobilization might be partially circumvented by mechanical BMMNC aspiration and subsequent direct administration. It is conceivable, however, that patients with few risk factors, and therefore an intact mobilization response, gain little additional benefit from BMMNC treatment. This is also in line with the findings from the CCTRN trials that personal bone marrow characteristics could explain infarct size reduction irrespective of cell therapy in both MI and heart failure.

Figure. A–D, Visualization of the interactions for (A) age, (B) weight, (C) baseline ejection fraction (EF), and (D) baseline end systolic volume (ESV). BMMNC indicates bone marrow mononuclear cells.
Variability in treatment success in clinical autologous stem cell trials is determined by 2 factors: the potency of the cell isolate and the disease state of an affected patient. This is in contrast to, for instance, medicinal therapy, where the variability in treatment response is theoretically solely dependent on the patient, as potency of different drug batches should ideally be nearly identical. Interestingly, the direction of effects from the identified risk factors, almost all besides age, pointed to a greater treatment response with an adverse risk factor profile. This finding is contrary to results from pre-clinical studies studying the cell product, which show that cardiovascular risk factors are associated with poorer proangiogenic capacity of human bone marrow cells in preclinical models. Preclinical studies have heretofore only been able to demonstrate a reduction in the proangiogenic potential of the BMMNC graft but supply little information on the recipient risk factor-fed hearts.

Importantly, the size of the REPAIR-AMI is insufficient to visualize all potential interactions that one might expect because interaction analyses need more participants to obtain adequate power than analyses of main effects in linear regression models. It is possible that this analysis is incomplete in identifying the effects of biological interactions that predict response in the general population of patients with MI and that the found interactions vary in effect size. There are many more (pre)clinical hints from other studies that might also have an effect on the response to cell therapy. Therefore, the effect of the specific combination of cardiac function, age, and weight is applicable to this data set and should not be applied to patient care yet. This analysis should be seen as a proof of concept and primarily hypothesis generating; the observed (combination of) independent predictors should be confirmed in other data sets and ideally be prospectively declared in larger trials (like the currently recruiting BAMI trial (NCT01569178; www.bami-fp7.eu) to generate the responder characteristics within the included population. Although adequately powered a priori for this analysis, there still is a risk of multiple testing here, as others before us analyzed this data set in the past. Most importantly, this analysis shows the estimation of a true effect compared with placebo treatment in a trial with a continuous outcome like EF.

MI and its aftermath can have a capricious course, blurring any effect of therapy to specific subgroups. Identifying responding populations through additional analyses might however be the next step toward optimal cell therapy in clinical care. In this article, we show a first step in identifying these subgroups using interaction models in a multivariable fashion. Future steps include prediction models for responder identification based on more retrospective and prospective data, to ultimately treat the patients who will benefit most from cell therapy.

Sources of Funding
This research was partially funded by a grant from the Alexandre Suerman program for MD/PhD students of the University Medical Center Utrecht, the Netherlands, and the Netherlands CardioVascular Research Initiative (CVON-HUSTCARE): The Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development, and the Royal Netherlands Academy of Science.

Disclosures
A.M. Zeiher is a founder and advisor of t2cure GmbH. The other authors report no conflicts.

References


**Key Words:** bone marrow cell transplantation clinical trial myocardial infarction
Responder Definition in Clinical Stem Cell Trials in Cardiology: Will the Real Responder Please Stand Up?
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Circ Res. 2016;119:514-518
doi: 10.1161/CIRCRESAHA.116.308733

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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