Rationale and design of the JUVENTAS trial for repeated intra-arterial infusion of autologous bone marrow-derived mononuclear cells in patients with critical limb ischemia

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Critical limb ischemia (CLI) continues to form a substantial burden on Western healthcare. Many patients still face amputation as a last treatment option. Autologous bone marrow (BM)-derived cell administration has emerged as a potential new treatment, but proof for sustainable clinical effects of BM-derived cell therapy in CLI is still lacking. The JUVENTAS (reJUVenating ENdothelial progenitor cells via Transcutaneous intra-Arterial Supplementation) trial is the first randomized, placebo-controlled, double-blinded clinical trial on repeated intra-arterial BM mononuclear cell (MNC) infusion in 110 to 160 CLI patients, designed to provide definite proof for the efficacy of stem cell therapy. Primary outcome is the incidence of major amputation at 6 months. Inclusion of patients is well underway. If BM-MNC cells therapy is beneficial, it could become a novel treatment to prevent amputation in patients with CLI. (J Vasc Surg 2010;51:1564-8.)

Critical limb ischemia (CLI) imposes a large burden on Western healthcare, as a considerable number of patients with CLI (~40%) are ineligible for surgical or radiological revascularization. The prognosis is poor,1 and with the lack of pharmacological treatments and other effective therapies, amputation is often the only treatment option left. Consequently, development of new revascularization therapies for CLI is of great importance. Bone marrow (BM)-derived endothelial progenitor cells (EPC) have been identified as a potential new therapeutic tool in the treatment of CLI.

Since the first observation of their presence in peripheral blood in the 1990s, increasing evidence indicates that EPC contribute to postnatal neovascularization by homing and incorporation into sites of new vessel formation.2,3 Encouraging results of small clinical studies on progenitor cell-based therapy have been reported in patients with CLI. Thus far, over 30 clinical studies have reported on the use of BM or peripheral blood (PB)-derived progenitor cells in patients with peripheral arterial occlusive disease (PAOD) or CLI (for an overview of design, patient number, route of administration, and outcome of these studies, see Sprengers et al4). Almost all studies reported beneficial results on clinical parameters. However, most of the studies have been small and lacked double-blind controls. Large, randomized, placebo-controlled trials are needed to evaluate the effects of cell-based therapy in CLI.

The JUVENTAS (reJUVenating ENdothelial progenitor cells via Transcutaneous intra-Arterial Supplementation) trial is an investigator-driven trial that examines the potential clinical effects of repeated intra-arterial infusion of BM-MNC in CLI patients. In addition, it studies the functional characteristics of BM-MNC obtained from CLI patients and will relate BM-MNC dysfunction to clinical outcome. This translational approach may lead to the identification of predictive assays or markers for therapeutic efficacy and may eventually yield strategies to improve therapeutic efficacy. To our knowledge, this is the first translational trial of its size that will investigate the clinical effects of intra-arterial infusion of BM-MNC in patients with CLI. This clinical update will cover the design of the...
JUVENTAS trial and discuss its decisions regarding unresolved issues on cell therapy in CLI patients.

**JUVENTAS TRIAL STUDY DESIGN**

The JUVENTAS trial has a randomized, double-blind, placebo-controlled design. A total number of 110 to 160 patients with proven chronic CLI or selected patients with severely invalidating intermittent claudication (claudicants on the verge of rest pain), who are not candidates for surgical or radiological revascularization (as decided by a multidisciplinary team of vascular surgeons and radiologists) will be included in the trial. Risk factors for cardiovascular disease are treated according to Dutch practice guidelines. Patients are screened for eligibility according to the criteria listed in Table. After obtaining written informed consent, patients are randomized by the Data Safety Monitoring Committee by means of a computerized randomization table to either repeated intra-arterial infusion of BM-MNC or placebo (a flow chart of the study design is shown in the Fig). The Gene and Cell Therapy Facility of the University Medical Center Utrecht is informed about the randomization result after BM aspiration. The steering committee and clinical staff remain blinded to the treatment allocation.

BM aspirates are obtained in all patients. A total volume of 100 ml BM is aspirated from the iliac crest under local anesthetic and conscious sedation according to local routine. Seven ml of BM aspirate is kept separately for fundamental research purposes. The remaining BM aspirate is used for purification of BM-MNC by density gradient centrifugation. After washing, the MNC is resuspended in a physiological salt solution containing 10% human serum albumin (HSA). For the study group, one-third of the remaining cells is prepared for direct infusion, while two-thirds of the cells are cryopreserved using 10% dimethylsulfoxide (DMSO) and stored for later infusions. For the control group, a placebo is prepared using autologous erythrocytes to match the color of the BM-derived cellular product in order to guarantee the double blinding procedure at the time of infusion. The steering committee and clinical staff remain blinded to the treatment allocation.

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Clinical evaluation is performed by the same investigator at baseline and at 2- and 6-month follow up. The primary outcome is incidence of major amputation (defined as being sited through or above the ankle joint) at 6

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**Table.** Inclusion and exclusion criteria of the JUVENTAS trial

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<tr>
<th>Inclusion criteria</th>
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<td>● Age &gt; 18 years</td>
<td>● History of neoplasm or malignancy in the past 10 years</td>
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<td>● Severe PAOD (Fontaine class IIb, III, and/or IV)</td>
<td>● Serious known concomitant disease with life expectancy of less than one year</td>
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<td>● Invalidating intermittent claudication</td>
<td>● (Anticipated) inability to obtain 100 ml of bone marrow aspirate</td>
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<td>● Persistent recurring rest pain requiring analgesia</td>
<td>● Known infection with human immunodeficiency virus (HIV), hepatitis B or hepatitis C virus</td>
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<tr>
<td>● Non-healing ulcers present for &gt;4 weeks without evidence of improvement in response to conventional therapies</td>
<td>● Follow-up impossible</td>
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<tr>
<td>● Ankle brachial index &lt; 0.6 or unreliable (non-compressible or not in proportion to the Fontaine classification)</td>
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<tr>
<td>● Not eligible for surgical or radiological revascularization</td>
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<td>● Written informed consent</td>
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months. Secondary outcomes are the incidence of minor amputations (sited more distal than the ankle joint), changes in the number and extent of leg ulcers, resolution of rest pain or improvement of pain-free walking distance, improvement of ankle-brachial index and transcutaneous oxygen pressure, and changes in quality of life. Standardized magnetic resonance imaging/angiography (MRI/MRA) imaging is performed at the time of inclusion and after 6-month follow-up to allow for objective measures for neovascularization (eg, collateral vessel formation, flow measurements, perfusion measurements).

ANALYSES

The sample size for the JUVENTAS trial is based on a 6-month risk of major amputation in patients with unreconstructed chronic critical leg ischemia of 42% and an estimated reduction of the risk of major amputation by BM-MNC infusion of 50%. Previously, predominantly Asian studies reported marked beneficial effects of BM-MNC infusion of 50%. For Western patients, who often have multiple risk factors, fewer data are available, but results seem more modest. A recent Dutch study showed relevant and sustained improvement in 15 out of 27 CLI patients. One uncontrolled Belgian study, in a similar population of 16 CLI patients with many cardiovascular risk factors, suggested that in these patients results are modest and restricted to the least affected patients. The estimated 50% reduction in risk of major amputations is a conservative estimate and takes into account that the effects in a Western, older population with multiple risk factors may be less than in the Asian population.

To allow for definite conclusions on the efficacy of cell administration in CLI patients, group sequential interim analysis will be performed. This statistical method allows for a varying number of patients to be included in a trial, depending on the difference in outcome between two groups. On average, fewer patients are needed in a trial if the expected difference in the primary outcome variable appears to be real. Assuming that BM-MNC infusion will reduce the risk of major amputation by 50%, it has been estimated that with a two-sided alpha of 0.05 and a power of 80%, approximately 110 to 160 patients need to be enrolled in the JUVENTAS Trial and followed for 6 months. If important clinical differences between groups become evident during an interim analysis, the trial will be stopped, and patients will be offered the best treatment available. If the observed benefit is ‘clearly’ larger, or if placebo treatment appears to be better than BM-MNC infusion, early termination of the trial may be recommended.

CURRENT STATUS

Recruitment of patients for the JUVENTAS Trial commenced in September 2006, and to date over 60 patients have been included in the trial. After a start-up phase, the speed of inclusion increased steadily, and recruitment of the 110th patient is currently expected by the end of 2011. If inclusion is prolonged to 160 patients, inclusion is expected to close by the end of 2012. Analysis and reporting is expected to be completed a half year after inclusion has ended. The trial has been submitted to the ClinicalTrials.gov trial register under number NCT00371371.

DISCUSSION

Although progenitor cell-based therapy has emerged as a promising new tool in the treatment of CLI, no definite proof about its efficacy is yet available, since the clinical studies thus far have been small and lacked double-blinded controls. The JUVENTAS trial has been designed to investigate the effects of intra-arterial infusion of BM-MNC in patients with CLI in a randomized, double-blind, placebo-controlled manner, and to provide additional evidence for efficacy and safety of cell-based therapeutic neovascularization.

With regard to cell therapy, many questions are still unanswered: for example, concerning the optimal cell population to be administered, the optimal route of administration, the optimal dose, the need for multiple treatments, and the impact of BM cell dysfunction.

Several uncontrolled studies, often in Asian populations, have showed that intramuscular (IM) administration of BM-MNC is feasible and safe and has potential beneficial clinical effects. Of the 10 studies (190 patients) in Western populations, five applied intra-arterial (IA) injection, either alone or in combination with IM injections. Several reasons for choosing IA administration over IM administration as the optimal delivery route can be identified: (a) preclinical data suggest that IA injection leads to improved survival of injected cells; (b) most CLI patients have multi-level disease, including the femoropopliteal tract and pedal arteries. These zones may be better reached by IA as compared with IM calf injections; (c) extensive experience exists and positive results have been reported on intra-coronary artery administration of BM-MNC in patients with myocardial ischemia (see meta analyses); (d) preclinical studies similar results for intra-arterial versus intramuscular injection on angiogenic activity were observed.

With regard to dosing, the JUVENTAS Trial chose to administer progenitor cells obtained from 100 ml BM, which can be aspirated under local anesthesia with a low risk of adverse events (none thus far) in a repeated infusion scheme. No criteria such as a minimal cell number, CD34+ cell number, and/or percentage have been defined. This strategy maximizes the amount of BM that is administered to the patient. In other clinical trials thus far, varying doses of injected MNC, with varying concentrations of CD34+ cells have been used. In studies on BM-MNC administration in CLI patients, amounts of aspirated BM ranging from 80 to 1000 ml have been reported, from which varying amounts of MNC and different fractions of CD34+ cells retrieved. All of those studies have reported beneficial effects on clinical outcome, and no consistent evidence of a dose-response in humans has been reported. In the JUVENTAS trial, all data on numbers of infused
BM-MNC, CD34+ cells, etc. are documented and will be related to study outcome.

Studies in myocardial ischemia have shown that only a limited number of cells are retained in the injured tissue. It was assumed that repeated administration of cells would lead to enhanced retainment of cells, and, therefore, a three times intra-arterial infusion scheme was chosen. No previous studies have reported on BM-MNC dose escalations or comparisons of different time points and frequencies of injection. A recent small dose-escalating study was unable to demonstrate differences between various dosages of intramuscularly transplanted GCSF-mobilized CD34+ cells, but encouraged larger randomized controlled trials on this topic.18

Several studies have demonstrated that circulating EPC are reduced and dysfunctional in the presence of risk factors for cardiovascular disease.19 Such functional impairment has been shown to extend to BM-MNC. BM-MNC obtained from patients with chronic ischemic heart disease has profoundly reduced neovascularization capacity.20 In PAOD, particularly in diabetes, reduced circulating EPC numbers and function have been reported.21 One small study showed lower circulating and BM EPC levels in patients with limb ischemia compared with controls, with a significant reduction in the mRNA expression level of EPC markers in BM-MNC, suggesting lower angiogenic potential.22 It has been suggested that the more modest response to BM-MNC therapy observed in Caucasians as compared with Asian patients may be related to the higher prevalence of cardiovascular risk factors and associated EPC dysfunction.5,9 A functional impairment of BM-MNC may limit the therapeutic potential of autologous BM cell therapy. The JUVENTAS Trial therefore includes a preclinical part that focuses on the functional characterization of the BM-MNC obtained from CLI patients. Functional characteristics of the BM-MNC will be related to clinical outcome. Such a translational approach may lead to the identification of predictive assays or markers for therapeutic efficacy and may eventually yield strategies to improve therapeutic efficacy.

CONCLUSIONS AND IMPLICATION

The JUVENTAS Trial will be the largest randomized, double-blind, placebo-controlled clinical trial yet conducted to evaluate the clinical effects of intra-arterial infusion of BM-MNC in patients with CLI. The results from this trial will further strengthen the evidence on the efficacy of cell-based therapy for CLI. If repeated intra-arterial infusion of autologous BM-MNC is beneficial, it could become a novel treatment to prevent amputation in patients with CLI.

AUTHOR CONTRIBUTIONS
Conception and design: RS, FM, MT, MV
Analysis and interpretation: N/A
Data collection: RS
Writing the article: RS, MV
Critical revision of the article: FM, MT, MV
Final approval of the article: RS, FM, MT, MV

Statistical analysis: N/A
Obtained funding: FM, MV
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REFERENCES

Final approval of the article: RS, FM, MT, MV

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COMMENTARY

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Over the last while, several investigators have explored the role of bone marrow-derived mononuclear cells in promoting angiogenesis or neovascularization in patients with critical limb ischemia (CLI) who have no other treatment option. The JUVENTAS (reJUVenating ENdothelial progenitor cells via Transcutaneous intra-Arterial Supplementation) trialists describe the background and rationale of this randomized study. There are a number of questions that permeate this and other similar studies.

Study subjects include patients with critical limb ischemia and severe claudication who are not candidates for revascularization as determined by a team of radiologists and surgeons. This is consistent with other studies, but what does it mean? Revascularization suitability is a criterion that is open to disagreement and variability. It certainly adds a subjective component to patient selection and can make it difficult to compare the results of different studies.

The study investigators’ decision to include severe claudicants raises some issues. First of all, some would argue that claudicants are more likely to be suitable for intervention and are less likely to have no revascularization options than those with CLI. Secondly, claudicants are less likely to undergo major amputation and, as this is the study’s primary outcome variable and the basis of the sample size calculation, the more claudicants included, the higher the chance of an underpowered study. According to the investigators, only three claudicants have been included in the over 60 subjects recruited to date, so this may not be a major issue.

Several practical questions remain as well, including mode of delivery. Is the best method of delivery intra arterial, as in JUVENTAS, or intramuscular, or a combination as used by other investigators? Additionally, what is the best method of determining neovascularization? Is it magnetic resonance angiography, as in this study, or angiography, duplex ultrasound, and/or ankle brachial indices, as used by others?

This is an important and exciting area of investigation involving patients who often have no alternative but major amputation. Hopefully, this randomized trial will clarify some of the issues regarding this form of therapy.

REFERENCE