Phase II Clinical Trial of CD34+ Cell Therapy to Explore Endpoint Selection and Timing in Patients With Critical Limb Ischemia

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**Background:** A prior phase I/IIa clinical trial provided evidence for safety, feasibility and potential efficacy of i.m. injection of granulocyte colony-stimulating factor (G-CSF)-mobilized CD34+ cells in patients with critical limb ischemia (CLI).

**Methods and Results:** A phase II trial of CD34+ cell therapy was conducted in patients with CLI to explore endpoint selection and timing. No-option CLI patients (n=11) underwent i.m. transplantation of G-CSF-mobilized CD34+ cells isolated by magnetic sorting. Ischemic rest pain scales improved from week 2 vs. baseline (P<0.05). Skin perfusion pressure (P=0.0175), transcutaneous partial oxygen pressure (P=0.0446) and pain-free walking distance (P=0.0056) improved from week 2, total walking distance from week 8 (P=0.0182) and toe brachial pressure index from week 12 (P=0.0174) vs. baseline. These parameters peaked at week 36 or 52. Rutherford’s category improved from week 24 vs. baseline (P=0.0065). CLI-free ratio serially increased and peaked (85.7%) at week 36. Serial change in Rutherford’s category correlated with that in Rest Pain Scale (P=0.0374), but not with that in any physiological parameters.

**Conclusions:** Ischemic rest pain scales and physiological parameters improved relatively early after cell therapy, then plateaued later accompanied by recovery from the CLI state. Rutherford’s category and CLI-free ratio at week 36 or later may be suitable endpoints in cell therapy clinical trials for CLI. Functional parameters should be evaluated independently of such clinical endpoints for ischemia severity. (Clinical Trial Registration: URL: https://dbcentre3.jmacct.med.or.jp/jmactr/Default.aspx. Unique identifier: JMA-IIA00022)  (Circ J 2014; 78: 490–501)

**Key Words:** CD34+ cell therapy; Clinical trial; Critical limb ischemia; Endothelial progenitor cells

Chronic critical limb ischemia (CLI) is defined as the end-stage of lower limb ischemia due to atherosclerotic peripheral arterial disease (PAD) or vasculitis including thromboangiitis obliterans (Buerger’s disease). The clinical manifestations consist of rest pain and/or skin ulceration or gangrene. The annual incidence of CLI is estimated to be 500–1,000 cases per million people in the developed countries. The prognosis of CLI patients is poor. The 1-year mortality and major amputation rate are reported to be 25% and 30%, respectively. Currently, revascularization of the ischemic limb with surgical bypass techniques or endovascular approaches is believed to be the best option for limb salvage. A total of 25–
40% of patients with CLI, however, are not candidates for either of these options due to a lack of autologous vein graft, extensive lesions in the tibial and peroneal arteries or medical comorbid-ity.\textsuperscript{1,2,3,4,5} The development of novel strategy for blood flow recovery is urgently needed for such no-option patients with CLI.

Bone marrow (BM)-derived endothelial progenitor cells (EPCs), which comprise a small fraction (0.1–2%) of total mononuclear cells (MNCs), are capable of promoting physiological and pathological neovascularization through proliferation, migration, homing and potentially differentiation into endothelial lineage.\textsuperscript{6} The therapeutic potential of EPCs has been established by a number of preclinical studies for hindlimb\textsuperscript{7}, myocardial\textsuperscript{8} and cerebral ischemia.\textsuperscript{9} Based on the preclinical data, early phase clinical studies have been performed that indicate potential effectiveness of transplantation of MNCs obtained from BM or peripheral blood (PB) for no-option patients with CLI.\textsuperscript{10,11} Onodera et al reported that treatment with a small number of harvested CD34+ cells, an EPC-enriched fraction, was a negative independent predictor of amputation and death following either BM- or PB-MNC implantation in patients with CLI.\textsuperscript{12} This suggests an important role of EPCs for therapeutic neovascularization and may provide a reasonable rationale for transplantation of EPCs purified from crude MNCs in patients with CLI.

Our group reported the short-term outcome after i.m. injection of granulocyte colony-stimulating factor (G-CSF)-mobilized CD34+ cells in no-option patients with CLI. The phase I/IIa clinical trial demonstrated the safety, feasibility and potential efficacy of CD34+ cell transplantation in the 12 weeks following the cell therapy.\textsuperscript{13} Furthermore, we reported that the safety and efficacy of CD34+ cells may be sustained for up to 4 years after cell therapy in patients with CLI.\textsuperscript{14} Recently, in the ACT34-CLI study, a double-blind, randomized, placebo-controlled, phase I/IIa pilot clinical trial, 28 patients with CLI were randomized to receive i.m. injection of 1×10^6 (low-dose) or 1×10^6 (high-dose) cells/kg of mobilized autologous CD34+ cells or an equal volume of diluent. A favorable trend towards improvement of amputation-free survival rate was observed in the cell-treated groups, especially in the high-dose group, compared with control group at 6 and 12 months after treatment.\textsuperscript{15} Clinical experiences in previous phase I/II studies provide suggestive evidence for the benefit of G-CSF-mobilized CD34+ cell therapy for CLI. The precise time course of various efficacy parameters after CD34+ cell therapy and correlation of serial improvement of the clinical severity parameters with that of functional or subjective parameters, however, have never been fully investigated.

In this phase II trial, we examined various endpoints for the clinical disease severity, rest pain scales, limb blood flow, exercise tolerance and quality of life (QoL) at multiple time points after CD34+ cell therapy to explore appropriate endpoints for future clinical trials in patients with CLI.

Methods

Study Design and Enrollment Criteria

This phase II clinical trial was designed as a single-arm, non-randomized study. This study protocol conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the Institute of Biomedical Research and Innovation. The clinical trial notification was approved by the Pharmaceuticals and Medical Device Agency in Japan. This study was performed in accordance with Good Clinical Practice (GCP) for Medical Devices based on the use of the Isolex device for selecting the cell product for clinical use.

The inclusion criteria were (1) atherosclerotic PAD or Buerger’s disease with >70% luminal stenosis in the leg arteries on digital subtraction angiography; (2) >6 months since the onset of lower limb ischemia; (3) CLI category 4 or 5 on the Rutherford scale; (4) failure of or no indication for transluminal angioplasty/stenting or bypass surgery; (5) age 20–80 years; and (6) written informed consent. The exclusion criteria, which were mainly implemented to exclude patients considered to be at potentially elevated risk for G-CSF treatment, apheresis or use of CD34+ cells, and to permit optimal evaluation of efficacy, are given in Table S1.

Treatment

All CLI patients enrolled in this study received s.c. G-CSF to mobilize EPCs from BM. The dose of G-CSF (Filgrastim; Gran, Kyowa Hakko Kirin, Japan) was 5 μg/kg per day for 5 days. G-CSF was not given for WBC ≥75,000μl. Leukopheresis (AS. TEC204; Fresenius HemoCare, Bad Homburg, Germany) was performed to harvest PB MNCs on day 5. The leukopheresis product was kept at a concentration of ≤2×10^6 cells/ml in phosphate-buffered saline with 1% human serum albumin and 0.41% sodium citrate at 2–8°C overnight until the immuno-magnetic separation of CD34+ cells was started using Isolex 300M Magnetic Cell Selection System version 2.5 (Baxter Healthcare, Deerfield, IL, USA). The purity of the isolated CD34+ cells was quantified on fluorescence-activated cell sorting (FACS) using CD34-specific and CD45-specific monoclonal antibodies (Becton, Dickinson and Company, San Jose, CA, USA).

Cell transplantation was performed under spinal or general anesthesia. Patients with bilateral CLI underwent cell transplantation into both limbs. CD34+ cells dissolved in 10 ml of physiological saline were injected i.m. into 40 sites (0.25 ml/site) of each leg with critical ischemia. The treatment points consisted of 30 sites in the calf muscle, 6 sites in the sole muscle, and 4 sites in the intertorse muscle. The target dose was 1×10^6 cells·kg\textsuperscript{-1} (body weight)·limb\textsuperscript{-1}. When the CD34+ cell yield was less than the target dose, all of the isolated cells were used.

Endpoints

Because this study was conducted to explore endpoints and the timing of evaluations, a primary endpoint was not predefined. Instead, data of various parameters regarding safety and efficacy of CD34+ cell therapy were extensively collected. When cell transplantation was performed into bilateral limbs, the data from the more severely ischemic limb at baseline were used for follow up comparison.

Clinical Endpoints

The severity of lower limb ischemia in either each patient or each transplanted leg was assessed on Rutherford’s category and Fontaine’s stage at baseline, week 2, 4, 8, 12, 24, 36 and 52. The CLI-free ratio was defined as the percentage of patients or limbs with Rutherford’s category ≤3.

Major adverse clinical events were defined as death due to any cause, major amputation, and unplanned minor amputation of the treated leg. Incidence of these clinical events was recorded until week 52.

Ischemic rest pain was evaluated at baseline, week 2, 4, 8, 12, 24, 36 and 52 by Visual analogue Scale (VAS).\textsuperscript{16} Wong-Baker’s FACES pain rating scale\textsuperscript{17} and Rest Pain Scale.

Functional Endpoints

As for the physiological examinations, we measured the following parameters in the treated legs twice at each time point (>24 h but <2 weeks apart); ankle brachial pressure index (ABPI), toe brachial pressure index (TBPi; Form PWV/ABI; Omron Colin, San Antonio, TX, USA), transcutaneous partial oxygen pressure (TcPo2; PO-850; Sumitomo Electric System Solutions, Tokyo, Japan) at room air, skin perfusion pressure (SPP; LASORDP PV2000; Vasamedics, St.
For safety monitoring, all adverse events (AEs) including serious AEs were collected, regardless of cause. Physical and laboratory examinations including hematological and biochemical tests were performed during the treatment period and at each follow-up visit (week 2, 4, 8, 12, 24, 36 and 52). To detect pathological angiogenesis in the retina, fundus oculi examination was performed before and after (week 2 and week 52) CD34+ cell transplantation. Medical Dictionary for Regulatory Activities (MedDRA), version 14.0, was used for classification of each AE.

Statistical Analysis
Case registration, data management and statistical analysis were performed at Translational Research Informatics Center, an in-
dependent data center. Monitoring and auditing were performed for system validation and data quality assurance.

Data are given as mean±SD for continuous variables and median with range for ordered categorical variables. Serial changes of Rutherford’s category were analyzed using Wilcoxon’s signed-rank test. The linear mixed model was applied in order to evaluate longitudinal variation, and the differences between baseline and each time point after cell therapy were assessed on Dunnett-Hsu’s test for multiple comparisons. Overall and event-free survival ratios were analyzed using Kaplan-Meier methods. The serial change of each efficacy parameter after cell therapy was evaluated using the slope of the estimated regression line, namely, \( y = a + bt \) where \( y \) is the value of an efficacy variable, \( t \) is time, \( a \) is the constant when \( t \) equals zero, and \( b \) is the slope coefficient. Spearman’s rank correlation was used to examine the correlation of the slopes of the regression \( (b) \) between Rutherford’s scale and each efficacy parameter. Analyses were performed using SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

**Results**

**Patients**

A total of 16 patients with CLI were enrolled to participate in this study from July 2008 to June 2010. Among these patients, 5 were excluded before CD34+ cell treatment. Finally, 11 patients were registered and received the treatment (Figure 1).

Baseline patient characteristics are summarized in Table 1. Seven patients (63.6%) had Buerger’s disease and 4 (36.4%) had PAD. Two patients with Buerger’s disease and 2 patients with PAD received cell transplantation in both limbs. As a result, 15 limbs in 11 patients received cell therapy. Five of the 11 patients (45.5%) were Rutherford 5 while 6 patients (54.5%) were category 4. Five of 15 limbs (33.3%) were Rutherford’s category 5 and 10 (66.7%) were Rutherford’s category 4. Nine patients (81.8%) were past smokers and no current smokers were enrolled. All patients received at least 1 prostanoid or antiplatelet drug throughout the study.

**Outcome of CD34+ Cell Treatment**

The mean dose of G-CSF was 330.8±51.1 μg per day. There were no patients in whom WBC count was ≥75,000/μl during the G-CSF treatment period. The total apheresis product was (3.0±0.8)×10^{10} cells and that of transplanted CD34+ cells was (6.5±3.7)×10^{10} kg^{-1}·limb^{-1}. On FACS analysis the purity, recovery and viability of the CD34+ fraction following magnetic sorting were 84.8±11.9, 69.1±16.4 and 97.1±1%, respectively. The number of transplanted CD34+ cells was (3.5±1.5)×10^{10} per patient, (9.0±6.3)×10^{10}/kg, (4.6±2.8)×10^{10}/limb and (6.5±3.7)×10^{10}·limb^{-1}·kg^{-1}.

**Efficacy Evaluation in All Patients**

Following CD34+ cell injection, Rutherford’s category significantly improved at week 24 (median, 3.0; range, 2.0–5.0) vs. baseline (median, 4.0; range, 4.0–5.0; \( P<0.01 \)) and the significant change was sustained up to week 52 (Figure 2A). The CLI-free ratio serially increased and peaked at week 36 (80.0%; 95% confidence interval: 26.2–87.8; Figure 2B).

To evaluate the severity of ischemic rest pain, we used the VAS, Wong-Baker’s FACs pain rating scale and the Rest Pain Scale. All of these parameters significantly improved beginning at week 2 compared with baseline. Improvement of the VAS peaked at week 36 and that of Wong-Baker’s FACs pain rating scale and the Rest Pain Scale peaked at week 52 (Figures 2C–E). Furthermore, complete pain relief was achieved in 4 out of 10 patients (40.0%) at week 12, 5 patients (50.0%) at week 24 and in 7 patients (70.0%) at week 52. All functional parameters including ABPI, TBPI, SPP, TcPO2, TWD and PFWD were measured twice at each time point, and the mean and the higher of these data were evaluated. With regard to the mean, ABPI, which was <0.8 at baseline in only 4 out of 11 patients, did not significantly change after the cell therapy (data not shown). TBPI significantly improved from week 12 compared with baseline and peaked at week 52. SPP and TcPO2 significantly increased from week 2 relative to baseline and peaked at week 52 (Figures 3A–C). TWD and PFWD significantly increased from week 8 and 2, compared with baseline, respectively. These parameters for exercise tolerance peaked at week 52 (Figures 3D,E). Analysis of the higher data points for the functional parameters found a similar serial change to that of the means (Figure 4).

One patient with Buerger’s disease underwent planned minor amputation at day 6 after starting G-CSF and 1 patient with PAD underwent major amputation at week 8. It was impossible to validly assess the serial change of ulcer area in the amputated patients because the target lesion for area measurement changed after limb amputation (Figure S1).

These results suggest that rest pain scales start to improve early after CD34+ cell therapy (from week 2), followed by depen-
Figure 2. Clinical improvement during the 52-week follow up. Serial changes in (A) proportion of Rutherford’s category (0–6); (B) critical limb ischemia (CLI)-free ratio; (C) Visual Analogue Scale (VAS; mean±SD); (D) Wong-Baker’s FACES (WBF) pain rating scale (median with range); and (E) Rest Pain Scale (median with range) following CD34+ cell transplantation in patients with CLI (n=11 at week 0–4; n=10 at week 8–52). *P<0.05 vs. baseline; **P<0.01 vs. baseline.
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Figure 3. Serial changes in the means of the functional parameters including (A) toe brachial pressure index (TBPI); (B) skin perfusion pressure (SPP); (C) transcutaneous partial oxygen pressure (TcPO2); (D) total walking distance (TWD); and (E) pain-free walking distance (PFWD) during the 52-week follow-up period following CD34+ cell transplantation in patients with critical limb ischemia (n=11 at week 0–4; n=10 at week 8–52). *P<0.05 vs. baseline; **P<0.01 vs. baseline. Data given as mean±SD.
Figure 4. Serial changes in the higher data points of functional parameters including (A) toe brachial pressure index (TBPI); (B) skin perfusion pressure (SPP); (C) transcutaneous partial oxygen pressure (TcPO2); (D) total walking distance (TWD); and (E) pain-free walking distance (PFWD) during the 52-week follow-up period following CD34+ cell transplantation in patients with critical limb ischemia (n=11 at week 0–4; n=10 at week 8–52). *P<0.05 vs. baseline; **P<0.01 vs. baseline. Data given as mean±SD.
Figure 5. Serial changes in quality of life (QoL) scores of 8 subscales in SF-36v2 including (A) Physical functioning; (B) Role-Physical; (C) Bodily Pain; (D) General Health; (E) Vitality; (F) Social Functioning; (G) Role-Emotional; and (H) Mental Health during the 52-week follow-up period after CD34+ cell transplantation in patients with critical limb ischemia (n=11 at week 0–4; n=10 at week 8–52). *P<0.05 vs. baseline; **P<0.01 vs. baseline. Data given as mean±SD.
Correlation Between Efficacy Parameters

Spearman’s rank correlation for the time-related slope of the regression lines were analyzed between Rutherford’s category and other efficacy parameters including rest pain scales, functional parameters and subscales in SF-36v2 during the follow-up period in all patients. Regarding the ischemic rest pain scales, a significant correlation between Rutherford’s category and Rest Pain Scale was observed ($\rho=0.6333; P<0.05$). Regarding the physiological parameters, there was no significant correlation between Rutherford’s category and each other parameter ($\rho=0.0364; P=0.9155$; $\rho=0.0566; P=0.0827$). As for the health-related QoL, a significant inverse correlation was confirmed between Rutherford’s category and Vitality ($\rho=−0.6546; P<0.05$). There were no significant correlations between Rutherford’s category and the other subscales in SF-36v2 ($\rho=−0.6000; P=0.0510$).

These data suggest that serial improvement of rest pain scales but not functional parameters may correlate with that in clinical severity endpoints for CLI.

Correlations of Serial Improvement Markers in All Treated Limbs

The time-related slope in Rutherford’ category significantly correlated with that in Wong-Baker’s FACES pain rating scale ($\rho=0.5689; P<0.05$) and Rest Pain Scale ($\rho=0.5989; P<0.05$), but not any physiological parameters or health-related QoL in all treated limbs ($\rho=−0.5909; P=0.0556$).

These results suggest that the efficacy parameters in treated limbs improve in a similar time course in all patients.

Evaluation of Health-Related QoL

Among the 8 subscales in SF-36v2, Physical Functioning, Bodily Pain, Vitality, Role-Emotional and Mental Health significantly improved from week 4 after cell therapy compared with baseline. Role-Physically significantly improved beginning at week 8 compared to baseline. Finally, all of the 8 subscales were significantly improved at week 36 compared with baseline (Figure 5).

These outcomes provide evidence that CD34+ cell therapy improves health-related QoL in patients with CLI.

Table 2. Correlations in Serial Improvement Markers in All Patients

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<th>Rutherford’s category</th>
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<td>Rest Pain Scale</td>
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<td>QoL scores of 8 subscales in SF-36v2</td>
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<td>Mental Health</td>
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PFWD, pain-free walking distance; QoL, quality of life; SPP, skin perfusion pressure; TBPI, toe brachial pressure index; TcpO2, transcutaneous partial oxygen pressure; TWD, total walking distance; VAS, Visual Analogue Scale; WBF, Wong-Baker’s FACES.
moderate AEs were transient and resolved without sequelae. No cerebrovascular or cardiovascular events were observed during the study period in all patients. Fundus oculi examination indicated no pathogenic angiogenesis in the retina at week 4 and 52 after CD34+ cell therapy in all patients.

**Discussion**

Design and execution of clinical trials in CLI patients are challenging, in part because of the lack of consensus on relevant endpoints. Guidelines or recommendations for adequate endpoints were recently proposed for clinical trials of pharmacotherapy, endovascular intervention and bypass surgery in patients with CLI. The Basel PAD Clinical Trial Methodology Group recommended conventional endpoints assessing ischemic rest pain, ulcer healing and amputation rates as well as composite endpoints including cardiovascular morbidity, amputation and all-cause mortality for pharmacotherapy in CLI. The DEFINE Group proposed appropriate endpoints such as sustained clinical improvement/deterioration based on Rutherford stage, hemodynamic improvement by ABPI, mortality and need for unplanned amputations for endovascular interventions in patients with CLI. The Society for Vascular Surgery proposed objective performance goals for bypass surgery in CLI: major amputation or any major vascular re-intervention defined as major adverse limb event (MALE) and freedom from perioperative death or any MALE (MALE+POD) as key endpoints for revascularization therapy. No guidelines of clinical trial endpoints, however, have been proposed for cellular therapy in CLI. To establish the endpoints for cell therapy trials, various aspects of cell therapy, as distinct from conventional revascularization, should be considered. As for EPCs, newly formed blood vessels after cell therapy are mainly capillaries or arterioles, but not arteries. Therefore, blood flow recovery might be slower after cell therapy compared with conventional revascularization. In addition, the mechanism of action of cell therapy is more complicated than conventional therapies, in which mechanical revascularization is the primary mechanism. Transplanted stem/progenitor cells may not only directly differentiate into endothelial lineage cells for vasculogenesis but also secrete cytokines/growth factors inducing angiogenesis, vasodilatation or anti-inflammation. Such multiple biological actions of EPCs may result in improvement of clinical and functional parameters in a unique time course. To clarify this clinically important issue, in the present phase II study, various parameters for clinical disease severity, rest pain scale, ulcer size, limb blood flow, exercise tolerance and QoL were evaluated at frequent intervals. Also, it is unclear which biological action of EPCs may most contribute to recovery from CLI. With regard to mechanism of action, correlation of serial improvement of clinical severity parameters with that of functional parameters for rest pain, blood perfusion, exercise tolerance or QoL were evaluated in this study.

The results of this phase II study almost reproduce those of our previous phase I/IIa trial, indicating the safety, feasibility and potential effectiveness of CD34+ cell transplantation for CLI patients. In addition, the present study shows that rest pain scales started to improve early after CD34+ cell therapy (from week 2), functional parameters including TBPI, SPP, TcPO2, TWD and PFWD increased from week 2 to 12, and health-related QoL also improved from week 4. Finally clinical severity of limb ischemia including Rutherford’s category and CLI-free ratio improved in the later phase (from week 12 to 24). Although the major trend in serial improvement of these parameters is similar in the present and previous studies, the clinical disease parameters improved later in the present study than in the previous study (from week 4). The reason for the different result is unclear, but CD34+ cells were transplanted into a single limb in all patients in the previous study, whereas 4 of 11 patients received the cells into both limbs in the present study. Lower cell dose in selected patients in the present study might affect the results of the statistical analysis. The time course of the clinical parameters would be carefully monitored in the upcoming phase III clinical trial. Taken together with the previous recommendation of a key endpoint for endovascular therapy trials, these results strongly indicate that clinical disease severity could be a valid primary endpoint for trials investigating CD34+ cell therapy in CLI. In the present study, both rest pain scales and most functional parameters significantly improved from week 2 to 12. The rest pain scales, however, drastically improved in contrast to the modest increase of blood flow recovery. Although the mechanism of excellent improvement of ischemic rest pain remains to be investigated, anti-inflammation activity by the paracrine action of CD34+ cells in addition to the modest increase of blood flow might be involved in this phenomenon. Another intriguing finding is that most parameters peaked at week 36–52, indicating that the therapeutic benefit of CD34+ cells may be durable through to the end of year 1.
after cell transplantation. As for the appropriate timing of each parameter assessment, clinical disease severity would be evaluated 6 months or later after CD34+ cell therapy, whereas examination of rest pain scales and blood flow parameters would be started as early as at week 2–4. As for the correlation of serial improvement of clinical severity with that of functional parameters, the time-related slope of Rutherford’s category significantly correlated with that of rest pain scales, but not blood flow parameters or most of the QoL parameters. It is possible that improvement of Rutherford’s category correlated with that of rest pain scales, because the presence or absence of ischemic rest pain directly relates to the diagnosis of Rutherford’s category. The present results also suggest that functional parameters for limb blood flow may be evaluated independently of clinical endpoints for ischemic severity. The discordance between clinical endpoints and functional and surrogate endpoints was similarly reported in a gene therapy trial for therapeutic angiogenesis in CLI.28

In this study, we measured the functional parameters twice at each time point and evaluated the mean and the higher of these 2 data points, because transient vasospasm especially in patients with Buerger’s disease might lead to underestimation of limb blood flow. The serial change of the higher data point, however, was similar to that of the mean. These results suggest that only a 1-time measurement at each time point may be sufficient for the assessment of functional parameters. Regarding the ulcer area, it was impossible to validly assess the serial change in patients undergoing planned minor amputation, because the target lesion for area measurement changed after limb amputation and anastomosis. To overcome this issue, baseline ulcer area should be measured immediately after a planned amputation, not before starting G-CSF.

As a limitation of the present study, the placebo effect could not be evaluated due to the non-randomized and non-placebo-controlled study design. Also, the number of subjects was relatively small. Detailed serial changes in various parameters after implantation of other types of cells such as BM-derived or adipose tissue-derived cells are unclear. Further clinical investigations are warranted to clarify these issues.

Conclusions

The present phase II clinical trial provides important information in addition to the reconfirmation of the previous outcomes demonstrating the safety, feasibility and potential efficacy of G-CSF-mobilized CD34+ cell therapy for no-option patients with CLI. For the clinical trial of CD34+ cell therapy for CLI, Rutherford’ category and CLI-free ratio may be a suitable candidate of primary endpoint and would be evaluated at week 36–52. Rest pain scales and functional parameters should probably be assessed from week 2 to 4 after CD34+ cell therapy. On the basis of these results, our group is preparing for a larger phase III, randomized and controlled clinical trial, evaluating CD34+ cell therapy against standard of care.

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References


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Supplementary Files

Supplementary File 1

Table S1. Exclusion criteria
Table S2. Correlations of serial improvement markers in all treated limbs
Table S3. Possibly study-related AEs during 52-week follow-up

Figure S1. Serial change in skin ulcer size during the 52-week follow-up period after CD34+ cell transplantation in 4 treated limbs of 4 patients with Rutherford’s category 5.

Figure S2. Serial changes in (A) proportion of Rutherford’s category (0–6); (B) critical limb ischemia (CLI)-free ratio; (C) Wong-Baker’s FACES (WBF) pain rating scale (median with range); (D) Visual Analogue Scale (VAS; mean ± SD); and (E) Rest Pain Scale (median with range) following CD34+ cell transplantation in treated limbs (n=15 at week 0–4; n=14 at week 8–52).

Figure S3. Serial changes in the (A,C,E) means and the (B,D,F) higher values of functional parameters including toe brachial pressure index (TBPI), skin perfusion pressure (SPP) and transcutaneous partial oxygen pressure (TcPO2) during the 52-week follow-up period following CD34+ cell transplantation in treated limbs (n=15 at week 0–4; n=14 at week 8–52).

Please find supplementary file(s);