Interim analysis results from the RESTORE-CLI, a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells and placebo in patients with critical limb ischemia

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Objectives: **Cell therapy is a novel experimental treatment modality for patients with critical limb ischemia (CLI) of the lower extremities and no other established treatment options. This study was conducted to assess the safety and clinical efficacy of intramuscular injection of autologous tissue repair cells (TRCs).**

Methods: **A prospective, randomized double-blinded, placebo controlled, multicenter study (RESTORE-CLI) was conducted at 18 centers in the United States in patients with CLI and no option for revascularization. Enrollment of 86 patients began in April 2007 and ended in February 2010. For the prospectively planned interim analysis, conducted in February 2010, 33 patients had the opportunity to complete the trial (12 months of follow-up), and 46 patients had completed at least 6 months of follow-up. The interim analysis included analysis of both patient populations. An independent physician performed the bone marrow or sham control aspiration. The aspirate was processed in a closed, automated cell manufacturing system for approximately 12 days to generate the TRC population of stem and progenitor cells. An average of 136 ± 41 × 10^6 total viable cells or electrolyte (control) solution were injected into 20 sites in the ischemic lower extremity. The primary end point was safety as evaluated by adverse events, and serious adverse events as assessed at multiple follow-up time points. Clinical efficacy end points included major amputation-free survival and time to first occurrence of treatment failure (defined as any of the following: major amputation, death, de novo gangrene, or doubling of wound size), as well as major amputation rate and measures of wound healing.**

Results: **There was no difference in adverse or serious adverse events between the two groups. Statistical analysis revealed a significant increase in time to treatment failure (log-rank test, $P = .0053$) and amputation-free survival in patients receiving TRC treatment, (log-rank test, $P = .038$). Major amputation occurred in 19% of TRC-treated patients compared to 43% of controls ($P = .14$, Fisher exact test). There was evidence of improved wound healing in the TRC-treated patients when compared with controls at 12 months.**

Conclusions: **Intramuscular injection of autologous bone marrow-derived TRCs is safe and decreases the occurrence of clinical events associated with disease progression when compared to placebo in patients with lower extremity CLI and no revascularization options. (J Vasc Surg 2011;54:1032-41.)**

Approximately 1.1 million Americans suffer from peripheral artery disease (PAD), which is associated with a 5-year cardiovascular death risk of 20% to 30%.[1] Critical limb ischemia (CLI) is the severest form of PAD defined by severely impaired hemodynamics and chronic ischemic rest pain, ulcers/tissue loss, or gangrene. Mortality and morbidity due to CLI is high. Up to 20% of patients with CLI will die within the first 6 to 12 months; 2-year, 5-year, and 10-year mortality rates are approximately 35%, 70%, and 100%, respectively. As many as 40% to 50% of patients will undergo major limb amputation within 6 to 12 months.[2] The ability of this elderly

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patient population with many associated comorbidities to successfully rehabilitate and maintain an independent living status following major limb amputation is poor.

The treatment of CLI patients is multidisciplinary.[3] Underlying atherosclerosis is treated pharmacologically with lipid reduction, antiplatelet, and antihypertensive therapies. Wound care and surgical debridement is also integral to patient care.[4] Revascularization continues to be the mainstay of therapy, and this includes either open surgical procedures or percutaneous endovascular approaches.

For a substantial number of patients effective revascularization is not possible, due to location or extent of the disease or associated comorbidities precluding open surgery. Patients with CLI, who are unable to undergo successful revascularization, currently have no effective treatment options. There are currently no Food and Drug Administration (FDA)-approved therapies for CLI. Therapy for this "no-option" CLI patient population is limited to management of the associated comorbidities with intensive wound care, pain control, and eventual amputation of the limb. Thus, the no-option CLI patient represents a population with a serious and life-threatening disease with an unmet medical need. Given the limitations of current therapies and high rate of mortality, CLI quality of life in this patient population has been likened to terminal cancer.[5]

Biologic regenerative therapies, including stem cell therapy (autologous and allogeneic) and gene transfer are currently undergoing clinical investigation. In several clinical studies, administration of bone marrow cells (BMC) appears to have improved critical limb ischemia patient outcomes.[5-9] In adults, bone marrow is the reservoir for stem and progenitor cells that can differentiate into cells that repair or generate new blood vessels. Administration of cells by injection directly into the ischemic tissue has developed as a potential strategy to improve limb perfusion and limb salvage in the ischemic limb.

It is not clear which subsets of BMCs are optimal for treating CLI. Animal studies have demonstrated that various subsets of bone marrow cells are active in improving vascularization in limb ischemia models.[9] This suggests that a combination of cell types may provide enhanced benefit, by providing precursors of various lineages or by the interactions between them that may drive differentiation and proliferation. Tissue repair cells (TRCs) expanded from autologous bone marrow are a mixture of cell types.[10] They are composed of the same mesenchymal and hematopoietic progenitor cells found in bone marrow but have a greater number of certain cells, such as monocytes/macrophages, that have been reported to be central to neovascularization.[9] In vitro, the TRC production process supports growth of stem cells, progenitors and mature cells in mesenchymal, endothelial and myeloid lineages, while reducing lymphoid and erythroid precursors.[10,11] The RESTORE-CLI trial is a randomized, double-blind multi-center phase II study comparing intramuscular injection of TRCs against placebo in affected limbs of patients with CLI in patients without revascularization options. Interim safety and efficacy analyses are reported.

METHODS

Study design

The study "Use of Tissue Repair Cells (TRCs-Autologous Bone Marrow Cells) in Patients with Peripheral Arterial Disease to Treat Critical Limb Ischemia" (RESTORE-CLI) is a prospective, randomized, double-blinded, placebo-controlled multicenter study that compares intramuscular injections of expanded autologous bone marrow cells ("tissue repair cells" or TRCs) that are suspended in physiological electrolyte solution with injections of the same electrolyte solution without cells in patients with lower extremity CLI. The study was sponsored by Aastrom Biosciences Inc in Ann Arbor, Michigan. The protocol was reviewed by the Center of Biologics Evaluation and Research (CBER) of the Federal FDA and the institutional review boards of the participating centers. All participants provided written voluntary consent. An independent Data Safety Monitoring Board (DSMB), consisting of three expert physicians and one statistician who were not involved in any other aspect of the study, monitored the safety of participants in the study according to the DSMB charter specifically developed for RESTORE-CLI.

Eligible patients were men and women 18 to 90 years of age with a diagnosis of CLI of the lower extremities defined as persistent, recurring ischemic rest pain for at least 2 weeks and/or ulceration or gangrene of the foot or toe with absent palpable pedal pulses with toe systolic pressure $\leq$50 mm Hg or ankle systolic pressure $\leq$70 mm Hg. Patients with flat or barely pulsatile pulse volume recording (PVR) and higher systolic blood pressures could be included based on sponsor review. Patients with infrainguinal occlusive disease without acceptable options for revascularization as determined by the site principle investigator were confirmed by angiographic imaging or color flow duplex ultrasound within 6-months prior to randomization were eligible. Establishment of controlled blood pressure with antihypertensive therapy as necessary, adequate antiplatelet, and statin therapy was required prior to entry.

Main exclusion criteria were HbA_{1c} >10%; known aortoiliac disease with >50% stenosis; wound with exposed tendon or bone; known failed ipsilateral revascularization procedure within 2 weeks prior to randomization (defined as failure to restore adequate circulation, ie, the procedure did not achieve an increase in ankle-brachial Index (ABI) of 0.15 or more, substantial improvement in PVR, or clinical improvement); previous amputation of the talus or above in the target limb; infection of the involved extremity manifested by fever, purulence, and severe cellulitis; and active wet gangrenous tissue.

Patients were centrally randomized 2:1 (treatment: control). Visits were scheduled on day minus 14 (bone marrow or sham aspiration), day 0 (injection), days 3 and 7, and months 3, 6, 9, and 12.

Enrollment began at 22 clinical sites in the United States in April 2007 with 18 actually randomizing patients. The planned study population size was originally up to 150 patients. By November 2009, 33 patients had the opportunity to complete the trial (the 12-month follow-up visit) and were included in a prospectively planned interim analysis. The interim analysis was expanded to include 13 additional patients that had completed 6 months of follow-up at that time. Only the set of 33 patients completing 12 months and the set of 13 patients completing 6 months of follow-up by November 2009 were unblinded and included in the interim analysis and reported. Enrollment has been subsequently halted, and all enrolled patients will be followed until completing the 12 month efficacy end point.

Bone marrow aspiration, TRC production, and intramuscular injection

An independent physician (other than the physician performing TRC injections) aspirated approximately 50 mL bone marrow from the posterior iliac crest. Control patients underwent a sham aspiration that involved the insertion of an aspiration needle at the iliac crest without penetration of the iliac periosteum. The aspirate was shipped overnight to Aastrom Biosciences Inc for TRC production. Patients were dropped from the study if the bone marrow was determined to be unsuitable for ex vivo processing due to insufficient mononuclear cell number or if the TRC product did not meet production specifications for sterility and number of total and $CD90^+$ viable cells. In addition, patients could drop out between randomization and aspiration and treatment due to interceding medical events.

The TRC product was generated in a single-pass perfusion biochamber over approximately 12 days and then transported to the clinical site in a shipping container designed to maintain hypothermic storage conditions (between 0 and 12°C).[10] TRCs are a mixture of nucleated cells cultured from the patient's bone marrow with high viability. TRCs are primarily composed of two cell phenotypes: mesenchymal stem and progenitor cells defined by the $CD90^+$ cell surface marker, and hematopoietic and endothelial stem and progenitor cells, defined by the $CD45^+$ cell surface marker. The overall cell viability as measured by membrane integrity by dye exclusion was ≥70%. The cells are suspended in a physiological solution of HypoThermosol (BioLife Solutions, Bothell, Wash) and Isolyte (B. Braun, Bethlehem, Pa) supplemented with 0.5% human serum albumin (HSA) in a volume between 5.8 and 8.4 mL. Characteristics of TRCs from patients in the RESTORE-CLI interim analysis are presented in the Results section.

An average of 136 million total viable TRCs, of which 25 million were $CD90^+$, or electrolyte (control) solution was injected into 20 sites in the ischemic lower extremity. Injection sites were mapped by marking four circumferential linear bands around the lower third of the thigh, the greatest diameter of the patient's calf, and at one location proximal and one distal to greatest calf diameter; five injections of 0.5 mL were given along each band, at least 2.0 cm apart and 0.5 inches into the muscle, to include all muscle groups. The majority of patients also had four injections placed in the intermetatarsal spaces either on the dorsal or plantar surface. In this case, only four injections per linear band were administered.

Study end points

Safety evaluations. The primary end point of the study was safety, which included adverse events, aspiration site assessment, injection toxicities, and injection site assessments. Amputation rates and wound healing, while also safety end points, are described below in the Efficacy evaluations section.

Safety was assessed continually throughout the study via direct evaluation (including physical examination, vital signs measurement and electrocardiography) and by spontaneous reporting by the patient during study visits or telephone contacts. The DSMB reviewed safety data on a quarterly basis.

Efficacy evaluations. The efficacy of TRC treatment was assessed by secondary end points. Principal efficacy measures included time to first occurrence of treatment failure, amputation-free survival, incidence of major amputation, and wound healing. Study investigators made amputation decisions independently based on their clinical judgment.

The composite treatment failure end point was comprised of the following events: major amputation, death, doubling of wound total surface area from baseline (day 0), or occurrence of de novo gangrene. Major amputation was defined as amputation at or above the talus on the limb receiving injections. For a given patient, the time to first occurrence of treatment failure was defined as the earliest day at which any one of the treatment failure events occurred. For patients who did not experience any of the treatment failure events, their last day in the study was used to calculate event-free duration.

The duration of amputation-free survival was defined as the first day on which a major amputation or death was reported. For patients who did not experience a major amputation or death, their last day in the study was used to determine the event-free duration.

Wound healing was evaluated according to three separate assessments: severity using the Wagner Wound Scale, complete wound healing, and total surface area of wounds. Wagner classification is useful to measure wound depth whereas total surface area is useful to measure changes in more superficial Wagner 1 and 2 classification wounds. Only wounds present at entry into the study were evaluated by the Wagner Wound Scale and for complete wound healing. The total wound surface area was calculated as the sum of the surface area of each individual wound. For a wound that appeared after the baseline evaluation, the baseline surface area for that wound was defined as zero.

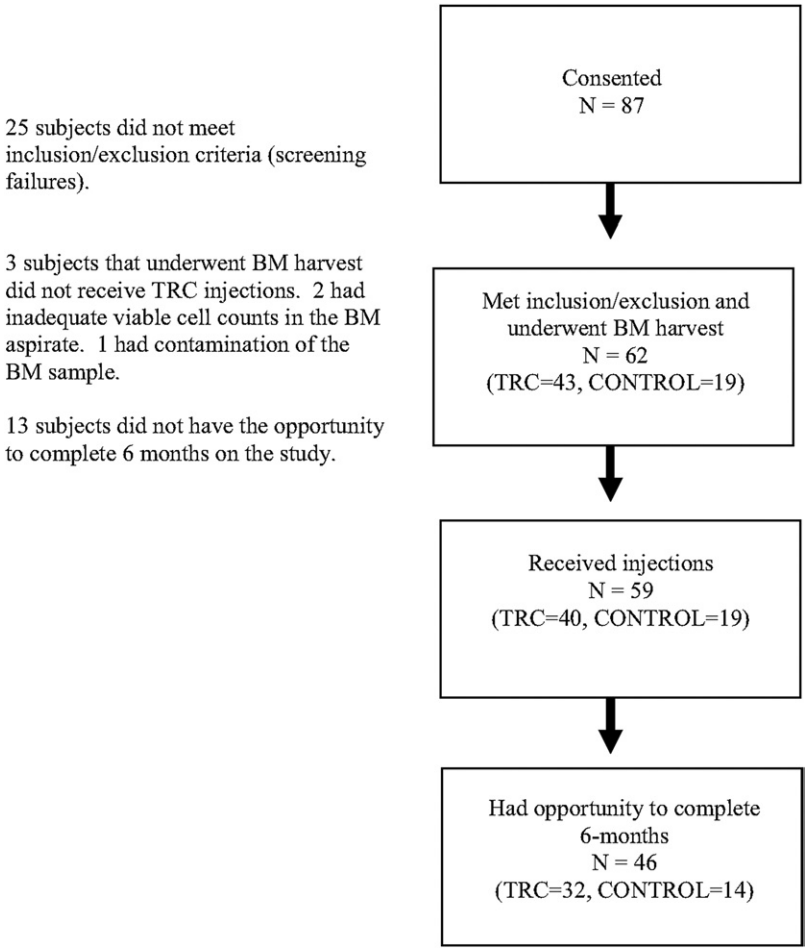

Fig 1. Patient flow diagram (based on interim analysis data up to November 2009).

Table I. Patient disposition for RESTORE-CLI interim analysis – 6-month population

Parameter	*TRC*	*Control*
Randomized, n (%)	32 (100)	14 (100)
Aspirated, n (%)	32 (100)	14 (100)
Treated, n (%)	32 (100)	14 (100)
Withdrew after treatment, n (%)	7 (22)	1 (7)
Reason for withdrawal, n (%)		
Withdrew consent	1 (3)	0 (0)
Death	1 (3)	1 (7)
Did not return to clinic	3 (9)	0 (0)
Loss to follow-up	1 (3)	0 (0)
Amputation of treated leg	1 (3)	0 (0)
Completed, n (%)	16 (50)	10 (71)
Continuing follow-up, n (%)	9 (28)	3 (21)

TRC, Tissue repair cells.

Statistical analysis

As a phase II trial the primary purpose of the trial was exploratory. The planned sample size was based on assuming 100 TRC patients and 50 control patients and a control composite primary event rate of 65% at 6 months with $\alpha = 0.05$, 2-sided, then there would be over 80% power to detect a 30% treatment effect (a TRC event rate of 39%).

Safety and efficacy data were summarized for the randomized and treated patient populations at 6 and 12 months posttreatment. The 6- and 12-month populations were defined as all study participants with the opportunity to complete 6 or 12 months of follow-up, respectively, as of November 2009.

Amputation-free survival and time to first occurrence of treatment failure were both summarized using Kaplan-Meier plots by treatment group; the *P* value from the log-rank test was provided for descriptive purposes. Major amputation rates at 6 and 12 months were analyzed using Fisher exact test.

The last-observation-carried-forward (LOCF) method was used for wounds removed due to an amputation at or above the wound location, for total wound surface area and Wagner Wound Scale category.

Measurements of wound severity were based on the Wagner Wound Scale categories. Statistical evaluations were based on the most severe wound (highest Wagner

Table II. Patients withdrawn: Time in study, reason for withdrawal, and contribution to efficacy data

Treatment	*Study day of withdrawal*	*Reason for withdrawal*	*Additional information*
TRC	63	Other: Amputation of injected leg	Below knee above talus amputation on treated side on day 31. No wound data reported or other events contributing to treatment failure composite.
TRC	407; Last relevant data (labs) reported at month 9/day 259	Investigator discretion: Missed visit 9; unable to return	No amputations reported. Wound data reported through month 9. No other events contributing to treatment failure composite.
TRC	210	Other: Lost to follow-up; certified letter sent	Above knee amputation on treated side on day 101. Wound data reported through day 7. No other events contributing to treatment failure composite.
TRC	197; Last relevant data (labs) reported at month 3/day 97	Other: Patient did not return to clinic; called 3 times; certified letter sent	No amputations were reported. No wound data reported. No events contributing to treatment failure composite.
TRC	207	Withdrew consent	Above knee amputation on treated side on day 32. Wound data reported through day 7. No other events contributing to treatment failure composite.
TRC	132	Death	Adverse event of cardiac failure congestive began on day 114 and ended in death on day 132. No amputations were reported. Wound data reported through month 3. Death was event contributing to treatment failure composite.
TRC	380; Last relevant data (labs) reported at month 9/day 254	Other: Patient did not return for visit 9	Wound data reported through month 9. No events contributing to treatment failure composite.
Control	37	Death	Adverse event of hypovolemic shock began on day 37 and ended in death on the same day. Wound data reported at baseline only.

TRC, Tissue repair cells.

score) present at day 0. The number and percent of patients in each Wagner scale category for each time point as well as the number and percent of patients with improving wounds, were based on the reduction of the Wagner score from baseline. Fisher exact test was used to analyze differences in the proportion of patients experiencing wound improvement between groups.

Complete wound healing was summarized as the number and percent of patients with wounds present at baseline whose wounds were healed at a given time point (wound size of 0 cm and Wagner score of 0 for each wound). Total wound surface area and change from baseline were summarized by descriptive statistics.

RESULTS

Patient enrollment and characteristics

The trial patient flow is shown in Fig 1. The disposition of the 46 patients in the 6-month population is shown in Table I. There were seven treatment group withdrawals due to withdrawal of consent (one patient), death (one patient), not returning to clinic for mandated assessments (three patients), loss to follow-up (one patient), and amputation of the injected leg (one patient; this was not a protocol allowable reason for withdrawal). In the control group, the one withdrawal was due to death. All patients who withdrew were included in all efficacy and safety analyses. Five of the seven withdrawals in the treatment group occurred after the 6-month time point. Four of seven withdrawals in the treatment group experienced a treatment failure efficacy end point prior to withdrawal. The one control withdrawal also experienced a treatment failure efficacy end point prior to withdrawal. The time point, reasons for patient withdrawal, and outcomes are shown in Table II. Baseline characteristics for the 46 patients are shown in Table III. There was no significant difference between the treatment and control group in any of the demographic parameters.

Analysis of bone marrow aspirate and TRC phenotype

The cell surface phenotype of cells from both the bone marrow aspirates and the TRC product was assessed by flow cytometry in 19 of the patients that received TRCs. The results, presented in Fig 2, are consistent with established phenotypic differences between unprocessed bone marrow mononuclear cells and culture expanded TRCs.[10] The total number of cells was decreased by more than half primarily due to loss of non-proliferative hematopoietic cells, including mature lymphocytes and granulocytes, which is reflected in the marked decrease in the number $CD45^+$ cells. In contrast, the $CD90^+$ mesenchymal cell population was expanded about 25-fold from 1 to 25 million cells. Monocytes, as defined by $CD14^+$ expression, were expanded by approximately twofold. The specific population of autofluorescent $CD14^+$ activated macrophages increased 12-fold, from 0.75 to more than 9 million. There was no

Table III. Patient demographics for RESTORE-CLI interim analysis – 6-month population

Baseline parameter	*TRC N = 32*	*Control N = 14*	P *value*[a]
Gender, n (%)			.17
Male	25 (78)	8 (57)	
Female	7 (22)	6 (43)	
Age, years			.76
Mean (SD)	68.8 (12.4)	65.9 (13.4)	
Smoking status, n (%)			.61
Never	5 (16)	2 (14)	
Current	7 (22)	5 (36)	
Past	20 (63)	7 (50)	
Alcohol consumption, n (%)			.44
Never	7 (22)	5 (36)	
Current	15 (47)	4 (29)	
Past	10 (31)	5 (36)	
BMI			.19
Mean (SD)	27.3 (5.0)	28.8 (5.8)	
Creatinine, mg/dL			.88
Mean (SD)	1.21 (0.38)	1.07 (0.32)	
Prior amputation below talus of treated limb, n (%)	5 (16)	2 (14)	1.00
Known diabetes, n (%)	14 (44)	9 (64)	.34
Known on dialysis, n (%)	0 (0)	0 (0)	NA
GFR, n (%)			NA
≤30	0 (0)	0 (0)	
>30	32 (100)	14 (100)	
ABI[b]			.50
N	26	10	
Mean (SD)	0.4 (0.2)	0.4 (0.2)	

ABI, Ankle-brachial index; *BMI,* body mass index; *GFR,* glomerular filtration rate; *NA,* not applicable; *SD,* standard deviation; *TRC,* tissue repair cells.

[a]*P* values from Fisher exact test for count data, from t-test for continuous data.

[b]Not all patients had ABI evaluations, since protocol allowed either ABI, toe pressure, or waveform measurement at baseline.

significant difference between diabetic and non-diabetic subjects in the ratio of TRCs to bone marrow aspirate cells, or in any of the cell subsets included in Fig 2 by *t* test with significance level = 0.05 (data not shown).

Safety outcomes

A summary of overall adverse events (AEs) is shown in Table IV. Nearly all patients reported AEs; the proportion of AEs in TRC-treated and control groups was consistent with the 32 to 14 patient randomization. The percentage of patients with serious adverse events (SAEs) was similar between the groups: 44% in TRC-treated and 57% in control patients. There was 1 death each in the TRC-treatment and in the control groups; neither was considered related to treatment. AEs reported by a total of 4 or more patients in the 6-month population are listed in Table V. Bone marrow aspiration and injection site toxicities were minimal.

Most severe AEs were determined by investigators to be "unrelated" or "unlikely related" to treatment, but rather to the underlying disease. Two events in treated patients were considered "possibly related" to TRC treatment. One patient experienced moderate cellulitis in the treated limb after bone marrow aspiration but prior to TRC injections. The second patient had a severe localized infection of the first toe of the treated limb recorded on study day 34 that resolved by study day 63.

Efficacy outcomes

All analyses were based on the intention to treat 6-month or 12-month populations, respectively. It should be noted that RESTORE-CLI was not statistically powered to demonstrate efficacy of TRC therapy. The interim efficacy analysis was designed to demonstrate feasibility and support the statistical design of larger clinical trials. The study was stopped early following the 6-month interim analysis due to a positive efficacy signal and the sponsor's plan to develop the phase III program.

Time to treatment failure. Treatment failure was defined as a composite end point of major amputation, death, doubling of wound size from baseline, or de novo occurrence of gangrene. Statistical analysis of this population revealed that the time to first occurrence of treatment failure was significantly longer in TRC-treated patients compared with control patients (Fig 3, log-rank test, $P = .0053$). Differentiation between the two groups appeared within the first 100 days of follow-up and was maintained through the remainder of the study. In the 6-month population, 11 of 14 control patients (79%) and 13 of 32 TRC-treated patients (41%) failed treatment by this definition (Fisher exact test, $P = .026$). The etiology of the first occurrence of treatment failure, as well as the total number of patients experiencing each type of failure, is listed in Table VI.

Amputation-free survival. Analysis of the 6-month population revealed that amputation-free survival was significantly longer in the TRC-treated patients compared with control patients (Fig 4, log-rank test, $P = .038$). As with time to treatment failure, differentiation between the distributions appeared within the first 50 days of follow-up and was maintained throughout the study. Median amputation-free survival times for control and TRC-treated patients had not been reached.

Major amputation. For the 6-month population at month 6, major amputation occurred in 43% of the control group compared with 19% in the treatment group ($P = .14$; Fisher exact test). There was no difference in above vs below the knee amputation between the groups. Below the knee amputation occurred in 66% of patients requiring major amputation in each group. For the 12-month population, major amputation occurred in 36% of the control group compared with 18% in the treatment group at both months 6 and 12 ($P = .39$; Fisher exact test).

Wound healing. Thirty-three patients had evaluable wounds at baseline that allowed efficacy assessment. Complete wound healing, defined as a Wagner score of 0 and wound size of 0 cm for each wound, was summarized as the percent of patients with wounds present at baseline for whom all wounds have healed at a given time point. Com-

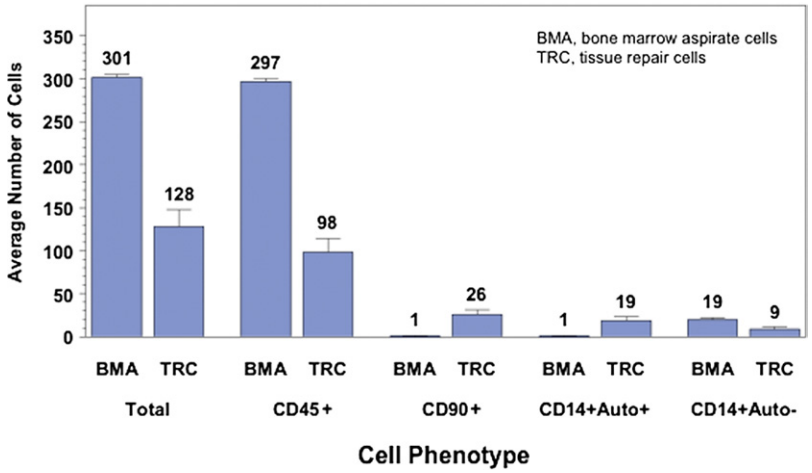

Fig 2. Phenotypic analysis of bone marrow aspirate and tissue repair cell (*TRC*) product samples. Flow cytometric analysis was performed on aspirate samples before automated single-pass perfusion culture and on the TRC products. Patients that had samples with complete phenotypic analyses for both aspirate and TRC samples were included (n = 19). Figures above the bars indicate the average number of cells in millions.

Table IV. Overview of safety – 6-month population

Parameter	*TRC N = 32*	*Control N = 14*
Patients reporting adverse events, n (%)	30 (94)	14 (100)
Number of adverse events	154	64
Patients with serious adverse events, n (%)	14 (44)	8 (57)
Number of serious adverse events	21	15
Number of deaths	1	1
Withdrawals due to adverse events (not including deaths), n (%)	0 (0)	0 (0)

TRC, Tissue repair cells.

Table V. Most frequently reported adverse events – 6-month population

Preferred term, n (%)	*TRC N = 32*	*Control N = 14*
Any adverse event	30 (94)	14 (100)
Pain in extremity	13 (41)	3 (21)
Skin ulcer	8 (25)	1 (7)
Nausea	5 (16)	3 (21)
Gangrene	4 (13)	3 (21)
Cellulitis	2 (6)	4 (29)
Diarrhea	4 (13)	1 (7)
Procedural pain	4 (13)	1 (7)
Localized infection	2 (6)	2 (14)

TRC, Tissue repair cells.

plete wound healing is shown for the 12-month patient population with wounds present at baseline in Fig 5. For the 12-month population at month 6, there were no differences in complete wound healing between the treatment and control groups. At month 12, complete wound healing was present in a greater percentage of the treatment group (31%) compared with the control group (13%); these differences were not statistically significant. Other measures of wound healing (Wagner Wound Scale, total wound surface area) did not show significant differences between groups.

DISCUSSION

RESTORE-CLI is the first placebo-controlled autologous stem cell trial to use expanded bone marrow mononuclear cells (TRCs) to treat no-option CLI patients. The study is designed to evaluate hard clinical end points that are consistent with recent recommendations for the conduct of trials in CLI[3] rather than surrogate end points. It is double-blinded and studies a larger subject population than previously reported cellular therapy studies in CLI. Interim analysis of this phase II trial has demonstrated that TRC therapy is safe and yields potential improvement in efficacy outcomes.

A unique feature of this autologous stem cell therapy is that TRCs are generated in a closed and automated culture system that expands the number of mesenchymal and monocytic stem and progenitor cells.[10] This enrichment is especially pronounced in cells that express the surface markers CD90, reflecting mesenchymal lineage, and CD14, a marker of monocyte/macrophage lineage. The increase in $CD14^+$ cells is almost entirely within the subset of activated autofluorescent cells. The TRC culture expansion process thus increases the number of both lineages of cells reported to be effective in treating CLI.

The precise mechanism(s) of action for TRCs in CLI are not yet established. It is unclear if the injected cells are directly involved in the angiogenic process or act as cytokine factories releasing multiple different growth factors that have a paracrine effect.

There are several putative mechanisms of activity of cell therapy in CLI. These mechanisms include (1) increased tissue vascularity; (2) increased tissue microperfusion at the

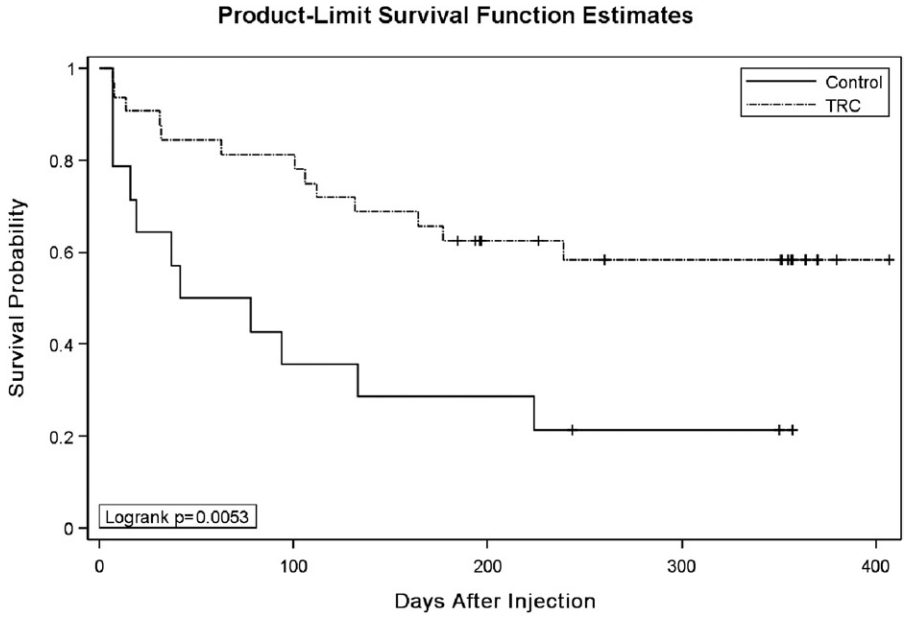

Fig 3. Kaplan-Meier survival plot of time to first occurrence of treatment failure, a composite end point of major amputation, doubling of wound total surface area, occurrence of de novo gangrene, or death. Most events occurred in control group patients within the first 100 days; median time to treatment failure was 60 days with 3 of 14 patients censored for the analysis. In the tissue repair cell (*TRC*)-treated group, the median time to treatment failure was not reached, with 19 of 32 patients censored for the analysis. Censored observations are indicated by "+" symbols. Number of subjects at risk in the TRC-treated group were 32 at day 0 and 26 at day 100 and 16 at day 200 and 13 at day 300. Number of subjects at risk in the control group were 14 at day 0 and 5 at day 100 and 4 at day 200 and 2 at day 300.

Table VI. Occurrence of composite treatment failure end point

Patient treatment group	*TRC (N = 32)*		*Control (N = 14)*	
Number that failed treatment	*13 (41%)*		*11 (79%)*	
Composite end point component N(%)	*Patients experiencing as first event*	*Total patients experiencing event*	*Patients experiencing as first event*	*Total patients experiencing event*
Major amputation	6 (19%)	6 (19%)	4 (29%)	6 (43%)
Death	1 (3%)	1 (3%)	1 (7%)	1 (7%)
Doubling in wound size	4 (13%)	6 (19%)	4 (29%)	6 (43%)
De novo gangrene	2 (6%)	4 (13%)	2 (14%)	2 (14%)

TRC, Tissue repair cells.

capillary level; (3) remodeling of fibrotic tissues to allow new capillary growth or increase interstitial fluid flow; and (4) modulation of the inflammatory response program from tissue destruction to wound healing. Since a combination of these mechanisms may be synergistic, the mixture of precursor cells in TRCs that includes mesenchymal, endothelial, and inflammatory cell precursors, may be important in their therapeutic function. For instance, the presence of $CD14^+$ macrophages may promote the dissolution of extracellular matrix in the perivascular interstitial space and thus enhance vessel sprouting by endothelial precursors.[9]

The study was performed primarily to assess safety and tolerability of TRC therapy. As expected in an autologous cell therapy, the administration of TRCs was safe and well tolerated. The number of patients reporting SAEs was similar in the TRC-treated group compared to the control group. RESTORE-CLI was not statistically designed for demonstration of efficacy, which was a secondary aim of the trial. In addition, the interim analysis reported here was performed in only 46 of the 86 subjects. Despite these limitations, the interim analysis revealed statistically significant differences in time to first occurrence of treatment failure and amputation-free survival between TRC-treated and control subjects. Wound healing differences, thought not statistically significant, favored TRC-treated patients at the 12-month but not the 6-month time point, consistent with a durable clinical benefit.

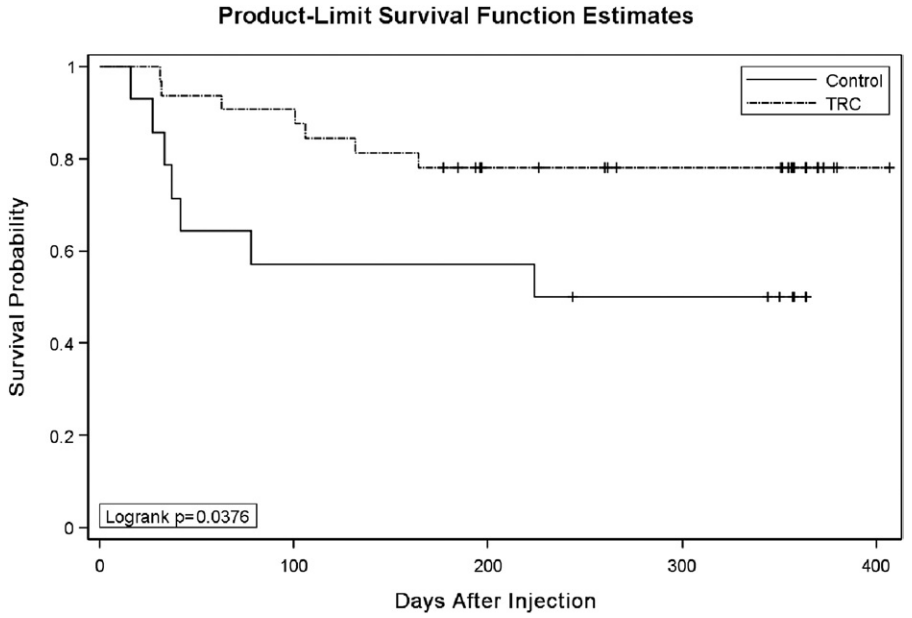

Fig 4. Kaplan-Meier survival plot of amputation-free survival. Most events occurred in control group patients within the first 50 days; median time to treatment failure was not reached, with 7 of 14 patients censored for the analysis. In the tissue repair cell (*TRC*)-treated group, the median time to treatment failure was not reached, with 25 of 32 patients censored for the analysis. Censored observations are indicated by "+" symbols. Number of subjects at risk in the TRC-treated group were 32 at day 0 and 29 at day 100 and 20 at day 200 and 16 at day 300. Number of subjects at risk in the control group were 14 at day 0 and 8 at day 100 and 8 at day 200 and 6 at day 300.

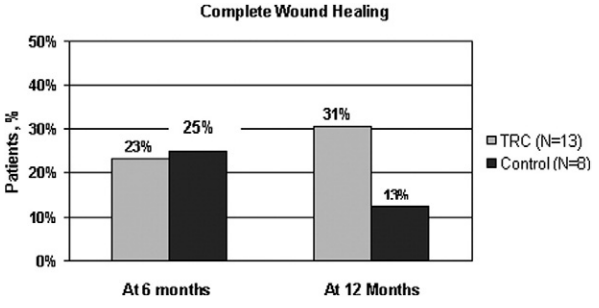

Fig 5. Complete wound healing rate in patients completing 12-months of follow-up at 6- and 12-month time points. There was no statistical difference between control and tissue repair cell (*TRC*)-patient groups at the 6-month time point ($P = 1.00$, Fisher exact test). At the 12-month time point, the greater incidence of wound healing in the TRC-treated group was also not statistically significant, due to the small sample size ($P = .61$, Fisher exact test).

Biologic therapy for CLI includes gene therapy and various stem cell therapy techniques. Gene therapy trials using hepatocyte growth factor (HGF),[12,13] vascular endothelial growth factor (VEGF),[14,15] or fibroblast growth factor (FGF)[16,17] have been performed. Early phase II trials using either FGF or HGF have shown potential in the treatment of CLI and are currently in pivotal trials. Potential advantages of gene therapy in CLI include an "off the shelf" therapy that can be delivered at the point of contact without the need for additional procedures such as bone marrow harvest. Potential disadvantages include concern over off-target angiogenesis with resultant progression of proliferative retinopathy and occult tumor growth.

Stem cell therapy using autologous stem cells is a newly developing therapy. Intra-arterial delivery[18] has been investigated as well as intramuscular injection. Potential techniques to deliver the autologous stem cells in sufficient numbers include direct bone marrow harvest and cell separation using a centrifuge at the point of treatment,[6,19,20] bone marrow mobilization using GM-CSF followed by plasmapheresis,[21] and the technique as described in this report in which cells are expanded within a bioreactor. Overall, there are fewer concerns with cell therapy regarding the risk of off-target angiogenesis compared with gene therapy. Potential advantages of the cell expansion technique reported here include the need to collect a relatively small amount of bone marrow under local anesthesia. Alternative techniques require harvesting up to 500 to 600 mL of bone marrow under general anesthesia. Other potential advantages of TRCs are that the expansion process enriches for the cell lineages thought to be important for angiogenesis and neovascularization and may reverse the suppressive effects of chronic medical conditions on bone marrow progenitors that may impair their regenerative function.[9]

A potential weakness of the current report is the relatively small number of patients assessed in the interim analysis. This has limited the ability to perform an in-depth

evaluation of some secondary end points, most important of which are hemodynamic measures. Although all patients had a toe pressure, ABI, or waveform at baseline, many patients could not have repeat studies due to digital amputation or calcified tibial arteries. As such there are insufficient numbers of patients with quantifiable hemodynamic measures to analyze these data.

In conclusion, interim analysis of 46 of 86 patients in RESTORE-CLI has demonstrated that intramuscular injection of bone marrow-derived TRCs is safe and well tolerated, and has demonstrated a significant improvement in amputation-free survival and time to first occurrence of treatment failure when compared with control subjects. These interim results suggest TRCs may be a future potential option for the treatment of CLI patients without revascularization alternatives. Based on these data, a larger pivotal trial evaluating the effect of TRCs on amputation-free survival in no-option CLI patients is planned.

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AUTHOR CONTRIBUTIONS

Conception and design: RP, AC, TH, RB, AL, TS, SW
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Overall responsibility: RP, SW

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