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PROTOCOL PAPER

Rationale and Design of Therapeutic Angiogenesis by Cell Transplantation Using Adipose-Derived Regenerative Cells in Patients With Critical Limb Ischemia

— TACT-ADRC Multicenter Trial —

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Background: Despite the growing knowledge regarding optimal treatments for critical limb ischemia (CLI), there are still a considerable number of patients who have to undergo major limb amputation. Intramuscular injection of autologous adipose-derived regenerative cells (ADRCs) in these patients has shown therapeutic potential in improving tissue ischemia, in both preclinical and initial pilot studies. Here, we present a clinical protocol for ADRCs use in a multicenter trial.

Methods and Results: The TACT-ADRC multicenter trial is a prospective, interventional, single-arm, open-labeled study at 8 hospitals in Japan, investigating the safety and feasibility of intramuscular injections of ADRCs and testing the hypothesis that this treatment promotes neovascularization and improves major amputation-free survival rates in patients with CLI who have no other treatment option. 40 patients with CLI will be enrolled and followed up from November 2015 to November 2020. Freshly isolated autologous ADRCs will be injected into the target ischemic limbs. Survival rate, adverse events, major limb amputation, ulcer size, 6-min walking distance, numerical rating scale, ankle–brachial pressure index, skin perfusion pressure and digital subtraction angiography will be evaluated at baseline and during 6 months' follow-up.

Conclusions: This trial will demonstrate whether implantation of autologous ADRCs is a safe and effective method for therapeutic angiogenesis, resulting in an improvement in major amputation-free survival rates in patients with CLI.

Key Words: Autologous adipose-derived regenerative cells; Critical limb ischemia; Multicenter clinical trial; Protocol paper; Therapeutic angiogenesis

Because approximately 202 million people are affected by lower extremity arterial disease worldwide, advanced cases of patients with critical limb ischemia (CLI) are a serious health problem, especially in developing countries.[1,2] Major lower extremity amputation surgery is often required for these patients, which significantly reduces their quality of life (QOL) and increases deaths from cardiovascular complications.[1,3] Although endovascular angioplasty or bypass surgery for these patients is recommended by guidelines,[1,4] we still have no effective optimal management strategies for patients with more severe disease, such as long calcification lesions below the knee in

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Table 1. Primary and Secondary Endpoints of TACT-ADRC Multicenter Trial
Primary endpoints
1) Safety assessment
a. Survival rate at perioperative period and 6 months' follow-up
b. Adverse events at perioperative period of ADRC-implantation procedure
c. Development of malignant neoplasm during 6 months' follow-up after ADRCs-implantation
d. Exacerbation of proliferative retinopathy or arthritis during 6 months' follow-up after ADRCs-implantation
2) Efficacy assessment
Amputation-free survival (AFS) rate compared with historical controls of conventional therapies or of BM-MNC implantation
Secondary endpoints
3) Improvement in
a. Ischemic ulcer size
b. Quality of life (QOL) using numerical rating scale (NRS)
c. Ankle-brachial pressure index (ABI)
d. Skin perfusion pressure (SPP)
e. Angiogenesis as assessed by digital subtraction angiography (DSA)
f. 6-min walking distance

ADRC, adipose-derived regenerative cells; BM-MNC, bone marrow-derived mononuclear cells.

Table 2. TACT-ADRC Multicenter Trial Inclusion Criteria
1. Male or female, aged 25–79 years
2. CLI caused by PAD, Buerger's disease, or CTD with ischemic symptoms including rest pain and/or established ischemic ulcers (Fontaine class III–IV), with no other conventional options, such as smoking cessation, drug therapy and revascularization
3. Providing informed consent

CLI, critical limb ischemia; CTD, connective tissue disease; PAD, peripheral arterial disease.

Table 3. TACT-ADRC Multicenter Trial Exclusion Criteria
1. Insufficient amount of adipose tissue
2. Withholding informed consent
3. Comorbidities with a life expectancy <1 year
4. Previous (within 5 years) or current history of neoplasm or clinically significant abnormality on prescreening examination
5. Ischemic heart disease without revascularization
6. Untreated diabetic retinopathy
7. Severe infection
8. Severe liver and/or renal dysfunction (except for patients under hemodialysis)
9. Severe hematologic disease
10. Pregnancy
11. Physician considers patient to be unsuitable for trial inclusion

patients with peripheral arterial disease (PAD), or distal diffuse lesions caused by Buerger's disease and connective tissue disease (CTD).[2,3,5] Therapeutic angiogenesis is an important strategy to rescue ischemic tissues in such patients who are not suitable for endovascular angioplasty or bypass surgery.[6,7]

Adipose-derived stem/regenerative cells (ADSCs or ADRCs) are a cell population that can be less invasively and easily obtainable from subcutaneous adipose tissues and can give rise to several cell lineages including fat, bone, cartilage, muscle and neurons, among others.[8] In addition, ADRCs can release multiple growth factors such as vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor, and chemokine stromal cell-derived factor-1 during regeneration.[9–12] Based on this evidence, ADRCs are a promising cell source and have already been applied in clinical regenerative medicine.[13] Initial pilot studies have reported that therapeutic angiogenesis with ADRC implantation for CLI might be safe under limited conditions of small sample size and single-center trials.[14,15] However, there are no trials that can resolve clinical concerns regarding ADRC treatment's safety, feasibility, and therapeutic efficacy in a multicenter setting.[16]

Accordingly, the present study was designed to validate the safety and feasibility of treatment of autologous ADRC implantation at 8 hospitals in different regions of Japan, and to test our hypothesis that it could be an effective strategy for therapeutic angiogenesis, resulting in an improvement in the major amputation-free survival rate in 40 treated patients compared with that in historical control patients with CLI.

Methods

The TACT-ADRC multicenter trial is a prospective, interventional, single-arm, open-labeled study performed at 8 hospitals in Japan. A total of 40 patients with CLI will be enrolled from November 2015 to June 2020. All procedures will be performed in accordance with the ethical standards of the institutional research committees and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study protocol was approved by Nagoya University certified committee for regenerative medicine (#NA8150011) and Tokai-Hokuriku Regional Bureau of Health and Welfare, Japan. The trial is registered in the UMIN Clinical Trials Registry (#UMIN000010143) and the Japan Registry of Clinical Trials (#jRCTb040190118).

Study Endpoints

The primary endpoints for the safety assessment are: (a)

Table 4. TACT-ADRC Multicenter Trial Schedule

Evaluation item	Pre	Therapy	Follow-up (total 6 months)			
			7 days	14 days	1 month	6 months (endpoint)
	Admission①	←	Admission②	→	Admission③	Admission④
Written informed consent	●					
Screening examination for malignant neoplasms	●				●	●
ADRC implantation		●				
Ulcer size	●		●	●	●	●
Numerical rating scale (NRS)	●		●	●	●	●
6-min walking distance	●		●	●	●	●
Ankle-brachial pressure index (ABI)	●		●	●	●	●
Skin perfusion pressure (SPP)	●		●	●	●	●
Transcutaneous oxygen pressure (TcPO2)	▲		▲	▲	▲	▲
Laser Doppler perfusion image (LDPI)	▲		▲	▲	▲	▲
Digital subtraction angiography (DSA)	●				●	●
Blood examination	●		●	●	●	●
Fluorescence activated cell sorting (FACS)	●		●	●	●	●
Adverse events		●	●	●	●	●
Ophthalmological consultation	●				●	●
Gynecological consultation	●				●	●

●, required; ▲, if possible.

survival rate in the perioperative period and at 6 months' follow-up, (b) adverse events in the perioperative period of the ADRC-implantation procedure, (c) development of malignant neoplasm during 6 months' follow-up after ADRC implantation and (d) exacerbation of proliferative retinopathy or arthritis during 6 months' follow-up after ADRC implantation. In addition, the primary endpoint for efficacy assessment is based on the major amputation-free survival rate after 6 months' follow-up following ADRC implantation. Moreover, secondary endpoints, as shown in **Table 1**, will be also evaluated.

Patients

Eligible patients are defined as: having CLI (defined as chronic ischemic rest pain, ulcers, or gangrene) caused by PAD, Buerger's disease, or CTD; having no other option for standard revascularization therapy; and aged between 25 and 79 years. All participants have to provide written informed consent. A summary of the inclusion and exclusion criteria are shown in **Table 2** and **Table 3**, respectively.

Prescreening Examination

Medical interview, physical examination, ophthalmological consultation to check for retinopathy, gynecological consultation for women to check for gynecological cancers, and further investigations (laboratory investigations (complete blood count, blood chemistry, urinalysis, fecal occult blood, and tumor markers [AFP, CEA, and CA19-9; PSA in addition for men]); chest and abdominal X-rays; ECG; abdominal ultrasound (AUS); esophagogastroduodenoscopy (EGD); mammography (MMG) for women only; head, chest, upper and lower abdomen computed tomography (CT); and exercise ECG or stress cardiac scintigraphy or coronary CT to evaluate the potential ischemic heart disease) comprise the prescreening examination for all candidates to assess their eligibility for participation in this study (refer to **Table 3**).

ADRC-Isolation and -Implantation Procedure

After passing the prescreening examination, candidates will undergo therapeutic angiogenesis with autologous ADRC transplantation. About 300 mL of adipose tissue is harvested during standard manual liposuction from the subcutaneous abdominal, breech and/or femoral regions using an aspiration syringe and cannula under general or partial anesthesia, according to the judgment of the anesthetist.[17] ADRCs are separated and extracted following the fully automated isolation steps of the Celution Systems (Cytori Therapeutics, Inc., Austin, TX, USA) for approximately 2 h.[17–19] The total cell number and viability

will be calculated using an automated cell counter (NucleoCounter NC-100, M&S TechnoSystems, Inc., Osaka, Japan). The freshly isolated ADRCs will be diluted with lactated Ringer's solution up to a total volume of 50 mL, and the remaining 90% of the cells excluding 10% for specimen storage will be implanted at 40–60 points into the ischemic muscles of the target limb using a 23- or 26-gauge needle.[6] If the patient is awake or semi-sedated with partial anesthesia during cell transplantation, peripheral nerve blocks of the affected limb will be used as well.

Follow-up Examination

Clinical assessment (survival rate, adverse events, major amputation-free survival rate, ulcer size [total of the minor axis×longer axis], 6-min walking distance, and QOL as evaluated using the NRS), laboratory tests (complete blood count, blood chemistry, fluorescence activated cell sorting [FACS] for detecting circulating CD34+ and CD133+ cells, and cytokines), ankle-brachial index (ABI), and skin perfusion pressure (SPP) will be performed at baseline, 7 and 14 days', and 1 and 6 months' follow-up. In addition, other laboratory data (urinalysis, fecal occult blood, and tumor markers [AFP, CEA, and CA19-9; PSA for men only]) will be obtained, and AUS, MMG (women only), head, chest, upper and lower abdomen CT scanning will be also performed at baseline, and 1 and 6 months' follow-up. EGD will be performed at baseline and 6 months' follow-up. Evaluation of angiogenesis will be performed using imaging: digital subtraction angiography (DSA) at baseline, and 1 and 6 months' follow-up, and if possible, laser Doppler perfusion imaging (LDPI) at baseline, 7 and 14 days', and 1 and 6 months' follow-up. Ophthalmological consultation to check for retinopathy, gynecological consultation for women to check for gynecological cancers, and exercise ECG or stress cardiac scintigraphy or coronary CT to evaluate potential ischemic heart disease will be performed, respectively, at baseline, and 1 and 6 months' follow-up. The summary of these study schedules is shown in **Table 4**.

Statistical Analysis

Statistical significance will be evaluated using unpaired Student's t-test for comparisons between 2 means. Statistical comparisons between baseline and follow-up data will be performed using repeated measures of variance in Prism 6 (GraphPad Software). A P-value <0.05 will be considered as statistically significant.

Results

The results are currently under investigation.

Discussion

CLI is a severe stage of PAD with pain at rest and/or ischemic ulcers or gangrene, and is associated with a high risk of subsequent major amputation, cardiovascular events and death.[1,3] CLI is reported to have a mortality rate of 20% within 6 months of the diagnosis.[20] In this study, we estimate a 96% of survival rate in participants with therapeutic angiogenesis following ADRC implantation based on our previous clinical trial.[6] As a historical control, the major amputation-free survival rate after 6 months in patients with conventional therapies is estimated to be 73.7% (threshold limb salvage rate) based on previous reports.[20–23] We estimate a major amputation-free survival rate of approximately 92% (expected limb salvage rate) in participants with therapeutic angiogenesis following ADRC implantation. Therefore, the necessary sample size of this study is estimated to be 40 with a 2-sided α level of 0.05 and statistical power of 80%.[24,25]

A previous study reported that an observed reaction to cell therapy for CLI was increased circulating endothelial progenitor cell (EPC) counts, and treatment-responders especially demonstrated continuous upregulation of circulating EPCs up to 28 days after treatment.[26] Thus, the present study is also designed to evaluate the kinetics of CD34+ and CD133+ cells in blood samples at prescribed time points after the treatment.

Study Limitations

First, for ethical reasons we cannot include a placebo control group. To account for this, we will compare our results with the outcomes of historical controls treated with conventional therapy,[21] and those for therapeutic angiogenesis following BM-MNC implantation in our previous reports.[6,7] Second, although the inclusion criteria are open to all ethnicities, the main study participants will be Asian, because all 8 medical institutions participating in the clinical trial implementation are located in Japan. Nevertheless, our trial should pave the way for emerging therapeutic angiogenesis by ADRC implantation as a strategy for ischemic limb salvage, and shed light on the treatment of CLI patients who have no other therapeutic options.

In conclusion, this study, the TACT-ADRC multicenter trial, will prove that implantation of autologous ADRCs is a safe and effective strategy for therapeutic angiogenesis that could improve the major amputation-free survival rates in patients with CLI.

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Conflicts of Interest

None declared.

Disclosures

Y.F., T.I., Y.J.A., K. Kuwahara, Y.K., and T.M. are members of the *Circulation Reports*' Editorial Team.

Appendix A

T.M. is the principal investigator for the TACT-ADRC multicenter trial.

The Safety Monitoring Board

Kazumasa Unno from Japanese Red Cross Nagoya Daini Hospital, Nagoya, Japan.

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