【2. 口頭発表】

1. 米満吉和
教育講演３：
「プラークバイオロジーから動脈硬化診療へつなぐトランスレーショナルリサーチ」
第 13 回　動脈硬化教育フォーラム 2013. 2.3.（京都）

2. 米満吉和
教育講演２：
「ナノ・サージェリー：外科視点からのトランスレーショナルリサーチ」
第 113 回日本外科学会定期学術総会 2013. 4.11-13.（福岡）

3. 米満吉和
特別講演１：
「我々の新しい医薬品創成の現状」
第 4 回「新しい医療」講演会（福岡市医師会主催）2013. 6.3.（福岡）

4. 米満吉和
特別講演：下肢血管障害の治療を考える
「下肢慢性動脈閉塞症に対する治療戦略：薬物療法を中心に」
下肢血管障害の治療を考える会　2013. 6.4.（福岡）

5. 米満吉和
イブニングセミナー：
「FGF-2 の生理活性を利用した RNA バイオ医薬品の開発：基礎と臨床」
第 5 回日本創傷治癒学会総会 2013. 7.11.（京都）

6. 米満吉和
シンポジウム７「血管再生研究の最前線」
「血管再生治療は PAD の clinical endpoint を達成できるか？」
第 21 回日本血管生物医学会 2013. 9.26-28.（大阪）

7. 米満吉和
シンポジウム「先端医療における血管新生の役割」
「血管再生治療は本当に有効か？ ～15 年間に及ぶ臨床評価の総括と今後の展望」
第 20 回九州血液血管研究会 2013. 10.19.（福岡）

8. Tanaka M, Fujino Y, Yonemitsu Y.
A pilot study of quality of life for patients with chronic critical limb ischemia after gene therapy.
ESGCT and SETGyC Collaborative Congress 2013. 10. 27. (Madrid, Spain)

9. Fujino Y, Tanaka M, Yonemitsu Y.
The efficacy and safety of DVC1-0101 for intermittent claudication secondary to peripheral artery disease: study protocol of a randomized phase IIb trial.
ESGCT and SETGyC Collaborative Congress 2013. 10. 27. (Madrid, Spain)

10. 米満吉和
特別講演
「がんの腹膜播種形成の分子・細胞メカニズム：なぜ、腹膜播種は難治性なのか？」
第 5 回群馬分子医学研究会　2013. 11.8.（前橋）

11. 米満吉和
市民セミナー「正しく知って欲しい「がんと免疫」、そして「がんワクチン」のこと」
ぐらんざ 15 周年記念ぐらんざ大学　2013. 12.1.（福岡）

12. 米満吉和
講演「iPS 細胞を使った再生医療と新薬開発」
福岡大学病院 市民・医学講座　2014. 2.1.（福岡）

13. Yoshikazu Yonemitsu.
Invited lecture: 'Dendritic cell-based cancer vaccine: current status of the conventional formulation and design & development of a novel autologous cell vaccine drug, pharmaceutical VACCELL® (vaccine cell)'
Symposium on Cancer Vaccines at Chung Shan Medical University 2014.3.29.
(Taichun, Taiwan)

IV. 研究成果の刊行物・別冊

本研究と最も密接に関係する以下の論文を抜粋する

1. Yonemitsu Y, Matsumoto T, Itoh H, Okazaki J, Uchiyama M, Yoshida K, Onimaru M, Onohara T, Inoguchi H, Kyuragi R, Guntani A, Shimokawa M, Ban H, Tanaka M, Inoue M, Zhu T, Hasegawa M, Nakanishi Y, Maehara Y.
   DVC1-0101 to treat peripheral arterial disease: Phase I/IIa, open-label, dose escalation clinical trial.
   Molecular Therapy 21: 707–714, 2013.

2. Tanaka M, Matsumoto T, Morisaki K, Kyuragi R, Fujino Y, Yoshida K, Yonemitsu Y, Maehara Y.
   Efficacy and safety of DVC1-0101 for intermittent claudication secondary to peripheral artery disease: study protocol of a randomized phase IIb trial.
   Journal of Clinical Trials, 3,3, 2013.

DVC1-0101 to Treat Peripheral Arterial Disease: A Phase I/IIa Open-label Dose-escalation Clinical Trial

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We here report the results of a Phase I/IIa open-label four dose-escalation clinical study assessing the safety, tolerability, and possible therapeutic efficacy of a single intramuscular administration of DVC1-0101, a new gene transfer vector based on a nontransmissible recombinant Sendai virus (rSeV) expressing the human fibroblast growth factor-2 (FGF-2) gene (rSeV/dF-hFGF2), in patients with peripheral arterial disease (PAD). Gene transfer was done in 12 limbs of 12 patients with rest pain, and three of them had ischemic ulcer(s). No cardiovascular or other serious adverse events (SAEs) caused by gene transfer were detected in the patients over a 6-month follow-up. No infectious viral particles, as assessed by hemagglutination activity, were detected in any patient during the study. No representative elevation of proinflammatory cytokines or plasma FGF-2 was seen. Significant and continuous improvements in Rutherford category, absolute claudication distance (ACD), and rest pain were observed ($P < 0.05$ to 0.01). To the best of our knowledge, this is the first clinical trial of the use of a gene transfer vector based on rSeV. The single intramuscular administration of DVC1-0101 to PAD patients was safe and well tolerated, and resulted in significant improvements of limb function. Larger pivotal studies are warranted as a next step.

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INTRODUCTION

Peripheral arterial disease (PAD) is a typical phenotype of progressive and systemic atherosclerosis, and causes disability of limb function (intermittent claudication (IC)) as well as serious pain or limb loss (critical limb ischemia (CLI)). PAD is estimated to affect more than 8.4 million individuals in the United States,[1] and its prevalence is increasing in Asian countries, including Japan. Even with the current standard therapies, subjects with severe PAD are left with immobility, intractable ischemia, ulceration, impaired wound healing, or the necessity of amputation. Therefore, the development of new therapeutics to rescue the limbs from critical ischemia, normalize limb function, and prevent the progression of PAD is urgently needed.

In contrast to interventional revascularization and bypasses that target the large- and medium-sized arteries, therapeutic angiogenesis modalities target the microvessels.[2] Soon after the discovery of angiogenic growth factors, investigators began to test proteins[3,4] and genes[5–8] for their ability to stimulate neovessels in subjects with PAD. However, the majority of the results of clinical trials of therapeutic angiogenesis have been disappointing, and importantly, the positive findings obtained in the small early trials were not confirmed in the larger, well-controlled multicenter trials. Nonetheless, since a number of preclinical evaluations in animal studies support the concept of therapeutic angiogenesis, the critical factors that affect its efficacy in clinical settings should be reconsidered in order to more fully investigate and test this intriguing therapy.

Among the published angiogenic trials for IC, only the TRAFFIC study—a well-controlled clinical trial for intraarterial injection of human recombinant basic fibroblast growth factor (bFGF/FGF-2)—could provide proof of concept; namely, a single-dose injection of bFGF/FGF-2 significantly improved the peak walking time of IC patients at day 90.[4] However, the TRAFFIC trial was terminated, because of the relatively limited clinical outcome as well as the mild-to-moderate adverse events (AEs), including hypotension and proteinuria, which may have been due to the systemic leakage of FGF-2. Therefore, a higher and sustained protein concentration of FGF-2 in local target muscles might be more effective and may have fewer side effects in clinical settings.

Recombinant Sendai virus (rSeV) vector is a powerful gene transfer agent[9] for the cytoplasmic transcription of therapeutic

**Deceased*

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genes. We previously showed that, in the ischemic muscles of mice, rSeV administration led to high-level expression of an angiogenic factor, FGF-2, with the levels being as much as 300-fold higher than at baseline.[10] At a higher dose, the ischemic murine muscles underwent severe congestion associated with markedly dilated postcapillary veins (Y. Yonemitsu *et al.*, unpublished observation), providing the first potential evidence of a toxic dose among the preclinical evaluations of angiogenic gene therapies. Using this system, we demonstrated that: (i) rSeV-mediated overexpression of FGF-2 showed better efficacy for limb salvage in mouse models with surgically induced severe limb ischemia than did administration of vascular endothelial growth factor (VEGF);[10] (ii) the biological and therapeutic effects of FGF-2 were dependent on the inducible expression of endogenous VEGF and hepatocyte growth factor (HGF) at an early phase during the angiogenic cascade[11] *via* a platelet-derived growth factor receptor-α/p70S6-mediated mechanism;[12] (iii) FGF-2 gene transfer induced not only angiogenesis but also lymphangiogenesis *via* the VEGF-C/VEGFR3 system;[13,14] and (iv) FGF-2 also stimulates transient inflammatory/arteriogenic responses *via* a monocyte/macrophage chemoattractant protein-1–mediated mechanism.[15] These results and the clinical information obtained by the TRAFFIC study strongly suggested that intramuscular injection of rSeV expressing FGF-2 could be an effective method for treating PAD patients.

Our new gene drug product, DVC1-0101, was designed as a RNA drug to induce cytoplasmic expression of native human FGF-2 *in vivo* to improve the walking performance of PAD patients. DVC1-0101 was based on a first-generation vector established by deleting the fusion (F)-gene (rSeV/dF) to render it nontransmissible.[16] We here report the results of the first-in-man, Phase I and IIa, open-labeled, single-center, dose-escalation clinical study of DVC1-0101 to treat PAD patients. This clinical study was designed to evaluate the safety and determine an effective dose of DVC1-0101 in PAD patients in whom revascularization was not considered a suitable option.

RESULTS

Baseline patient characteristics

The Japanese Guidelines for Clinical Trials of Gene Therapy issued by the Ministry of Health, Labor, and Welfare (MHLW) stipulate that only "no-option" patients should be enrolled as subjects in first-in-man gene therapy studies. Therefore, the Governmental Review Board recommended that this trial target cases of CLI without indications for any standard vascular interventions. In addition, because DVC1-0101 was designed to improve the walking performance of PAD patients, we made an effort to recruit subjects who could be tested on a treadmill.

A total of 18 patients with PAD who met the inclusion criteria were screened, and among them, 12 patients who did not meet the exclusion criteria were enrolled (**Table 1**). A total of 12 limbs, one limb per patient, were treated once with DVC1-0101 in a four dose-escalation fashion (**Supplementary Table S1**).

As shown in **Table 1**, the cohort consisted of 10 males and 2 females (mean age, 65 years; range, 48–82), including 10 cases of arteriosclerosis obliterans and 2 of thromboangitis obliterans (TAO, Buerger's disease; both cases were in Stage 4). Three patients had single (cases 103 and 403) or multiple (case 105) ischemic ulcers, and eight patients had relatively mild-to-moderate rest pain that did not prevent their taking a treadmill test. Nine patients had a smoking history, and enrollment was restricted to the patients who had ceased smoking at least 1 month before screening.

Primary end point: safety

Survival. Although one patient (case 105, Stage 1) was lost due to acute-on-chronic progression of preexisting interstitial pneumonitis ~2 years after treatment, no other death occurred over a

Table 1 Patients' baseline characteristics (n = 12)

					Clinical characteristics					
Cohort	Case number	Age	Gender	Smoking history	Target limb	Diagnosis[a]	Rest pain grade[b]	Ulcer diameter (mm)	Available for treadmill	Fontaine/ Rutherford
Stage 1	102	59	M	+	Right	ASO	2	—	Yes	III/II-4
	103	74	M	+	Left	ASO	4	23.1 (5th toe), 11.3 (ankle), 9.5 (foot sole)	No	IV/III-5
	105	66	M	–	Left	ASO	4	8.4 (5th toe)	No	IV/III-5
Stage 2	201	65	F	–	Right	ASO	2	—	Yes	III/II-4
	203	58	M	+	Left	ASO	2	—	Yes	III/II-4
	204	76	F	–	Right	ASO	2	—	No[c]	III/II-4
Stage 3	303	82	M	+	Right	ASO	2	—	Yes	III/II-4
	304	58	M	+	Right	ASO[d]	3	—	Yes	III/II-4
	305	83	M	+	Left	ASO	2	—	Yes[e]	III/II-4
Stage 4	401	48	M	+	Right	TAO	2	—	Yes	III/II-4
	403	54	M	+	Right	TAO	3	8.0 (4th toe)	Yes	IV/III-5
	405	57	M	+	Right	ASO	4	—	No[f]	III/II-4

[a]Diagnosis: ASO, arteriosclerosis obliterans; TAO, thromboangitis obliterans. [b]1: no pain without analgesic, 2: tolerable pain without analgesic, 3: tolerable with a few doses of analgesic(s), 4: multiple doses of analgesic(s), and 5: insufficient pain control with constant use of analgesic(s). [c]Available for treadmill after DVC1-0101 treatment. [d]A case of high aortic occlusion just below renal arteries. [e]Not available due to breathlessness 2 months after DVC1-0101 treatment. [f]Preexisting hemiplasia due to old cerebral infarction.

Table 2 Serious adverse events (SAEs) reviewed by the Data Safety and Monitoring Board and Health Sciences Council of the Japan Ministry of Health, Labor, and Welfare

Case number	SAE	Onset after treatment	Prognosis of event	Review result
102	Major amputation of treated limb (below knee)	3 years and 11 months	Healed stump	Not related, probably[a]
103	Major amputation of treated limb (below knee)	15 days	Healed stump	Cannot be denied
105	Minor amputation of treated limb (3–5 toes) + salvaging bypass	3 months	Healed stump	Not related, probably[a]
	Myelodysplastic syndrome	1 year and 8 months	No change	Not related, probably[b]
	Acute-on-chronic progression of preexisting interstitial pneumonitis	2 years and 1 month	Dead	Not related, probably[b]
201	Compression fracture of lumbar bone	3 years	Recovered	Not related (incidental)
204	Coronary bypass for preexisting coronary aneurysm	2 years and 10 months	Recovered	Not related (incidental)
403	Bleeding of gastric ulcer	1 year and 1 month	Recovered	Not related, probably[b]
	Minor amputation of treated limb (1st toe)	1 year and 4 months	Healed stump	Not related, probably[c]

Abbreviation: SAE, serious adverse event.
[a]Probably due to the natural course of the disease. [b]Probably incidental disease. [c]Probably due to the occlusion of a preexisting upstream bypass graft.

Table 3 Adverse events (AEs) categorized as grade 3 or 4 within 6 months of gene transfer

	AE	Cohort, cases (events) 1	2	3	4	Committee review result
Grade 4	No AE					—
Grade 3	Anemia (postoperative)	1 (1)	0	0	0	Not related
	γ-GTP ↑	1 (1)	0	0	1 (1)	Not related
	Hypoalbuminemia	1 (1)	0	0	0	Not related
	Decubitus ulcer	1 (1)	0	0	0	Not related
	Major amputation	1 (1)	0	0	0	Cannot be denied
	Minor amputation	1 (1)	0	0	0	Not related
	Limb-salvaging bypass	1 (1)	0	0	0	Not related
Total, cases (events)		3 (7)	0 (0)	0 (0)	1 (1)	

Abbreviations: AE, adverse event; GTP, glutamyl transpeptidase.

Table 4 Changes of Rutherford categories at 6 months after gene transfer

		Overall (n = 12)	Stages 2–4 (n = 9)
Improved	Marked (+3)	1	1
	Moderate (+2)	2	2
	Modest (+1)	4	4
	No change	2	1
Worsened	Modest (−1)	1	1
	Moderate (−2)	1	0
	Marked (−3)	1	0
	%Improvement	58.3% (7/12)	77.8% (7/9)
	P value	0.7744	0.1797

1-year period (the observation period ranged from 1 year and 1 month to 4 years and 8 months).

AEs. In the clinical protocol used, serious AEs (SAEs) are reported and reviewed by the Data Safety and Monitoring Board and followed by the Health Sciences Council of the Japan MHLW for 5 years after the protocol, and other AEs are carefully followed for 6 months after the gene transfer date. Nine SAEs occurred in six patients during the observation period (**Table 2**). No cardiovascular events or other SAEs that could be definitively attributed to gene transfer were observed.

A total of 136 AEs were observed (pretreatment = 16 events; post-treatment = 120 events) over the 6 months after the gene transfer. Among these, as shown in **Table 3**, there was no AE categorized as grade 4, and seven of eight AEs related to amputation in Stage 1 were grade 3 according to the Common Terminology Criteria for Adverse Events (CTCAE version 4.03, published by the U.S. National Cancer Institute, 14 June 2010: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). Another AE at Stage 4 was due to alcoholic liver damage. No dose-response relationship was observed.

Virus shedding. Urine and whole blood samples were collected from all of the patients and used to monitor the virus shedding in individuals by measuring the levels of genome copies using nested real-time reverse transcription-PCR as well as hemagglutination activity suggesting infectious activity of DVC1-0101 (assessed by the hemagglutination activity of chicken RBCs). As shown in **Supplementary Table S2**, neither the viral RNA genome nor infectious activity of DVC1-0101 was detected in urine samples. Three patients (cases 303, 401, and 405) exhibited transient persistence of the viral genome; however, no infectious activity was found in any samples, including those that tested positive for the RNA genome.

Proinflammatory reactions. All 12 patients were administered methylprednisolone hemisuccinate (Solu-medrol; 125 mg/day) *via* intravenous drip on day −1, day 0 (just before the vector injection), and day 1 in order to avoid an unexpected evocation of the innate immune response and allergic reactions to vectors. Administration of this cortisone has been shown to suppress the innate immune response but not antigen-specific acquired immune responses against the rSeV under experimental conditions.[17] The patients' laboratory parameters of systemic inflammatory reaction related to the vector injection and treatment are provided in **Supplementary Figure S1a**. In most of the patients, the WBC count increased transiently soon after

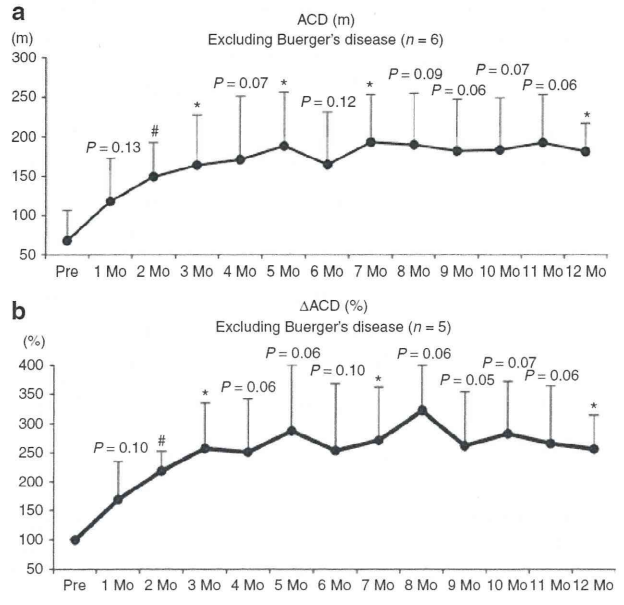

Figure 1 Time course of walking activity examined by treadmill. (**a**) Time course of absolute claudication distance (ACD; m, $n = 6$). (**b**) Change of ACD (ΔACD; %, $n = 5$). $^*P < 0.05$, $^\#P < 0.01$. The data for the two patients with Buerger's disease were excluded. Mo, month.

the gene transfer, and recovered to baseline by day 3. Since no corresponding increase of C-reactive protein or other proinflammatory cytokines was found, the increase of WBC counts was thought to be a result of the methylprednisolone treatment. In contrast, the levels of C-reactive protein and interleukin-6 were increased on days 7–14 on average; this finding was attributed largely to the marked elevation of these parameters in case 103, who underwent a major amputation after showing a septic reaction to a methicillin-resistant *Staphylococcus aureus* infection. Other proinflammatory cytokines, including interleukin-1β and tumor necrosis factor-α, did not show significant change during the trial.

Circulating angiogenic factors. To monitor the possible leakage into the blood circulation of exogenously expressed FGF-2 and its inducible and endogenous downstream angiogenic factors, VEGF and HGF,[12] we subjected the plasma and serum to analysis with specific ELISA systems. We used samples within 7 days of their collection, because our previous studies demonstrated that the expression of exogenous gene products peaked at 2 days and declined at around 7–14 days after gene transfer using multiple genes and vector constructs.[10,18]

As shown in **Supplementary Figure S1b**, there was no significant elevation in any angiogenic factors during the trial.

Other examinations related to safety. All patients underwent a retinal examination by independent ophthalmologists, cancer screening for tumor markers, and computed tomography scans of the brain and whole body as part of a follow-up examination 6 months after the gene transfer. No newly developed lesion was detected in any examination.

Secondary end point: efficacy-related parameters

Since CLI patients without indications for standard vascular interventions were eligible for this trial, we monitored the time course of clinical symptoms (Rutherford classification, rest pain, and ulcer healing) and other surrogates possibly related to the hemodynamics, including ankle-brachial pressure index and toe pressure index, laser Doppler perfusion images (LDPIs), foot-pad temperature assessed by thermography, and pulse-volume recording (PVR), at each patient visit for 6 months after the gene transfer. In addition, a flat treadmill test at 2.4 km/hour, terminated at 300 m to minimize the risk for cardiovascular complications, was added for the eight patients who were able to take the test ($n = 6$ for arteriosclerosis obliterans and $n = 2$ for TAO, **Table 1**).

Improvement of clinical staging (Rutherford classification). All CLI patients enrolled in this trial were categorized as Rutherford grade[19] II-4 or III-5. At the visit 6 months after the gene transfer, each patient's Rutherford category was re-evaluated and scored as +/−1 (improved/worsened one category), +/−2 (improved/worsened two categories), and +/−3 (improved/worsened more than three categories). The overall improvement rate was 58.3% ($n = 7/12$, $P = 0.7744$), and the improvement for Stages 2–4 was 77.8% ($n = 7/9$, $P = 0.1797$), as shown in **Table 4**.

Rest pain. Rest pain was scored based on the frequency of intake of analgesics, in most cases nonsteroidal anti-inflammatory drugs, within 24 hours of the interview, and was categorized as follows: 1, completely pain-free without analgesic; 2, feel pain but no need for analgesic; 3, feel pain and sometimes need analgesic(s); 4, use analgesic(s) constantly to control the pain; and 5, uncontrollable pain despite continuous use of analgesic(s). Two patients (cases 103 and 105) were excluded because of major and minor amputation, respectively.

Overall ($n = 10$), significant pain reduction was seen and continued for over 6 months ($P < 0.01$ or $P < 0.05$, Wilcoxon's signed-rank test), and six patients were completely pain-free during our observation (**Supplementary Figure S2a**).

Ischemic ulcers. The time course of a preexisting ischemic ulcer was recorded in three patients (cases 103 and 105 at Stage 1, and case 403 at Stage 4). No improvement in the size of the referenced ulcers was observed in the two Stage 1 patients, and complete healing was observed in the Stage 4 patient.

Ankle-brachial pressure index and toe pressure index. To avoid examiner-dependent biases, ankle-brachial pressure index and toe pressure index were measured by an automatic oscillometric system (VS-1500A; Fukuda Denshi, Tokyo, Japan). When the blood pressure of the target limb was too low to be detected, the theoretical lower limit, 10 mmHg, was used for calculation. As shown in **Supplementary Figure S2b**, no significant change was observed at any time point up to 6 months after the gene transfer.

LDPI and foot-pad temperature. We measured the blood flow ratio of both legs of each patient using a LDPI analyzer (Moor Instruments, Devon, UK). To minimize data variables due to ambient light and temperature, the LDPI index was expressed

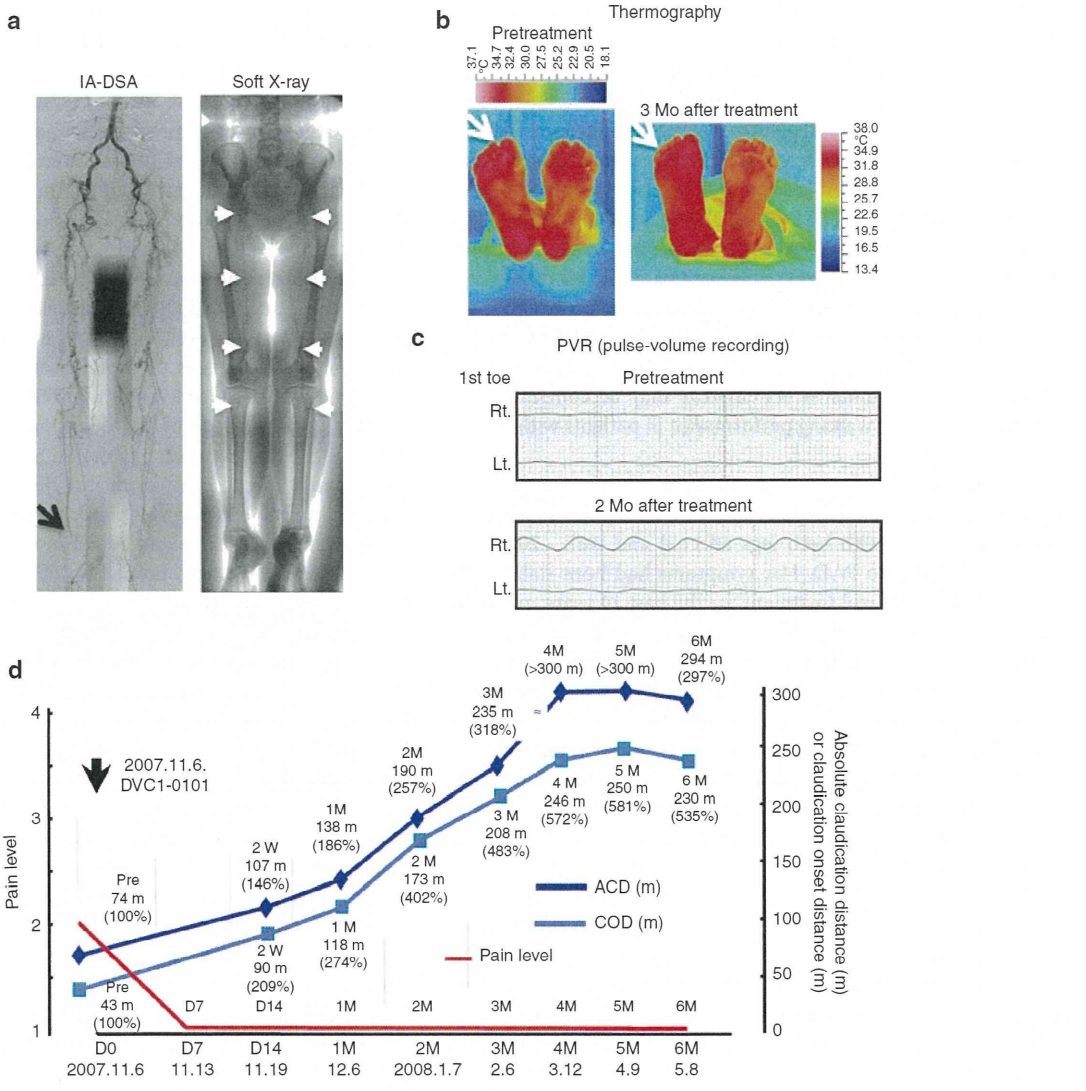

Figure 2 The case of a 65-year-old Japanese woman (case no. 201 at Stage 2). Both limbs were affected, with ~200 m of intermittent claudication since 1993 due to PAD. Her symptoms had been stable for 14 years, and then she developed rest pain in the right foot, which was treated by NSAIDs. (**a**) Her angiogram (left panel) demonstrated complete obstruction of the whole superficial femoral to popliteal arteries and all main arteries at the ankle level in both limbs. Severe calcification was seen throughout the superficial femoral to popliteal arteries on soft X-ray (right panel, white arrows). (**b**) A thermographic examination. A right limb-specific increase of foot temperature (white arrows) is apparent at 3 months after the gene transfer. (**c**) A pulse-volume recording (PVR). Right first toe-specific pulsation was seen. (**d**) Clinical course. Her rest pain completely disappeared within 1 week after the gene transfer, and her treadmill examination demonstrated the linear increase of absolute claudication distance (ACD) and claudication onset distance (COD) at 4 months after the gene transfer. These improvements of clinical symptoms have been maintained at 5 years post-gene transfer. IA-DSA, intraarterial-digital subtraction angiography; Mo, month; NSAID, nonsteroidal anti-inflammatory drug; PAD, peripheral arterial disease.

as the ratio of the blood flow in the treated limb to that in the contralateral limb. As shown in **Supplementary Figure S2c** (left graph), no significant change was observed as of 1 month after gene transfer.

The foot skin temperature of both limbs was measured by independent and blinded physicians (dermatologists in the hospital). The measurements were performed at constant room temperature (25°C), and a 60-minute interval was allocated for patient acclimatization after the removal of his or her socks and shoes. As shown in **Supplementary Figure S2c** (right graph), a significant decrease in the differences of foot-pad temperature (°C = treated limb – untreated limb) was observed at 1 month after the gene transfer ($P < 0.05$, paired t-test).

PVR. As shown in **Supplementary Table S3**, 10 patients (excluding the two amputated cases) underwent constant PVR measurements. Seven of the 10 patients (cases 102, 201, 204, 304, 305, 403, and 405) showed negative PVR values at pretreatment, and six subjects (excluding case 102) occasionally exhibited significant appearance of PVR values after the gene transfer.

Walking ability assessed by treadmill. Eight patients (n = 6 for arteriosclerosis obliterans and n = 2 for TAO) were eligible for the flat treadmill test; the test was performed at 2.4 km/hour and terminated at 300 m to minimize the risk of cardiovascular complications. Regarding the absolute claudication distance (ACD)-based walking performance, all six of the patients who did not have TAO demonstrated steady and significant improvement (**Figure 1a**). Changes of ACD (ΔACD) were also observed in five of these patients (one patient was excluded due to a lack of pre-treatment data because of very severe rest pain), and showed similar results (**Figure 1b**). Even when the data included the two cases of Buerger's disease (TAO), the trends of ACD and ΔACD were not influenced (**Supplementary Figure S3a,b**), suggesting that intramuscular injections of DVC1-0101 may be contributed to the improvement of walking performance in patients with PAD.

Case report

Figure 2 presents the findings for case 201, a 65-year-old Japanese woman at Stage 2 with both legs affected and with ~200 m of IC since 1993 due to PAD. Her symptoms had been stable with beraprost and cilostazol treatment for the past 14 years, and then she developed rest pain in the right foot, which was treated with nonsteroidal anti-inflammatory drugs. Her angiogram demonstrated complete obstruction of the whole superficial femoral to popliteal arteries and all main arteries at the ankle level in both limbs (**Figure 2a**, left panel, arrow). Severe calcification throughout the superficial femoral to popliteal arteries on soft X-ray (**Figure 2a**, right panel, white arrows) and insufficient size of both saphenous veins suggested the difficulty of surgical management; therefore, she was enrolled in the present trial on 22 October 2007.

She received DVC1-0101 at 30 sites of the right leg (10 sites at the upper and lower thigh and calf, respectively, for a total of 1.63×10^8 cell infection units (ciu)). Her experimental course was uneventful. A thermographic examination demonstrated a right leg-specific increase of foot temperature (**Figure 2b**, white arrows), and a PVR showed right first toe-specific pulsation as well (**Figure 2c**). Her rest pain completely disappeared within 1 week after the gene transfer, and her treadmill examination demonstrated a linear increase of ACD and claudication onset distance within 4 months after the gene transfer. These improvements of clinical symptoms have been maintained nearly 5 years later.

DISCUSSION

We here report the first-in-human Phase I/IIa dose-escalating clinical study of a new hFGF-2 gene expressing rSeV-based RNA gene drug, DVC1-0101, to treat PAD patients. The key findings of this study were: (i) the intramuscular administration of DVC1-0101, up to 5×10^9 ciu/60 kg, to an ischemic limb of CLI patients was feasible and well tolerated, and demonstrated no drug-related SAE within the 6-month study period; (ii) even though three patients transiently exhibited a significant amount of viral genome in whole blood, significant hemagglutinating activity was shown in neither urine nor whole blood; (iii) neither a serious inflammatory reaction nor significant leakage of angiogenic factors to the circulation was detected during the experimental course, except for one patient (case 103) who showed infected progressive gangrene; and (iv) representative and stable improvement was seen in the rest pain scores and ACD values. These findings suggest that DVC1-0101 may be safe and effective for the treatment of PAD patients, and indicate that further investigations are warranted.

Since the pioneering preclinical report describing the increase of capillary and collateral vessels after the administration of an angiogenic growth factor (VEGF) in preclinical animal models,[2] the clinical benefits of therapeutic angiogenesis to treat PAD have been intensively examined worldwide with the use of various proteins and genes. The majority of the early clinical studies on IC and CLI have suggested the possible clinical efficacy of therapeutic angiogenesis; however, almost all of the double-blinded and placebo-controlled trials of protein- or gene-based therapeutic angiogenesis have failed to show definitive clinical benefit.

At the initial stage of the development of DVC1-0101, we hypothesized that the peak expression and local concentration of the therapeutic gene in ischemic muscle might be a key factor for determining the efficacy; therefore, we used rSeV as the vector. In fact, using a severe ischemic limb model of balb/c *nu/nu* mice, we discovered that the combination of rSeV and FGF-2 showed a higher limb-salvaging effect compared with other combinations (*i.e.*, rSeV expressing FGF-1, VEGF, or angiopoietin-1, as well as plasmid DNA expressing VEGF, angiopoietin-1, or HGF; Y. Yonemitsu *et al.*, unpublished data). In addition, a preclinical dose-efficacy study demonstrated that 14.3- to 68.8-fold increase (compared with the baseline) in the local FGF-2 content that was obtained by administering 3×10^6 to 1×10^7 ciu/30 g mouse had the maximum limb-salvaging effect.[17] Such strong expression in muscles is likely to be the main reason for the safety advantage of rSeV in clinical settings, because the requirement of a lesser amount of vectors can avoid the vector-related activation of innate immunity, in addition to minimizing the leakage of vector particles into the blood circulation. The results of the present study support this notion.

Regarding the efficacy parameters, we employed a number of measurements, including hemodynamic surrogates, because there has been no definitive report indicating which parameter(s) are sensitive and optimal for determining the clinical outcomes of angiogenic trials. Importantly, the present findings suggest that DVC1-0101 may improve PAD patients' walking performance as examined by treadmill; however, we have to be cautious when interpreting this data, since it has been considered that the measurement of walking function in a number of previous angiogenesis trials was compromised by a placebo effect. First, the data in **Figure 1** were analyzed to suggest the possible efficacy in improving the walking function. Therefore, additional placebo-controlled studies with sufficient number of patients would be needed. Second, the placebo group usually exhibits ~30% of improvement of walking ability in therapeutic angiogenesis trials.[4,5,20] Such a placebo effect may be explained, for example, by some effects due to injection trauma for gene transfer and to immune-related responses against vectors. This may not be likely, however, because a similar level of placebo-related improvements was also observed by pharmacotherapies, including treatment with the first-line drug cilostazol.[20] In addition, the improvement of walking performance by DVC1-0101 obtained in this study reached ~240–300% (**Figure 1b**), suggesting much stronger activity than that seen by

cilostazol.[21] Since walking performance based on a treadmill test is recognized as a feasible end point in PAD trials for IC,[21,22] the next well-organized placebo-controlled study may make it clear whether DVC1-0101 is truly effective for patients with IC.

In summary, we observed no major adverse consequences of DVC1-0101 treatment during over the months of follow-up period after 30 direct intramuscular injections to patients with CLI. The dose-escalation schema were completed as planned. Further studies with large numbers of patients and a placebo control will be needed to establish both the safety and efficacy of DVC1-0101.

MATERIALS AND METHODS

DVC1-0101 (rSeV/dF-hFGF2): an F-gene–deleted nontransmissible rSeV vector expressing the human FGF-2 gene. DVC1-0101 is an F-gene–deleted nontransmissible rSeV based on a Z-strain encoding negative-stranded full-length complementary RNA of human FGF-2 at the upstream of gene encoding N-protein (rSeV/dF-hFGF2). The DVC1-0101 used in the present study was constructed at BioReliance (Starling, UK) in accordance with good manufacturing practice regulations that meet the good manufacturing practice standards of the USA, European Union, and Japan. In brief, LLC-MK2 cells were transfected with a plasmid mixture containing each plasmid: pSeV+18/dF-hFGF2, pGEM-NP, pGEM-P, and pGEM-L. The transfected cells were maintained for 3 hours, then washed and incubated for 60 hours in minimum essential medium containing ara-C. The cells were collected and lysed, and the lysate solution was incubated on the stably F-expressing LLC-MK2 cells in a 24-well plate. Twenty-four hours later, the cells were washed and incubated in minimum essential medium containing ara-C and trypsin. Purification was achieved using column chromatography and filtration to concentrate the vector. The virus yield is expressed in ciu, as described previously.[16]

Study design. The trial was conducted as a single-center Phase I/IIa, open-labeled, dose-escalating study investigating the safety, bioactivity, and potential clinical benefits of single intramuscular injections of DVC1-0101. The study design, protocol, and informed consent forms were approved by the institutional ethical committees, the Health Sciences Council of the Japan MHLW, and Biosafety Committees according to national regulations. A Data Safety and Monitoring Board provided an independent review of the safety data and of accordance of the defined inclusion criteria for each patient. This study was conducted, recorded, and reported in compliance with the principles of good clinical practice regulations with the support of the Clinical and Translational Research Center of Kyushu University Hospital and an external Contract Research Organization (EPS, Tokyo, Japan).

Patient cohort. Patients were enrolled when they (i) had chronic CLI including rest pain or ischemic ulcer that was resistant to standard medication for at least 2 weeks, (ii) were not candidates for surgical or catheter revascularization based on usual practice standards, (iii) did not have cancer or a history of cancer within the prior 5 years, (iv) did not have any significant vital organ dysfunction, (v) did not have any immunosuppressive agent, (vi) did not have any active inflammatory disease, and (vii) did not have proliferative or unstable retinopathy. The patient eligibility criteria and possible ethical discussion points of this trial based on the Declaration of Helsinki were confirmed by an independent committee for gene therapy clinical trials and the institutional review board at Kyushu University, which was approved by the MHLW. Patients participated in the trial on an outpatient basis, except for ~2 weeks just before and after the gene transfer. The baseline characteristics of the patients are summarized in **Table 1**.

Treatment and follow-up. On day 0, 30 intramuscular injections were performed in a single leg under bolus epidural anesthesia (0.5 ml/injection = 15 ml/patient). The sites of injection differed at each administration, and were selected according to angiogram findings and the investigator's discretion (**Supplementary Table S1**). Patient follow-up to collect the assessments was done monthly up to 6 months (±7 days at each visit), and the follow-up to monitor the occurrence of SAEs was continued to 5 years after the gene transfer. Rest pain was scored based on the frequency of intake of analgesics within 24 hours of each interview (mainly concerning non-steroidal anti-inflammatory drugs) and was categorized as 1: completely pain-free without analgesic, 2: feel pain but no need for analgesic, 3: feel pain and sometimes need analgesic(s), 4: use analgesic(s) constantly to control the pain, and 5: uncontrollable pain even though constant use of analgesic(s).

Virus shedding. Hemagglutinating activity was determined using chicken RBCs as described previously.[23] For the detection of genome copies, total RNA was extracted from whole blood or urine samples at each time point. The target viral sequence was first amplified by reverse transcription--PCR using the following primer set: forward, 5′-ATCACTGCCACCCAGAAGACT-3′; reverse, 5′-ACCAGGAAATGAG-CTTGACAA-3′. Real-time monitoring of the amplification of target genes was done by a Sequence Detection System, model 7000 (Applied Biosystems, Tokyo, Japan), according to the manufacturer's instructions for Taq-Man methods. The oligonucleotide sequences of the PCR primers and TaqMan probes were as follows: forward, 5′-ACTTGGATCCAAAACAGGACCTGGG-3′; reverse, 5′-TGTA TCGAAG-GTGCTCAACAACCCG-3′; and probe: 5′-FAM-CATCGCGG CCGCAGATCTTCAC-GATGGC-TAMRA-3′. The detection limit was 0.1 genome copy/μl for all assays.

ELISA. Angiogenic factors in serum and plasma were determined using Quantikine Immunoassay systems for human FGF-2, VEGF, and HGF (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Data are expressed as pg/ml.

Statistical analysis. All data are expressed as means ± SD. The data were examined using paired *t*-tests, when appropriate, in comparison with baseline values. A probability value of $P < 0.05$ was considered significant. All data were analyzed by the independent Data Center at Kyushu University Hospital.

SUPPLEMENTARY MATERIAL

Figure S1. Time course of proinflammatory markers and circulating angiogenic factors.
Figure S2. Time course of rest pain and hemodynamic surrogates.
Figure S3. Time course of walking activity examined by treadmill.
Table S1. Patients cohort.
Table S2. Virus shedding.
Table S3. Pulse-volume recording.

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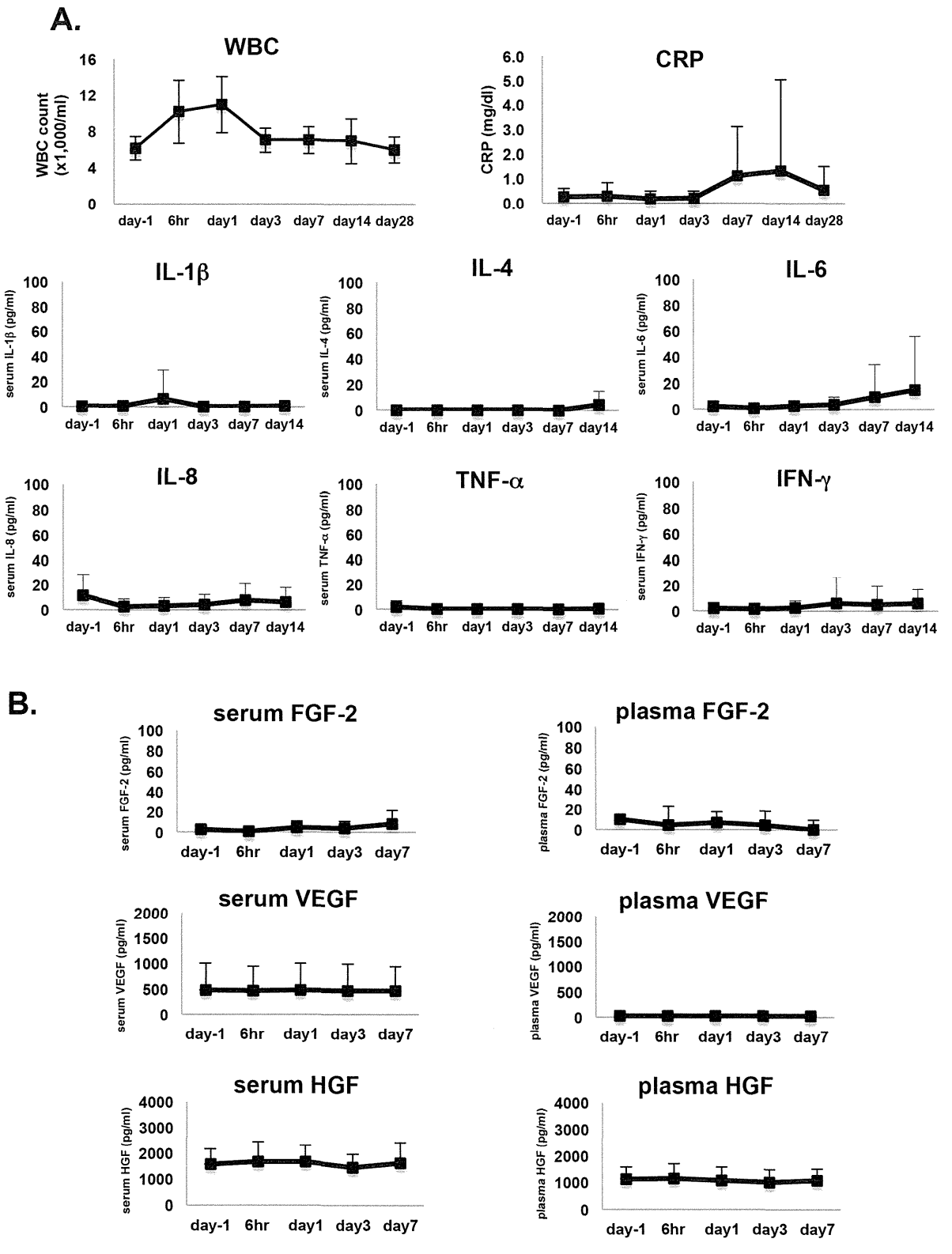
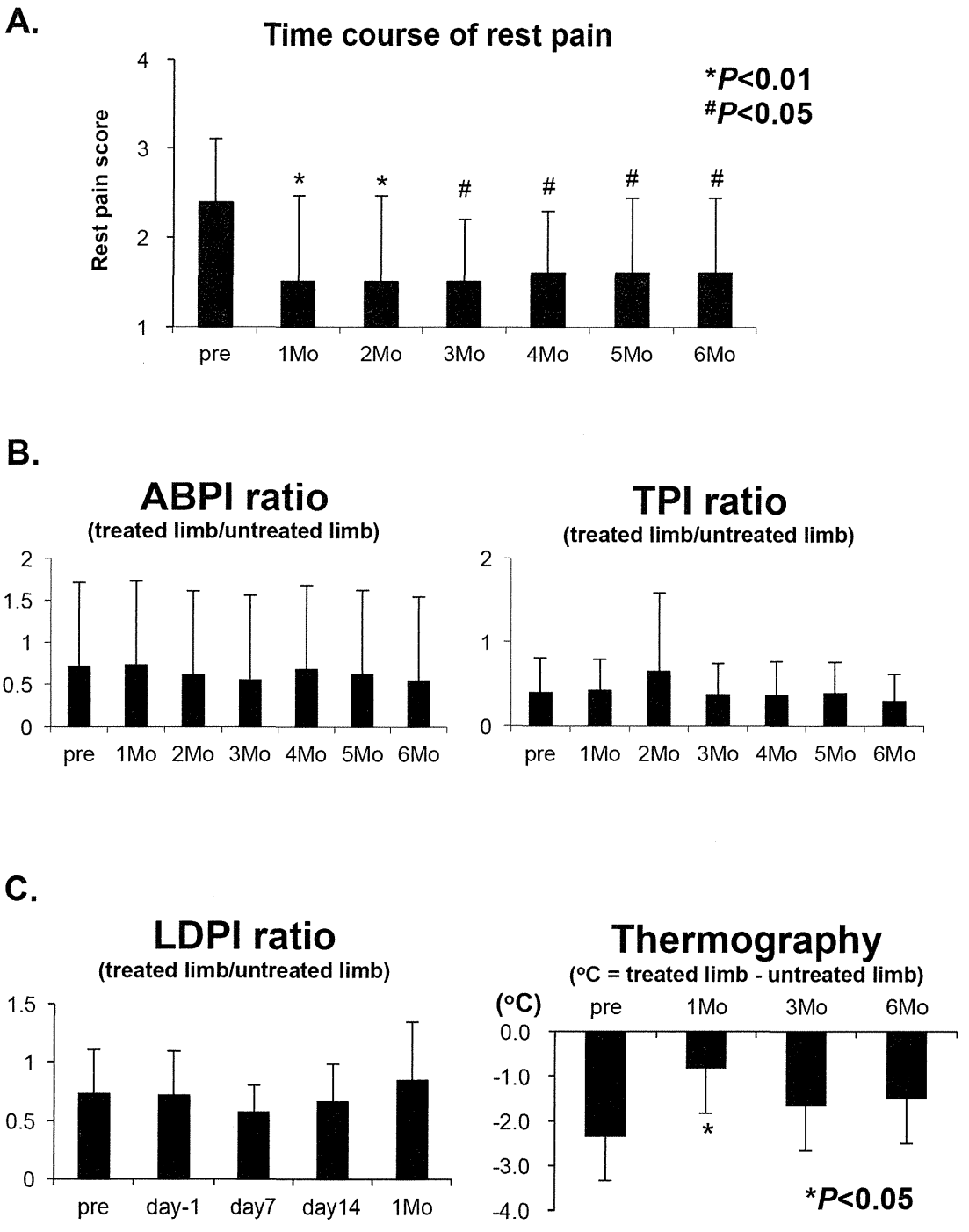
Supplementary Figure S1.

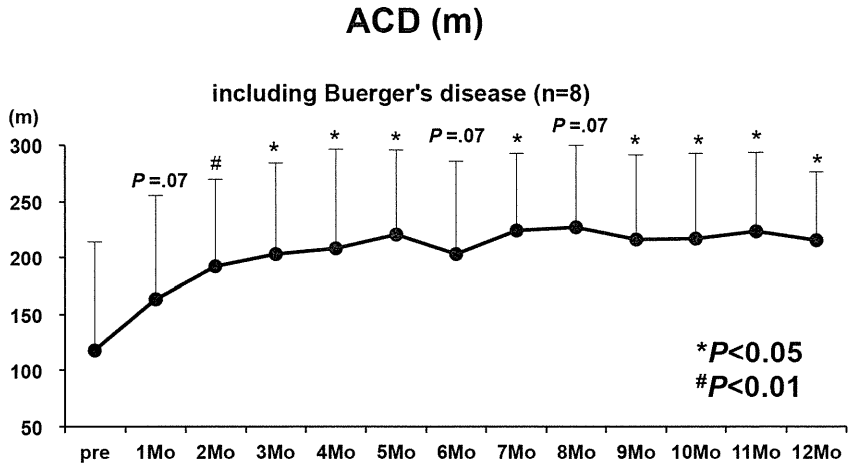


Supplementary Figure S2.



Supplementary Figure S3.

A.



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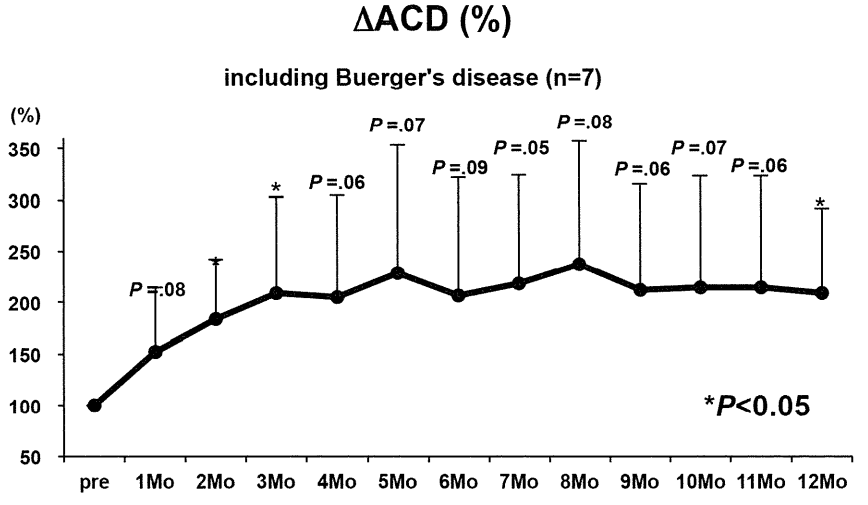

Legends for Supplementary Figures.

Supplementary Figure S1.

A. Time courses of inflammation/immune response-related parameters, including white blood cell count (WBC), serum C-reactive protein (CRP) level, serum interleukin-1β (IL-1β) , serum IL-4, serum IL-6, serum IL-8, serum tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Blood samples were collected at each time point, and subjected to laboratory examination. Each proinflammatory cytokine level was determined by specific ELISA as manufactures' instructions.

B. Time courses of circulating angiogenic factors, including serum and plasma FGF-2, VEGF, and HGF. Blood samples were collected at each time point, and subjected to the specific ELISA as manufactures' instructions.

Supplementary Figure S2.

Time courses of rest pain score (a), ankle-brachial pressure index (ABPI) and toe pressure index (TPI) (b), laser Doppler perfusion index (LDPI) ratio, and thermography (c), related to efficacy. $^{*}p<0.01$, $^{\#}p<0.05$.

Supplementary Figure S3.

Time course of A: absolute claudication distance (ACD; m, n = 8) and B: Change of ACD (ΔACD: %, n = 7). $^{*}p<0.05$.
The data of the two patients with Buerger's disease were included.

Supplementary Table S1. Patients Cohort

cohort (dose/60kg)	case No.	treatment				
		total dose (ciu/head)	injection sites (30 injections)			
			Upper thigh	Lower thigh	Calf	Foot pad
Stage 1 ($5x10^7$ ciu)	102	$5.21x10^7$	10	10	10	0
	103	$5.25x10^7$	7	8	10	5
	105	$3.98x10^7$	0	12	18	0
Stage 2 ($2x10^8$ ciu)	201	$1.63x10^8$	10	10	10	0
	203	$2.11x10^8$	10	10	10	0
	204	$1.79x10^8$	10	10	10	0
Stage 3 ($1x10^9$ ciu)	303	$0.87x10^9$	0	9	21	0
	304	$1.20x10^9$	0	14	16	0
	305	$1.07x10^9$	6	9	15	0
Stage 4 ($5x10^9$ ciu)	401	$7.70x10^9$	0	0	30	0
	403	$6.35x10^9$	0	6	20	4
	405	$4.98x10^9$	0	14	16	0

Supplementary Table S2. Virus Shedding

Genome copies (gc/μl): urine

Case No.	pre	D0 (6 hrs)	D1	D3	D7	D8 - 15
102	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
103	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
105	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
201	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
203	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
204	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
303	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
304	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
305	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
401	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
403	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
405	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

Genome copies (gc/μl): whole blood

Case No.	pre	D0 (6 hrs)	D1	D3	D7	D8 - 15
102	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
103	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
105	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
201	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
203	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
204	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
303	<0.1	≥0.1	≥0.1	<0.1	<0.1	<0.1
304	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
305	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
401	<0.1	≥0.1	≥0.1	<0.1	<0.1	<0.1
403	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
405	<0.1	≥0.1	≥0.1	≥0.1	≥0.1	<0.1

Hemagglutinating activity: urine

Case No.	pre	D0 (6 hrs)	D1	D3	D7	D8 - 15
102	(-)	(-)	(-)	(-)	(-)	(-)
103	(-)	(-)	(-)	(-)	(-)	(-)
105	(-)	(-)	(-)	(-)	(-)	(-)
201	(-)	(-)	(-)	(-)	(-)	(-)
203	(-)	(-)	(-)	(-)	(-)	(-)
204	(-)	(-)	(-)	(-)	(-)	(-)
303	(-)	(-)	(-)	(-)	(-)	(-)
304	(-)	(-)	(-)	(-)	(-)	(-)
305	(-)	(-)	(-)	(-)	(-)	(-)
401	(-)	(-)	(-)	(-)	(-)	(-)
403	(-)	(-)	(-)	(-)	(-)	(-)
405	(-)	(-)	(-)	(-)	(-)	(-)

Hemagglutinating activity: whole blood

Case No.	pre	D0 (6 hrs)	D1	D3	D7	D8 - 15
102	(-)	(-)	(-)	(-)	(-)	(-)
103	(-)	(-)	(-)	(-)	(-)	(-)
105	(-)	(-)	(-)	(-)	(-)	(-)
201	(-)	(-)	(-)	(-)	(-)	(-)
203	(-)	(-)	(-)	(-)	(-)	(-)
204	(-)	(-)	(-)	(-)	(-)	(-)
303	(-)	(-)	(-)	(-)	(-)	(-)
304	(-)	(-)	(-)	(-)	(-)	(-)
305	(-)	(-)	(-)	(-)	(-)	(-)
401	(-)	(-)	(-)	(-)	(-)	(-)
403	(-)	(-)	(-)	(-)	(-)	(-)
405	(-)	(-)	(-)	(-)	(-)	(-)

Supplementary Table S3. Pulse-volume Recording

Case No.	limb		pre	1Mo	2Mo	3Mo	4Mo	5Mo	6Mo
102	treated	R	(-)	(-)	(-)	(-)	(-)	(-)	(-)
	contralateral	L	++	++	++	++	++	++	++
103	treated	L	(-)	Not available due to major amputation					
	contralateral	R	++						
105	treated	L	(-)	(-)	Not available due to minor amputation				
	contralateral	R	++	++					
201	treated	R	(-)	(-)	+	(-)	(-)	+	(-)
	contralateral	L	(-)	(-)	(-)	(-)	(-)	(-)	(-)
203	treated	L	++	++	++	+	+	+	++
	contralateral	R	++	++	++	+	+	+	++
204	treated	R	(-)	++	(-)	+	+	+	+
	contralateral	L	++	++	++	++	++	++	++
303	treated	R	+	+	*1	+	+	(-)	+
	contralateral	L	+	++	+	+	+	+	+
304	treated	R	(-)	+	+	+	(-)	(-)	(-)
	contralateral	L	++	++	++	++	++	++	++
305	treated	L	(-)	(-)	(-)	(-)	(-)	(-)	+
	contralateral	R	+	++	++	++	++	++	++
401	treated	R	+	(-)	(-)	(-)	(-)	+	+
	contralateral	L	++	++	++	++	++	++	++
403	treated	R	(-)	+	+	(-)	+	+	(-)
	contralateral	L	++	++	++	++	++	++	++
405	treated	R	(-)	+	+	+	+	++	+
	contralateral	L	++	++	++	++	++	++	++

*1: not available

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Clinical Trials

Protocol Open Access

Efficacy and Safety of DVC1-0101 for Intermittent Claudication Secondary to Peripheral Artery Disease: Study Protocol of a Randomized Phase IIb Trial

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Abstract

Background: We have developed a new gene transfer vector based on nontransmissible recombinant Sendai virus expressing the human fibroblast growth factor-2 gene (DVC1-0101) to treat peripheral arterial disease. A phase I/IIa open-label four dose-escalations clinical trial for critical limb ischemia was completed. We concluded that DVC1-0101 is safe and well tolerated, and resulted in significant improvement of limb function. We present the protocol of the next phase of our study.

Methods: We plan to conduct a phase IIb clinical trial, which will be a randomized, placebo-controlled, parallel design, single-dose blinded and single center clinical trial in Japan. This study will enroll 60 patients diagnosed with PAD with intermittent claudication. Subjects who meet eligibility criteria will be randomized to receive a single dose of either placebo, 5×10^9 ciu/limb of DVC1-0101, or 1×10^9 ciu/limb of DVC1-0101 administered by direct intramuscular injection. The participation length in this trial for subjects will be approximately 12 months with nine visits. The primary endpoints are to evaluate the efficacy of DVC1-0101 versus placebo on peak walking time, and to evaluate the safety and tolerability of two dosage levels of DVC1-0101. The secondary endpoints are 1) to evaluate the effect of DVC1-0101 on claudication onset time, measured by a treadmill test and quality of life, measured using the Walking Impairment Questionnaire, 2) to determine the effect of DVC1-0101 on qualifying limb hemodynamics, and 3) to explore the pharmacodynamics of DVC1-0101 by evaluating biomarkers.

Discussion: The results of this trial will provide insights into the potential of DVC1-0101 for improving walking activities. The results will also help with the design of a possible phase III study.

Keywords: Sendai virus vector; Gene therapy; Fibroblast growth factor-2; Study protocol; Peripheral arterial disease; intermittent claudication; DVC1-0101

Introduction

In today's aging society in Japan, the total number of deaths from atherosclerotic diseases (e.g., atherosclerosis obliterans, ischemic heart disease, and cerebro cardiovascular disease) exceeds the number of deaths from malignant neoplasms [1]. Therefore, there is an urgent need to take measures against atherosclerotic diseases to improve public health. The prevalence of Peripheral Arterial Disease (PAD), which is mainly due to atherosclerosis obliterans, is increasing in Japan. PAD is often asymptomatic or observed as numbness in the early stage (Grade I based on the Fontaine classification). However, as PAD progresses, patients' Quality Of Life (QOL) decreases because of limited walking ability (Intermittent Claudication (IC), Grade II based on the Fontaine classification) associated with pain in the lower legs during walking. If untreated, IC progresses to Critical Limb Ischemia (CLI) in approximately one of four patients within 5 years [2], and induces pain at rest (Grade III based on the Fontaine classification) and ischemic ulcer/gangrene in the legs (Grade IV based on the Fontaine classification), resulting in substantial deterioration in the QOL. This affects the life prognosis of patients who are forced to be bedridden for a long time. This issue has become a cause of medical economic pressure associated with long-term medical care.

The prognosis of limb ischemia has greatly improved because of bypass surgery techniques, intra-postoperative management, artificial blood vessel material, endovascular treatment, and drug treatment. Based on the results of previous studies showing that the 5-year survival rate is approximately 40% for CLI and 70% to 80% for IC, the most important objectives for the treatment of PAD are (1) avoiding amputation and (2) preventing progression from IC to CLI [3]. However, in patients with chronic arterial occlusion who have bypass surgery to the popliteal artery or the lower leg, the 5-year patency rate is approximately 40%. This result suggests that such treatment is not yet sufficiently effective. In addition, there are many cases where bypass surgery is not indicated because of the presence of severe lesions in the peripheral region. Additionally, patients may not tolerate bypass surgery owing to a poor general condition associated with advanced arteriosclerosis in other organs. For CLI patients, mortality is as high as 20%, and major amputation is required within 1 year in 40% of patients [4]. There are many cases of complications with diabetes mellitus, which is an important risk factor of arteriosclerosis, because arteriosclerosis obliterans is a consequence of arteriosclerosis. Bypass surgery or endovascular treatment may not be indicated for patients with arteriosclerosis obliterans because they often have concurrent diabetic nephropathy requiring chronic dialysis. Additionally, they are often in poor health because of complications derived from diabetes

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mellitus accompanied by diffuse narrowing and advanced calcification. There is no effective treatment option available for such patients. Therefore, every year, tens of thousands of patients are estimated to gradually progress to CLI, resulting in amputation. The survival prognosis for patients who have had amputation is poor. According to the inter-society consensus for the management of PAD (TASC II), the mortality rate is twice as high as that for breast cancer, and is comparable with that of malignant tumors, such as colon cancer and Hodgkin's lymphoma [3].

The most effective treatment of chronic arterial occlusion is surgery or endovascular treatment. However, there is no choice but drug treatment for patients for whom these invasive treatments are not indicated. Currently, there is no drug with proven efficacy for the treatment of CLI. Cilostazol and naftidrofuryl (not marketed in Japan) have been demonstrated to be effective in the treatment of IC in several large-scale clinical studies. Cilostazol is recommended as the first-line drug for the treatment of IC. Cilostazol has been reported to improve claudication distance by approximately 50%, but there is no evidence to support the effectiveness of cilostazol in preventing the progression from IC to CLI [3]. Given these circumstances, there is an urgent need to develop more effective techniques for the treatment of chronic arterial occlusion.

Therapeutic angiogenesis has been proposed [5] and clinically evaluated as a new treatment option for chronic arterial occlusion since the end of the 1990's [6]. To date, techniques of therapeutic angiogenesis using (1) recombinant proteins, (2) bone-marrow/blood cells, and (3) genes have been clinically tested. Sanofi-Aventis has been developing gene therapy products for the treatment of CLI using human Fibroblast Growth Factor-1 (FGF-1) expression plasmid. Additionally, Anges MG has been developing gene therapy products using Hepatocyte Growth Factor (HGF) expression plasmid. However, candidate drugs using recombinant proteins or genes have not yet succeeded in demonstrating efficacy in the treatment of IC.

In view of the current development of therapeutic angiogenesis techniques, we have attempted to investigate these problems through basic research. As a result, we have obtained the following findings from experiments using pathological animal models. (1) Gene expression is increased 50 to 500-fold by intramuscularly injecting the recombinant virus vector (SeV) that we developed compared with a technique using a plasmid. (2) The results of examinations comparing various genes related to angiogenesis as candidate therapeutic genes indicate that only the FGF-2 gene has a wide margin of safety without significant adverse drug reactions, and exerts a potent effect of relieving limb ischemia below the margin of safety. (3) FGF-2 activates the signaling system in different ways from other angiogenic factors. FGF-2 also strongly induces endogenous angiogenic factors, such as Vascular Endothelial Growth Factor (VEGF) and HGF downstream [7,8].

Previous Study

Based on the above data, researchers at Kyushu University Hospital planned a gene therapy clinical study in CLI patients with normal renal function using F gene-deleted non-transmissible recombinant Sendai virus vector expressing the human FGF-2 gene (rSeV/dF-hFGF2 [Development code: DVC1-0101]). Case registrations were started in April, 2006, after obtaining approval on January 31, 2006, from the Minister of Health, Labour and Welfare, and the Minister of the Environment in Japan (open-label, four-stage dose-escalation study corresponding to phase I/IIa in terms of study design). In total, 12 subjects, consisting of three subjects in the first stage (5×10^7 ciu/60 kg (ciu = cell infectious unit)), three subjects in the second stage (2×10^8 ciu/60 kg), three subjects in the third stage (1×10^9 ciu/60 kg), and three subjects in the fourth stage (5×10^9 ciu/ 60 kg), completed the scheduled administration of test product and observation period for 6 months. The gene therapy clinical study completion report was submitted to the Minister of Health, Labour and Welfare in Japan in March 10, 2011. The results suggested that DVC1-0101 can be used safely because it is well tolerated in patients and only affects the general condition of patients to a relatively small extent. At doses for the second stage (2×10^8 ciu/60 kg) or greater, DVC1-0101 appears to contribute to improvement of walking function and pain at rest among other efficacy endpoints [9].

Objectives

The primary objective of the study is to investigate the safety and clinical efficacy of DVC1-0101 (1×10^9 ciu/leg, 5×10^9 ciu/leg) in patients with IC. We also aim to examine the dose-response relationship using the rate of improvement in walking function as an indicator.

Methods/Design

Design

The DVC1-0101 trial is designed as a phase IIb investigator-initiated, randomized, centrally-registered, double-blinded, single center, and dose-response clinical trial of gene therapy versus placebo in patients with IC. The trial has a parallel-arm design with 1:1:1 allocation to the experimental intervention groups and the control intervention group. A full flow diagram for the study is shown in Figure 1.

Participants and setting

Eligible participants will include adults aged 40–80 years with arteriosclerosis obliterans accompanied by limited walking function due to IC (absolute claudication distance <200 m; Grade IIb and partly Grade III based on the Fontaine classification) [10]. The details of inclusion and exclusion criteria are shown in Table 1. A total of 60 subjects will have investigational product administered at a single center, Kyushu University Hospital, in Japan (Table 2).

Endpoints

In this study, a progressive load treadmill test shall be used to detect changes in walking function because this is more accurate than any other test [9]. The test condition is that the slope shall be progressively increased from 0 degrees by 2% every 2 minutes at a constant speed of 3.2 km/h. The Quinton® Q-STRESS® TM55 (Cardiac Science, Waukesha, WI, USA) will be used for the treadmill test.

Primary endpoints

- Rate of increase in absolute claudication distance (ACD)
- ACD
- Peak walking time
- Initial claudication distance (ICD)
- Claudication onset time

Secondary endpoints

- Measurement of oxygen dynamics in the leg muscles by near-infrared spectroscopy after a treadmill load test
- Proportion of subjects in whom readministration was not required