Safety Evaluation of Clinical Gene Therapy Using Hepatocyte Growth Factor to Treat Peripheral Arterial Disease

Ryuichi Morishita, Motokuni Aoki, Naotaka Hashiya, Hirofumi Makino, Keita Yamasaki, Junya Azuma, Yoshiki Sawa, Hikaru Matsuda, Yasufumi Kaneda, Toshio Ogihara

Abstract—Therapeutic angiogenesis using angiogenic growth factors is expected to be a new treatment for patients with critical limb ischemia (CLI). Because hepatocyte growth factor (HGF) has potent angiogenic activity, we investigated the safety and efficiency of HGF plasmid DNA in patients with CLI as a prospective open-labeled clinical trial. Intramuscular injection of naked HGF plasmid DNA was performed in ischemic limbs of 6 CLI patients with arteriosclerosis obliterans (n=3) or Buerger disease (n=3) graded as Fontaine III or IV. The primary end points were safety and improvement of ischemic symptoms at 12 weeks after transfection. Severe complications and adverse effects caused by gene transfer were not detected in any patients. Of particular importance, no apparent edema was observed in any patient throughout the trial. In addition, serum HGF concentration was not changed throughout the therapy period in all patients. In contrast, a reduction of pain scale of more than 1 cm in visual analog pain scale was observed in 5 of 6 patients. Increase in ankle pressure index more than 0.1 was observed in 5 of 5 patients. The long diameter of 8 of 11 ischemic ulcers in 4 patients was reduced >25%. Intramuscular injection of naked HGF plasmid is safe, feasible, and can achieve successful improvement of ischemic limbs. Although the present data are conducted to demonstrate the safety as phase I/early phase IIa, the initial clinical outcome with HGF gene transfer seems to indicate usefulness as sole therapy for CLI. 

Key Words: clinical trials ■ DNA ■ blood vessels ■ safety ■ vascular diseases

The clinical consequences of peripheral arterial disease include pain on walking (claudication), pain at rest, and loss of tissue integrity in the distal ischemic limbs. Although development of beneficial drugs and intervention devices do contribute to the treatment of this disease, critical limb ischemia is estimated to develop in 500 to 1000 individuals per million per year. In a large proportion of these patients, the anatomical extent and the distribution of arterial occlusive disease make the patients unsuitable for operative or percutaneous revascularization. Thus, the disease frequently follows an inexorable downhill course. Recent progress in molecular biology has led to the development of gene therapy as a new strategy to treat a variety of cardiovascular diseases. Most of the studies have used vascular endothelial growth factor (VEGF), also known as vascular permeability factor. Indeed, beneficial effects of therapeutic angiogenesis using VEGF gene transfer have been reported in human patients with critical limb ischemia and myocardial ischemia. On the other hand, we have focused on hepatocyte growth factor (HGF), because HGF is a potent angiogenic growth factor in mice, rat, and rabbit ischemia models. Even in high-risk conditions for atherosclerosis such as a diabetic and high lipoprotein (a) concentration model, overexpression of HGF is enough to stimulate collateral formation to treat ischemic symptoms. Unexpectedly, the mitogenic activity of HGF has been reported to be more potent than that of VEGF in human aortic endothelial cells in vitro as well as a rabbit hindlimb ischemia model in vivo. Based on our previous data, we designed a human clinical trial of gene therapy for peripheral artery disease using HGF gene as an open-labeled study. Despite this limitation of the trial, we demonstrate that intramuscular injection of naked HGF plasmid is safe and feasible as subanalysis of phase I/IIa.

Methods

Plasmid DNA

The pVAX1 plasmid vector (3.0 kb) is commercially produced by Invitrogen Corporation (Carlsbad, Calif) and was selected for HGF construct because it incorporated elements to be in compliance with...
TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (Sex)</th>
<th>Disease</th>
<th>Fontaine Classification</th>
<th>Rutherford Classification</th>
<th>Risk Factors Classification</th>
<th>Previous for Arteriosclerosis</th>
<th>DSA/MRA Treatment</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>57 (M)</td>
<td>ASO</td>
<td>III</td>
<td>II-4</td>
<td>Smoking, HT</td>
<td>7 Bypass grafts (occluded)</td>
<td>SFA, POP, PT, PN occlusion</td>
</tr>
<tr>
<td>2</td>
<td>42 (M)</td>
<td>ASO</td>
<td>IV</td>
<td>III-II</td>
<td>Smoking, HT, DM CRF (HD)</td>
<td>2PTA Sympathectomy</td>
<td>SFA, POP, PT, AT occlusion</td>
</tr>
<tr>
<td>3</td>
<td>48 (M)</td>
<td>Buerger</td>
<td>IV</td>
<td>III-5</td>
<td>—</td>
<td>Sympathectomy</td>
<td>SFA, POP, AT, PT, PN occlusion</td>
</tr>
<tr>
<td>4</td>
<td>64 (F)</td>
<td>Buerger</td>
<td>III</td>
<td>II-4</td>
<td>—</td>
<td>Prostaglandin therapy</td>
<td>SFA, POP, AT, PT, PN occlusion</td>
</tr>
<tr>
<td>5</td>
<td>67 (M)</td>
<td>Buerger</td>
<td>IV</td>
<td>III-5</td>
<td>—</td>
<td>Prostaglandin therapy</td>
<td>AT, PT, PN occlusion</td>
</tr>
<tr>
<td>6</td>
<td>69 (M)</td>
<td>ASO</td>
<td>IV</td>
<td>III-5</td>
<td>HT, DM</td>
<td>Bypass graft (occluded)</td>
<td>POP, AT, PN occlusion</td>
</tr>
</tbody>
</table>

HT indicates hypertension; DM, diabetes mellitus; CRF, chronic renal failure; HD, hemodialysis; SFA, superficial femoral artery; POP, popliteal; AT, anterior tibial artery; PT, posterior tibial artery; PN, peroneal artery.

In this initial single-center phase of a prospective open-labeled study, patients could be enrolled if they (1) had chronic critical limb ischemia, including rest pain or nonhealing ischemic ulcers, for a minimum 4 weeks; (2) were resistant to conventional drug therapy at least for >4 weeks after hospitalization; (3) were not candidates for surgical or percutaneous revascularization based on usual practice standards; (4) did not have cancer or a history of cancer; and (5) did not have severe unstable retinopathy. Objective documentation of ischemia, including a resting ankle brachial index (ABI) of less than 0.6 in the affected limb on 2 consecutive examinations performed 1 week apart was necessary. Patients were observed for 4 weeks under conventional drug therapy to confirm that their clinical symptoms and objective parameters were not improved. Selection criteria was confirmed by an independent committee for assessment and evaluation of clinical gene therapy at Osaka University, which was approved by the Ministry of Welfare and the Ministry of Education (Science and Culture). After confirmation, 6 patients (5 male and 1 female, aged 57.8±4.5 years) with arteriosclerosis obliterans (ASO; n=3) or Buerger disease (n=3) graded as Fontaine III or IV underwent direct intramuscular gene transfer of naked plasmid DNA encoding HGF. The characteristics of the patients are shown in Table 1.

Intramuscular Injection of Naked Plasmid DNA Encoding Human HGF

Each patient received an intramuscular injection of naked plasmid DNA encoding HGF gene regulated by the cytomegalovirus promoter/enhancer. The preparation and purification of the plasmid from cultures of HGF-transformed E coli was performed at Qiagen, Inc using kanamycin. The purified plasmid was transported to the human gene therapy laboratory at Osaka University Medical School and stored in vials at −20°C. Some of them were pooled for quality control analysis and sequence check. No vectors were used to deliver plasmid.

First, test intramuscular injection of small dose (test injection; 0.4 mg plasmid DNA) was performed to examine acute or subacute allergy to plasmid DNA (Table 1, available online at http://www.hypertensionaha.org). After confirmation of no allergic reaction or anaphylaxis, a therapeutic dose (2 mg) of naked HGF plasmid DNA was intramuscularly injected 2 weeks after test injection. Five hundred micrograms of HGF plasmid DNA was diluted in a sterile saline solution up to 3 mL, and 4 aliquots (total 2000 μg/12 mL) were administered into the calf or distal thigh muscles of the ischemic limbs by direct intramuscular injection under echosonographic guidance. Four injection sites were selected arbitrarily, according to the angiographic findings and the available muscle mass. The injection sites of plasmid DNA are summarized in Figure 1. Four weeks after the initial injection, a second injection (2000 μg) was similarly administered, giving a total dose of 4000 mg plasmid DNA per patient.

Patient Follow-Up and Assessment

Patients were followed-up by physical examination (including change in ischemic ulcers), blood analysis, visual analog scale (pain scale), SF-36 (quality-of-life scale), transcutaneous PO2 (TcPO2), and measurements of ABI and toe pressure index (TPI) weekly during the first 12 weeks, every other week for the next 8 weeks, monthly for the next 12 weeks, and every 3 months up to 2 years after the first injection of plasmid DNA (Table 1). All patients received usual and full drug therapy for peripheral arterial disease including antplatelet drugs, which were not changed throughout this trial. Ischemic ulcers and necrotic lesions were managed in a standard fashion, and antibiotics were prescribed as necessary. ABI and TPI were measured using the toe plethysmographic wave or Doppler wave with an IMEX (GETS Brothers). TcPO2 was calculated before and after supply of O2 (5 L/min×5 minutes) (Sumitomo-modenkko Hitche Inc). Intravascular digital subtraction angiography, magnetic resonance angiography, and, if possible, computerized tomographic angiography were performed within 1 month before the first injection and 4 weeks after each treatment and 3 months after.
the final intramuscular injection. To allow a precise comparison between the intra-arterial angiographic findings that were observed before and after gene therapy, meticulous attention was paid to the volume of contrast used and the timing of image acquisition to be certain that comparison of identical phases was made. To evaluate angiographic improvement fairly and precisely, the contrasted untreated leg was used as a control reference. Rest pain was evaluated objectively by a visual analog scale. During the study period, the development of malignant tumors and progression of retinopathy were carefully investigated by various examinations. All of the values were checked by the Evaluation Committee without the trial investigators to avoid bias.

ELISA was performed at baseline and weekly for up to 12 weeks after the initial treatment. Antecubital venous blood was taken during the morning, 7:00 to 9:00 AM, after an overnight fast. Serum was immediately separated by centrifugation at 4°C and stored at −20°C until assay. Serum HGF concentration was assayed using a recently developed enzyme immunoassay for use in humans.20,21

Statistical Analysis
All values are expressed as mean ± SEM. ANOVA with subsequent Bonferroni test was used to determine the significance of differences in multiple comparisons. Values of P<0.05 were considered statistically significant.

TABLE 2. Results of HGF Gene Therapy

<table>
<thead>
<tr>
<th>No.</th>
<th>Severe Complication</th>
<th>ABI</th>
<th>Pain Scale</th>
<th>Limb Status</th>
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<tr>
<td>1</td>
<td>None</td>
<td>0.50 → 0.63</td>
<td>6.0 → 5.5</td>
<td>Reduction of rest pain reduction</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>—</td>
<td>8.0 → 6.0</td>
<td>Reduction of rest pain reduction</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>0.29 → 0.42</td>
<td>6.0 → 4.0</td>
<td>Reduction of rest pain reduction</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>0.53 → 0.66</td>
<td>8.0 → 7.0</td>
<td>Reduction of rest pain reduction</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>0.35 → 0.67</td>
<td>6.0 → 0.5</td>
<td>Reduction of rest pain reduction</td>
</tr>
<tr>
<td>6</td>
<td>Cerebral infarction (Catheter-induced)</td>
<td>0.46 → 0.60</td>
<td>7.0 → 2.0</td>
<td>Reduction of rest pain reduction</td>
</tr>
</tbody>
</table>

Results
Safety Evaluation
We initially evaluated the safety of HGF gene therapy. In particular, we focused on the (1) allergic reaction against plasmid DNA, (2) incidence of angiogenesis-related disease such as tumor, and (3) other severe complications. To identify the allergic reaction, we used the test injection of a small amount of plasmid DNA. However, none of the test initial and second therapeutic injection of human HGF plasmid DNA induced an allergic or anaphylactic reaction. Throughout the gene therapy periods, there were no signs of systemic or local inflammatory reactions. No critical side effects related to gene therapy were seen. To date, development of tumors or progression of diabetic retinopathy has not been observed in any patient transfected with HGF plasmid DNA during the trial. Two-month follow-up studies showed no evidence of the development of neoplasm or hemangioma. In addition, no significant increase in serum HGF concentration was observed throughout the gene therapy periods (Figure 1). We also measured the plasma level of plasmid HGF DNA. As expected, at 1 week after transfection plasmid DNA could not be detected in the plasma, whereas at 1 day after transfection, low level of plasmid DNA could be detected by polymerase chain reaction. Although 1 patient developed signs of cerebral infarction immediately after angiography during the trial period (Table 2), the committee determined that this incident was related to the catheter at angiography, and there was no relationship with the gene therapy. To date, no change in visual acuity has been observed in any patient treated with plasmid HGF gene transfer. It is noteworthy that no edema has been observed in this trial, although transient lower-extremity edema was reported with clinical gene therapy using the VEGF gene because of an increase in vascular permeability.

Efficacy of HGF Gene Therapy
The efficacy of angiogenesis induced by plasmid DNA was also evaluated, although the patient number was small in this open-labeled trial. Unfortunately, it is, in fact, hard to detect distinct angiogenesis because the technique of angiography cannot visualize vessels <200 μm. Nevertheless, the improvement in digital subtraction angiography (DSA) findings...
was shown in 2 of 6 treated ischemic limbs. Figure 2 demonstrates DSA findings in a patient with Buerger disease (No. 3). A large vessel, which is indicated by an arrow, was newly observed (Figure 2a to 2c). Although it is not clear whether this vessel was new or not, it is possible that an increase of new microvessels led to the recanalization. This recanalization was also confirmed by serial magnetic resonance angiograms (data not shown). DSA of another patient with Buerger disease (No. 5) showed a marked increase in peripheral blood flow and formation of new blood vessels (Figure 3a and 3b).

To evaluate the functional improvement of HGF gene therapy, we also measured ABI during gene therapy. Although ABI could not be measured in 1 patient because of uncompressible severely calcified vessels, ABI was significantly increased from 0.426±0.046 (n=5) at baseline (before administration) to 0.626±0.071 (P=0.0155; n=5) at 4 weeks after the second injection and to 0.596±0.046 (P=0.0360; n=5) at 8 weeks after the second injection (Figure 4). The absolute value of systolic ankle pressure was significantly increased in 5 limbs after gene transfer, whereas ankle pressures of untreated limbs were not significantly changed (data not shown). Also, TPI, which could be measured only in 2 patients (Nos. 1 and 3), tend to be increased (data not shown), accompanied by an improvement of ABI. However, TPI was not measured in 4 patients (No. 2, 4, 5, and 6) because of ischemic ulcers on the great toes of their ischemic legs. When an increase in ABI of >0.1 was assumed to be an improvement, according to the standard of Rutherford, 5 of 5 patients (100%) showed a positive response. In addition, transcutaneous PO$_2$ is an indicator of the effectiveness in terms of angiogenesis and increase in blood supply in targeted ischemic lesions, we also measured TcPO$_2$. As shown in Table 3, the change in TcPO$_2$ after O$_2$ stimulation was significantly increased at 8 weeks compared with baseline (P<0.05).

To evaluate the effects of HGF gene therapy in clinical symptoms, we used the change in the ischemic ulcer and visual analogue scale. In this trial, a total of 11 ischemic ulcers were found in 4 patients. As shown in Figure 5, 2 of 11 ulcers completely disappeared. Considering an improvement of ischemic ulcers of more than 25% to be evaluated as positive, 8 of 11 ulcers (72%) improved. Typical examples of

TABLE 3. Transition of TcPO$_2$

<table>
<thead>
<tr>
<th>Week</th>
<th>0 Week</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre O$_2$</td>
<td>58.7±2.8</td>
<td>60.2±5.9</td>
<td>45.8±11.3</td>
<td>68.3±8.0</td>
</tr>
<tr>
<td>Post O$_2$</td>
<td>80.3±6.7</td>
<td>83.2±5.4</td>
<td>69.3±17.4</td>
<td>99.5±13.4*</td>
</tr>
<tr>
<td>Δ (Post-pre)</td>
<td>21.7±4.5</td>
<td>23.0±3.7</td>
<td>23.5±9.0</td>
<td>31.2±6.4*</td>
</tr>
</tbody>
</table>

*P<0.05 vs 0 week.
the change in ischemic ulcers in patients with Buerger disease (No. 3 and 5) are shown in Figure 6. Three of 4 patients demonstrated an improvement of the maximum ischemic ulcer diameter of >25% (efficacy rate 75%). Also, we evaluated resting pain using a visual analog scale, as a standard method for the evaluation of pain, where 0.0 cm means “pain free” or no pain, and 10 cm means more severe pain. As shown in Figure 7, pain was significantly improved in a time-dependent manner.

Discussion

The natural history of critical limb ischemia has been well documented to have an inexorable downhill course.1 The inclusion criteria for this study were drafted to restrict treatment to patients in whom the natural history of critical limb ischemia had been established previously. Especially, this trial excluded patients who demonstrated an improvement of clinical symptoms after hospitalization and pharmacological treatment, to identify the effects of HGF gene therapy on clinical symptoms. In this series of 6 patients, 6 developed critical limb ischemia despite having undergone many vascular surgical reconstructions. Furthermore, because HGF had not been administered previously as recombinant protein or gene therapy, no data were available from any source to indicate either the safety or bioactivity of any dose of plasmid human HGF DNA. Accordingly, the design of this phase I/early phase IIa trial, approved by the Japanese Government, was conducted as a nonrandomized consecutive treatment series based on the animal experiments.15–18,22–24 The previous articles reported the detection of human HGF protein in mouse experiments (control; not detected, HGF: 1.05 ± 0.08 ng/g tissue), rat experiments (control; not detected, HGF: 1.95 ± 0.42 ng/g tissue15,24), and rabbit experiments (control; not detected, HGF: 2.08 ± 0.39 ng/g tissue15,24).

In all patients, treatment was shown to achieve clinically significant modulation of the recipient phenotype. Noninvasive studies documented hemodynamic evidence of improved limb perfusion that satisfied the outcome criteria proposed to assess the results of surgical reconstruction or percutaneous revascularization.25 Absolute ankle pressure increased in 5 limbs after gene therapy. ABI increased from 0.426 ± 0.046 at baseline to 0.596 ± 0.046 at 12 weeks after the first injection. To put this in perspective, an increase in ABI of >0.1 is considered indicative of a successful surgical or percutaneous intervention.25 This increase in ABI was similar to the results of VEGF trials.5,6 To our knowledge, such an improvement has not previously been achieved spontaneously or with medical therapy in patients with critical limb ischemia.

Figure 5. Change in size of ischemic ulcers in 4 limbs after intramuscular injection of HGF plasmid DNA. Baseline indicates 4 weeks before test injection; Pre, just before test injection; 4 weeks, 4 weeks after first injection (just before second injection); 8 weeks, 8 weeks after first injection (4 weeks after second injection); 12 weeks, 12 weeks after first injection (8 weeks after second injection). *P<0.05 vs baseline.

Figure 6. Typical examples of change in ischemic ulcers in patients with Buerger disease (patient Nos. 3 and 5). Pre-GT indicates 4 weeks before test injection; 8 weeks, 8 weeks after first injection (4 weeks after second injection).

Figure 7. Reduction of visual analog scale after intramuscular injection of HGF plasmid DNA. Baseline indicates 4 weeks before test injection; Pre, just before test injection; 4 weeks, 4 weeks after first injection (just before second injection); 8 weeks, 8 weeks after first injection (4 weeks after second injection); 12 weeks, 12 weeks after first injection (8 weeks after second injection). *P<0.05 vs baseline.
Similarly, angiographic demonstration of newly visible collateral vessels, accompanied here by noninvasive evidence of improved blood flow, confirmed these improvements, although the quantification of angiograms is quite difficult. Indeed, previous reports have indicated that current methods used to perform diagnostic contrast angiography cannot provide images of arteries measuring <200 μm in diameter.26 The conventional angiographic techniques used in this study may have failed to depict the full extent of angiogenesis achieved after HGF gene transfer. Rest pain was significantly improved from baseline (before treatment) to 12 weeks after transfection as assessed by visual analog scale. Ischemic ulcers healed or improved markedly by >25% in 3 of 4 patients; this included 3 patients recommended for below-knee amputation in whom successful limb salvage was achieved. Given the poor prognosis for patients with chronic critical limb ischemia, in whom the possibility of spontaneous improvement is remote,1 the outcome in this initial cohort is thus encouraging. Nevertheless, the present study cannot fully eliminate the placebo effects. Further studies in a randomized placebo-double blinded manner are necessary to demonstrate the effectiveness of HGF gene therapy.

Unlike previous reports using VEGF gene showing that VEGF protein level demonstrated a transient peak in the systemic circulation 1 to 3 weeks after gene transfer, our trial did not demonstrate an increase in serum HGF concentration during gene therapy. It is noteworthy that there was no evidence of edema in the patients transfected with the human HGF gene, in marked contrast to a VEGF trial in which 60% of patients developed moderate or severe edema in a phase II/IIa trial. What is the difference between HGF and VEGF? We believe that one of the distinguishing features of HGF is that it stimulates the migration of vascular smooth muscle cells (VSMCs) without the replication of VSMCs, whereas VEGF does not stimulate either the migration or proliferation of VSMCs because of its lack of receptors in VSMCs. The initial event in angiogenesis induced by VEGF is the migration of endothelial cells, leading to the sprouting of blood vessels. Later, the migration of VSMCs occurs because of the release of platelet-derived growth factor, followed by the migration of endothelial cells. However, a delay in the maturation of blood vessels might exist in the case of angiogenesis induced by VEGF. In contrast, HGF simultaneously stimulated the migration of both endothelial cells and VSMCs. Thus, the blood vessels may mature at an earlier time point, thereby avoiding the release of blood-derived cells into the extracellular space, although further studies might be necessary to examine the angiogenic properties of various angiogenic growth factors including HGF, VEGF, and fibroblast growth factor.

Regarding the safety of HGF gene therapy, previous work established that transgene expression using plasmid DNA is limited to <30 days in animal models of limb ischemia.27,28 In contrast, it appears that in both animals and humans, collateral vessel development sufficient to restore limb perfusion to satisfactory resting levels occurs within this time interval. The cessation of gene expression beyond this time point can be considered to constitute an inherent safety feature of HGF gene transfer that protects the organism from indefinite constitutive expression of an angiogenic growth factor. In addition, the circulating level of HGF is elevated in patients with hypertension, peripheral arterial disease, and myocardial infarction,21,29,30 although the present study demonstrated no increase in serum HGF concentration during gene therapy. Potential side effects, such as cancer or diabetic retinopathy due to an increase in plasma HGF secreted from transfected cells, would be minimized in gene therapy using intramuscular injection of HGF into ischemic muscle. Nevertheless, these findings are preliminary and do not establish the long-term safety of HGF. Clearly, further clinical studies of alternative dosing regimens of gene therapy with randomized placebo-controlled trials will be required to define the efficacy of this therapy.

Acknowledgments
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**Figure I.** Injection of human HGF plasmid into ischemic hindlimb.

Red cross demonstrates the first injection, and blue cross demonstrates the second injection of human HGF plasmid DNA.

**Table I.** Schedule of Examinations for Year 2.
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<thead>
<tr>
<th>TABLE I.</th>
<th>Preliminary injection (0.4mg)</th>
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<th>2mg</th>
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<td>Pre observation period</td>
<td>Observation period</td>
<td>Week</td>
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<td>3</td>
<td>1</td>
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<td>Brain magnetic resonance imaging</td>
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<td>Ankle-brachial index/Toe pressure index</td>
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<td>Ophthalmologic examination</td>
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<td>Stomach endoscope</td>
<td>○</td>
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※ : Evaluation
Fig. 1a

A) Arterial blood flow of the lower extremities receiving gene therapy

B) Arterial blood flow in healthy individuals

Case 10101

Case 10102

Case 10103

- X: Vascular occlusion site
- 1st dose
- 2nd dose
- Site of angiography
Fig. Ib

A) Arterial blood flow of the lower extremities receiving gene therapy

B) Arterial blood flow in healthy individuals

A) Vascular occlusion site

× 1st dose
× 2nd dose

Site of angiography

Case 10104

A) Vascular occlusion site
× 1st and 2nd doses (same sites)

Site of angiography

Case 10105

A) Vascular occlusion site

Case 10106