therefore, is an essential requirement to develop more efficient and safer DES in the future.

Platelet-derived growth factor (PDGF), expressed by VSMC, plays a central role in the pathogenesis of restenosis. Mechanical forces, such as stent-induced overstretch, stimulate VSMC expression and release of PDGF in animals[7, 8] and humans[9, 10]. Imatinib mesylate is an inhibitor for c-Abl tyrosine kinase, c-Kit receptor kinase, and PDGF receptor tyrosine kinase[11, 12] and is approved for the treatment of patients with chronic myeloid leukemia. It has been shown that c-Kit-positive progenitor cells can differentiate into α-actin-positive VSMCs and may contribute to neointima formation[13]. It has also been reported that c-Abl tyrosine kinase is involved in angiotensin II-induced VSMC hypertrophy[14]. Imatinib is reported to be a significantly more potent inhibitor of VSMC proliferation than other inhibitors of PDGF receptor (AGL-2043), with $IC_{50} < 10$ nM[15]. In contrast, imatinib has little effect on vascular endothelial cell growth factor receptor tyrosine kinase or endothelial cell proliferation[15]. These data provide a rationale for the use of imatinib mesylate in the prevention of neointima formation associated with in-stent restenosis as a VSMC-specific molecular-targeting drug.

Prior studies have reported that systemic oral administration of imatinib inhibited balloon injury-induced neointima formation in rats[11] when dosages beyond the clinical norm were used (50 mg/kg per day). In contrast, imatinib had no effect on in-stent neointima formation in rabbits when administered at a clinically relevant dosage (10 mg/kg per day)[16]. Recent clinical studies in humans have detected no beneficial effects of the oral administration of imatinib (600 mg/day for 10 days)[17] on in-stent restenosis. These data suggest that systemic administration of imatinib at clinical dosages may not be sufficient to antagonize PDGF-induced vascular responses. Furthermore, it was reported the polymer-coated stents with imatinib (600 μg/stent) had no effect on neointima formation in a porcine coronary in-stent stenosis model[15]. This was probably because of unsuitable release characteristics of imatinib from polymer-coated stents. It is suggested that the present polymer coating DES technology is not useful for coating water-soluble drugs such as imatinib. Therefore, preventing in-stent restenosis via imatinib-mediated PDGF-R signaling blockade requires a new efficient drug delivery system. We previously succeeded in developing bioabsorbable polymeric nanoparticles (NP) formulated from the polymer poly (DL-lactide-co-glycolide) (PLGA)[18], and in formulating a NP-eluting stent by cation electrodeposition coating technology[19]. This NP-eluting stent system provided an effective means of delivering NP-incorporated drugs or genes that target intracellular proteins involved in the pathogenesis of in-stent neointima formation.

Therefore, we hypothesized that imatinib-NP-eluting stent can be an innovative therapeutic strategy for preventing in-stent neointima formation *in vivo*. We used a porcine coronary artery in-stent stenosis model and investigated whether imatinib-NP-eluting stent attenuates in-stent neointima formation without adverse effects on arterial healing processes *in vivo*.

Materials and Methods

Vascular Smooth Muscle Cell Proliferation Assay

Human coronary artery VSMCs (Lonza, Walkersville, MD, USA) were cultured and placed into 48-well culture plates (5000 cells per well; BD). Proliferation was stimulated by the addition of PDGF at 10 ng/mL (Sigma, Tokyo, Japan)[20]. Various concentration of imatinib (Novartis Pharma) at 0.1, 1, and 10 μM, imatinib-loaded PLGA NP (PLGA at 0.5 mg/mL containing imatinib at 10 μM), or vehicle alone was added to the wells, and four days later, the cells were fixed with methanol and a single observer counted the number of cells/plate.

Endothelial Cell Proliferation Assay

Human umbilical vein endothelial cells (HUVEC) were obtained, cultured, and used between passages 4 to 8[21]. Recombinant human VEGF165 (10 ng/mL; R&D) or PDGF at 10 ng/mL was added to the basal medium, and cells (7500 cells per well) were incubated in the presence or absence of imatinib, imatinib-NP, or vehicle for 4 days in 48-well culture plates. Cell count assay was performed as stated above.

Preparation of NP-Eluting Stents by Cationic Electrodeposition Coating Technology

A lactide/glycolide copolymer (PLGA) with an average molecular weight of 20,000 and a lactide to glycolide copolymer ratio of 75:25 (PLGA7520; Wako Pure Chemical Industries, Osaka, Japan) was used as wall material for the NP. Chitosan was used to coat the surface of PLGA NP. Polyvinylalcohol (PVA-403; Kuraray, Osaka, Japan) was used as a dispersing agent. PLGA NP incorporated with the fluorescent marker fluorescein isothiocyanate (FITC; Dojindo laboratories, Kumamoto, Japan) or with imatinib (purchased from a pharmacy) were prepared by a previously reported emulsion solvent diffusion method in purified water[19, 22, 23].

The mean particle size was analyzed by the light

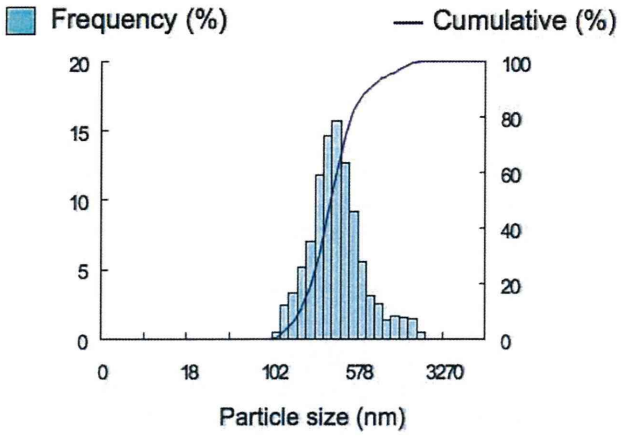

Fig. 1. Particle size distribution of imatinib-incorporated PLGA nanoparticles in water.

scattering method (Microtrack UPA150; Nikkiso, Tokyo, Japan). A sample of nanoparticulate suspension in distilled water was used for particle size analysis. The average diameter of FITC- and imatinib-incorporated NP was about 200 nm. Size distribution was similar between FITC-NP and imatinib-NP (see **Fig. 1**). FITC- and imatinib-encapsulated PLGA NP contained 5.0% (w/w) FITC and 8.3% (w/w) imatinib, respectively. The zeta potential of the NP as measured by Zetasizer Nano Z (Malverm, America) was +6.7 and +10.0 mV, respectively.

The 16 mm-long stainless-steel, balloon-expandable stents (Multilink) were ultrasonically cleaned in acetone, ethanol, and demineralized water. The cationic electrodeposited coating was prepared on cathodic stents in NP solution at a concentration of 5 g/L in distilled water with a current maintained between 2.0 and 10.0 mA by a direct current power supply (DC power supply; Nippon Stabilizer Co, Tokyo, Japan) for different periods under sterile conditions[19]. The coated stents were then rinsed with demineralized water and dried under a vacuum overnight. This electrodeposition coating procedure produced a coating of approximately 250 ± 40 μg of the polymer NP per stent and 21 ± 8 μg of imatinib per stent (n=12). The surface of some NP-coating stents were observed with scanning electron microscopy (JXM8600; JEOL, Tokyo, Japan).

Prior to experimental use, non-coated bare metal and NP-coated stents were mounted mechanically over the 3-mm balloon for implantation in the coronary artery. These balloon-mounted stent sets were sterilized using ethylene oxide.

Animal Preparation and Stent Implantation

All *in vivo* experiments were reviewed and approved by the Committee on Ethics in Animal Experiments, Kyushu University Faculty of Medicine, according to the Guidelines of the American Physiological Society. This study also conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Domestic male pigs (Kyudo, Tosu, Japan; aged 2 to 3 months and weighing 25 to 30 kg) received oral aspirin (330 mg/day) and ticlopidine (200 mg/day) until euthanasia from 3 days before the stent implantation procedure. Animals were anesthetized with ketamine hydrochloride (15 mg/kg, IM) and pentobarbital (20 mg/kg, IV). They were then intubated and mechanically ventilated with room air. A preshaped Judkins catheter was inserted into the carotid artery and advanced to the orifice of the left coronary artery. After systemic heparinization (100 IU/kg) and intracoronary administration of nitroglycerin, coronary angiography of the left coronary artery was performed using contrast media (iopamidol 370®) in a left oblique view with an angiography system (Toshiba Medical, Tokyo, Japan). Animals were divided into 3 groups, which underwent deployment of either a non-coated bare metal stent (2 week; n=4 for Western blot analysis, 4 week; n=10 for angiographic, histopathological and intravascular ultrasound analyses), FITC-incorporated NP-eluting stents (4 week; n=10 for angiographic, histopathological and intravascular ultrasound analyses), or imatinib-incorporated NP-eluting stents (2 week; n=4 for Western blot analysis, 4 week; n=10 for angiographic, histopathological and intravascular ultrasound analyses), to either the left anterior descending (LAD) or the left circumflex coronary (LCx) arteries.

A segment with a mean coronary diameter of 2.5 mm was selected by using quantitative coronary angiography (Toshiba Medical, Tokyo, Japan) with a stent-to-artery ratio of approximately 1.1 to 1.2 (**Table 1**). A balloon catheter mounted with a stent was then advanced to the pre-selected coronary segments for deployment over a standard guidewire. The balloon catheter was inflated at 12 atm for 60 seconds once and thereafter deflated, and was then slowly withdrawn, leaving the stent in place.

Quantitative coronary angiography (Toshiba Medical, Tokyo, Japan) was performed before, immediately after, and 4 weeks after stent implantation to examine the coronary arterial diameter at stented and non-stented sites. An image of a Judkins catheter was used as the reference diameter. Arterial pressure, heart

rate, and ECG were continuously monitored and recorded on a recorder.

Intravascular Ultrasound

Intravascular ultrasound imaging (IVUS) was performed to assess the extent of neointima formation *in vivo* 4 weeks after stent implantation. Imaging was performed using a 40 MHz ultrasonic imaging catheter (Ultra cross; Boston Scientific, Boston, USA) and an automatic pullback device, and the studies were recorded on 1/2-inch high-resolution s-VHS tapes for off-line volumetric assessment. Because of the limited availability of IVUS probes, IVUS was performed 7 and 8 pigs in FITC-NP and imatinib-NP stent groups, respectively.

Histopathological Study

Four weeks after the coronary angiographic study, animals were euthanized with a lethal dose of sodium pentobarbital (40 mg/kg intravenously), and histological analysis was performed. The left coronary artery was perfused with 10% buffered formalin at 120 mm Hg and fixed for 24 hours. The stented artery segments were isolated and processed as described previously[24]: The segment was cut at the center of the stent and embedded in methyl methacrylate mixed with n-butyl methacrylate to allow for sectioning through the metal stent struts. Serial sections were stained with elastica van Gieson and with hematoxylin-eosin (HE). The neointimal area, the area within the internal elastic lamina (IEL), and the lumen area were measured by computerized morphometry, which was carried out by a single observer who was blinded to the experimental protocol. All images were captured by an Olympus microscope equipped with a digital camera (HC-2500) and were analyzed using Adobe Photoshop 6.0 and Scion Image 1.62 Software. The injury, inflammation, and re-endothelialization scores were determined at each strut site, and mean values were calculated for each stented segment[25].

Western Blot Analysis

For *in vitro* study, protein was extracted from cultured VSMC, and protein expression was analyzed using antibodies against human PDGF receptor-β (0.1 mg/mL; R&D Systems Inc.), phospho-PDGF receptor-β (0.5 mg/mL; R&D), or anti-actin (Sigma).

For *in vivo* study, animals were euthanized with a lethal dose of sodium pentobarbital (40 mg/kg intravenously) two weeks after stent implantation when the neointima was modestly formed, and Western blot analysis was performed. Protein was extracted from frozen arterial tissues excised from stented coronary arterial segments (LAD or LCx) and non-stented normal coronary arterial segments (right coronary artery). Cell extracts (20 μg) were resolved on 10% reducing SDS-PAGE gels and blotted onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Protein expression was analyzed using antibodies against MAP kinase (ERK1/2) (0.5 mg/mL; R&D Systems Inc.), phospho-ERK1/2 (1:2000; Cell Signaling), or anti-actin (Sigma). Immune complexes were visualized with horseradish peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL) using the ECL Plus system (Amersham Biosciences). Western blot analysis was performed with sequential antibodies and was detected with the ECL Detection Kit (Amersham).

Statistical Analysis

Data are expressed as the means ± SE. Statistical analysis of differences between two groups was performed with the unpaired t-test, and the statistical analysis of differences among three or more groups was assessed using ANOVA and multiple comparison tests. P values < 0.05 were considered significant.

Results

In vitro Effects of Imatinib on Proliferation of Vascular Smooth Muscle Cells and Endothelial Cells

We previously reported (1) the *in vitro* time course of FITC release from FITC-incorporated NP, and (2) highly efficient and stable delivery of NP into the cytoplasm of SMC and endothelial cells[19, 21, 26, 27]. In the present study, we examined *in vitro* effects of imatinib and imatinib-NP. As reported by others[15], imatinib attenuated the PDGF-induced proliferation of human coronary arterial SMC in a dose-dependent manner (**Fig. 2A**). Imatinib-NP also prevented the PDGF-induced responses of SMC. Western blot analysis showed that in human coronary artery SMC, both imatinib and imatinib-NP inhibited PDGF-induced phosphorylation of PDGF receptor-β in a dose-dependent manner (**Fig. 2B**). In contrast, neither imatinib nor imatinib-NP affected VEGF- and PDGF-induced proliferation of human endothelial cells (**Fig. 2C**).

Effects of Imatinib-NP-Eluting Stent on Neointima Formation 4 Weeks After Stent Implantation

Three animals (2 in control bare metal stent group and 1 in FITC-NP-eluting stent group) died suddenly between weeks 3 and 4; therefore, these animals were excluded from angiographic and histopathological analyses. These analyses were performed in 27

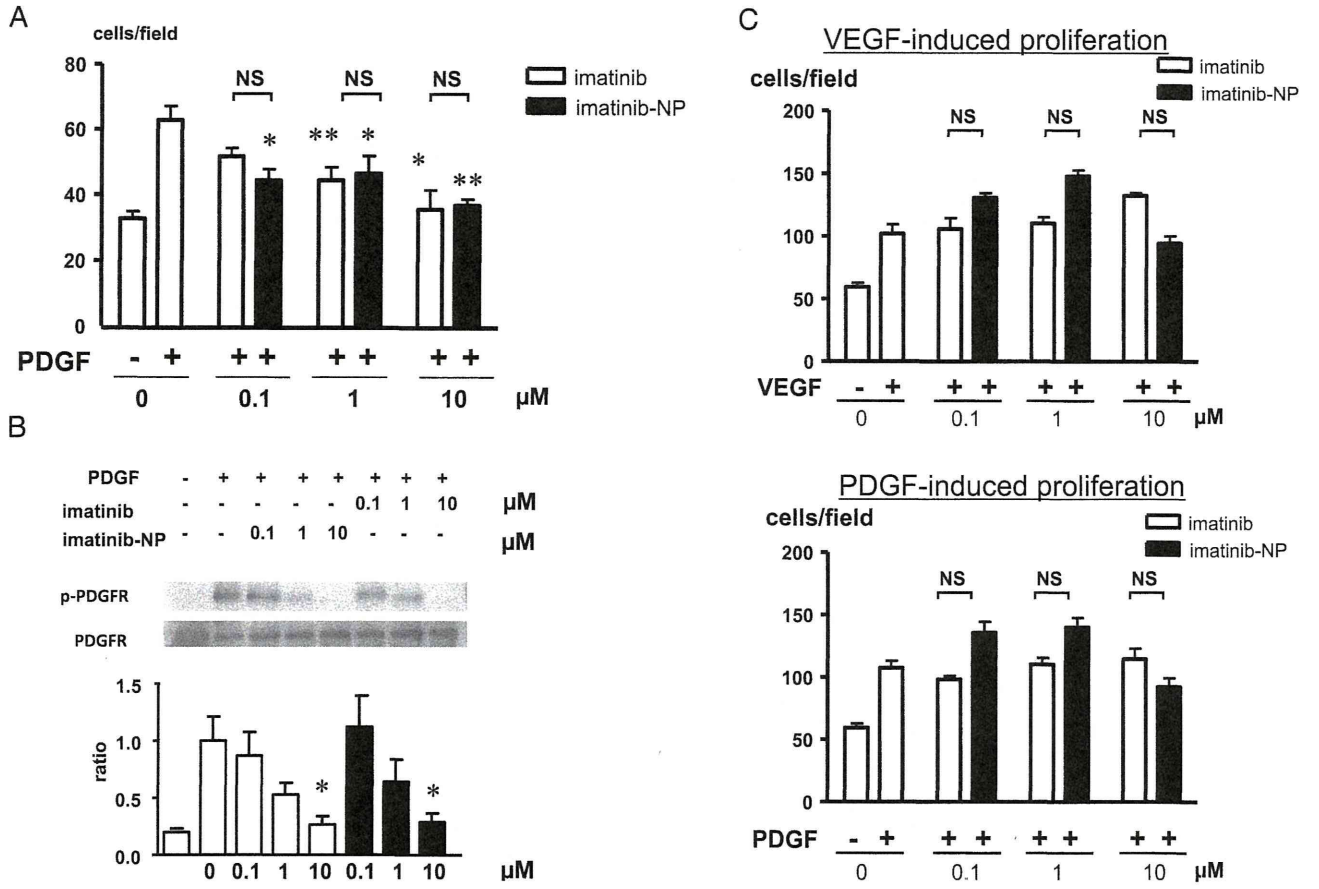

Fig. 2.

A, Effects of imatinib and imatinib-NP on PDGF-induced proliferation of human coronary artery SMCs. Data are the mean ± SEM ($n = 8$ each). *$p < 0.001$ versus PDGF-induced response by two-way ANOVA and Dunnett's multiple comparison tests.

B, Effects of imatinib and imatinib-NP on PDGF-induced activation (phosphorylation) of PDGF receptor-β in human coronary artery SMC. Densitometric analysis of protein expression (p-PDGFR/PDGFR ratio) is also shown as a bar graph ($n = 5$ each). *$p < 0.05$ versus the PDGF-induced response by one-way ANOVA and Dunnett's multiple comparison tests.

C, Effect of imatinib on the VEGF- and PDGF-induced proliferation of human umbilical vein endothelial cells ($n = 8$).

Table 1. Coronary artery diameter before, immediately after, and 4 weeks after stent implantation in porcine coronary artery

	Bare metal control stent ($n = 8$)	FITC-NP-eluting stent ($n = 9$)	imatinib-NP-eluting stent ($n = 10$)	p value
coronary diameter before stent implantation	2.21 ± 0.06	2.25 ± 0.05	2.32 ± 0.05	0.33
coronary diameter immediately after stent implantation	2.63 ± 0.08	2.65 ± 0.05	2.70 ± 0.04	0.70
stent-to-artery ratio immediately after stent implantation	1.19 ± 0.03	1.18 ± 0.03	1.17 ± 0.02	0.74
coronary diameter 4 weeks after stent implantation	1.48 ± 0.14	1.49 ± 0.12	2.06 ± 0.16	0.009

Data are the mean ± SEM. *$p < 0.01$ versus coronary diameter before stent implantation, †$p < 0.01$ versus coronary diameter immediately after stent implantation, #$p < 0.01$ versus bare metal control stent group.

pigs (8 in control bare metal stent group, 9 in FITC-NP-eluting stent group, and 10 in imatinib-NP eluting stent group).

Quantitative coronary arteriography revealed that (1) there was no significant difference in the coronary diameter before and immediately after stent implantation and the stent-to-artery ratio among the 3 groups; and (2) the coronary diameter was less in

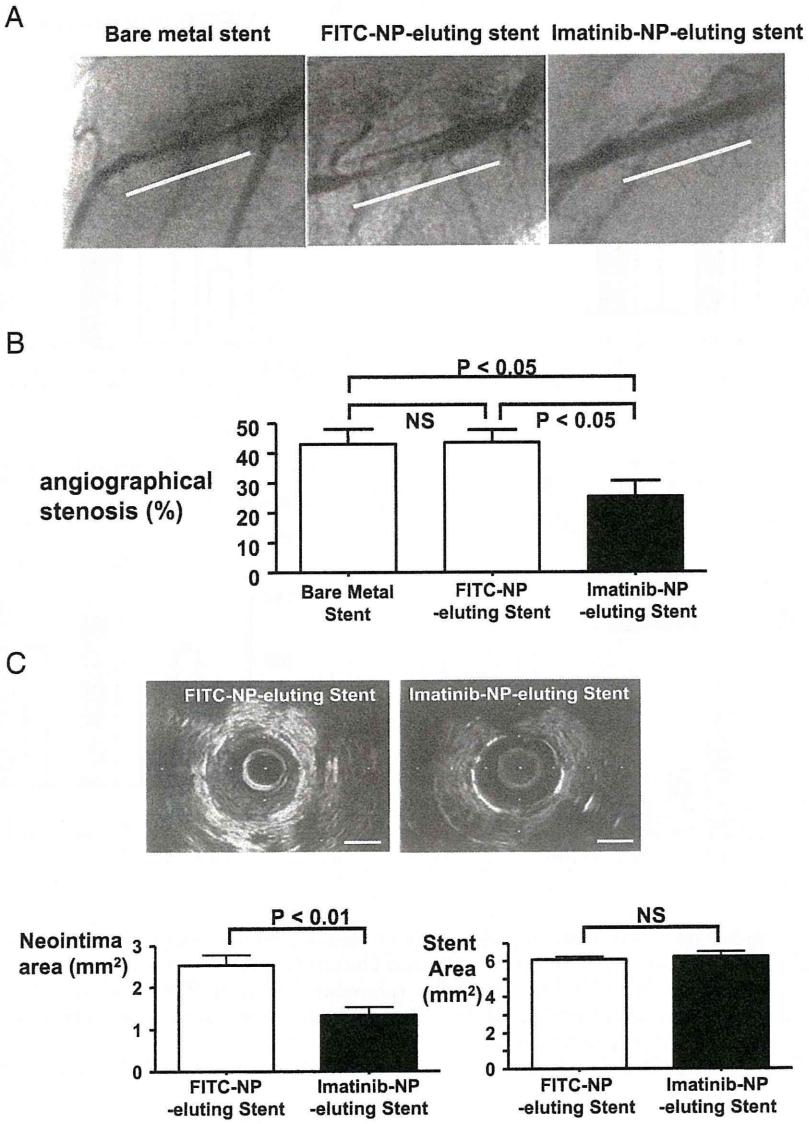

Fig. 3. Coronary arteriography and in-stent stenosis 4 weeks after stent implantation.

A, Representative coronary arteriographic images of stented segments in the left anterior descending coronary artery in bare metal, FITC-NP-eluting, and imatinib-NP-eluting stent groups. White bars in the images denote stented segments.

B, Angiographically-examined in-stent stenosis in bare metal ($n=8$), FITC-NP-eluting ($n=9$), and imatinib-NP-eluting ($n=10$) stent groups.

C, Intravascular ultrasound cross-section images and the summary of neointima formation (neointima area and stent area) in FITC-NP-eluting ($n=7$) and imatinib-NP-eluting ($n=8$) stent groups. Bar=1 mm.

the control bare metal and FITC-NP-eluting stent sites than in the imatinib-NP-eluting stent sites 4 weeks after stenting (**Table 1**). Thus, angiographically, in-stent stenosis was less in the imatinib-NP group than in the control and FITC-NP group (**Fig. 3A and B**).

Intravascular ultrasound imaging (IVUS) could be performed in FITC-NP ($n=7$) and imatinib-NP stent ($n=8$) groups, which demonstrated that the extent of neointima formation was significantly less at the imatinib-NP stent site than at the FITC-NP-stent site (**Fig. 3C**).

Histological analysis demonstrated that a signifi-

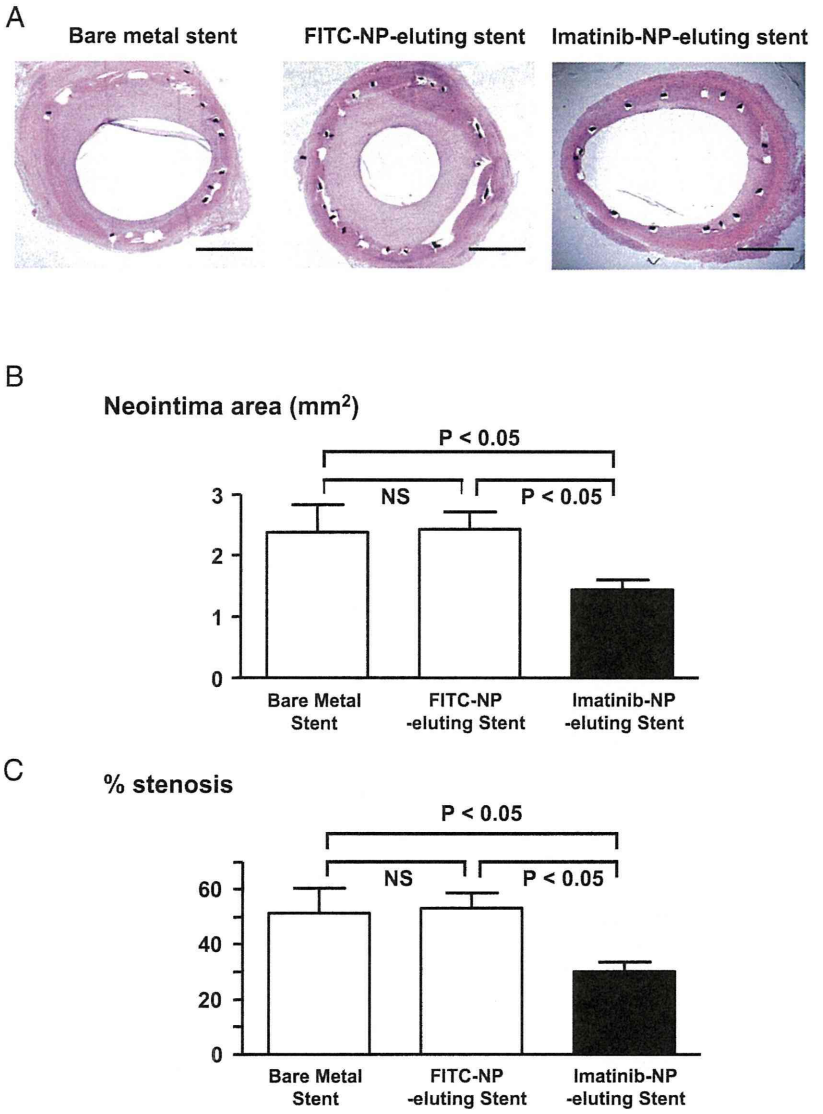

Fig. 4. Histopathological analysis of in-stent neointima formation 4 weeks after stent implantation.

A, Coronary artery cross-sections from the bare metal stent, FITC-NP-eluting stent, and the imatinib-NP-eluting stent groups. Tissue was stained with hematoxylin-eosin. Bar = 1 mm.

B, The neointima area at bare metal stents ($n = 8$), FITC-NP-eluting stents stents ($n = 9$), and the imatinib-NP-eluting stents ($n = 10$). NS = not significant. For statistical analysis, one-way ANOVA and Bonferroni's multiple comparison tests were performed.

C, The % stenosis [100 × (area of internal elastic lamia - neointima area)/area of internal elastic lamia] at bare metal stents, FITC-NP-eluting stents and the imatinib-NP-eluting stents. NS = not significant.

cant in-stent neointima formed similarly at the non-coated bare metal stent and FITC-NP-eluting stent sites. Quantitative analysis demonstrated a significant reduction in neointima formation at the imatinib-NP-eluting stent site (**Fig. 4**). In contrast, there were no significant differences in IEL and EEL areas among all 3 groups (**Table 2**). A semiquantitative histological scoring system demonstrated no significant difference in the injury score and inflammation score among the 3 groups (**Table 3**). Endothelial cell linings were

Table 2. Histopathological analysis of in-stent neointima formation 4 weeks after stent implantation in porcine coronary artery

	Bare metal control stent ($n=8$)	FITC-NP-eluting stent ($n=9$)	imatinib-NP-eluting stent ($n=10$)	p value
Area within the internal elastic lamina (IEL), mm^2	4.56 ± 0.11	4.54 ± 0.09	4.84 ± 0.14	0.13
Area within the external elastic lamina (EEL), mm^2	5.72 ± 0.18	5.76 ± 0.10	5.96 ± 0.14	0.40
Lumen area, mm^2	2.18 ± 0.38	2.11 ± 0.24	3.41 ± 0.23**	0.003

Data are the mean ± SEM. *$p<0.05$, **$p<0.01$ versus control bare metal stent.

Table 3. Re-endothelialization, injury score, and inflammation score 4 weeks after stenting

	Bare metal control stent ($n=8$)	FITC-NP-eluting stent ($n=9$)	imatinib-NP-eluting stent ($n=10$)	p value
Re-endothelialization score	3 ± 0	3 ± 0	3 ± 0	1.0
Injury score	1.75 ± 0.09	1.79 ± 0.09	1.88 ± 0.08	0.57
Inflammation score	1.70 ± 0.14	1.62 ± 0.08	1.75 ± 0.06	0.63

Data are the mean ± SEM.
The re-endothelialization score was defined as the extent of the circumference of the arterial lumen covered by endothelial cells and was scored from 1 to 3 (1=25%; 2=25% to 75%; 3 ≥75%)[23].
The injury score was determined at each strut site, and mean values were calculated for each stented segment[23]. In brief, a numeric value from 0 (no injury) to 3 (most injury) was assigned: 0=endothelial denudate, internal elastica lamina (IEL) intact; 1=IEL lacerated, media compressed, not lacerated; 2=IEL lacerated, media lacerated, external elastica lamina (EEL) compressed, not lacerated; and 3=media severely lacerated, EEL lacerated, adventitial may contain stent strut. The average injury score for each segment was calculated by dividing the sum of injury scores by the total number of struts in the examined section.
The inflammation score took into consideration the extent and density of the inflammatory infiltrate in each individual strut[23]. With regard to the inflammatory score for each individual strut, the grading is: 0=no inflammatory cells surrounding the strut; 1=light, noncircumferential inflammatory cells infiltrate surrounding the strut; 2=localized, moderate to dense cellular aggregate surrounding the strut noncircumferentially; and 3=circumferential dense inflammatory cells infiltration of the strut. The inflammatory score for each cross section was calculated in the same manner as for the injury score (sum of the individual inflammatory scores, divided by the number of struts in the examined section).

observed equally in the 3 groups (**Table 3**).

Effects of Imatinib-NP-Eluting Stent on Protein Expression of MAP Kinase *in vivo*

Western blot analysis was performed in another set of animals, which underwent deployment of both a bare metal stent and imatinib-NP-eluting stent to either LAD or LCx. On day 14 post-stenting, the neointima, and the media and adventitia were harvested. Protein expression of the phosphorylation of ERK was significantly less at the imatinib-NP-eluting stent site than at the bare metal stent site (**Fig. 5**). In contrast, no significant changes were found in phosphorylated ERK expression in the media and adventitia.

Discussion

We here report the first successful development of imatinib-NP-eluting stents with a newly invented cation electrodeposition coating technology. Importantly, this NP-mediated drug delivery platform is able to carry hydrophilic agents such as imatinib, which offers advantages over the current stent-coating technology. We here showed that (1) imatinib-NP caused the cell-specific targeting of VSMC proliferation associated with inhibition of the target molecules of imatinib (phosphorylation of PDGF receptor-β) *in vitro*; (2) imatinib-NP showed no negative effects on the proliferation of endothelial cells *in vitro*, and (3) imatinib-NP-eluting stent effectively attenuated in-stent stenosis (neointima formation) by about 50% as compared to bare metal stents and FITC-NP eluting stents in porcine coronary arteries without apparent negative effects on the endothelial healing process *in vivo*.

We and others previously showed that (1) the PLGA NP was taken up by cultured SMC mainly via endocytosis, and retained stably in the intracellular space[18, 19, 21, 26, 28]. It is likely that after cellular or tissue uptake of NP, NP slowly releases the encapsulated drug (imatinib in this case) into the cytoplasm or

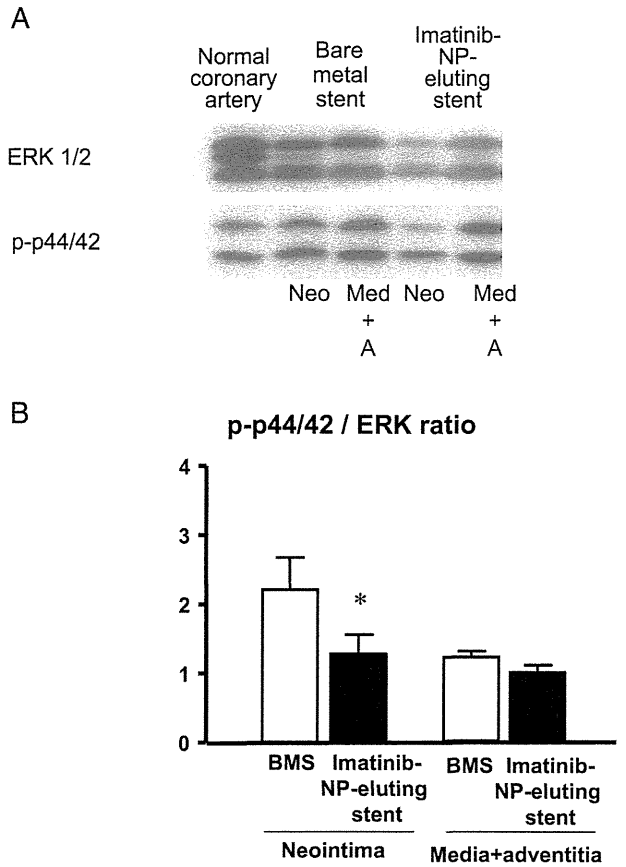

Fig. 5. Protein expression of MAP kinase (p-ERK1/2/ERK 1/2) 14 days after stenting.

A, Photographs of immunoblots of tissues from neointima (neo) and media plus adventitia (Med + A) from normal coronary artery sites (NCA), bare metal stent sites, and imatinib-NP-eluting stent sites.

B, Densitometric analysis of protein expression ($n=4$ each). $^*p<0.05$ versus bare metal stent site.

extracellular space as PLGA is hydrolyzed, resulting in prolonged delivery of imatinib into the stented coronary artery. In this regard, we recently reported that this bioabsorbable polymeric NP-eluting stent system has unique aspects in vascular compatibility and an efficient drug delivery system (stable delivery of NP into the neointima and medial layers until day 28 after deployment of a NP-eluting stent), compared to a dip-coated polymer-eluting stent[19].

In contrast to our present findings, prior studies failed to demonstrate the inhibitory effect of imatinib on in-stent neointima formation in rabbits (oral administration at 10 mg/kg per day for 6 weeks)[16], pigs (600 μg/stent)[15], and patients (oral administration at 600 mg/body per day for 10 days)[17]. The estimated dose of imatinib loaded on our NP-eluting stent was 21 ± 8 μg/stent, which is markedly lower than the doses used in these prior studies; therefore, it is likely that the inhibition of in-stent neointima formation is mediated by slower release and longer retention of imatinib at the imatinib-NP-eluting stent site in this porcine coronary artery model. To confirm this hypothesis, we tried to measure local tissue concentrations of imatinib immediately after and 6 hours after deployment of a imatinib-NP-eluting stent by the HPLC system as a preliminary experiment, which was under the limit of detection (1 ng/mL). Local concentrations of imatinib after deployment of imatinib-NP-eluting stent are unclear; however, our present data (**Fig. 5**) demonstrated that the attenuation of in-stent neointima formation by an imatinib-NP-eluting stent was associated with inhibition of the downstream signal of PDGF receptor (ERK) *in vivo*. Therefore, our present data provide evidence that PDGF receptor signaling blockade by an imatinib-NP-eluting stent may be a promising means for preventing in-stent neointima formation *in vivo*.

An impaired arterial healing process has been demonstrated to be a major histopathological feature in arteries exposed to currently marketed DES in experimental animals[29, 30] and in humans[4-6]. In this study, neither FITC- nor imatinib-NP-eluting stents had apparent effects on inflammation, injury, and re-endothelialization in porcine coronary arteries *in vivo*, suggesting that this NP-eluting stent system may not impair the healing process and endothelial regeneration in this model. Collectively, these data on vascular compatibility support the notion that this bioabsorbable PLGA NP-eluting stent system could be applied to human subjects. One limitation of this interpretation is that we did not compare delayed endothelial healing effects between our NP-eluting stent and current DES devices. In this respect, we do not know whether this approach may have an advantage over currently marketed first-generation DES devices. Future studies are needed to prove this point. Another limitation is that this study was performed in normal pigs without pre-existing atherosclerotic coronary lesions, although this porcine coronary artery model is regarded as an appropriate and standard preclinical study model[31]. A long-term efficacy study is also needed.

We and others have reported that monocyte-mediated inflammation induced by monocyte chemoattractant protein-1 (MCP-1) plays a central role in the pathogenesis of neointima formation[24, 32-36] and in atherogenesis[37, 38]. If imatinib and anti-MCP treatment exert their effects through different pathways, it would be interesting to examine whether combined

blockade of PDGF-R and MCP-1 would have additive inhibitory effects on in-stent stenosis.

In conclusion, blockade of PDGF signaling by imatinib-NP inhibited the proliferation of VSMC with no adverse effects on endothelial cells *in vitro*, and an imatinib-NP-eluting stent attenuated in-stent neointimal formation in porcine coronary arteries *in vivo*. This molecular-targeting NP-eluting stent system may be an innovative platform for delivering agents that target future diagnosis and treatment of atherosclerotic vascular disease.

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Disclosures

Dr. Egashira hold a patent on the results reported in the present study. The remaining authors report no conflicts.

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臨床評価　39巻2号　2011

九州大学　各拠点からの報告

「血管内皮細胞選択的ナノDDS技術」を活用した低侵襲ナノ医療の開発

The development of vascular endothelial cell selective nanotechnology based drug delivery system for less invasive nanotherapy

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1. 開発目標

試験物はピタバスタチン封入PLGAナノ粒子製剤，対象疾患は重症末梢動脈疾患（閉塞性動脈硬化症等）です（Table 1）．最終目標は本製剤を医薬品として市販化，医療として定着させることです（Table 2）．出口は医師主導治験によるPOC（proof of concept）取得です．出口に至る現時点での主なハードルはGLP基準の安全性試験，安定性試験の実施およびGMPでの製造です．現在，本製剤の安全性試験，薬物動態試験および筋注製剤の製剤設計と安定性試験を実施中です．GMP製造のためのスケールアップには既に成功し，GMP施設への技術移管も完了し，来年度（2011年度）は本格的に治験薬GMPの製造を開始します．

2. 開発スケジュール・知財確保状況

本年度（2010年度）末までに安定性試験および安全性試験の最終報告書が上がってくる予定です（Fig. 1）．治験薬の製造については昨年度（2009

Table 1　「血管内皮細胞選択的ナノDDS技術」を活用した，重症虚血性疾患に対する革新的低侵襲ナノ医療を実現するための探索的橋渡し研究ならびに臨床試験
— 平成22年度成果報告会 —

プロジェクト責任者：　江頭　健輔
（九州大学大学院医学研究院　循環器病先端医療研究開発学）
（NEDO橋渡し研究プロジェクトリーダー，スーパー特区分担プロジェクトリーダー）
プロジェクトマネージャー：　中野　覚
連携企業：　興和株式会社

試験物名称：ピタバスタチン封入PLGAナノ粒子製剤
対 象 疾 患：重症末梢動脈疾患（閉塞性動脈硬化症等）

*1　写真と筆頭著者は，発表者．
*2　プロジェクト責任者．

Table 2　開発目標

①開発の最終目標
ピタバスタチン封入PLGAナノ粒子製剤を医薬品として市販化
医療として定着

②当該プロジェクトの「出口」
医師主導治験によるPOC取得

③「出口」へ至る主なハードル
GLP基準の安全性試験と安定性試験の実施およびGMP製造
1. ピタバスタチン封入PLGAナノ粒子製剤（以下本ナノ製剤）のGLP基準安全性試験
2. 本ナノ製剤の筋注製剤の製剤設計と安定性試験
3. 本ナノ製剤の治験薬GMP製造

④「出口」へ至る現時点での到達点と解決策
1. 本製剤の安全性試験，薬物動態試験，および筋注製剤の製剤設計と安定性試験を実施中．GMP製造施設から治験施設への輸送を鑑み，冷凍保存（-20℃）で18ヶ月，冷蔵保存（4℃）で12ヶ月，の製剤安定性が確認された．
2. 本ナノ製剤製造のGMP製造のためのスケールアップに成功（本橋渡し研究プログラム）
3. GMP製造施設への技術移管完了，来年度は治験薬GMP製造開始

年度）より試作を開始しており，平成23（2011）年度中には医師主導治験を開始したいと思っています．最終的には平成33（2021）年度の市販を目指します．

3. 非臨床試験成績

マウス急性モデル，ウサギ慢性下肢虚血モデルにおいて，本製剤の有効性は既に実証し，国際的なトップジャーナルに既にアクセプトされました（Table 3）．

Table 3　マウスおよびウサギ下肢虚血モデルで本製剤の有効性を実証

Integrative Physiology/Experimental Medicine

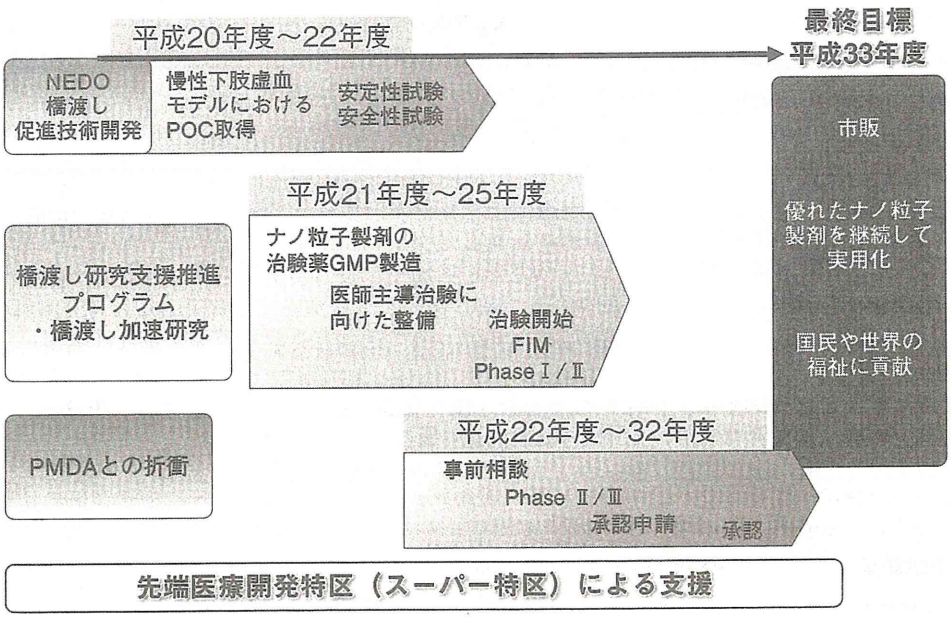
Therapeutic Neovascularization by Nanotechnology-Mediated Cell-Selective Delivery of Pitavastatin Into the Vascular Endothelium

Arterioscler Thromb Vasc Biol. 2009；29：796-801.

Nanoparticle-mediated endothelial cell-selective delivery of pitavastatin induces functional collateral arteries (therapeutic arteriogenesis) in a rabbit model of chronic hind limb ischemia

J Vasc Surg. 2010；52：412-20.

Fig. 1　開発スケジュール（ロードマップ）



また，霊長類であるカニクイザルの重症下肢虚血モデルを用い，単回投与群，3日間連続投与群，6日間連続投与群を設け，CTによる分子イメージングを行い，側副血行路数を測定しました．投与後8週では，溶媒対照群に比べて3日間連続投与群，6日間連続投与群において側副血行路数が有意に増加するという結果が得られました(Fig. 2)．

安定性試験は，本製剤の室温で6ヶ月まで影響ないという結果が得られています．また，GLP下で行った安全性試験では，ラット，イヌの筋肉注射でいずれも重篤な副作用は認められていません．安全性薬理試験も実施しましたが，特に問題となる副作用は認められませんでした．

4. 治験実施計画の概要・実施体制

医師主導治験で，first-in-manになります(Table 4)．試験デザインはまだ案の段階でfixしていませんが，筋肉内投与に関するPhase Ⅰ/Ⅱa臨床試験，安全性と用量設定を行うスタディを実施する予定です．単施設，用量漸増試験，現在1用量4症例，4用量として合計16症例を検討しています．

Fig. 2　スタチン封入ナノ粒子3日間および6日間連続投与により側副血行路（血管新生）が有意に発達（サル）

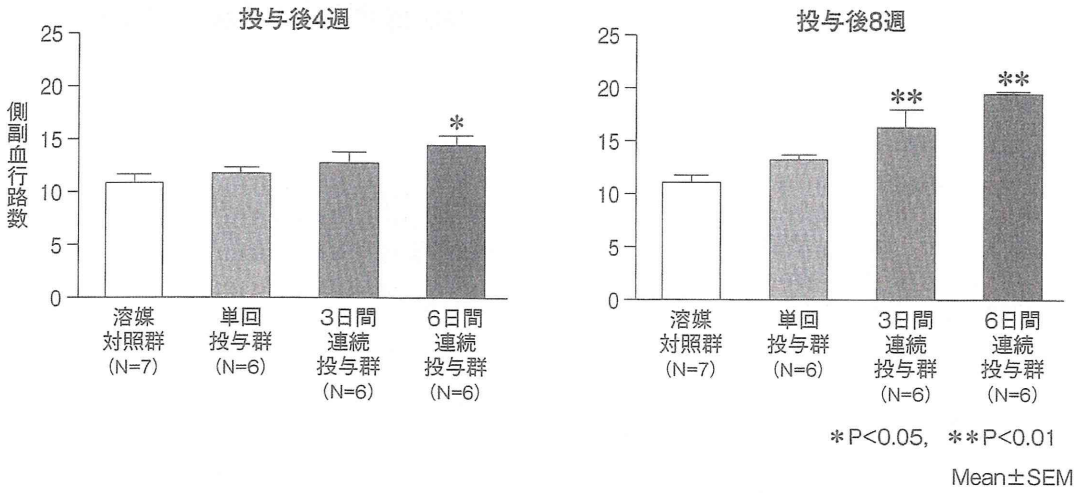


Table 4　臨床試験の概要 (1/2)

①試験の枠組み　医師主導治験

②試験の段階　First-in-man

③プロトコルコンセプト

背景・Rationale：本ナノ製剤筋肉内投与によりピタバスタチンは血管内皮細胞選択的に送達されて薬効を発揮することが疾患モデル（ラット，ウサギ，霊長類）において明らかになった．全身経口投与と比較して格段に少ない用量(300分の1)で有効性を発揮した．これらの成果から，より効果的で且つ低副作用のナノ医療を実用化できると考えられる．ピタバスタチン経口製剤（リバロ錠，4mg/body/日）のヒトにおける安全性は確立．

試験デザイン：

1. 本ナノ製剤の筋肉内投与に関するPhase Ⅰ/Ⅱa臨床試験（安全性と用量設定）：単施設，用量漸増試験，1用量4症例，4用量として計16症例
2. 本ナノ製剤の筋肉内投与に関するPhase Ⅱb/Ⅲ臨床試験（POC試験）：偽薬対照・二重盲検・群間比較，ピタバスタチン封入ナノ粒子製剤筋肉内投与群と対照群の比較：1群20症例，計40症例

適格基準：重度の糖尿病性網膜症患者，担癌患者，透析患者を除外

主要／副次エンドポイントは（Table 5），救肢率，Fontaine分類の変化，Rutherford分類の推移，血行動態を考えています．治験実施計画書および治験薬概要書第1版が完成，治験薬GMP基準下での治験薬試作品が完成し，前臨床試験はすべて終了しています．臨床試験の障害は，適応基準を満たす患者さんのリクルートだと考えています．これを解決するため，先ほど内山麻希子先生が報告したシーズ[*3]を実施した米満吉和先生方の血管外科チームの協力を得る予定です．

「自ら治験を実施する者」は前原喜彦先生（九州大学消化器・総合外科学教授）です（Table 6）．以下Table 6に示すようなスタッフで実施を予定しています．

本ナノ粒子製剤について，興和株式会社と連携し九州大学病院高度先端医療センターの支援のもと治験を実施し，日本や世界の医療として定着させることを目指しています（Fig. 3）．

Table 5　臨床試験の概要（2/2）

主要／副次エンドポイント：
救肢率，Fontaine分類の変化，Rutherford分類の推移，血行動態

臨床試験の準備／進捗状況
医師主導治験実施計画書ドラフト完成，治験薬概要書ドラフト完成
治験薬GMP基準下での治験薬試作品完成，前臨床試験は全て終了

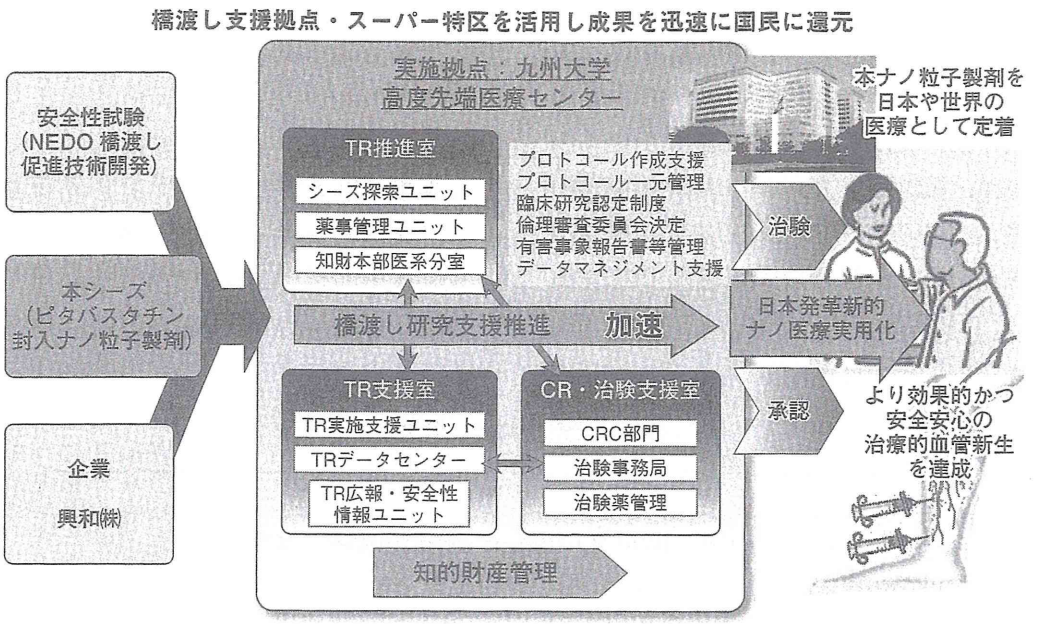
臨床試験の障害とその解決策（費用調達も含む）
障害：臨床試験の適応基準を満たす患者のリクルート
解決策：
①先行するシーズ（TR1：高性能国産新規RNAウイルスベクターによる虚血肢治療製剤の開発）を実施した血管外科チームの協力を得る
②循環器内科も患者の動員を支援

Table 6　医師主導治験実施組織編成表（H22.10.）

＜TR08＞	所属・責任者名	所属・担当者名
自ら治験を実施する者	消化器・総合外科学・前原喜彦	
プロジェクト責任者	循環器内科学・江頭健輔	
プロジェクトマネジメント	循環器内科学・中野　覚	
薬事	TR推進室・内山麻希子	－
データマネジメント	TR支援室・下川元継	－
統計解析	デジタルメディシン・イニシアティブ 岸本淳司	医療情報部・徳永章二
モニタリング	がん先端医療応用学講座・江見泰徳	－
CRC	高度先端医療センター・菊武恵子	高度先端医療センター 田中理子，中尾雅文
安全性情報管理	医療情報部・徳永章二	TR支援室・北島奈緒子
記録保存／文書管理	高度先端医療センター・中西洋一	－
メディカルライティング	－	－
信頼性保証／監査	呼吸器科・高山浩一	血液・腫瘍内科・馬場英司

[*3] 内山麻希子，米満吉和，松本拓也，岡崎　仁，吉田久美，中西洋一，前原喜彦．高性能国産新規RNAウイルスベクターによる虚血肢治療用バイオ製剤の開発．臨床評価．2011；39(2)：293-9.

Fig. 3　本橋渡し支援拠点における実施体制



＜質疑応答＞

清水　プロトコルと概要書が既に1版というのはGCP管理上少々問題があるのではと思いますが.

中野　第1版と申しましたが，第0.1版のドラフトです．現在，九州大学学内で米満先生方，専門家の先生方にレビューがようやく回った状況で，1版として確定したものではありません.

清水　多分今後苦労されると思いますが，版管理の状態をしっかりしておかないと，治験に行くときに監査で，第1版から全部記録があるかという話になってしまうので，0コンマ何版というのを導入されることを強くお勧めします.

中野　どうもありがとうございます.

福島　SOPは揃っていてGCPの適格性調査もクリアされているので，私は大丈夫だと思っています．1点確認したいのですが，九州大学でなぜ2つの下肢血管再生を行うのか皆さんひょっとして疑問に思われるといけませんが，私がお聞きしているのは，適応が違う．つまり先ほどの米満先生の開発されたウイルスベクターによるもの[*3]は間歇性跛行，より軽症のもので，先生のところはより重症のクリティカルなケースを対象にするというわけですね.

中野　はい，そうです.

福島　そういうことで，皆さんご理解いただければと思います.

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