

Protocol - 897

**Phase I/II multi-center, open label, dose -
escalation study to evaluate the safety and
tolerability of DVC1-0101 administered
intramuscularly in subjects with stable
peripheral artery disease**

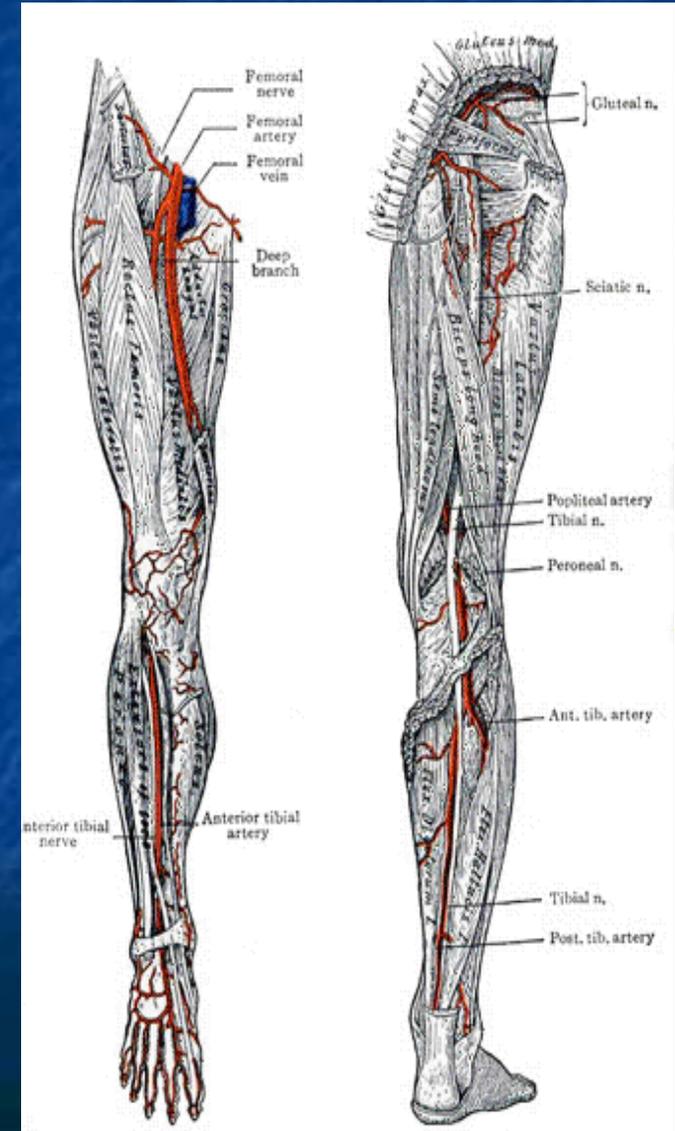
PI : Brian H. Annex, M.D.,
Professor of Medicine
Duke University, Durham, North Carolina

Sponsor: DनावेC Corporation, Tsukuba, Japan

Thanks: Dr. Yoshi Yonemitsu Kyushu University

Peripheral Arterial (Obstructive) Disease: PA(O)D

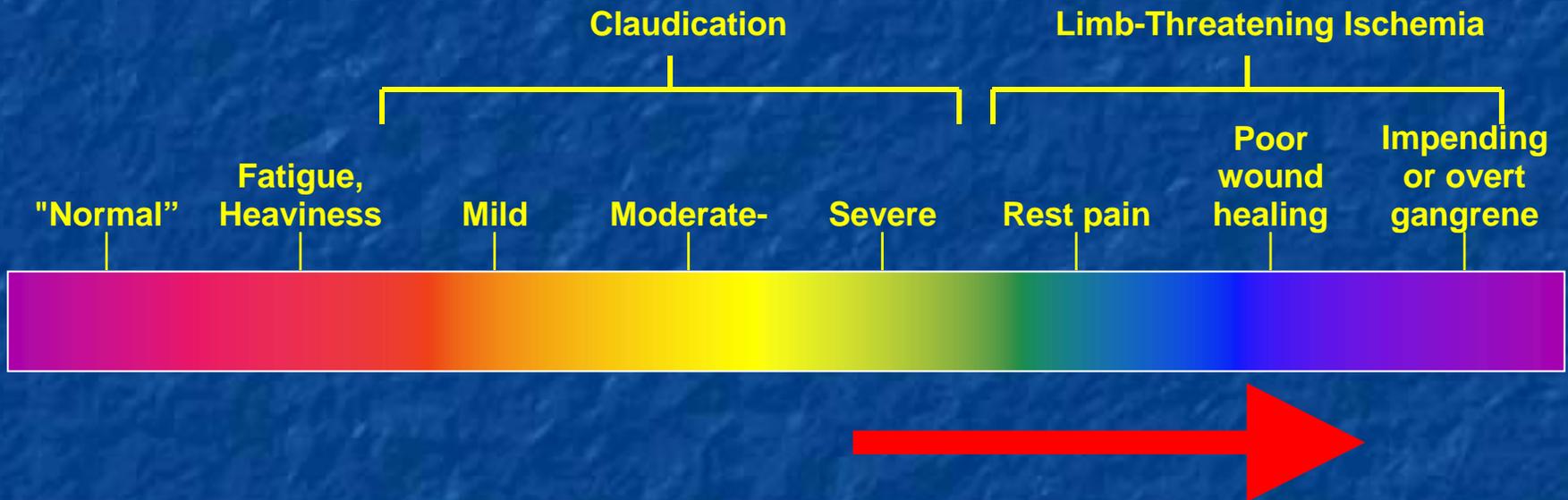
- Presence of a stenosis or occlusion in a major vascular bed other than the heart. Most frequently in the aorta or arteries of the (lower) limbs.



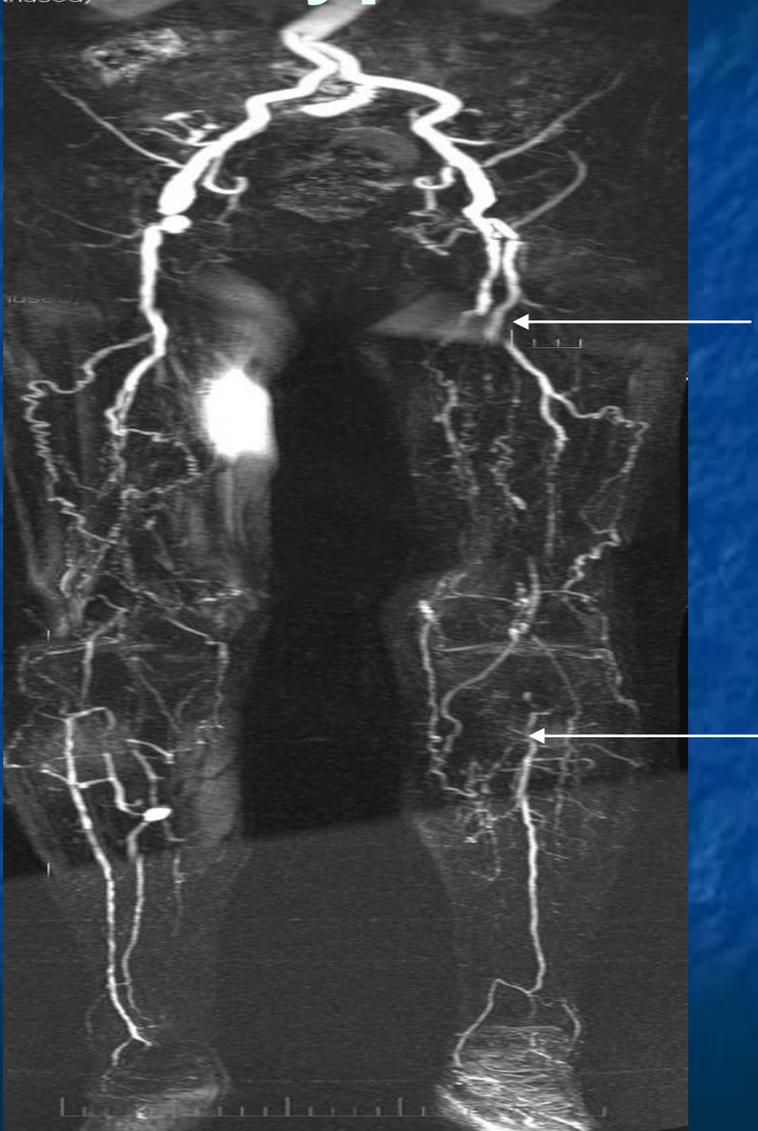
Atherothrombotic Diseases in US

	Prevalence (millions)	Incidence (millions)
Coronary heart disease	13.2	1.2
Cerebrovascular disease	4.8	0.7
Peripheral arterial disease	8.0–12.0	—

Spectrum of Peripheral Artery Disease (PAD) with Two Major but Distinct Clinical Manifestations



What can be done in PAD given this MRA of “Typical” Patient with PAD:



Peripheral Arterial Disease: Current Therapies

- **Medical therapies in PAD are directed toward modifying the underlying atherosclerotic risk factors.**
- **Exercise training is the treatment of choice but has limited availability and the mechanism of exercise in PAD is unknown.**
- **Cilostazol – FDA approved for claudication but contraindicated in CHF**
- **No therapies are available to increase blood flow to the ischemic limb.**

Angiogenesis

Growth and proliferation of new blood vessels from pre-existing vascular structures.

Therapeutic Angiogenesis

*The growth of new blood vessels to
TREAT disorders of inadequate
tissue perfusion.*

FGF-2: Rationale for Choice of Agent

FGF-2:

- Well known angiogenic growth factor.
- Promotes endothelial proliferation in-vitro and both angiogenesis and arteriogenesis in vivo. Well established in multiple models.
- Has moderate affinity for the extracellular space and therefore is not “expected” to significantly leak into systemic circulation

Protein/Gene Therapy Trials in PAD (IC or CLI) CAD With FGF

Agent	Disease	Patient	Route	Reference
FGF-2	PAD	16	IV (2ug/kg X 6 weeks)	Cooper (2001)
FGF-2	PAD/IC	190	IA/Protein (30ug/kg)	Lederman (2002)
FGF-2	PAD/IC	19	IA/Protein	Lazarous (2000)
FGF-2	CAD	330	IC/Protein	Simons (2004)
FGF-2	CLI	7	Hydrogel/Protein	Marui (2007)
pFGF-1	CLI	51	IM/Plasmid	Camerota (JVS)
pFGF-1	CLI	125 EU	IM/Plasmid	Nikol ACC
pFGF-1	CLI	70 US	IM/Plasmid	Henry

Articles

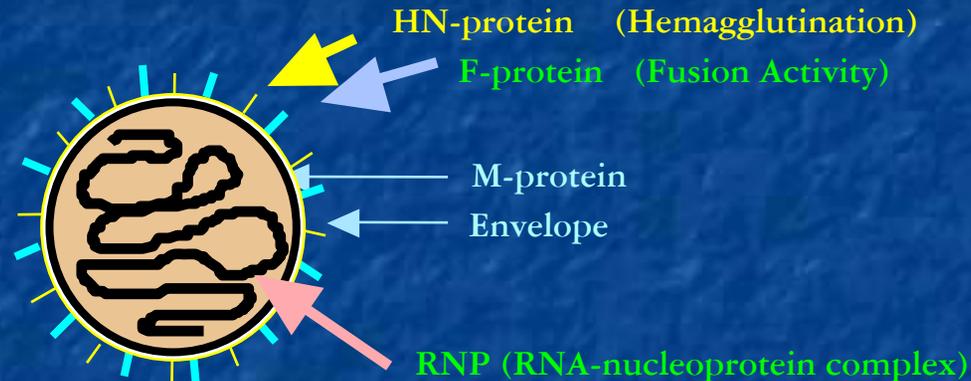
Therapeutic angiogenesis with recombinant fibroblast growth factor-2 for intermittent claudication (the TRAFFIC study): a randomised trial

*Robert J Lederman, Farrell O Mendelsohn, R David Anderson, Jorge F Saucedo, Alan N Tenaglia, James B Hermiller, William B Hillegass, Krishna Rocha-Singh, Thomas E Moon, M J Whitehouse, and Brian H Annex, for the TRAFFIC investigators**

Features of Gene Transfer and Expression via Recombinant Sendai virus vector



200nm



Sendai Virus (SeV)

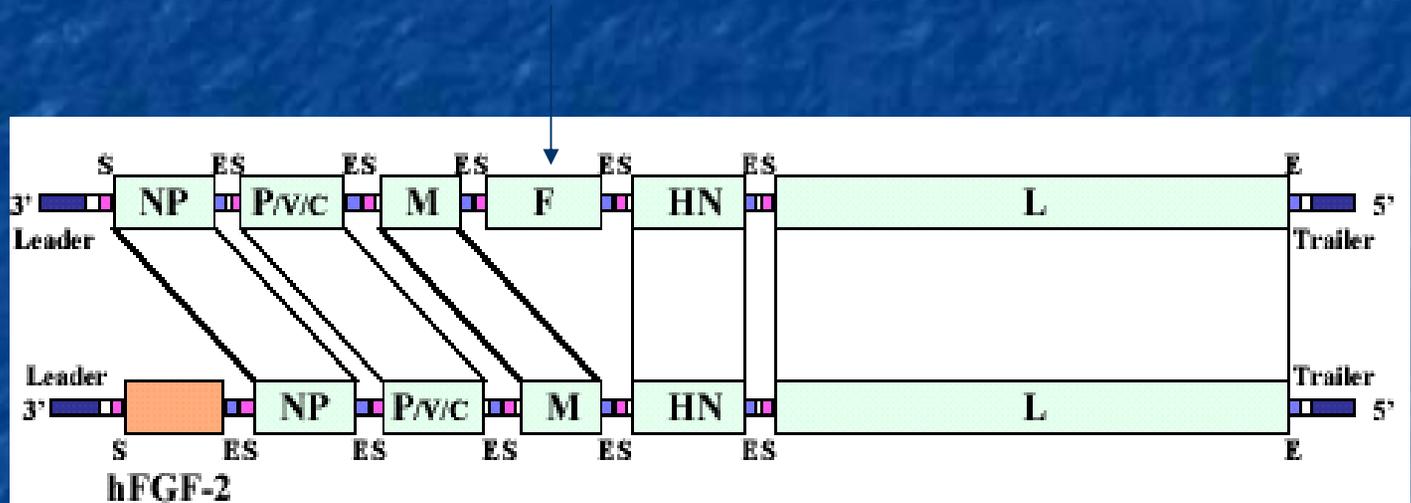
- 1) single (-) strand RNA genome
- 2) cytoplasmic transcription (no requirement of nuclear import)
- 3) very high level of transgene expression
- 4) non-pathogenic virus for human
- 5) no DNA phase, no obvious path for intergration

Rationale for Choice of Vector:

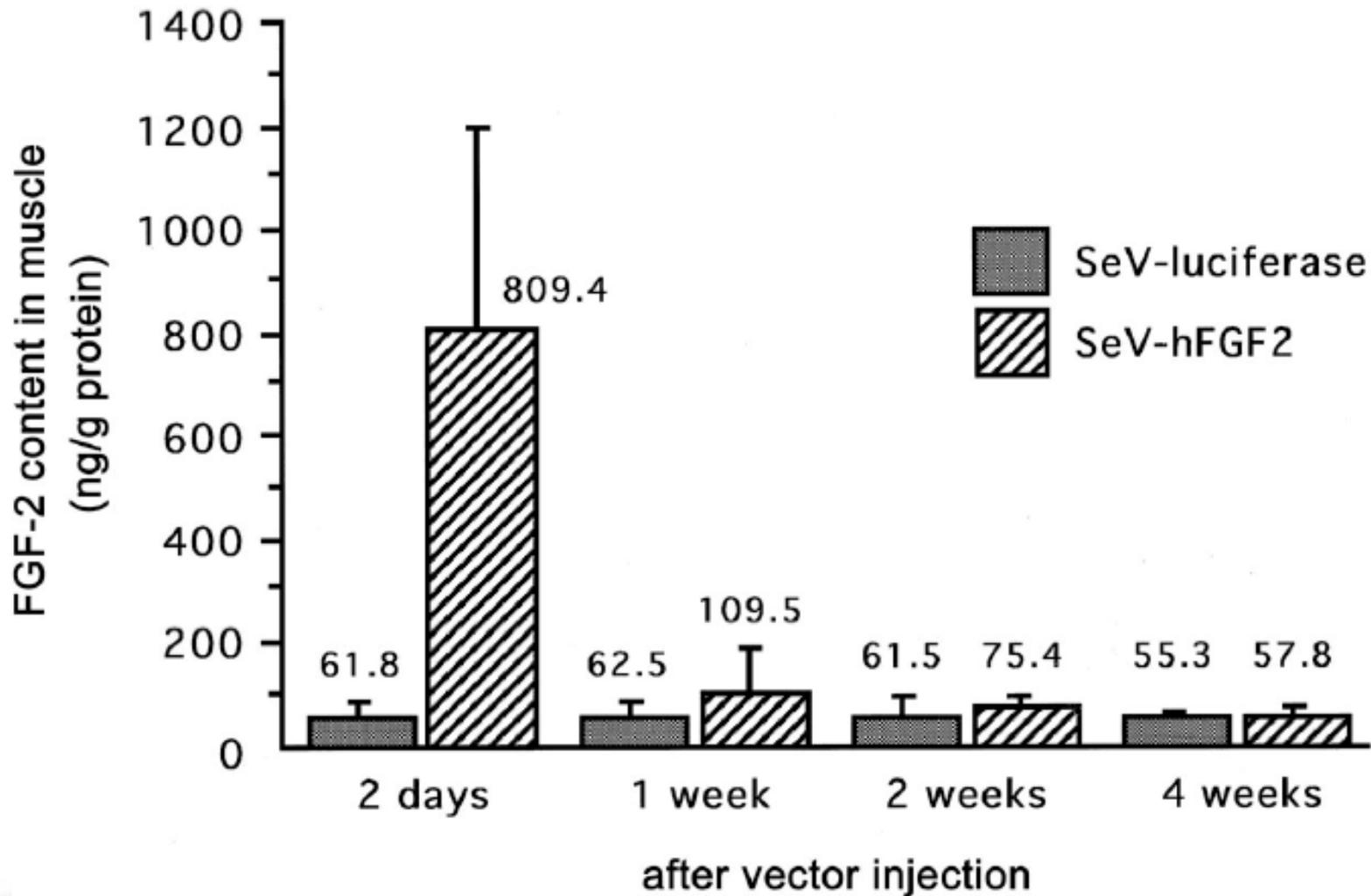
Schematic structure of SeV and SeV/dF-hFGF2

Wild-type SeV

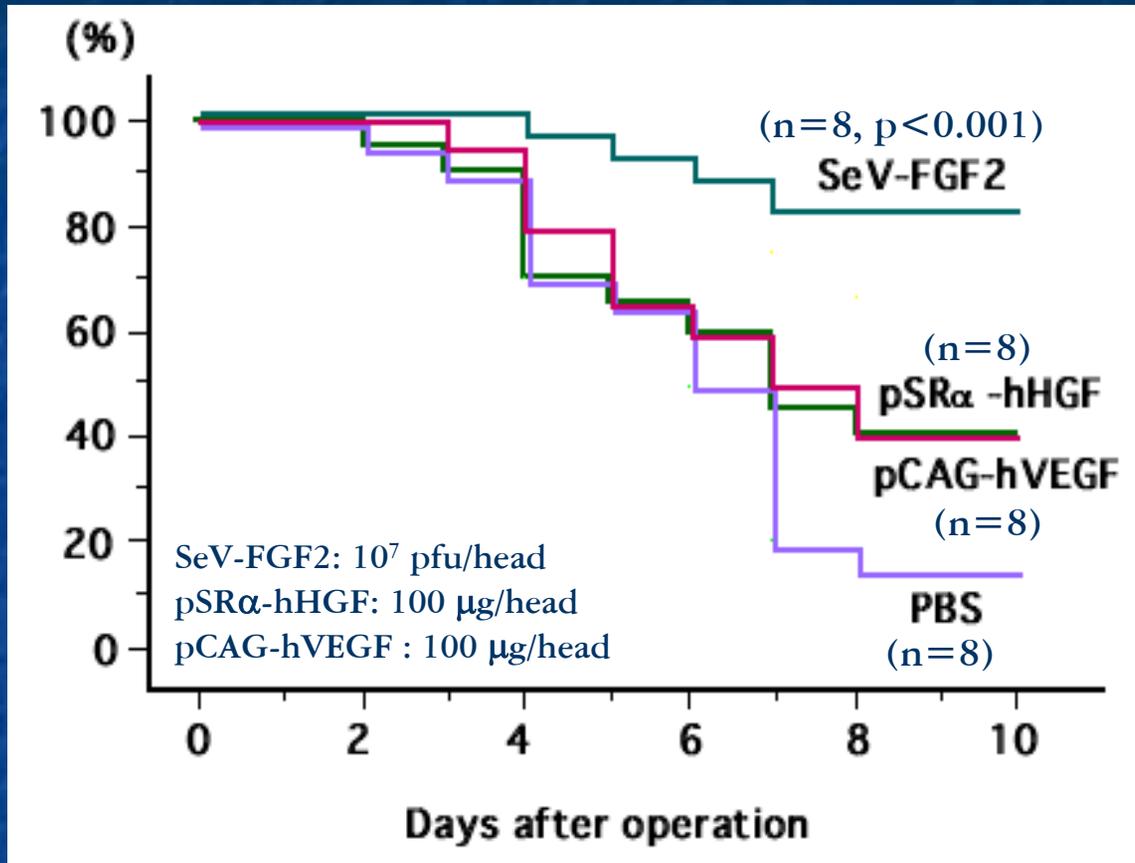
SeV/dF-hFGF2



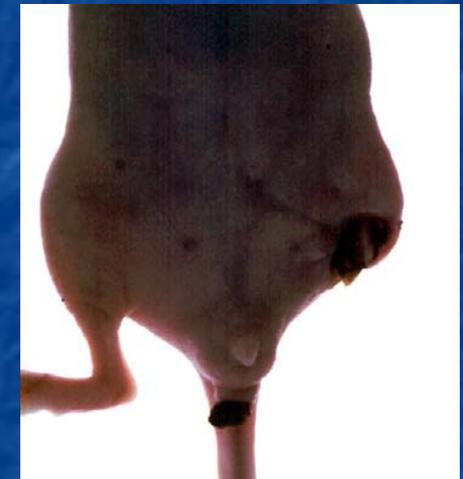
Time Course of FGF2 Expression After IM injection of Control Vector (right) or SeV -hFGF2 (left) in Rabbit Poor Runoff Hind Limbs



Murine Model of Critical Limb Ischemia

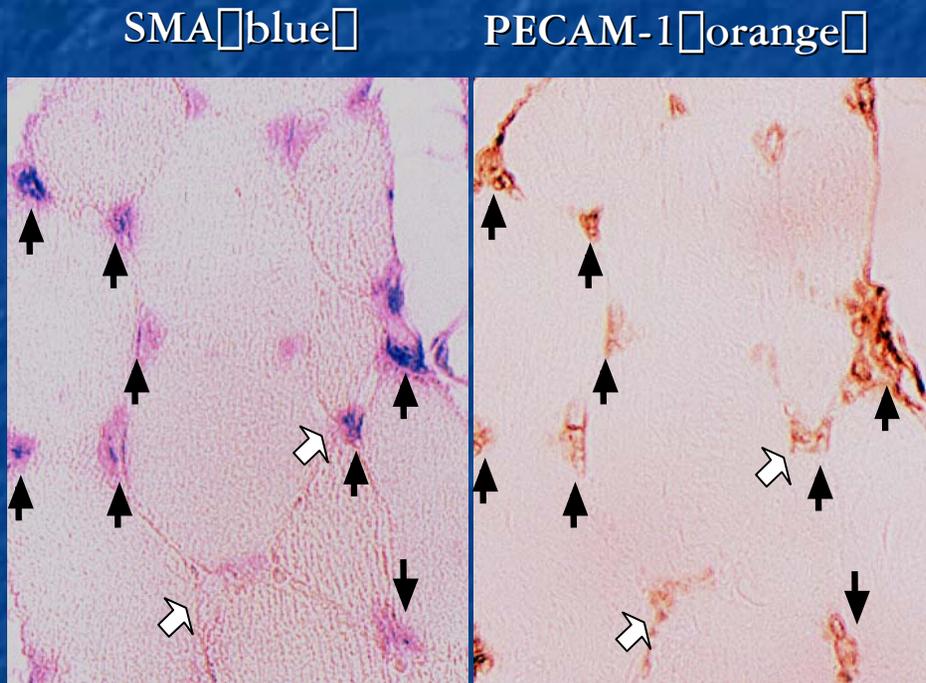


*Balb/c nu/nu mice
auto-amputation model*

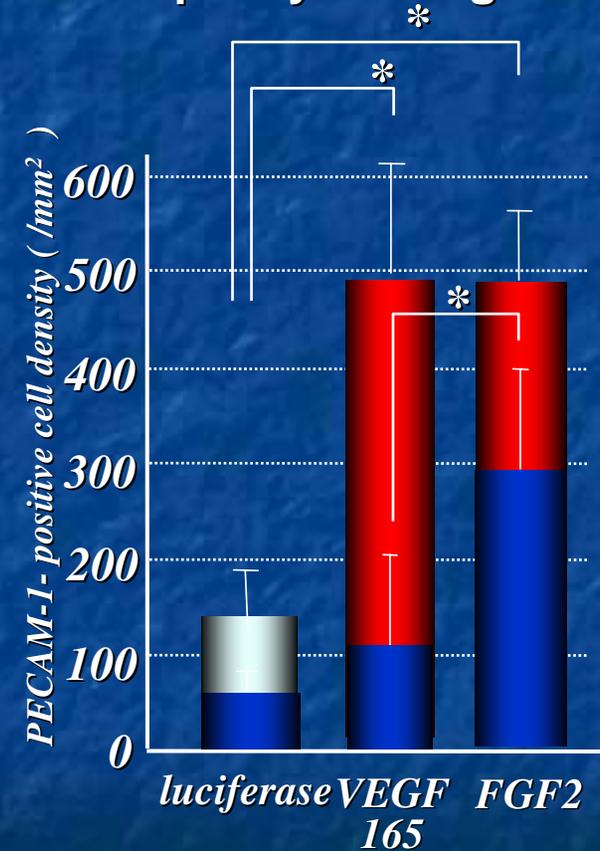


FGF-2: their effects on the maturation of capillaries

Both increase the number of capillaries, however, those by FGF-2, but not by VEGF, associate pericyte lining



White arrow: immature vessel
Black arrow: mature vessel



* $P < 0.01$

List of Toxicity Studies Performed with DVC1-0101

Rat ¹⁾	5x10 ⁸ , 5x10 ⁹ CIU/kg	Single	i.m.	Acute toxicity; MTD>5x10 ⁹ CIU/kg
Rat ¹⁾	5x10 ⁸ , 5x10 ⁹ CIU/kg	Single	i.v.	Acute toxicity; MTD>5x10 ⁹ CIU/kg
Rat ²⁾	3.67x10 ⁹ CIU/kg	Single	i.m.	Acute toxicity; Slight inflammation at the injected site
Rat ³⁾	1x10 ⁸ , 4x10 ⁸ , 4x10 ⁹ CIU/kg	Repeat, 14d	i.m.	Subacute toxicity; MTD>4x10 ⁹ CIU/kg
Monkey*	5x10 ⁸ , 5x10 ⁹ CIU/kg	Single	i.m.	Acute toxicity; Inflammation at the injection site
Monkey ³⁾	7x10 ⁷ , 7x10 ⁸ CIU/kg	Repeat, 14d	i.m.	Subacute toxicity; Inflammation & temporal vacuole formation at the injected muscle
Mouse ³⁾	3.3x10 ⁷ , 3.3x10 ⁸ , 3.3x10 ⁹ CIU/kg	Repeat, 14d	i.m.	Short-term carcinogenicity study; No carcinogenicity
Mouse*	1x10 ⁹ CIU/kg	Single	i.m.	Local irritability; Slight inflammation at the injection site
Mouse ³⁾	4x10 ⁸ , 4x10 ⁹ CIU/kg	Single	i.m.	Safety Pharmacology; No apparent change
Dog ³⁾	2x10 ⁸ , 2x10 ⁹ CIU/kg	Single	i.m.	Safety Pharmacology; No apparent change
Guinea pig ³⁾	4x10 ⁸ , 8x10 ⁸ CIU/kg	5 times	i.p., i.v.	Hypersensitivity; No hypersensitivity

1) GLP study in UK, 2) GLP study in USA, 3) GLP study in CN, *: Non-GLP study in JPN

List of Biodistribution Studies Performed with DVC1-0101

Rat ¹⁾	1.5x10 ⁸ CIU/kg	Single	i.m.	Injected muscle & spleen (+) until 4d
Rat ¹⁾	5x10 ⁸ CIU/kg	Single	i.v.	Blood, heart, lung, spleen, ovary (+) in early phase
Monkey ¹⁾	5x10 ⁸ CIU/kg	Single	i.m.	Heart & lymphnode (+) in early phase
Rat ²⁾	1x10 ⁸ , 4x10 ⁸ , 4x10 ⁹ CIU/kg	Repeat, 14d	i.m.	No viral genome detected
Monkey ²⁾	7x10 ⁷ , 7x10 ⁸ CIU/kg	Repeat, 14d	i.m	Muscle (+) in high dose group on day 15

1) GLP study in UK, 2) GLP study in CN

2-week observation period

SeV RNA genome persistency (single i.m. injection)

Rat: Day 4 (+) at inj. Muscle, Spleen, Day 7 (-)

(1.5×10^8 ciu/kg, no clinical abnormality)

Monkey: Day 4 (+) at Heart, Local lymph node, Day 7 (-)

(5×10^8 ciu/kg, no clinical abnormality)

Mouse: On going (advised by FDA)

(5×10^9 ciu/kg)

Clinical Dose Selection -- Maximum Dose would be safe

Ratio of Animal / Human Dose

Rat Acute Toxicity study: NOAEL at 3.7×10^9 ciu/kg (**37-fold**)

Mouse Safety Pharmacology study: No AE at 4×10^9 ciu/kg (**40-fold**)

Rabbit Efficacy study: No AE at 3.3×10^8 ciu/kg (**3.3-fold**)

Monkey Acute Toxicity study: No AE at 5×10^9 ciu/kg (**50-fold**)

Mouse Acute Toxicity & biodistribution (advised by FDA): On going

NOAEL: No observed adverse effect dose, AE: adverse effect

Proposed Human Trial **#897**: Max Dose 1×10^{10} ciu/man (1×10^8 ciu/kg)

1×10^{10} ciu/man (1×10^8 ciu/kg) is thought to be safe.

Human Equivalent Dose (HED)

In ischemic mouse muscle, FGF-2 production increased when 1×10^6 ciu/mouse.
{Dec amputation in CLI model}.

Mouse Body Weight = 30 g

Effective Dose = 3×10^7 ciu/kg

Human Weight = 60 Kg yielding 2×10^9 ciu/human

Modifying/Uncertainty Factor mouse to man 10 – 12.3 yielding 2×10^8 ciu/human

Converting to Human Equivalent Dose from Mouse Dose (mg/kg)

<u>Modifying Factor or Uncertainty Factor</u>	<u>Ref</u>	<u>Year</u>
Divide Animal Dose by	12.3	FDA(CDER) 2005
	12	MHLW 1998
	10	WHO EHC170 1994

Assuming systemic exposure

Current Status and Observed Adverse Events

(Japan PAD Trial for DVC1-0101 - since 4.1.2006)

Stage 1
5x10⁷ ciu/60 kg
(Completed)



Stage 2
2x10⁸ ciu/60 kg
(Ongoing)



Clinical course (Stage 1)

Case No.	1M	3M	6M	Recent
102 (Fontaine III)	improved	improved	improved	improved (21 Mo)
103 (Fontaine IV)	Grade 3/ SAE: Major amputation at day 15 (alive)			
105 (Fontaine IV)	improved	Grade 3/ SAE: Toe amputation on 3 Mo (alive)		

Adverse events (Stage 1): 45 events within 6 Mo

Grade 1.	43	Grade 3.	2	(Study related=0)
Grade 2.	0	Grade 4.	0	

Clinical course (Stage 2)

Case No.	1M	3M	6M	Recent
201 (Fontaine III)	improved	improved	Not yet	- (alive)
203 (Fontaine III)	improved	Not yet	Not yet	- (alive)
204 (Fontaine III)	Injection of DVC1-0101: 3.11.2008			

Stage 3
1x10⁹ ciu/60 kg



Stage 4
5x10⁹ ciu/60 kg



Efficacy assessments (Open Label)

(Japan PAD Trial for DVC1-0101 - since 4.1.2006)

Tread-mill test

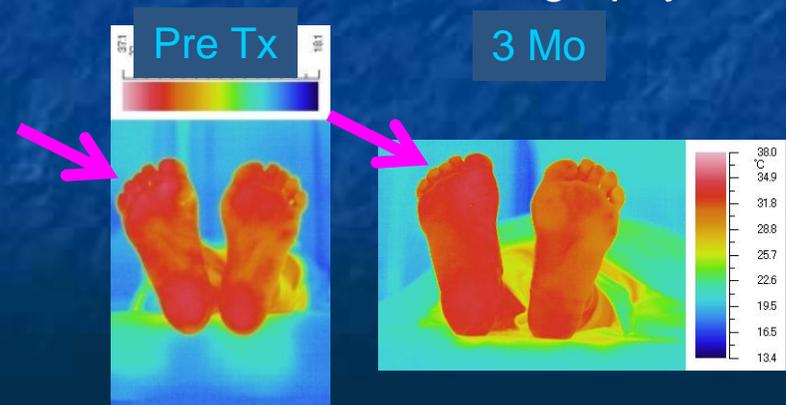
Case 102 (Stage 1)	pre	1Mo	3Mo	6Mo
PWD (m)	67	113 (169%)	117 (175%)	121 (180%)
PFWD (m)	38	86 (228%)	110 (289%)	92 (242%)

Case 201 (Stage 2)	pre	1Mo	3Mo	6Mo
PWD (m)	74	138 (186%)	235 (318%)	Not yet
PFWD (m)	43	118 (274%)	208 (483%)	Not yet

Case 201: Pulse-volume recording (PVR)



Case 201: Thermography



Courtesy from Kyushu University Hospital

Protocol #897

Open-label, multi-center, dose escalation study

4 cohorts

N=4 patients/cohort

Sites with prior experience in PAD and Gene Transfer

Dose will vary but not injection volume

Primary Endpoint

- Safety and tolerability of escalating doses of DVC1-0101
 - Measure frequency, severity, and duration of treatment-emergent AEs
 - Monitor clinically significant changes in safety laboratory parameters.
 - Freedom major cardiovascular events.
 - Freedom from drug related SAE

Protocol #897

Secondary Endpoints

- **Pharmacokinetics (SeV RNA genome)**
- **Pharmacodynamics (hFGF-2 and VEGF-A)**
- **Local (injection site)**
- **Preliminary efficacy**
 - **Hemodynamics (ankle brachial index: ABI, toe brachial index: TBI)**
 - **QOL (Walking Impairment Questionnaire) patients with IC**
 - **Resting pain intensity (Visual Analogue Scale) patients with CLI**
 - **Limb retention (major and minor amputation)**

Protocol #897

Inclusion Criteria

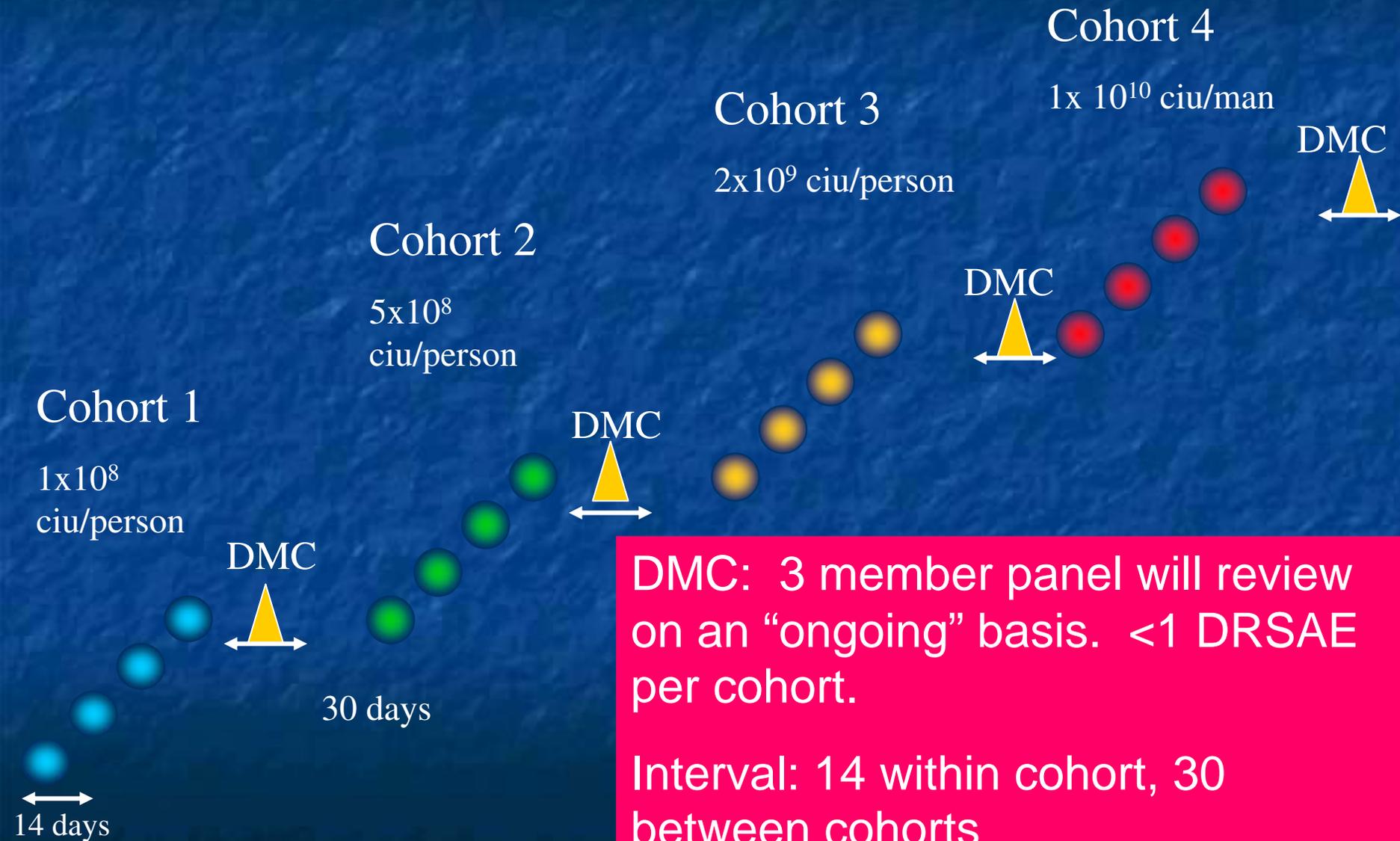
- 80 years old > Males and Females > 40 years old
- Fontaine Stage II (symptom limiting claudication) or Stage III (ischemic rest pain)
- Stable symptoms for 3 Mo before screening
- Resting ABI < 0.60 or TBI < 0.40 (if resting ABI > 1.30)
- In compliance with cancer screening guidelines
- Patients with ongoing fever will be excluded. {active infection}

Protocol #897

Exclusion Criteria

- Fontaine IV (pedal necrosis or ulceration)
- Planned limb revascularization
- Performed limb revascularization
- Prior major or minor amputation
- MI, unstable angina, stroke, TIA
- Buerger's disease
- Abnormal renal or liver function
- Type I diabetes or type II diabetes with HbA1c >10%
- Proliferative retinopathy
- Presence or history of cancer
- Pregnant women
- LFT > 2X ULN

Dose Escalation Scheme in Protocol #897



Protocol Alternatives That Where Considered

1. Randomized, multi-center, double-blinded, placebo-controlled, dose-escalation study with N=6-8 patients/cohort active:placebo = 2:1
2. Dosing only claudication or dosing only CLI.
3. Does this meet the criteria of Phase I/II
 - dose escalation
 - pre and post comparison
 - establish dose for next study
 - narrow group

Summary

DVC1-0101 (SeV/dF-hFGF2):

- (1) Single Stranded Modified Cytoplasmic RNA virus vector (No DNA phase No genetic toxicity)**
- (2) Lack of F gene limits/eliminates spreading**
- (3) High efficiency of hFGF-2 expression in cells**
- (4) Localized expression (injection site)**
- (5) Safety margin in animal toxicity studies (mouse, rat, guinea pig, dog, monkey)**

Supplemental slides:

Questions of

Dr. Murphy

Dr. Weber

Dr. Zaia

Dr. Fan

#1: Prior experience with hPIV-1

How will it impact on the efficacy of DVC1-0101?

#2: Prior experience with hPIV1

Is it likely to elicit a systemic or local accelerated response?

#1: Pre-existing immunity

DVC1-0101 may overcome pre-existing immunity and prove to be effective.

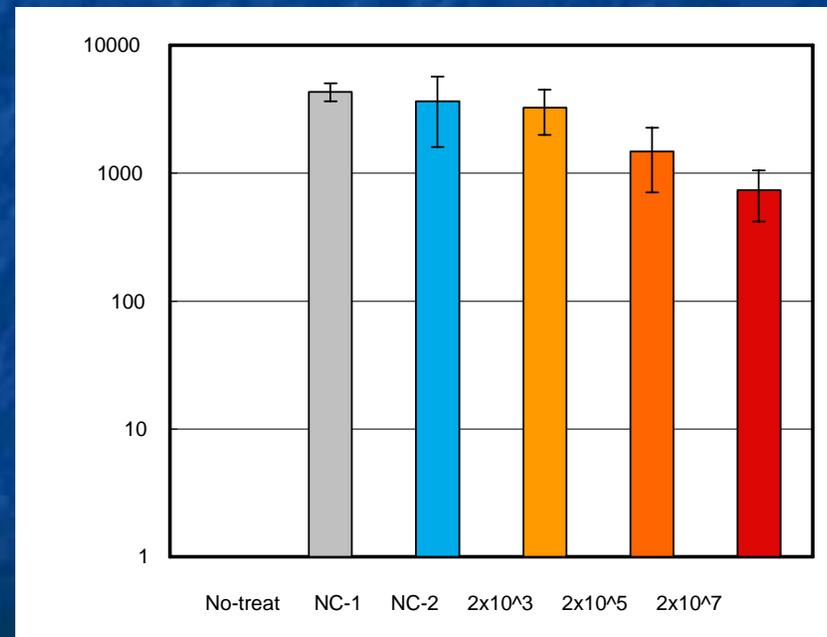
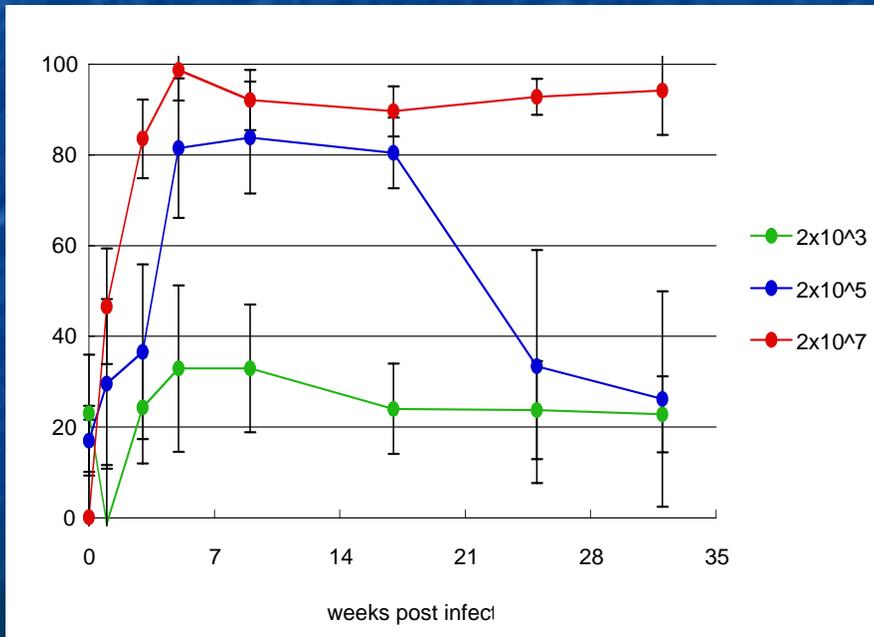
- Human: Serum INFgamma increase and beneficial effect after i.m. administration of DVC1-0101 to a patient (JPAD Clinical Study, 2nd stage, at Kyushu University Hospital).
- Human: Adults and children can be reinfected by hPIV1 (Chanock et al. 1963, Marx et al. 1999).
- Human: Ab increase after administration of wtSeV as hPIV1 vaccine (Slobod et al. 2004).
- Monkey: SIVgag-specific CTL boosted by second i.n. challenge of SeV/dF-SIVgag vaccine in the presence of neutralizing Ab (Matano et al. 2007).
- Mouse: Significant expression of luciferase by SeV/dF-Luci administered i.n. in the presence of neutralizing Ab (Kuwahara et al. 2003).

#1: Influence of anti-SeV immunity on gene expression by second SeV vector challenge 23

Anti-SeV antibody affected gene expression of 2nd challenge (2.5×10^8 ciu/kg) in mice. Gene expression levels were 90%, 40% and 20% of the age-matched control in inverse proportion to the priming dose levels (1×10^5 , 10^7 , 10^9 ciu/kg).

Anti-SeV neutralizing antibody (x20 plasma)

Gene expression rate comparing with NC-2 (Age-matched naïve control)



#2: Accelerated immune response

Accelerated immune response at the injection site of DVC1-0101 is unlikely because no serious immunological responses are reported in the following studies.

- Human: Adults with pre-existing hPIV1 immunity challenged i.n. with live attenuated SeV (Slobod et al., 2004).
- Rat/Monkey: Repeat daily i.m. administration of DVC1-0101 for 14 days. (Internal Reports: Zuo et al. 2006, Koujimoto et al. 2007).
- Guinea pigs: **Hypersensitivity study**. Sensitized with DVC1-0101 by repeated i.p. inoculation (4×10^8 ciu/kg, every two days, 5 times), challenged i.v. with DVC1-0101 (8×10^8 ciu/kg). (Internal Report: Zuo et al. 2005).

#1-1 : 4 patients / cohort

#1-2 : Stopping rule

#1-3 : Grading severity of adverse events

#2: The Highest Clinical Dose (1×10^{10} CIU/ patient)
Is it safe?

#3: 2-week observation period prior to the next dose

#1-1: 4 patients, -2: Stopping rule, -3: Grading of AE

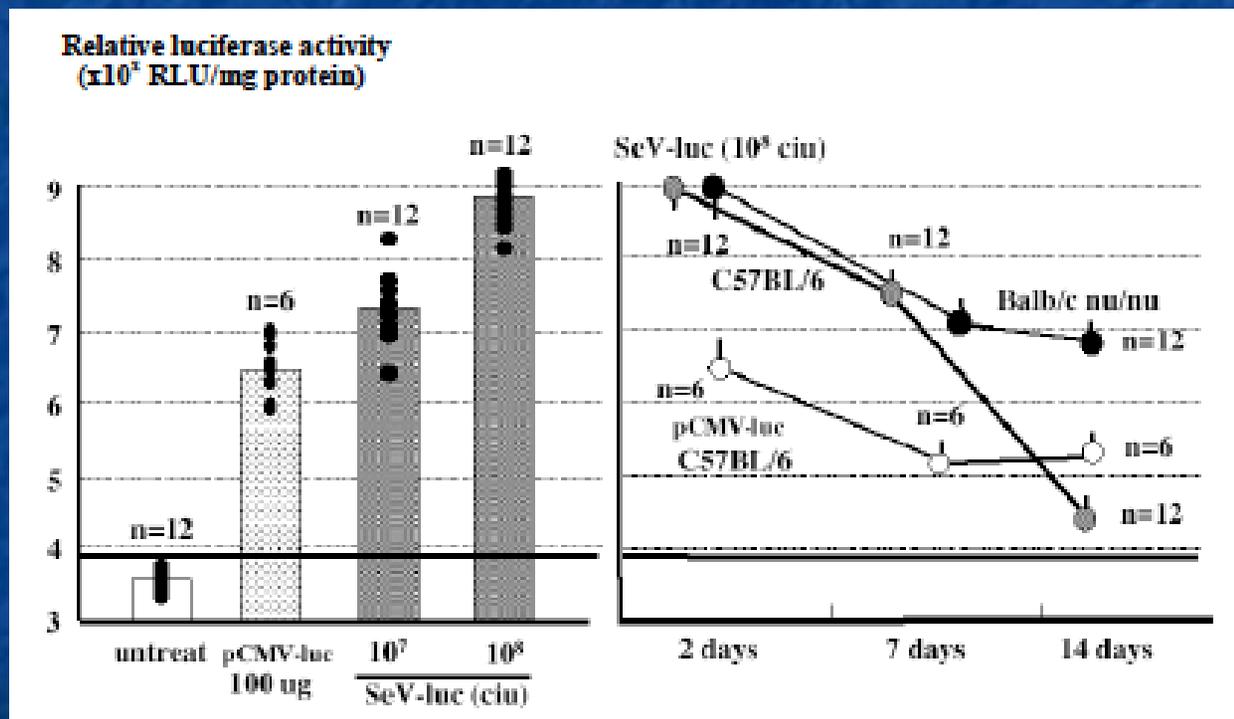
#1-1: Consistent with similar other studies.

#1-2: Will prohibit the dose escalation if **one** patient in a cohort developed a serious study-related adverse event.

#1-3: Will use NCI Common Toxicity Criteria.

#3: 2-week observation period

Gene expression (SeV-luc) level on Day 14 is less than 1/10000 of the peak level on Day 2 in normal mice (C57BL/6).



(Masaki et al: Circ Res, 2002)

#4 : More frequent pregnancy tests

→ Will perform additional pregnancy test on 1 and 3 M visit.

#5 : Upper age as exclusion criterion

→ Will set upper age of 80 years.

#6 : Two birth control method

→ Will follow the IRB's recommendation.

#7 : Pain relief medication

→ Will follow the judgment of PI.

#8 : Cancer information to whom

→ Will follow the judgment of patient's primary care physician.

#9 : Definition "chronic use of NSAIDS

→ Definition of "Chronic" will be longer than 5 days.

#10 : DMC detail

→ At least a vascular specialist, a cardiologist and an infectious disease physician will be included.

#11 : In Phase I and II trials, hFGF-2 was safe

→ Will revise more accurately.

#12 : Endometrial biopsy

→ Will be removed.

#13 : Potential to cause proteinuria

→ Risk of proteinuria should be lower than i.a. or i.v. trials. But the protocol will be modified.

#14 : Injection of local anesthetic

→ Local anesthetic will not be injected.

#15 : Side effect of gadolinium (contrast medium)

→ Risk and measures will be described in the protocol.

From Dr.Weber (informed consent form : ICF)

ICF#1: (Research) study

→ Will add “research” before “study”.

ICF#2: Quantitate specific risks of colonoscopy

→ Will be updated to indicate the risk.

ICF#3: Radiation amount on mammography

→ Will be updated to indicate the amount of radiation.

ICF#4: Any benefits for patients?

→ Will remove the statement as suggested by the reviewer.

ICF#5: HIV, Hepatitis B , Hepatitis C are reportable diseases

→ Will include.

From Dr.Weber (informed consent form : ICF)

ICF#6: 12 weeks birth control

→ Will modify the statement as direction.

ICF#7: Need to report any pregnancy to investigator

→ This is included.

ICF#8: 60 days or 12 weeks as birth control period

→ Will use 60 days.

ICF#9: Amount of compensation

→ \$400 will be offered and will be prorated.

ICF#10: Circumstances to obtain Informed consent

→ A patient's legally authorized representative may consent on behalf of the patient.

#1-1: 2 weeks observation period prior to next dose

→ Will extend to 30 days.

#1-2: Definition of DLT

→ Will use an objective AE grading scale.

#2: New subject can be treated every 2 days

→ Will extend to 14 days.

#3: Multi-center trial? or only at Duke?

→ A few centers will be required.

#4: Replacement of subjects

→ Will follow them and add another patient.

#5: ALT and AST limits 3 x ULN

→ Will lower to 2 x ULN.

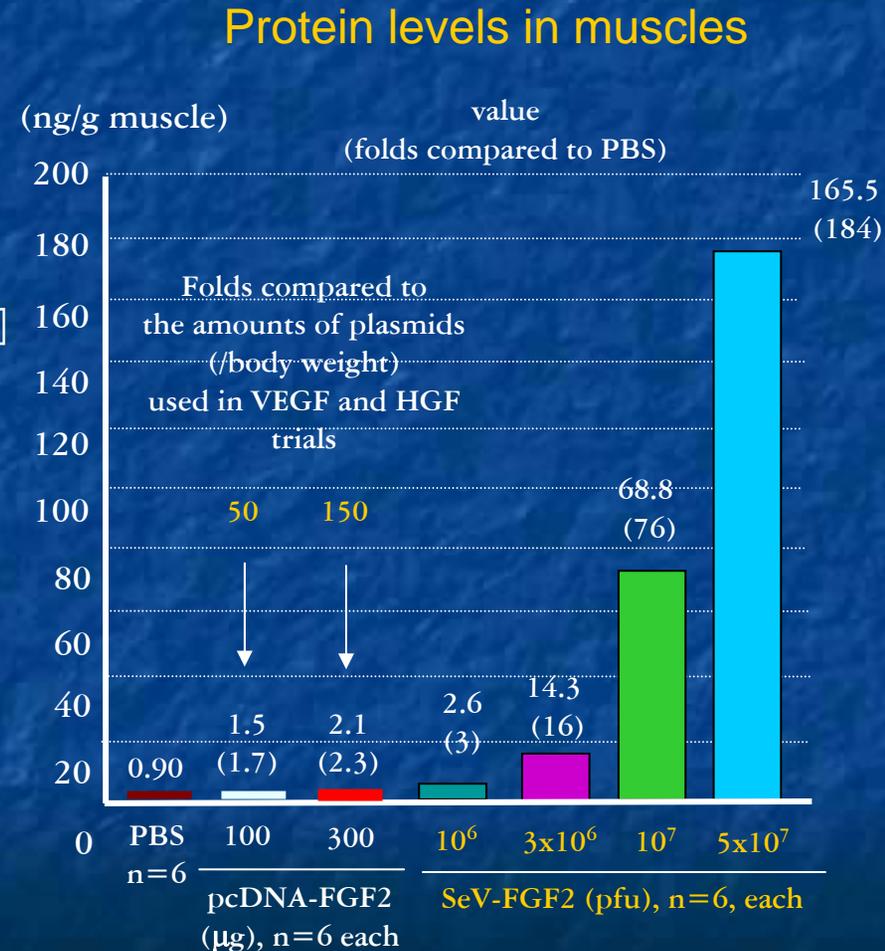
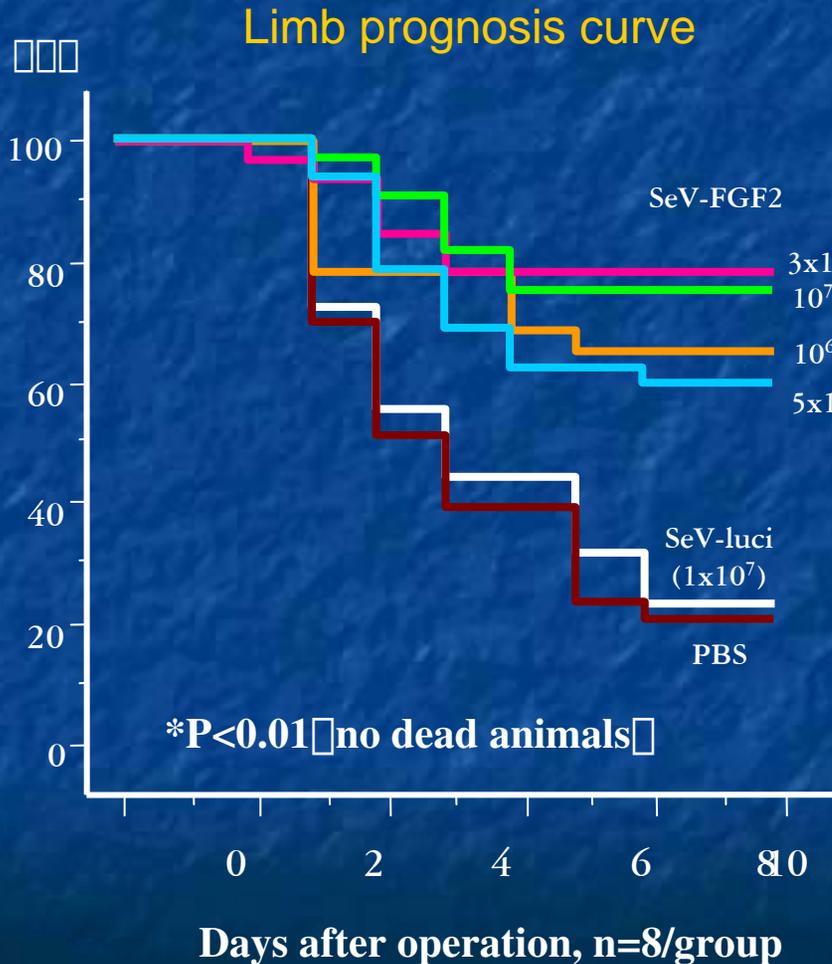
#6: Phase I/II study?

→ Shall fall into a Ph I/IIa category.

#7: Appendix M 2.2.2.2 minimal level of gene expression

#7: Minimum level of gene expression

Dose escalation studies for the relationship between FGF-2 protein level in muscles and limb prognosis



#7: Minimum level of gene expression

- **Effective gene expression level** of FGF-2 in the **mouse** autoamputation model was **2.6 to 14.3 ng/g muscle** (vs the **baseline level of 1 ng/g muscle**) when 1×10^6 to 3×10^6 ciu/head (= 3.3×10^7 to 1×10^8 ciu/kg) of SeV-FGF2 was administered.
- **pcDNA-FGF2** administration at the doses of 100 or 300 ug/head resulted in 1.7 to 2.3-fold increase in the level of FGF-2 (= **1.5 - 2.1 ng/g muscle**) in the mouse experiment.
- These plasmid doses correspond to **50 to 150-times** the doses of pHGF and pVEGF found effective in human trials. Thus, **pcDNA-FGF2 may be assumed to be effective even at the dose of 2 ug/head**.
- If the above is true, SeV-FGF2 or DVC1-0101 administration at a dose 100 times less than the effective dose of 1×10^6 ciu/head found in the mouse experiment can be assumed effective. Thus, **1×10^4 ciu/head** (= **3.3×10^5 ciu/kg = 2×10^7 ciu/man**) is expected to be effective.
- Therefore, the first dose, **5×10^7 ciu/man, is proposed**.

#8: Injection volume

→ Injection volume will remain constant.

#9: Lower level of FGF-2 in females?

→ There was no significant difference between genders.

- #1: Could co-infection with another enveloped virus spread SeV by pseudotyping?
- #2: Is active infection with HPIV-1 exclusion criterion?
- #3: Discrimination between anti-SeV and anti-HPIV-1 antibodies

Spread of DVC1-0101 *in vivo* by pseudotyping is unlikely.

In vitro pseudotyping of SeV/dF by hPIV1 is possible (Bernloehr et al. 2004). However, **barriers exist *in vivo*** to prevent spreading of DVC1-0101 by pseudotyping;

(1) A specific protease is needed to activate Fusion protein (F) (Nagai 1995)

(2) Different route of administration/infection separating two viruses;

hPIV1: Airborne infection, mostly confined to respiratory system because;

- Progeny virions bud only from the apical side of infected cells (Tashiro et al. 1992)
- Unable to infect basal cells of the respiratory epithelium (Massion et al. 1993).

DVC1-0101: intramuscular injection, infection mostly confined to muscle because;

- Progeny virions lack F protein, incapable of further infection
- Mostly inactivated in blood stream (Yonemitsu et al. unpublished data)
- i.v. administrated wt SeV does not infect or replicate in the lung (Bitzer et al. 2003)

Method: **ELISA** (developed originally for SeV)

Sample: **pre- and post-injection sera**

Operationally regard the elevation of antibody level after DVC1-0101 administration as the immune response specific to SeV vector.