Intramuscular Gene Transfer of Stromal Cell-Derived Factor-1 using plasmid JVS-100 Induces Therapeutic Angiogenesis in a Rabbit Model of Peripheral Vascular Disease

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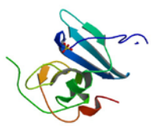
Abstract

Peripheral vascular disease (PVD) affects approximately 12 million Americans and is associated with significant morbidity and mortality. Patients with advanced PVD who are poor candidates for revascularization have 6-month amputation and mortality rates as high as 40% and 20%, respectively. JVS-100 is comprised of non-viral DNA plasmid engineered to transiently express non-modified human Stromal cell-Derived Factor-1 (SDF-1). SDF-1 is a strong chemoattractant of endogenous organ specific and bone marrow derived stem cells and progenitor cells to the site of tissue damage, which promotes tissue preservation and blood vessel development. Re-stimulating SDF-1 expression by gene transfer into ischemic muscle has a high therapeutic potential for treatment of ischemic disease because it has the potential to regenerate vasculature repair in organs damaged by ischemia. We have previously demonstrated that injection of JVS-100 into pigs with heart failure due to myocardial infarction increased cardiac vessel density and improved cardiac function through 90 days post-treatment. We hypothesized that the vasculogenic and stem cell homing properties of SDF-1 could also provide therapeutic benefit in PVD. In this study, we tested the safety and efficacy of JVS-100 delivery to ischemic limbs in an established rabbit PVD model.

Background



SDF-1

- JVS-100 currently being tested in Phase I Heart Failure Study
- 8 kDA chemokine, receptor is CXCR4
- Expressed in most tissues, including skin[4]
- Improves cardiac function after acute myocardial infarction[5]
- Mobilizes/recruits hematopoietic stem cells to injured tissue[1]
- SDF-1 is increased in wounds after trauma[3]
- SDF-1 therapy in diabetic wounds increased healing[2]
- Increased SDF-1 expression stimulates blood vessel formation in damaged tissue

Critical limb Ischemia (CLI) in the US

- 125,000-250,000 patients per year
- Affects 1% of Americans over 50 (>1 million in US)
- 1 yr mortality rate of patients with CLI is 25%
- Only 20-30% of patients are undergoing treatment, of which 30% may require amputation
- 150,000 amputations per year in the US

Hypothesis:

Direct injection of JVS-100 to increase SDF-1 expression in a rabbit model of hind limb ischemia will increase blood flow.

Methods

Plasmid DNA for all pre-clinical studies was manufactured by **Aldevron LLC** (North Dakota)

SDF-1 ELISA was performed using the R&D Systems (Minneapolis, MN) SDF-1 ELISA kit.

MSC transwell migration assay was performed using p12 rat MSCs incubated on 12 uM fibronectin coated transwells. HEK 293 cells were plated in the bottom well and transfected with JVS-100 using Fugene. Migration was assessed over a 4 hour period 3 days post-transfection.

Rat HLI model for time course of JVS-100 expression was performed at Cleveland Clinic animal facility over 4 weeks. Male Lewis rats were anesthetized and a longitudinal incision in the medial thigh from the inguinal ligament to the knee joint, exposing the femoral artery, which was ligated and removed. Animals were allowed to recover for 10 days, then anesthetized and directly injected with 1.0, 2.0 or 4 mg/ml of the luciferase version of JVS-100 (vector backbone with luciferase cDNA) in 0.2 ml at 4 sites along the hindlimb. *In vivo* bioluminescence was measured after injection of 125 mg/kg using the Xenogen imaging system for 3 minute exposure.

***Ex vivo* bioluminescence demonstrated pDNA expression in normal rabbit hind limb**
Normal Adult New Zealand white rabbits were injected at 4-8 sites with up to 8 mg of the luciferase version of JVS-100 in 1.0 ml per injection site. A 4-0 suture was used to identify each injection site. 3 days post-injection, animals were sacrificed, muscles (gastrocnemius, adductor, semi-membranous) excised. Muscle was incubated in 125 mg/ml luciferin for 7 minutes and imaged with the Xenogen imaging system for 3 minute exposure.

Standard model of rabbit hind limb ischemia and ante-mortem follow-up.
New Zealand white rabbits (n=5/group, 2-3 males/females per group) underwent unilateral femoral artery ligation and 10-days post ligation received 4 or 8 mg JVS-100 or 4 mg of control (luciferase) plasmid via 8 direct intramuscular 0.5 ml injections to the ischemic limb. Safety endpoints were evaluated at 60 days post-injection and included histopathology and biodistribution. Clinical pathology was assessed in all groups at 60 days post-injection. Efficacy was measured by % change in angiographic score compared to control at 30 and 60 days post-treatment.

Study design of JVS-100 Safety and Efficacy in a rabbit model of hindlimb ischemia

Group	# animals	pDNA/ Dose	# Injection sites	Volume/ Site	pDNA/ site
1	5	Control (4mg pLuc)	8	0.5 ml	0.5 mg
2	5	4 mg			0.5 mg
3	5	8 mg			1.0 mg

Results

JVS-100 Peak expression in muscle 3-8 days post-injection

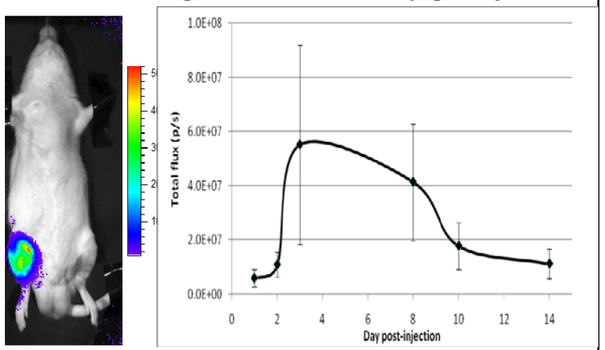


Figure 1. Time course of JVS-100 vector expression in a rodent HLI model. A. Image of luciferase expression in ischemic rat leg 3 day post-injection. B. Time course of mid dose (2 mg/ml) JVS-100 vector expression in ischemic rat leg (n=4). Data are +/- SEM.

Results

JVS-100 vector expression 3 days post-injection into rabbit hindlimb

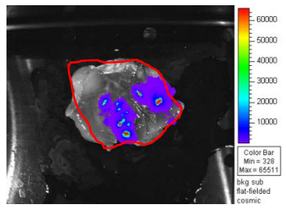


Figure 2. Day 3 *ex vivo* bioluminescence of JVS-100 vector with luciferase cDNA. Bioluminescence 3 days post-injection of rabbit hind limb muscle with luciferase version of JVS-100. 2 mg pDNA was injected into each of 4 sites (8 mg total) into a normal rabbit hind limb. Injection volume = 1 ml/site. Red outline is area of injections as determined by suture identification at time of injection.

SDF-1 stimulated vasculogenesis in ischemic rabbit hindlimbs

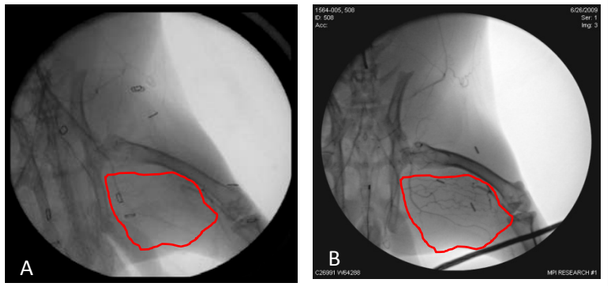


Figure 3. JVS-100 increases SDF-1 expression and stimulates vasculogenesis. Example of Angiograms and scoring of ischemic hindlimb of rabbit at baseline (A) and 30 days post-injection (B) with 1 mg/mL of JVS-100(B,D). Red outline in A and B indicate approximate area of scoring grid.

JVS-100 increased angiographic score in ischemic rabbits

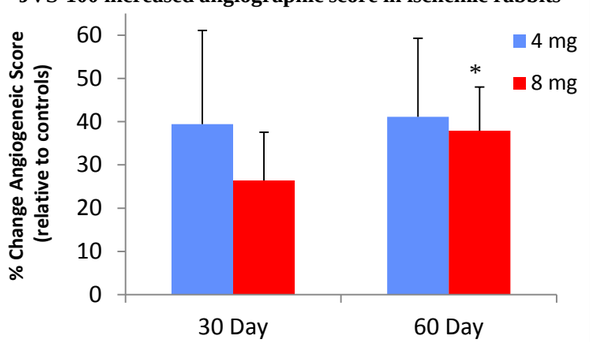


Figure 4. JVS-100 increases angiographic score 30 and 60 days post-injection.
% change in angiographic score 30 and 60 days post-injection with JVS-100, normalized to control group. * = P<0.05 vs. control. Data shown as averages +/- SEM.

Summary and Conclusions

- JVS-100 expresses functional SDF-1 which induces MSC migration
- Peak SDF-1 expression occurs 3 days post muscle injection
- 8 injections of 1 or 2 mg/ml JVS-100 induced therapeutic benefit
- JVS-100 increased angiographic score 30 and 60 days post-injection ($p < 0.05$ at 60 days)
- Direct injection of JVS-100 improves angiogenesis in rabbits with CLI.
- These results indicate that treatment of PVD by an SDF-1 encoding plasmid, JVS-100, is safe and promotes increased vessel density in a rabbit PVD model that is sustained for at least 60 days.
- This data has led to the initiation of a Phase II clinical trial evaluating JVS-100 safety and efficacy after delivery to Rutherford Class 4 and 5 patients with critical limb ischemia.

Proposed Clinical Program in CLI

JVS-100 Phase IIa Study allowed under US IND for treatment of Critical Limb Ischemia (CLI):

- 48 subject, double-blind, placebo controlled trial
- Single dose escalation, 4 cohorts
- Rutherford Class 4 & 5
- Safety and efficacy assessments with 12 month follow-up
- Safety Endpoints : Adverse Events, ECG, PE/Vitals, Labs
- Efficacy Endpoints: Amputations, survival , ulcer healing Rutherford class, VAS,QOL,TBI,VAS,QOL,ABI,TBI,TcP02

Phase IIa Clinical Dosing Regimen

Co-hort	# Pts	DNA /Dose	# Inj. Sites	DNA Conc.	Vol. per Site	DNA/ Site	Randomization
1	12	4 mg	8	1 mg/ ml	0.5 ml	0.5 mg	Randomized 3:1 (9 Tx, 3 control)
2	12	8 mg	16		0.5 ml	0.5 mg	Randomized 3:1 (9 Tx, 3 control)
3	12	8 mg	8		1 ml	1 mg	Randomized 3:1 (9 Tx, 3 control)
4	12	16	16		1 ml	1 mg	Randomized 3:1 (9 Tx, 3 control)

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