

## Abstract

Peripheral vascular disease (PVD) affects approximately 12 million Americans and is associated with significant morbidity and mortality. PVD prevalence increases dramatically with age, affecting approximately 20% of Americans age 65 and older. Patients with advanced PVD whom are ineligible for reconstruction or in whom prior revascularization has been unsuccessful have 6-month amputation and mortality rates as high as 40% and 20%, respectively. Despite its prevalence and cardiovascular risk implications, only 20 to 30 percent of PVD patients are undergoing treatment. JVS-100 comprises a non-viral DNA plasmid engineered to transiently express 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 PVD because, unlike current treatments that focus on alleviating symptoms, SDF-1 has the potential to regenerate vasculature and provides an opportunity to repair and retain function in degenerating limbs. We have previously demonstrated that injection of JVS-100 (SDF-1 plasmid DNA) into a large animal model of chronic heart failure increased heart function for at least 90 days post-injection. In this study, rabbits (n=5/group) underwent a unilateral femoral artery ligation and 10-days post ligation received 4 or 8 mg JVS-100 via 8 intramuscular injections to the ischemic limb. Blood flow was measured by angiography at 30 and 60 days post-treatment. JVS-100 treated animals showed improved blood flow at both time points compared to vector injected control animals, which showed decreased blood flow. Importantly, benefits were observed at 30 days with significant (p<0.05) benefits sustained at 60 days. These results indicate that patients with PVD may benefit from treatment with JVS-100.

## Background

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- Also known as CXCL12
  - 8 kDa chemokine, receptor is CXCR4
  - Expressed in most tissues, including skin<sup>1</sup>
  - Improves cardiac function after acute myocardial infarction<sup>5</sup>
  - Mobilizes/recruits hematopoietic stem cells to injured tissue<sup>1</sup>
  - SDF-1 is increased in wounds after trauma<sup>3</sup>
  - SDF-1 therapy in diabetic wounds increased healing<sup>2</sup>
  - 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 µm fibronectin coated transwells. HEK 293 cells were plated in the bottom well and transfected with JVS-100 using Eugene. 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-membranosus) excised. Muscle was incubated in 125 mg/ml luciferin for 7 minutes and imaged with the Xenogen imaging system for 3 minute exposure.

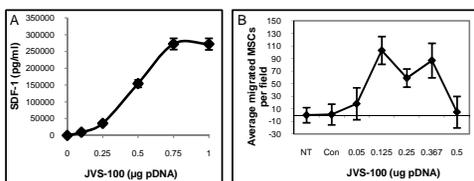
**Standard model of rabbit hind limb ischemia and ante-mortem follow-up.**

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

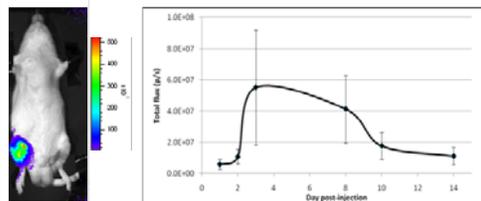
### JVS-100 expresses hSDF-1 and induces dose-dependent MSC migration



**Figure 1. SDF-1 ELISA and rat MSC transwell migration assay.** (A) SDF-1 ELISA of supernatant from HEK293 cells 3 days post-transfection with JVS-100 pDNA (B). Transwell migration assay of rat MSCs to SDF-1 secreted from HEK293 cells transfected with JVS-100. Shown is average +/- SEM.

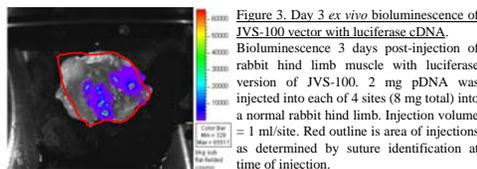
## Results

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



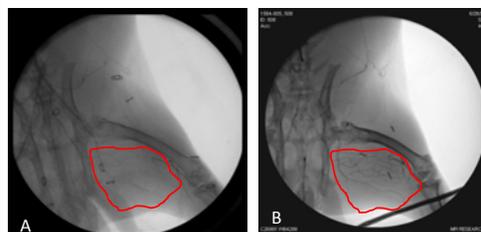
**Figure 2.** Time course of JVS-100 vector expression in a rodent HLI model. A. Image of luciferase expression in ischemic rat leg 3 day post-injection. B. Time course of mid dose (2 mg/ml) JVS-100 vector expression in ischemic rat leg (n=4). Data are +/- SEM.

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



**Figure 3.** 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.

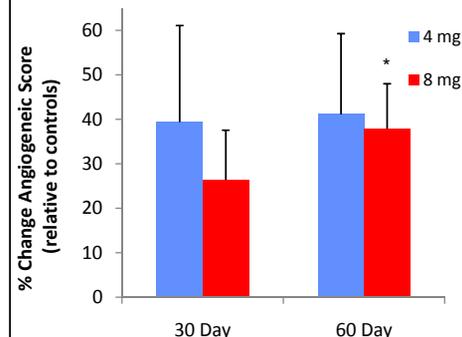
### SDF-1 stimulated vasculogenesis in ischemic rabbit hindlimbs



**Figure 4.** 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.

## Results

### JVS-100 increased angiographic score in ischemic rabbits



**Figure 5.** 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.

Please see Poster #426 for information on JVS-100 and treatment for HF

### References:

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