Intra-myocardial administration of JVS-100 promotes cardiac benefit and improves angiogenesis in a porcine model of heart failure

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Abstract

JVS-100 comprises a non-viral DNA plasmid engineered to transiently express human Stromal-cell Derived Factor 1 (**SDF-1**). SDF-1 triggers a number of protective molecular cascades that are both anti-inflammatory and anti-apoptotic. Furthermore, **SDF-1** is a strong chemoattractant of stem cells and progenitor cells that promote tissue preservation and blood vessel development. Previous studies have demonstrated that **SDF-1** expression is increased in the myocardium after a myocardial infarction, but expression lasts for less than a week, and therefore the induced stem cell homing response quickly fades. This short duration of **SDF-1** expression reduces the potential for tissue repair and suggests that therapeutic interventions which prolong the ability of **SDF-1** to stimulate the stem cell homing process may be beneficial for patients that have damaged heart tissue. A 57-pig safety and efficacy study evaluated cardiac functional response, toxicity and bio-distribution in a porcine model of heart failure after treatment with escalating doses of JVS-100. All enrolled pigs had a left ventricular ejection fraction (EF) <40% and a left ventricular end systolic volume (LVESV) >56.7 ml as measured by echocardiography 30 days post-infarct at which time they received intra-myocardial injections of Dextrose (control), or JVS-100 at doses of 7.5 mg (low), 30 mg (mid) or 100 mg (high). At 60 days post-therapy, JVS-100 improved LVEF and LVESV at low and mid doses. JVS-100 promoted vasculogenesis at all doses relative to controls, with significant increases at the low and mid doses at 30 days post-injection. Importantly, increased vessel density (>200 vessels/field) correlated with improved cardiac function. The difference in change in LVESV between vessel density groups (<200 vessels/field: 5.9±11.5 ml vs. >200 vessels/field: -8.6±13.4 ml) was statistically significant ($p<0.05$) at 30 days post-injection, and the difference in change in LVEF (<200 vessels/field: -0.9±10.2% vs. >200 vessels/field: 7.5±5.5%) approached statistical significance ($p=0.052$). No JVS-100 dose was associated with signs of toxicity, adverse effects on clinical pathology or histopathology. JVS-100 was distributed primarily to the heart with negligible amounts found in non-cardiac tissues. The product was essentially cleared from all organs within 90 days of treatment. These findings indicate that the no observable adverse effect level (NOAEL) for JVS-100 in the pig model of ischemic heart failure was 100 mg. Based on these results, the FDA has allowed an IND to initiate an open label, 16 subject Phase 1 dose-escalation study to evaluate the initial safety of JVS-100 to treat heart failure in subjects with ischemic cardiomyopathy (NYHA Class III with prior myocardial infarction).

Methods

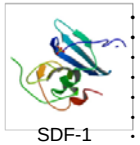
Plasmid DNA for all pre-clinical studies was manufactured by **Aldevron LLC** (North Dakota)
SDF-1 ELISA was performed using the R&D Systems (Minneapolis, MN) SDF1 ELISA kit.
MSC transwell migration assay was performed using p12 rat MSCs incubated on 12 uM fibronectin coated transwells. HEK 293 cells 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.

Porcine heart failure study was conducted by MPI Research (Mattawan, MI) using the Biocardia Helical Infusion Catheter (pictured left). Study design is described in Table 1. Animals received an anterior MI 30 days prior to injection with JVS-100. Pigs having an ejection fraction <40% and an LVESV >56.7 ml as measured by echocardiography 30 days post-infarct were enrolled. Efficacy, Biodistribution and toxicology assessed at 3, 30 and 90 days post-injection.

Table 1. Study Design: JVS-100 Therapy for Chronic Heart Failure in Pigs

Group	Treatment	Number of Animals			
		Dosing	Necropsy		
		Day 0	Day 3	Day 30	Day 90
		M/F	M/F	M/F	M/F
1	Control (20 injection sites of 1 ml each)	6/6	2/2	2/2	2/2
2	0.5 mg/site X 15 injection sites of 1 ml each (7.5 mg total)	7/8	3/2	2/3	2/3
3	2 mg/site X 15 injection sites of 1 ml each (30 mg total)	8/7	2/3	3/2	3/2
4	5 mg/site X 20 injection sites of 1 ml each (100 mg total)	8/7	2/3	3/2	3/2
Study Article:	JVS-100 for injection				
Dose Administration:	Direct LV intramyocardial injection by BioCardia Helical Infusion Catheter at 15-20 peri-MI sites				
Endpoints:	**Primary:** Change in LVESV from baseline measured by echocardiography **Secondary:** Safety endpoints, including organ histopathology, clinical tox and additional echocardiographic parameters (LVEF, LVEDV)				
Follow-up Duration	3 months (90 days)				
Statistical Methods:	One-way ANOVA with Dunnett's correction or Student's t-test with Bonferroni correction				

Background



- Also known as CXCL12
- 8 kDA chemokine, receptor is CXCR4
- Upregulated in damaged tissue, 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]

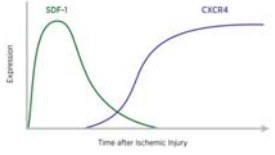


Figure 1. SDF-1 and CXCR4 expression after an ischemic injury.
SDF-1 expression is temporarily increased after an injury but soon fades, whereas the primary SDF-1 receptor, CXCR4, is continually upregulated in ischemically damaged tissue.

Hypothesis:
Re-introducing SDF-1 expression in a chronically damaged heart using naked plasmid DNA gene transfer will increase cardiac function.

Results

JVS-100 expresses hSDF-1 and induces dose-dependent CXCR4⁺ cell migration

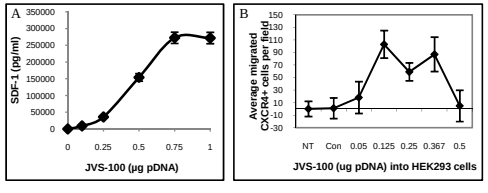


Figure 2. SDF-1 ELISA and rat MSC transwell migration assay.
(A) SDF-1 ELISA of supernatant from HEK293 cells 3 days post-transfection with JVS-100 pDNA (B). Transwell migration assay of rat CXCR4+ stem cells to SDF-1 secreted from HEK293 cells transfected with JVS-100. Shown is average +/- SEM.

Results

JVS-100 improves cardiac function in pigs with heart failure

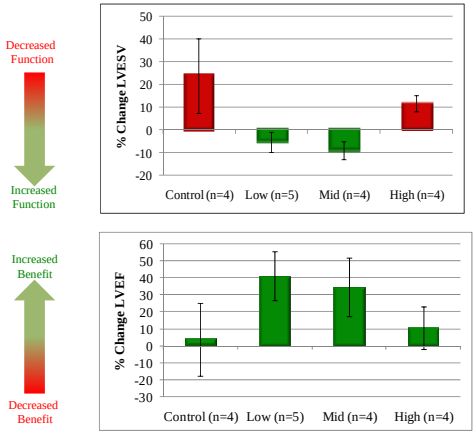


Figure 3. JVS-100 improves LVESV and LVEF in pigs with chronic heart failure.
(A) Left Ventricular End-Systolic Volume (LVESV) and (B) Left ventricular Ejection Fraction (LVEF) and were measured in pigs 60 days after JVS-100 injection. Shown is average +/- SEM.

JVS-100 cardiac biodistribution 90 day post-injection

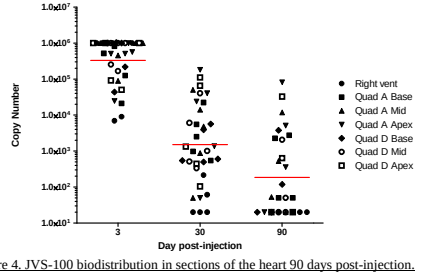
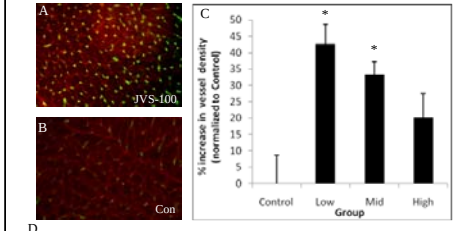


Figure 4. JVS-100 biodistribution in sections of the heart 90 days post-injection.
JVS-100 biodistribution 90 days post-cardiac injection, n = 4-6 per day. Red bar, mean per day. NQ = Not Quantifiable; sample tests above assay limit of detection but below quantifiable range of assay; > 20 copies / µg and < 50 copies / µg; LLOD = Lower than Limit of Detection; < 20 copies SDF-1 / µg. Red bar = grand mean.

Results

JVS-100 increases vasculogenesis 30 days post-injection



D

Average Absolute Functional Change from Baseline at 30-days

Vessels per Field	Sample Size	LVESV	LVEF
< 200	10	5.92	-0.9
> 200	6	-8.6	7.5
p-Values		0.037	0.051

Figure 5. JVS-100 increased vessel density 30 days post-injection.
Increased cardiac vessel density in (A) Mid dose compared to (B) control animals. (C) Myocardial vessel density was increased 30 days after treatment with JVS-100. % increase in vessels/field normalized to control group. * = P<0.05 vs. control. *Red = myocytes; green = endothelial cells.* Data shown as averages +/- SEM. (D) Increased cardiac function at 30 day post-injection is related to increased cardiac vessel density.

Summary and Conclusions

Key safety and efficacy findings of the JVS-100 Heart Failure Study:

- JVS-100 expresses functional SDF-1 protein and can induce dose-dependent migration of CXCR4+ stem cells
- Low and mid dose JVS-100 increased LVEF and decreased LVESV 60 days post-injection in a pig model of chronic heart failure
- JVS-100 increased vessel density 30 days post-injection
- Increased cardiac function correlated with increased vessel density
- < 99.9999% of JVS-100 was cleared from the heart 90 days post-injection
- There were no JVS-100 related deaths during the study – the NOAEL is 100mg
- These data demonstrate that JVS-100 is safe and effective in attenuating heart failure progression in a porcine model of ischemic cardiomyopathy.

References:
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Please see Poster #438
For information on JVS-100 and treatment for CLI