
Human Gene Transfer Protocol # 0910-1004

entitled: *An Open Label Dose Escalation Study to Evaluate the Safety of a Single Escalating Dose of ACRX-100 Administered by Endomyocardial Injection to Cohorts of Adults with Ischemic Heart Failure*

Presenters:

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Doug Losordo, M.D.

SDF-1 BIOLOGY AND MECHANISMS OF ACTION

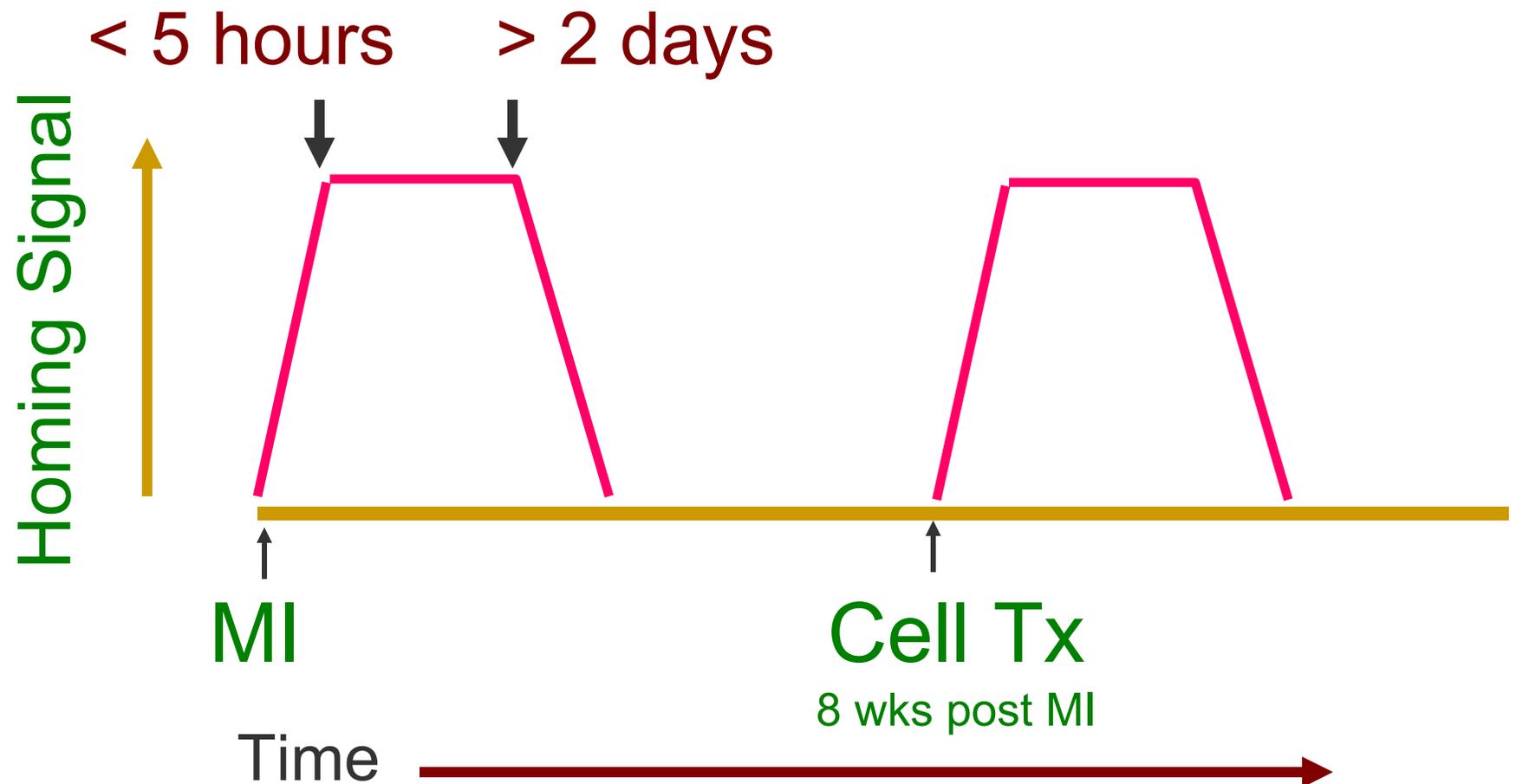
Hypothesis

The Penn Laboratory has uncovered a natural but inefficient stem cell based repair process in mammals that attempts to repair tissue damage following an injury.

This repair process occurs in a broad range of organs including the heart, brain, kidney and wounds following ischemic injury.

Reactivation of this repair system will result in tissue repair through recruitment of endogenous stem cells to the damaged organ

Characteristics of putative myocardial stem cell homing factor

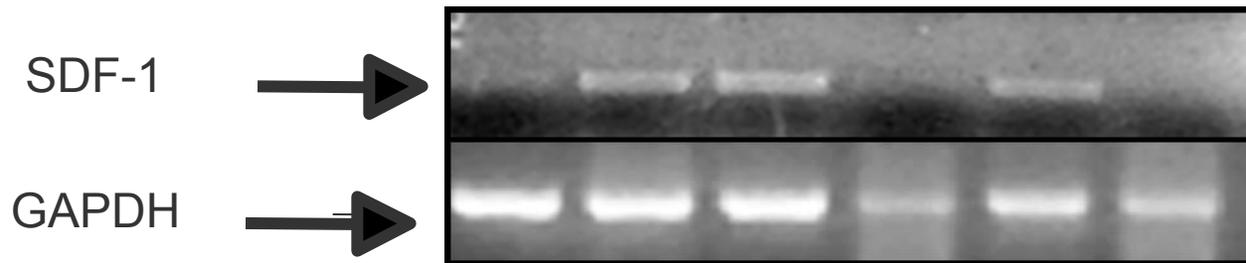


Orlic, et al. Nature 2001;410:701-705; Kocher, et al. Nat Med 2001;7:430-436.

SDF-1 mediates stem cell homing following a myocardial infarction

Stromal Cell-Derived Factor –1

	Hours			Days		
Time after MI:	0	1	24	7	30	30
Transplantation:	-	-	-	-	+	-



RT-PCR of 500 ng of total RNA for 40 cycles

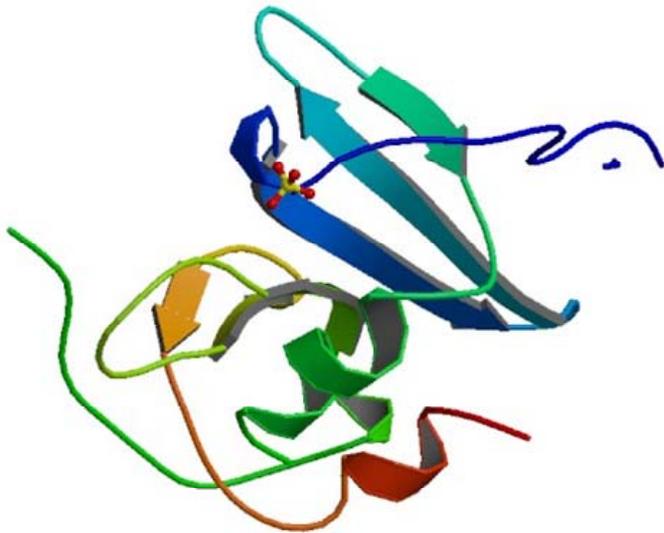
Stromal Derived Factor 1 (SDF-1)

A Natural “Repair Signal”

SDF-1 naturally attempts to repair tissue after injury but levels are not sufficient to see “full benefit”



By prolonging or reestablishing SDF-1 expression post-injury Juventas will maximize repair



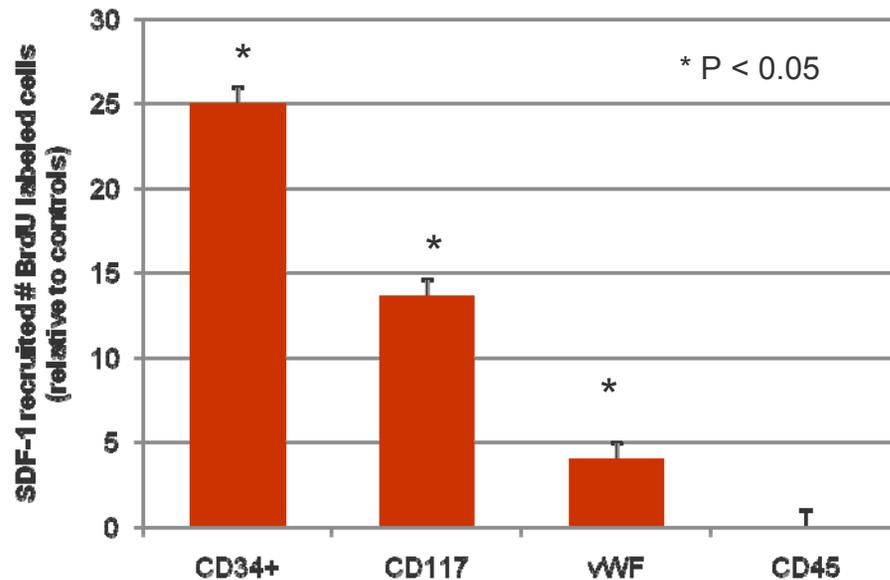
■ Mechanisms of Action

- Inhibits cell death
- Recruits stem cells that repair tissue
- Promotes blood flow

Effect of SDF-1 on cardiac repair

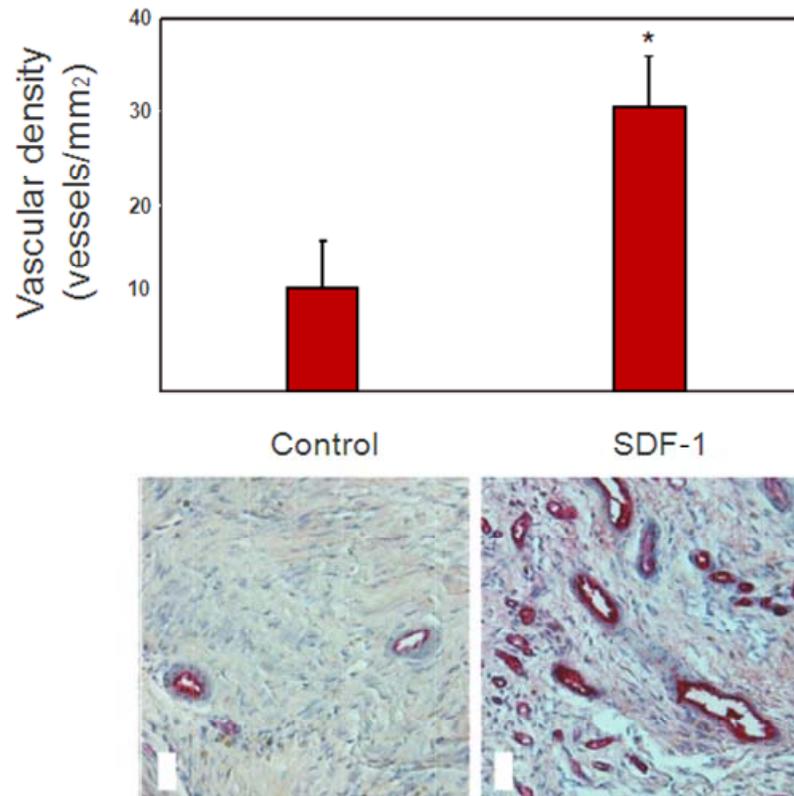
- We have studied mechanism of action using cell based gene transfer
 - SDF-1 over-expressing cardiac fibroblasts delivered 2 mo after AMI
 - AdSDF-1:SKMB delivered 2 mo after AMI
 - SDF-1 over-expressing MSC delivered 1 day after AMI
- Other groups have used
 - Cell based SDF-1 gene transfer
 - Protein based SDF-1 delivery using matrices or nanopeptides

SDF-1 Recruits CD34⁺ and CD117⁺ Cells to Damaged Tissue



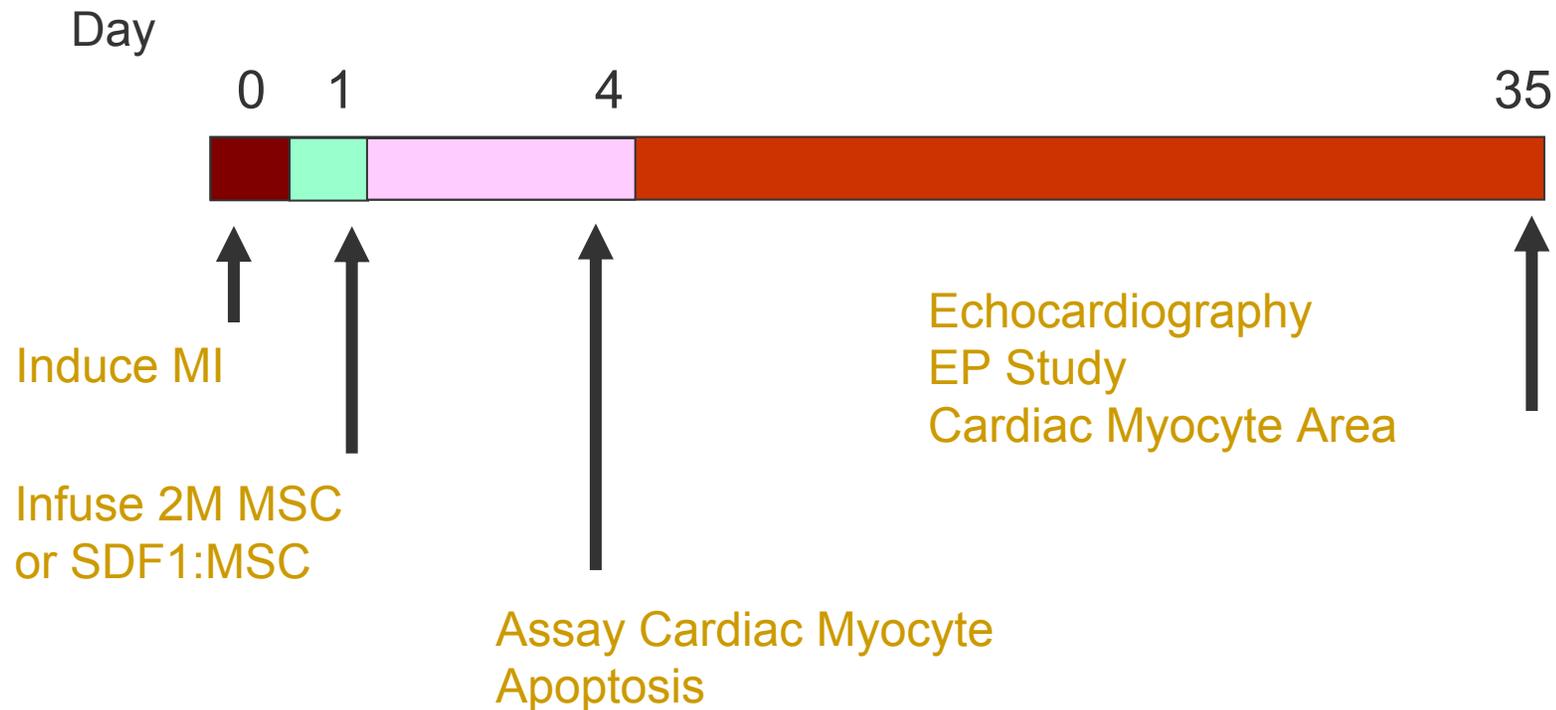
- SDF-1 recruits endogenous HSCs to damaged tissue when delivered 2 months following a myocardial infarction in rat models

SDF-1 is Vasculogenic

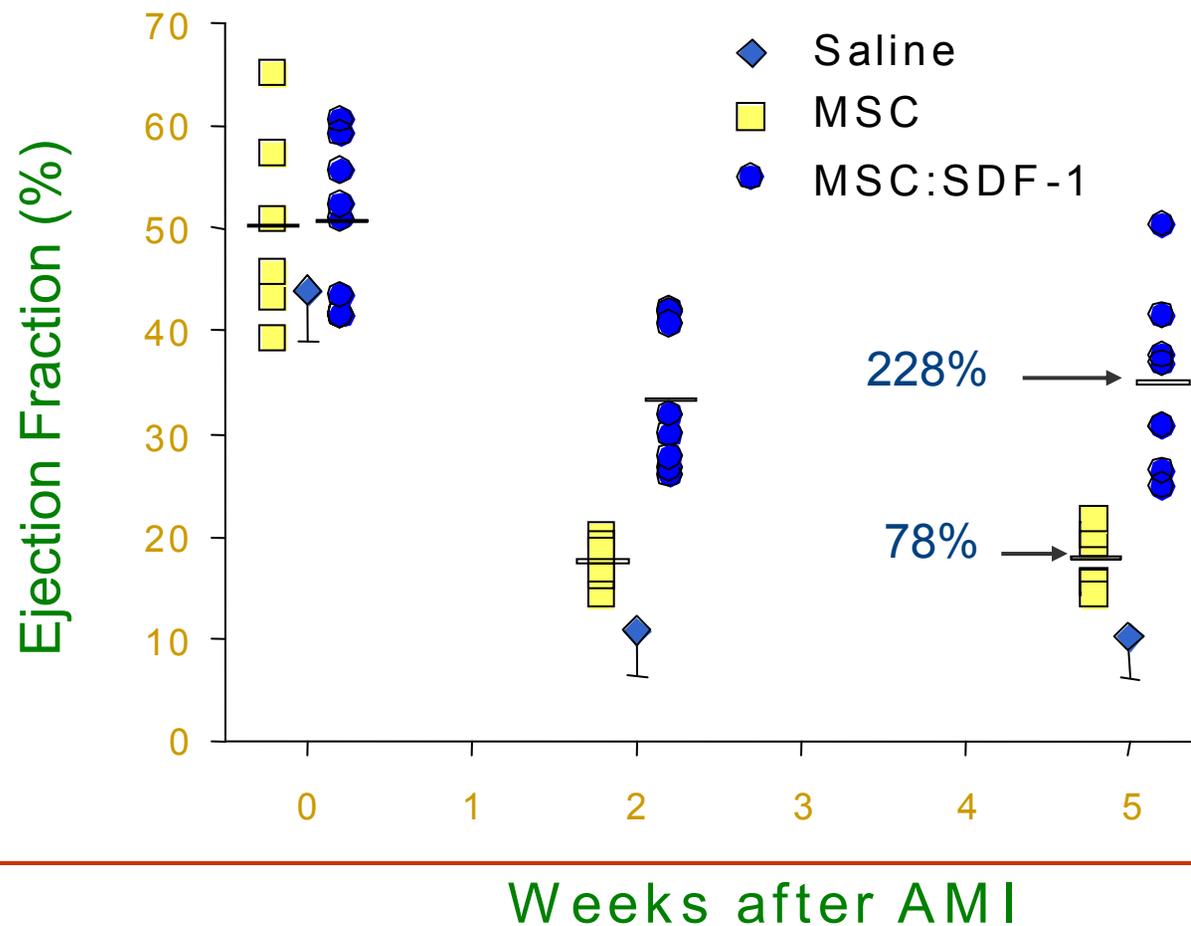


- SDF-1 promotes new blood vessel growth that is sustained for at least 2 months following treatment in rat and porcine models

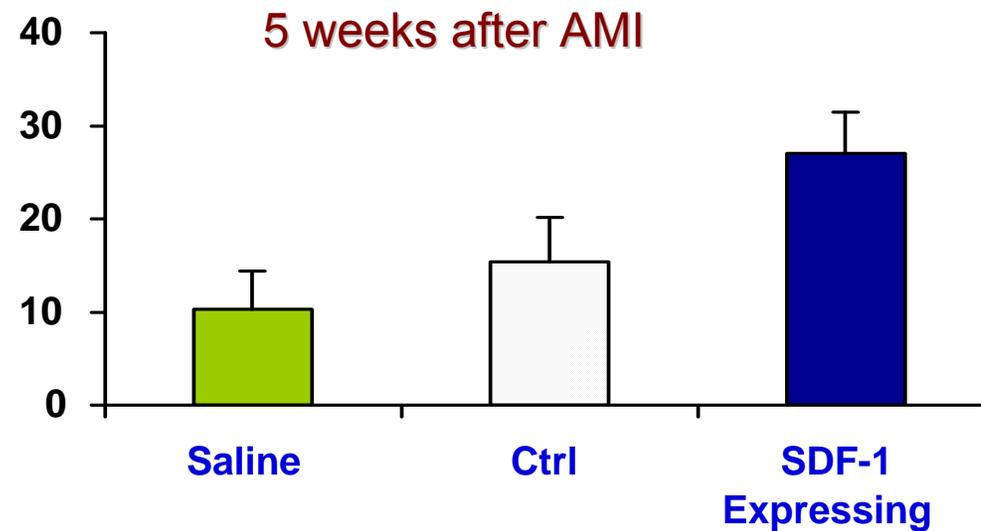
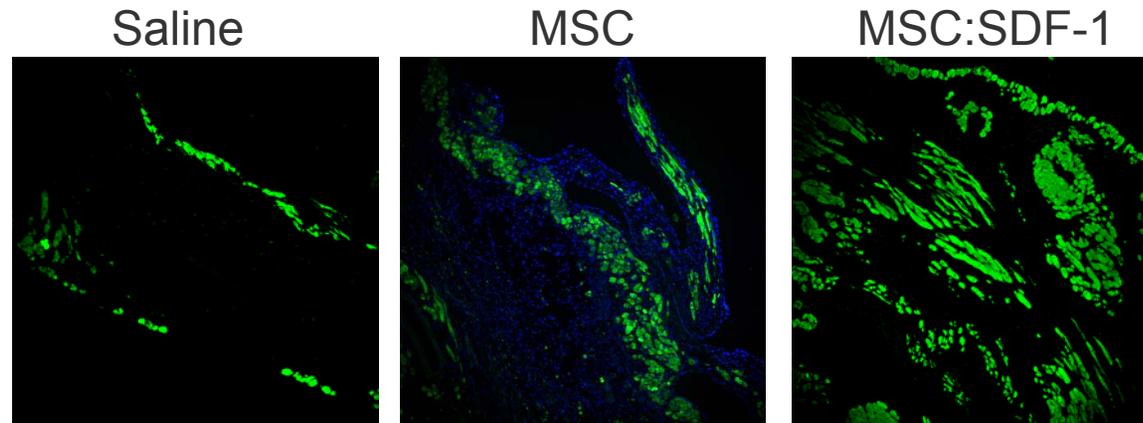
Effect of extending SDF-1 expression after Acute MI



SDF-1 over-expression improves cardiac function in Acute MI



SDF-1 preserves cardiac myocytes

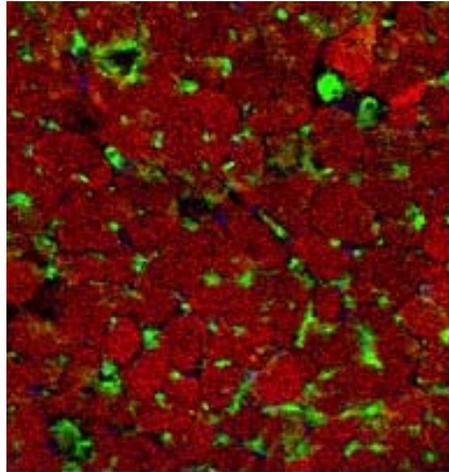


Cardiac
Myosin

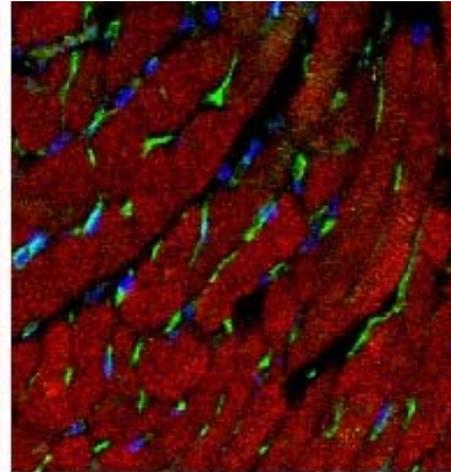
MSC Cell Type

Injured cardiac tissue express CXCR4

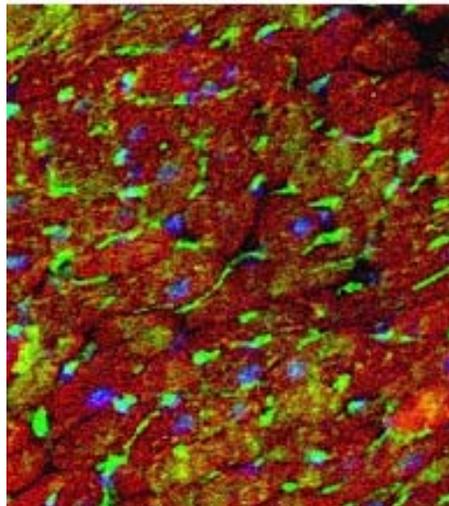
12 h



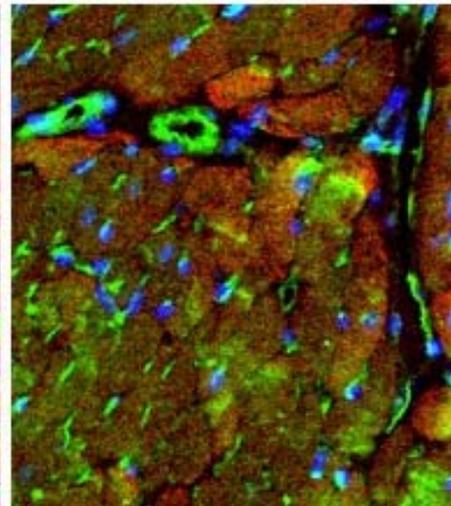
24 h



48 h

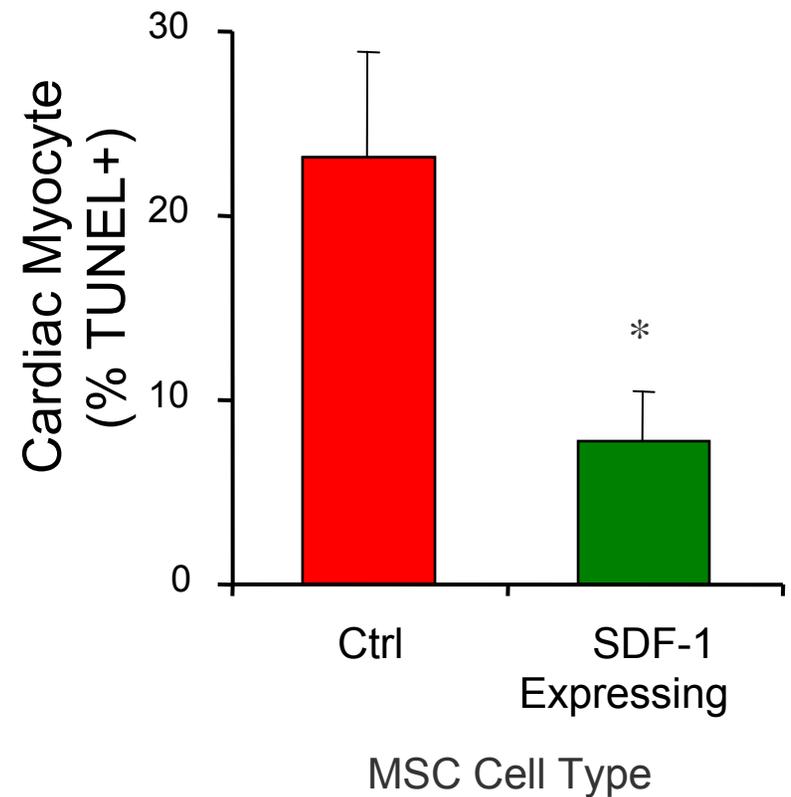
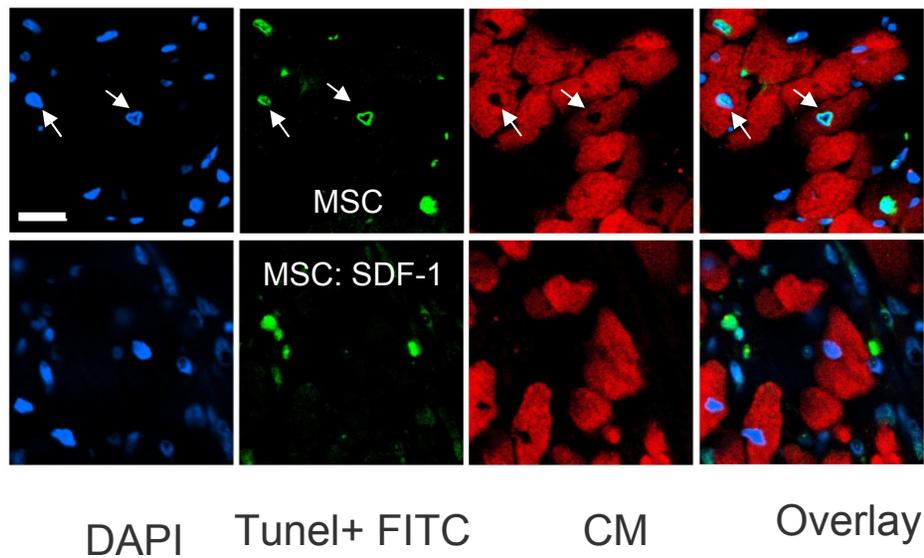


72 h



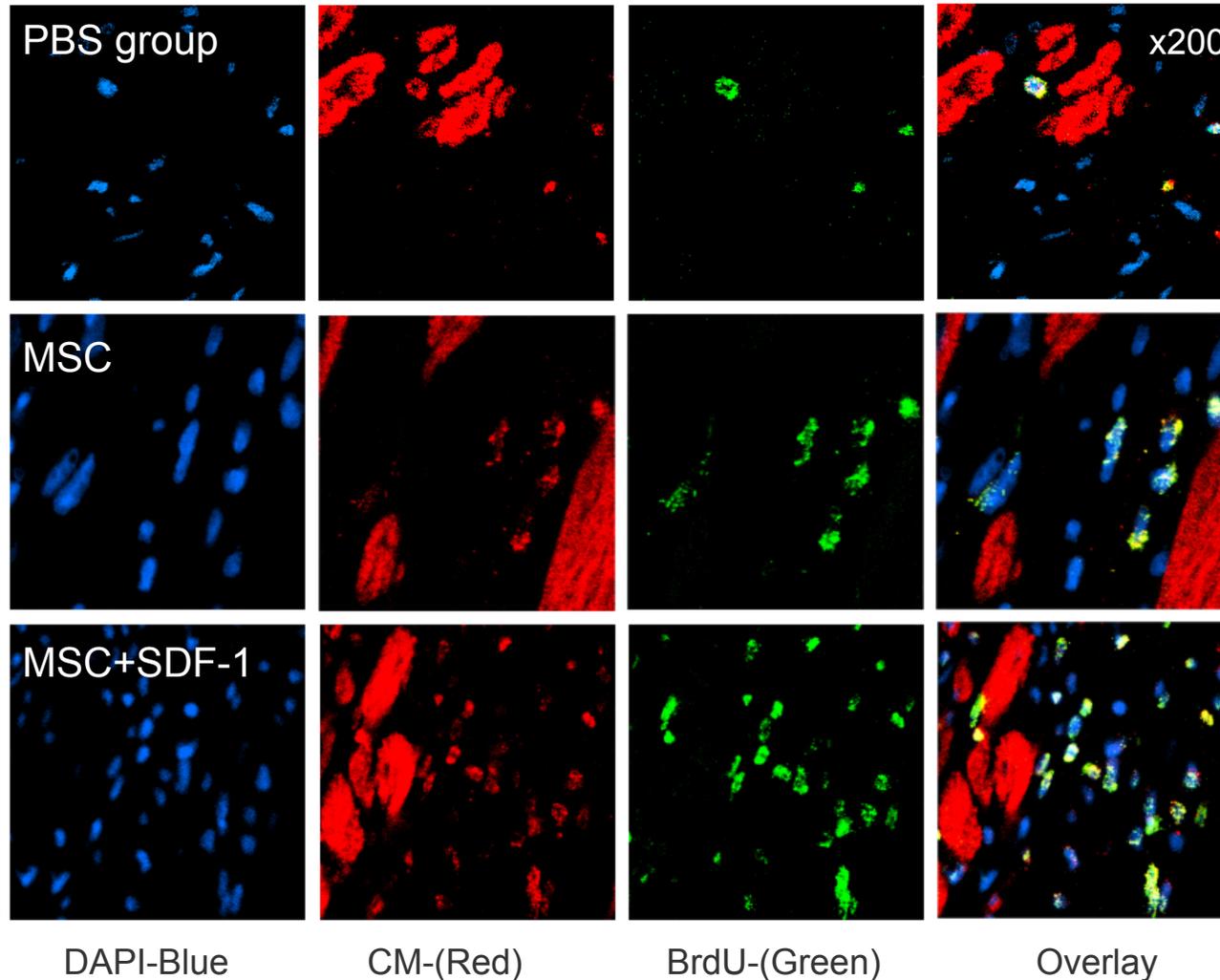
Troponin I
CXCR4

SDF-1 decreases TUNEL+ cardiac myocytes 4d after AMI

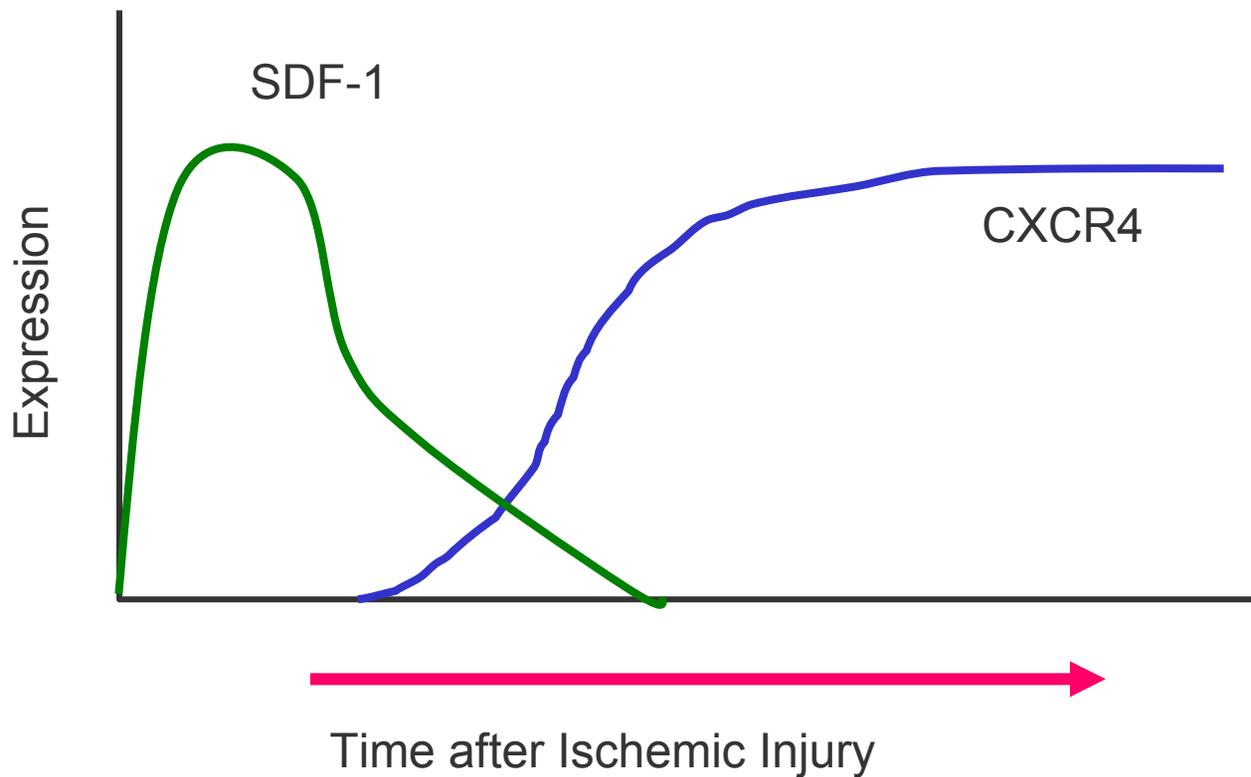


Infarct Border zone, 1000 cells quantified

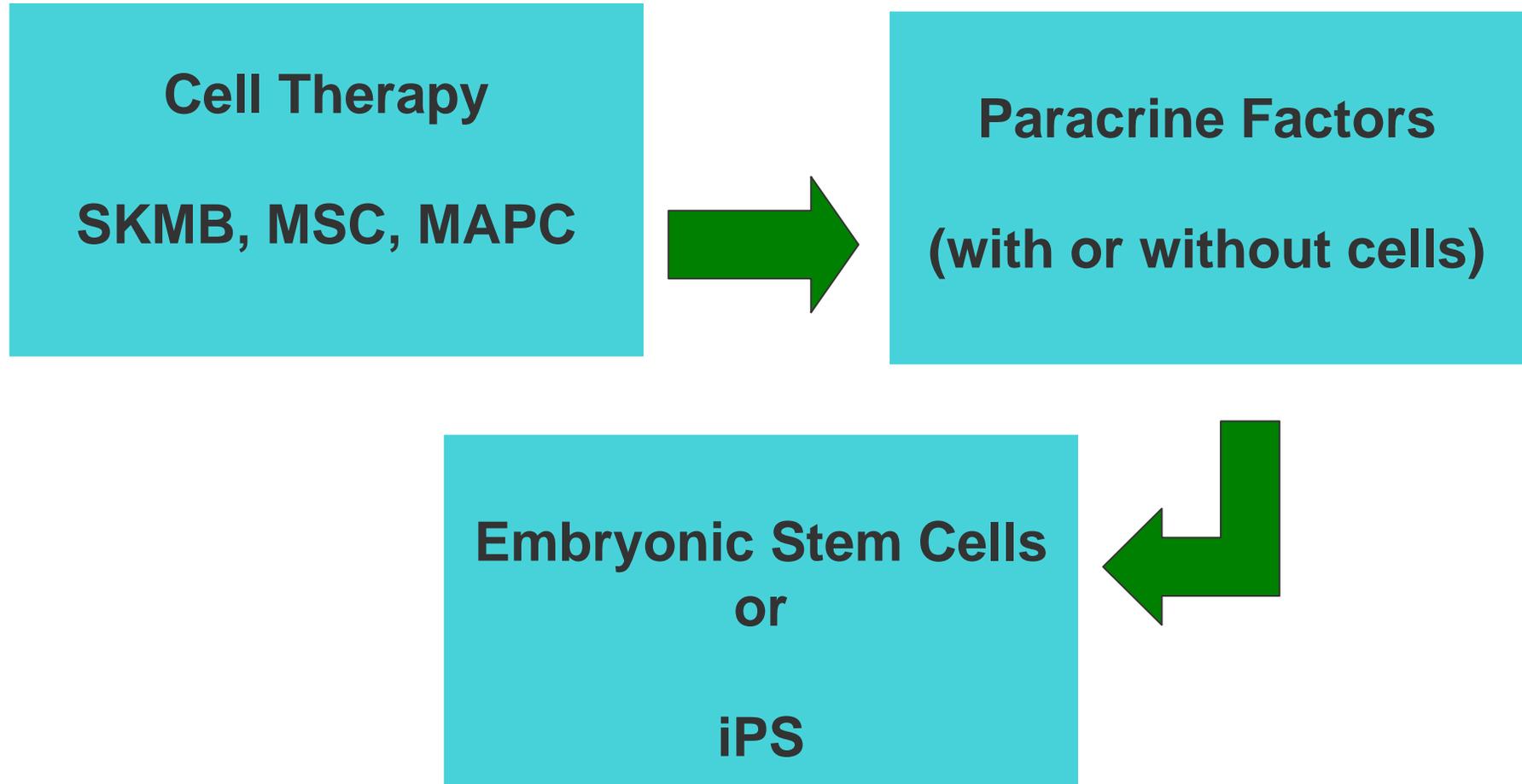
Myocytes are not derived from bone marrow or cardiac stem cells



Cardiac repair is promoted through re-alignment of the SDF-1:CXCR4 axis



Evolution of CV regenerative medicine

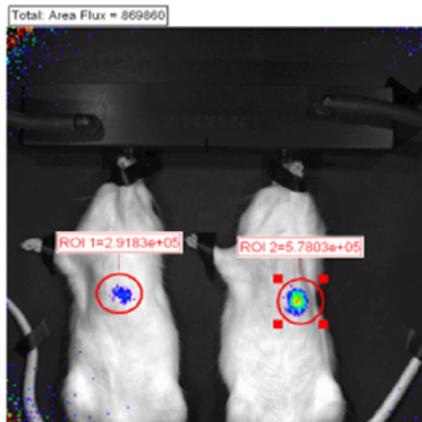
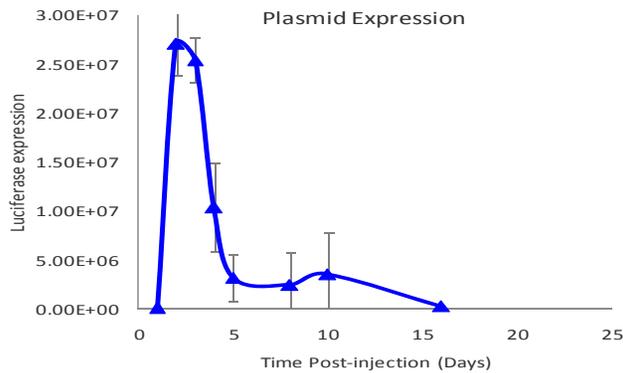


Benefits of non-viral vector delivery

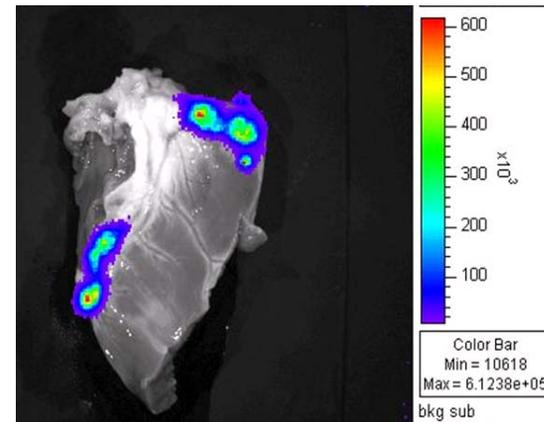
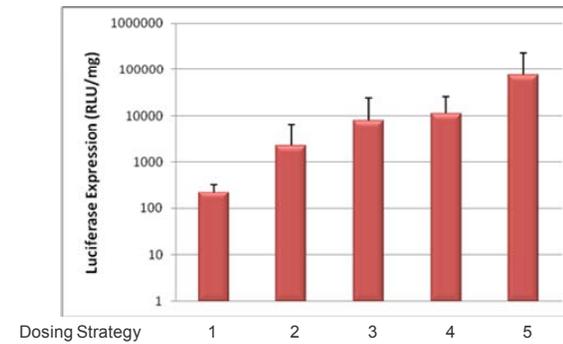
- **Non-viral vectors are safe in humans**
 - 11 clinical trials in ischemic cardiac or peripheral artery disease
- **Therapeutic advantages compared to protein/viral vectors**
 - Smaller dose compared to protein (reduces toxicity concerns)
 - Extends the half-life and prolonged bioavailability
- **Cost-effective**
 - Estimated cost significantly less than protein or viral vector
 - Long shelf-life

Optimized delivery strategy to overcome previous gene therapy challenges

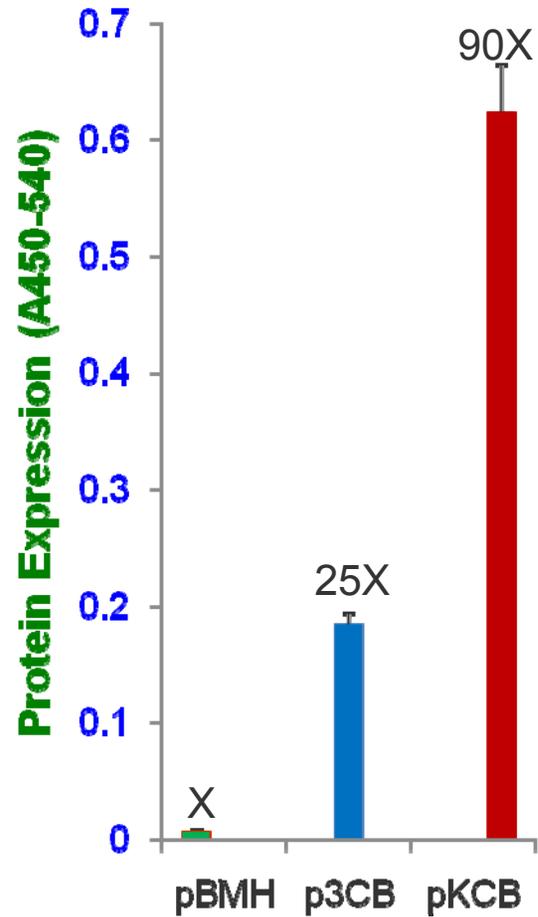
Targeted transient expression system



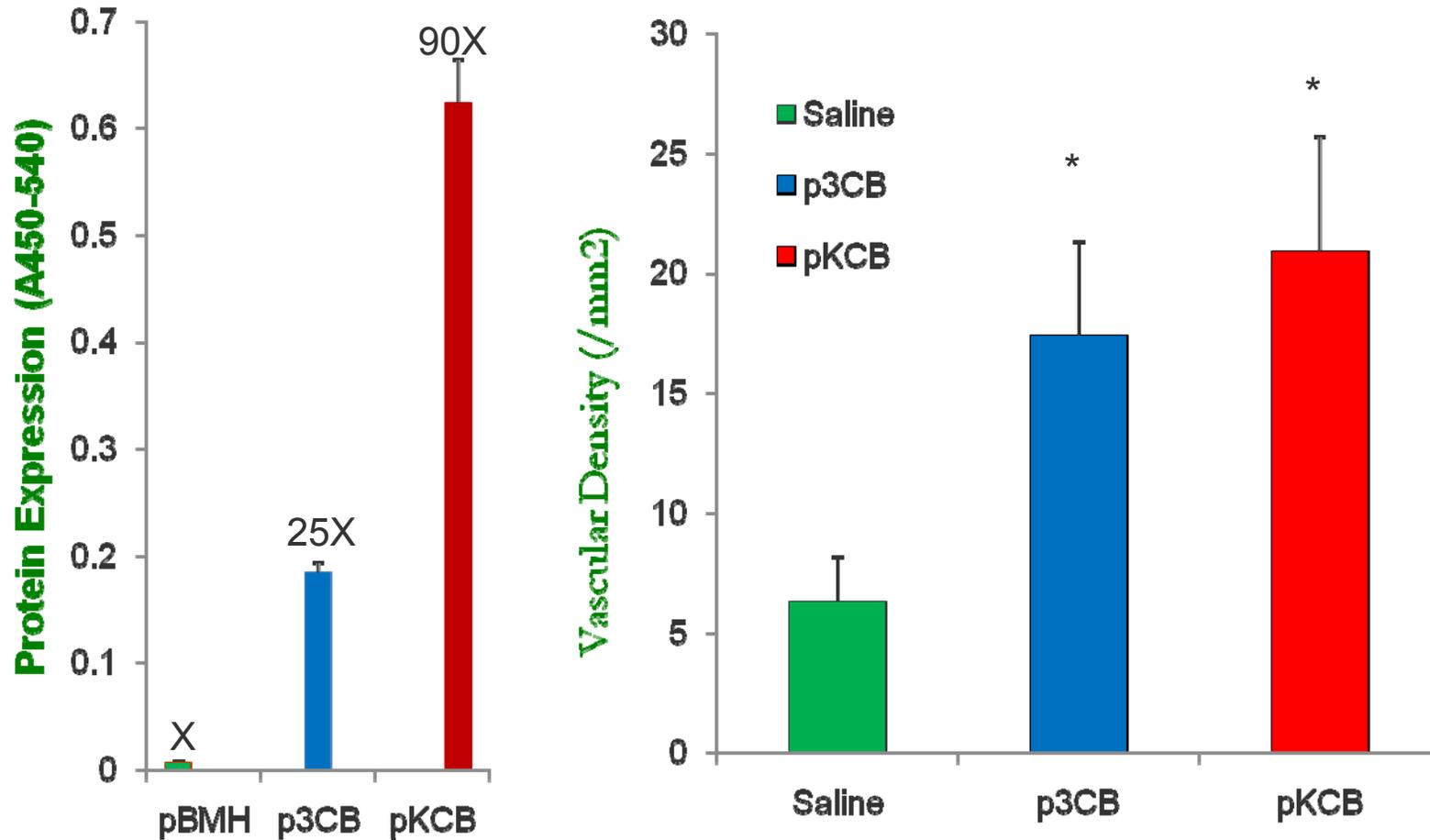
Dosing strategy results in therapeutically relevant tissue expression



There is a SDF-1 dose response in rats

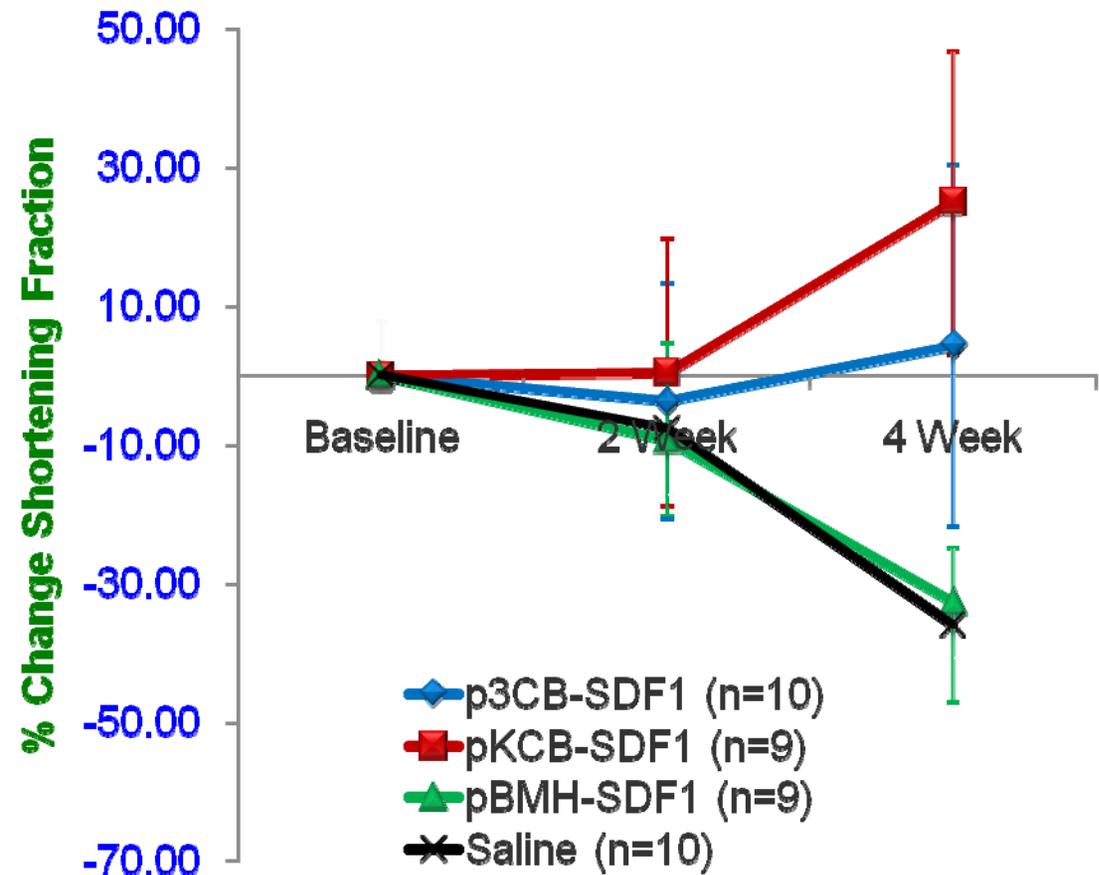
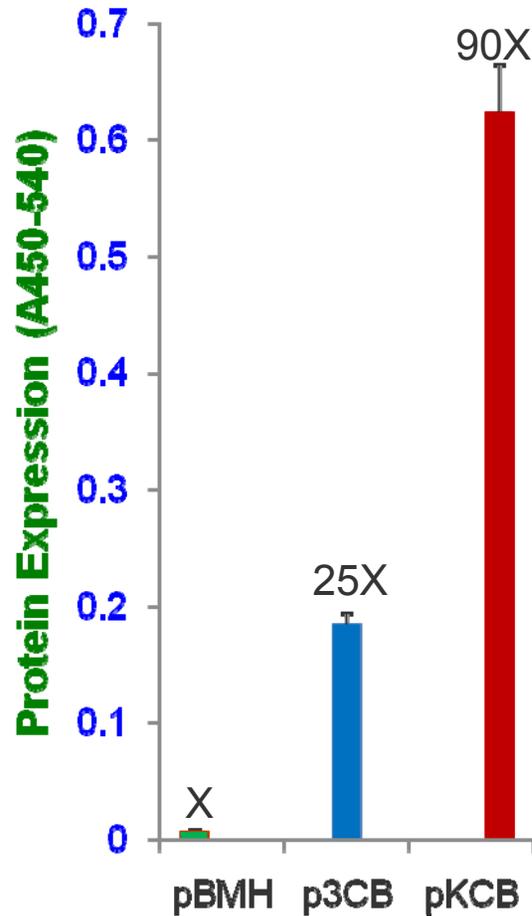


There is a SDF-1 dose response in rats

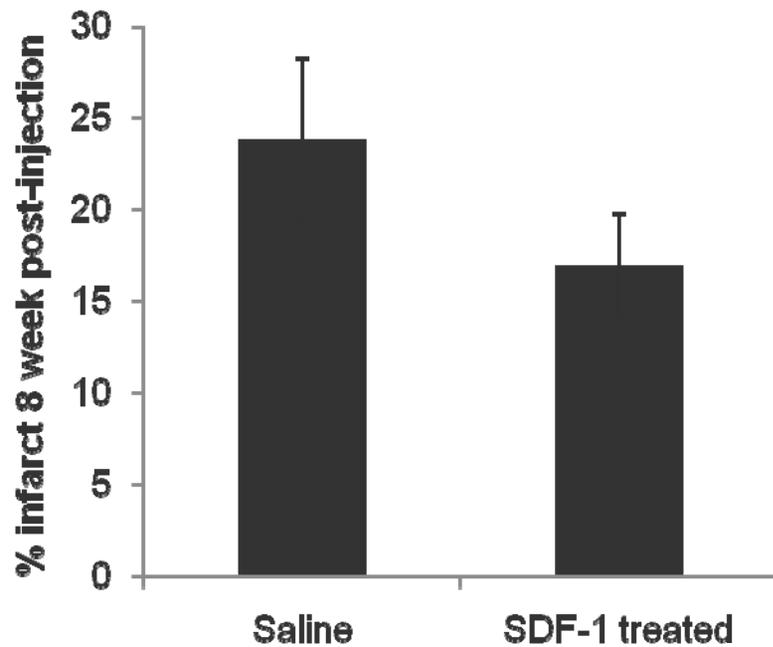


10 weeks after SDF-1 Plasmid

There is a SDF-1 dose response in rats



SDF-1 plasmid therapy decreases infarct size 8 weeks post-treatment



Saline



SDF-1 treated

infarct

SDF-1 pilot study in pigs

AWMI model in Pig

90 min Balloon
occlusion of LAD

EF ~35%

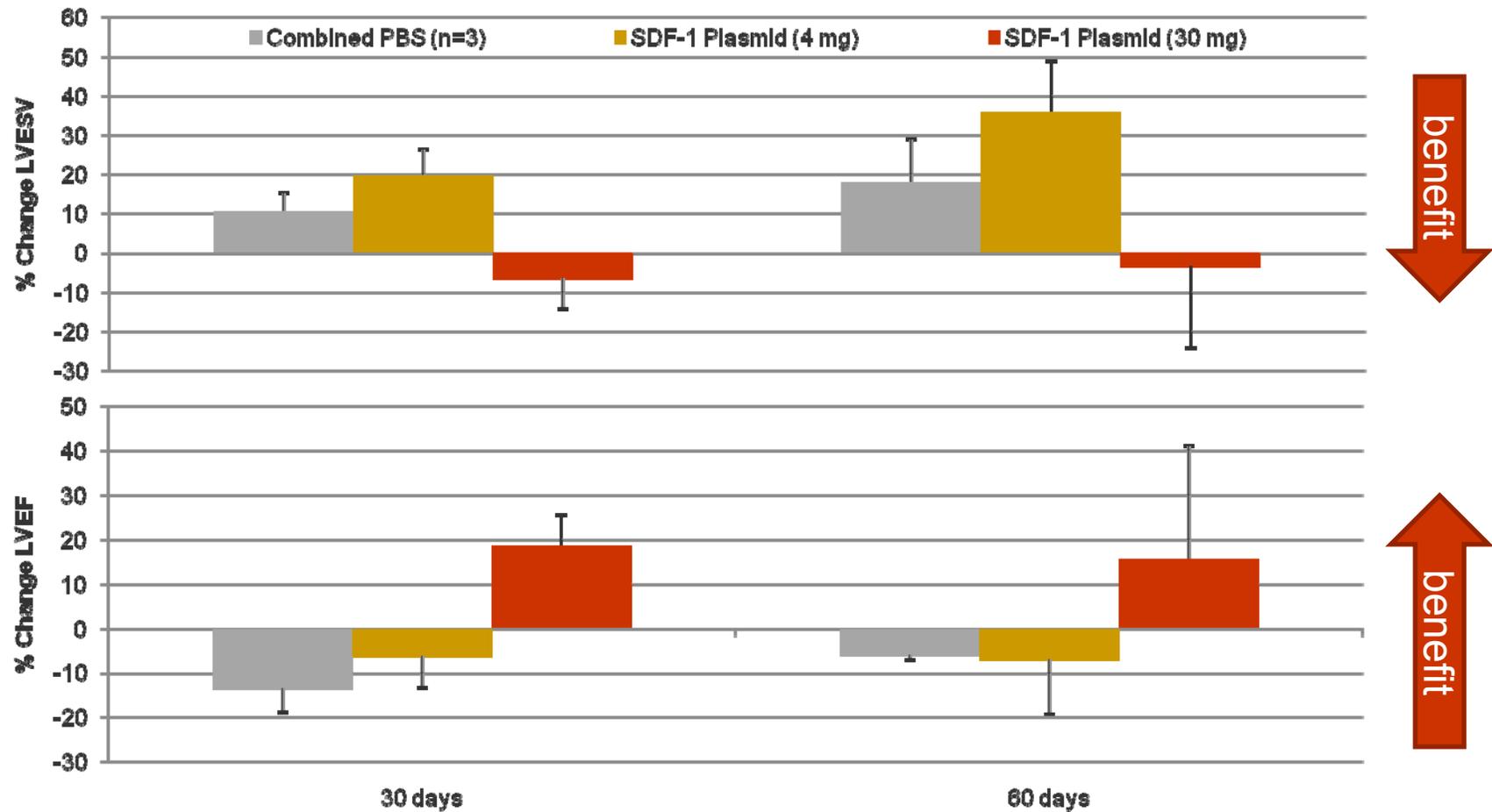
SDF-1 plasmid delivered 1-
3 month after AMI

Endoventricular injection
using Biocardia Catheter

Echo 30 day after injection



SDF-1 promotes cardiac benefit in CHF porcine models



SDF-1 PIVOTAL PRE-CLINICAL STUDY RESULTS

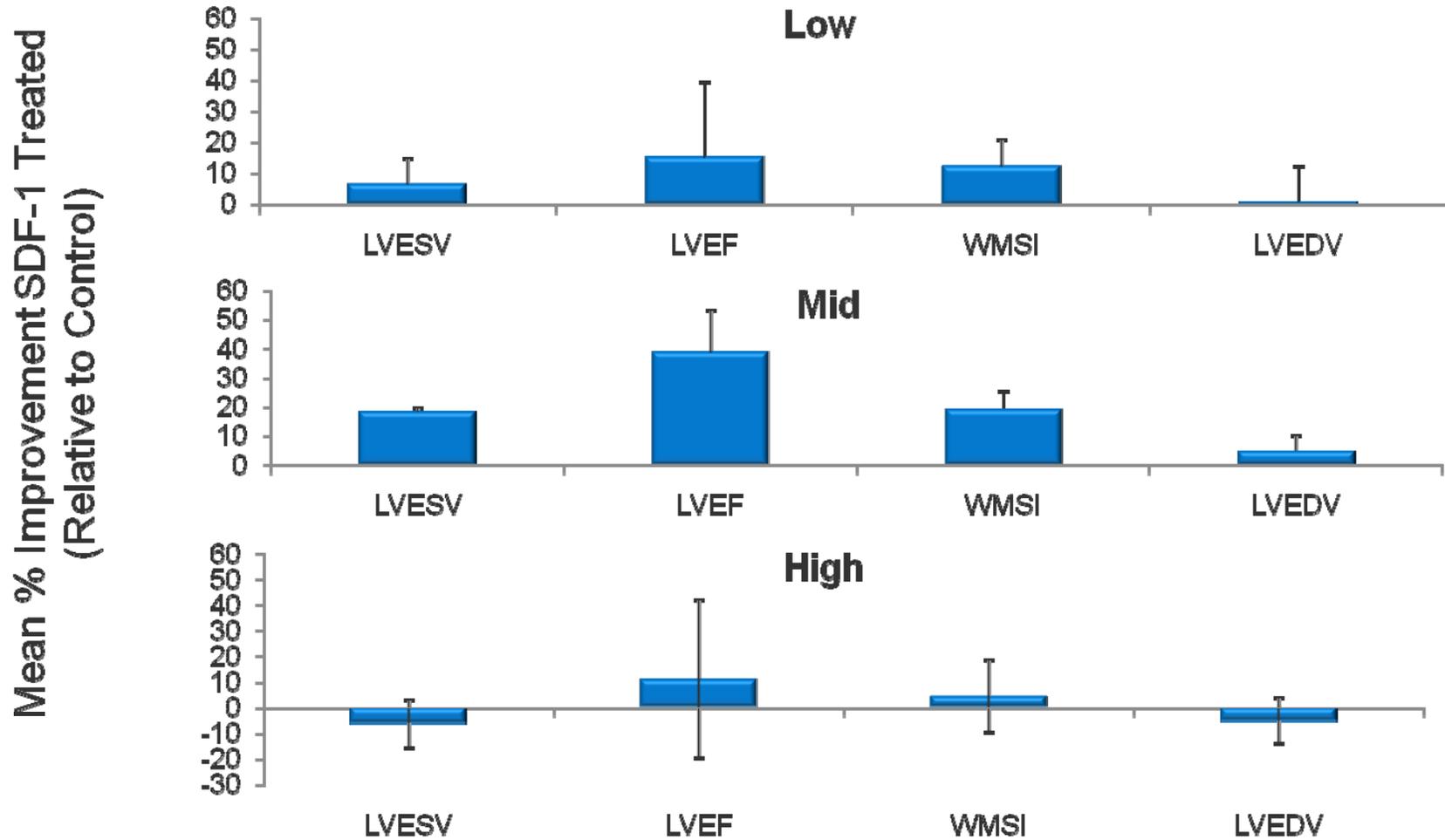
SDF-1 Pivotal study design

- Combined 57-pig safety, biodistribution and efficacy study
- Toxicology: Histopathology, SDF-1 serum levels, clinical pathology
- Biodistribution: Plasmid persistence, expression and integration measured in cardiac tissue, Persistence in non-cardiac tissues* in accordance with FDA recommended guidelines for gene therapy
- Efficacy: Echocardiography at baseline, 30, 60 and 90 days and vasculogenesis measured on animals sacrificed at 30 days.

Dose	Sacrifice Point			Total per group
	3 days	30 days	90 days	
Vehicle (1 ml @ 20 sites)	N=4	N=4	N=4	12
500 µg/site X 15 injection sites (7.5 mg DNA)	N=5	N=5	N=5	15
2 mg/site X 15 injection sites (30 mg DNA)	N=5	N=5	N=5	15
5 mg/site x 20 injection sites (100 mg DNA)	N=5	N=5	N=5	15

* Brain, kidney, liver, lung, mesenteric lymph nodes, spleen, ovaries

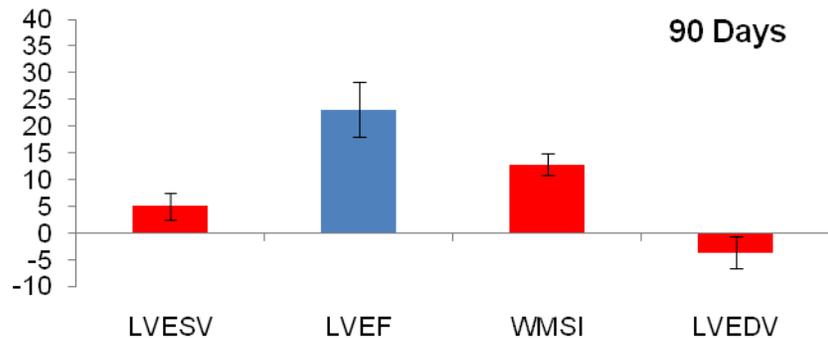
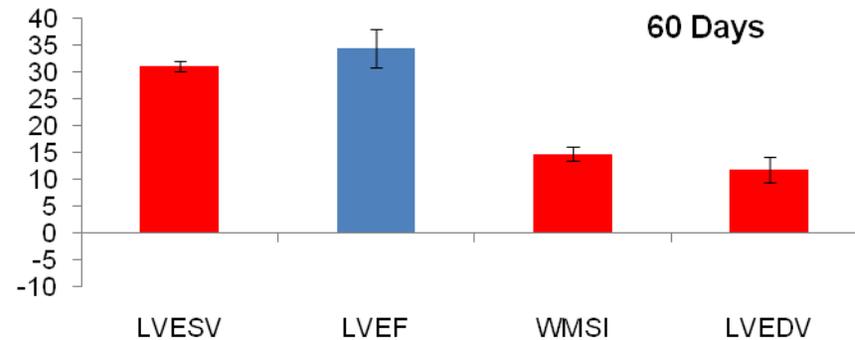
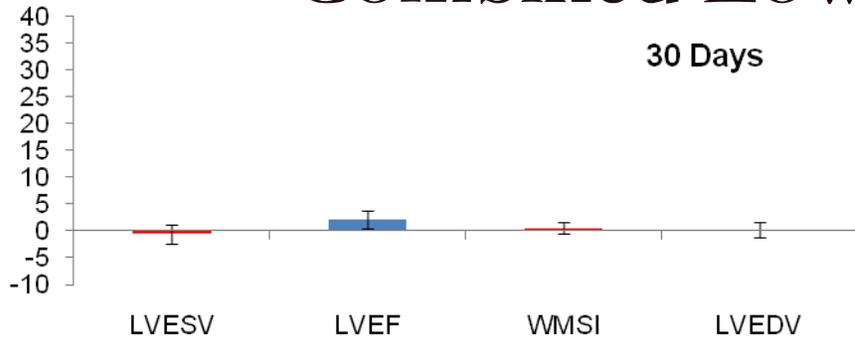
Efficacy Summary (90-day data)



Data excludes statistical outliers > 2 SD from mean

Combined Low and Mid Dose

Mean % Improvement SDF-1 Treated (Relative to Control)



AWMI model in Pig

90 min Balloon occlusion of LAD

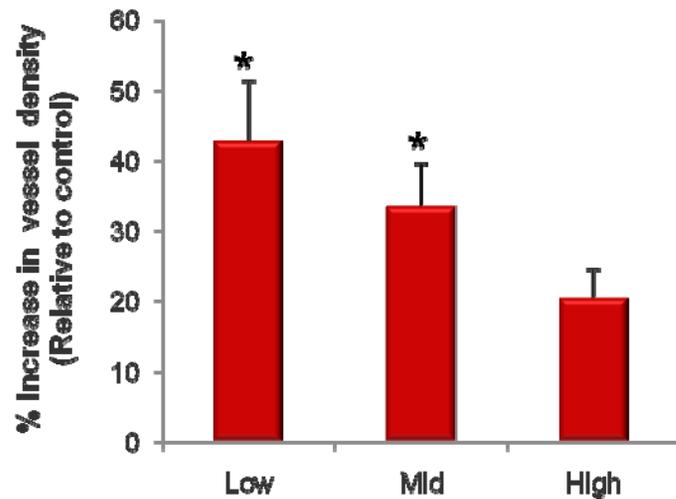
EF \leq 40%, LIVESV > 57 ml

SDF-1 plasmid delivered 1 month after AMI

Endoventricular injection using Biocardia Catheter



Vasculogenesis Summary



- All doses showed increased vasculogenesis
- Statistically significant for Low/Mid doses
- Number of blood vessels correlates significantly with improving function measured by LVESV and LVEF

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

*p<0.05 vs. control

Toxicology Summary

- Histopathology performed on tissues taken from hearts of SDF-1 treated pigs showed no signs of toxicity compared to control at any dose.
 - The low and mid dose treated animals showed signs of accelerated healing compared to the control group at 30 days post-injection.
 - There were no biologically significant clinical pathology changes in the SDF-1 treated animals compared to controls, and all values were within the normal range for Yorkshire pigs.
 - Based on the results, no doses showed any safety concerns and 100 mg is the no adverse effect level (NOAEL).
-

SDF-1 Plasmid Bio-distribution Summary

Plasmid Persistence

- Low and Mid dose shows low level plasmid persistence in heart at 90 days
- High dose results show significant plasmid in heart at 90 days.
- > 99% of product is cleared from heart at 90 days for target clinical doses
- Plasmid not present in non-cardiac tissue at 90 days.

Plasmid Expression

- High dose results show moderate expression in most samples
- Mid dose results show minimal expression in a few samples
- Low dose results show minimal to moderate expression in most samples

Plasmid Integration

- 3 samples (1 Low, 2 Mid) required testing per FDA suggested guidelines
- Potential for integration calculated to be less than 100x the predicted rate for spontaneous mutation

Pivotal Study Conclusions

- 100 mg (high-dose) is the maximum tolerable dose tested
 - 30 mg (mid-dose) is the maximum therapeutic dose tested
 - Potentially wide range for therapeutic efficacy
 - Improvements in vasculogenesis correlate with functional improvements in key echocardiography measurement
 - For target therapeutic doses (< 30 mg), SDF-1 plasmid is still present at low levels in proximity to injection sites but persistence does not result in prolonged therapeutic expression of protein or risk of integration into host genome.
-

SDF-1 CLINICAL TRIAL DESIGN

Phase 1 clinical study synopsis

- 3 center clinical trial
 - Northwestern, Columbia, Princeton Baptist
- Primary inclusion criteria (n=16 patients)
 - NYHA III, Prior MI, EF \leq 40%
- SDF-1 plasmid delivery via Biocardia Helical injection catheter
 - 3 escalating doses, No placebo
- Primary Endpoint
 - Major Adverse Cardiovascular Events (MACE) @ 30 days
- CRO
 - Accelovance (Rockville, MD)
 - Medical monitor: Gil Price, MD

Phase 1 Clinical Study

Dosing Schedule

Dosing Schedule

	# sites	Conc. (mg/ml)	Vol./ site (ml)	TOTAL DNA (mg)
Low (n=4)	15	0.33	1	5
Mid (n=6)	15	1	1	15
High (n=6)	15	2	1	30

Minimum Time Between Enrollments within a dose group

- 7 days for first 3 enrollments
- 3 days for all others

Minimum Time Between Enrollments when escalating to next dose

- >2 weeks
 - 14 day safety data from all subjects
 - DSMC approves dose-escalation

Inclusion Criteria

- NYHA Class III
 - Ischemic cardiomyopathy without ACS within 6 months
 - Significant WMA
 - At least 3 consecutive segments of abnormal wall motion by echo
 - LVEF $\leq 40\%$
 - Mitral regurgitation $\leq 2+$
 - Wall thickness > 0.5 cm
 - ICD
 - Up-to-date ACS cancer screening: all tests negative
 - Diabetics have no proliferative retinopathy
 - Informed consent
 - ≥ 18 years of age

 - Stable optimal medical therapy
 - ACE inhibitor or ARB, and Beta-blocker for 90 days with stable dose for 30 days
 - Diuretic in patients with evidence of fluid retention
 - ASA
 - Statin
 - Aldosterone-antagonist at physician's discretion
-

Exclusion Criteria

Diabetes related

- Patients with uncontrolled diabetes defined as HbA1c >9.0%

Electrophysiology related

- Persistent atrial fibrillation
- BIV pacemaker/ICD implant within the last 3 months
- Patients with $\geq 40\%$ RV univentricular pacing

SDF-1-associated disease related

- History of cancer (with exception of basal cell carcinoma)

Cardiac structure related

- History of Aortic Valve Regurgitation >2
- Moderate/Severe Aortic stenosis (AVA <1.5 cm²)
- Aortic aneurysm >3.8 cm

Other risk factors

- eGFR < 30 ml/min
- Abnormal liver function test (LFT)
- Previous organ transplant
- LV thrombus

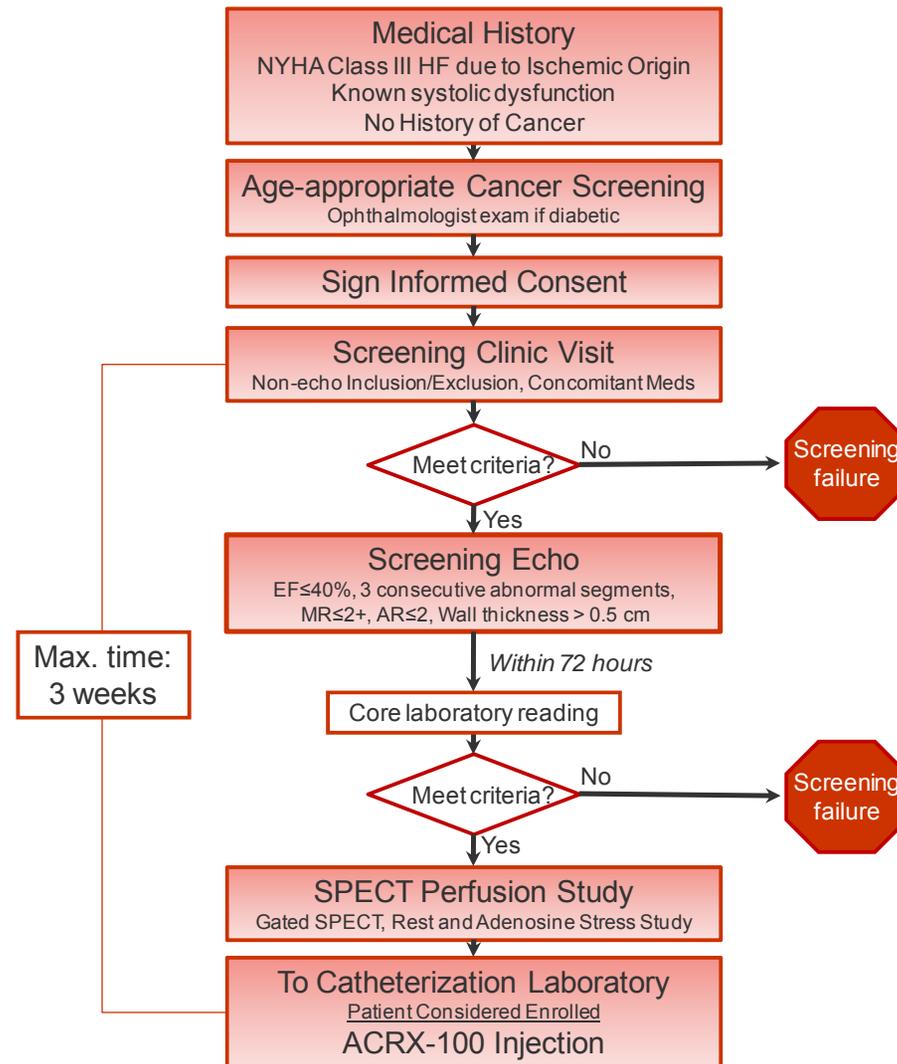
Clinical testing related

- Inability to undergo SPECT imaging

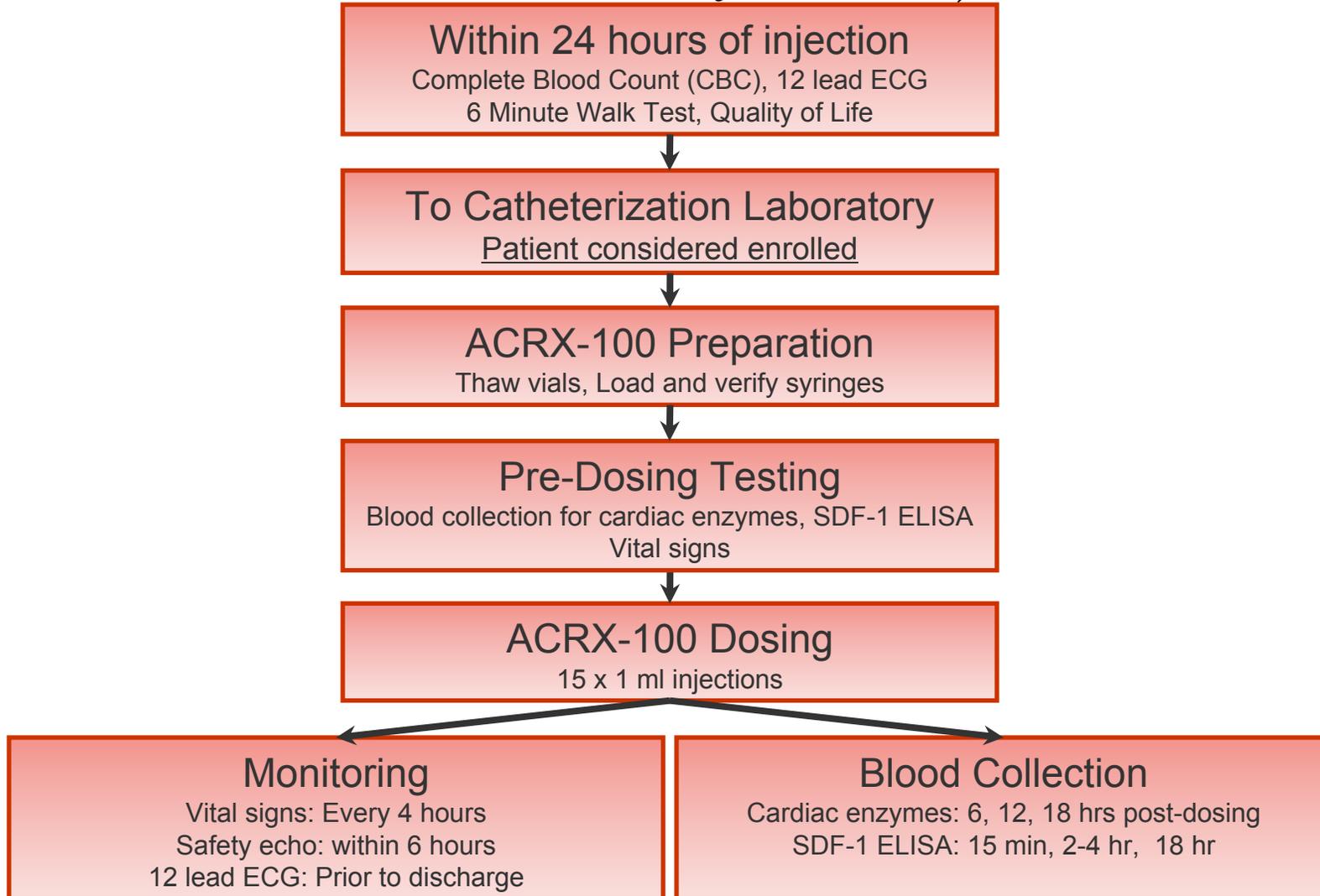
Standard

- No planned revascularization in ensuing 30 days
 - Life expectancy < 1 year
 - Participation in another clinical trial
 - Pregnancy
 - Breast feeding
 - History of drug abuse
 - HIV+
 - Hepatitis B/C +
 - Physician discretion
-

Patient Flow



Procedure Flow Day of Injection



Phase 1 Follow-up Schedule: Safety

Test	Injection Procedure			Follow up Date (Relative to injection)				
	Within 3 wks pre-Dosing	24 hrs Pre-Dosing	24 hrs Post-Dosing	3 d	7 d	1 m	4 m	12 m
Vital Signs/PA	X	X	every 4 h	X	X	X	X	X
MACE/AEs			X	X	X	X	X	X
12 LEAD ECG	X	X	Pre-discharge	X	X	X	X	X
Serum SDF-1 Protein			BL, 15 min, 2-4 h, 18 h	X	X	X		
Cardiac Enzymes			every 6 h					
CBC	X	X		X	X	X	X	X
Safety Echo			<6 hr					
AICD monitoring	X					X	X	X
Con. Med.	X			X	X	X	X	X
SDF-1 Ab blood draw	X				X	X	X	X

Safety monitoring

- A Data Safety Monitoring Committee (DSMC) will oversee safety of the trial
 - Any stopping rule triggers DSMC review

- Stopping rules
 - One or more subjects experiences an event that is serious, unexpected and the relationship to ACRX-100 cannot reasonably be excluded.
 - One or more subjects experience an event that is life-threatening/ causes death, and relationship to ACRX-100 cannot reasonably be excluded.
 - Two or more subjects enrolled to date experience a severe (Grade 3):
 - ACRX-100 related adverse event.
 - adverse event related to the delivery of ACRX-100 by the catheter.
 - cardiac adverse event.

Safety monitoring (cont'd)

■ Stopping rules (cont'd)

- If a single incidence of any of the below occurs, without regard to causality:
 - Death within 24 hours of ACRX-100 injection
 - Pericardial tamponade within 48 hours of ACRX-100 injection
 - Stroke or transient ischemic attack within 48 hours of ACRX-100 injection
 - New onset sustained ventricular arrhythmias within 14 days
 - New onset myocardial infarction within 48 hours to 4 weeks

- If a single incidence of any of the below occurs, and the relationship to the injection procedure cannot reasonably be excluded
 - RBBB, LBBB, or complete AV block within 7 days of ACRX-100 injection

Phase 1 Follow-up Schedule: Efficacy

		Injection Procedure		Follow up Date (Relative to injection)				
Test	Within 3 wks pre-Dosing	24 hrs Pre-Dosing	24 hrs Post-Dosing	3 d	7 d	1 m	4 m	12 m
Functional Echo	X					X	X	X
SPECT	X						X	
NYHA	X				X	X	X	X
Quality of Life		X			X	X	X	X
Six Minute Walk		X			X	X	X	X

Questions
&
Discussion
