Pre-Clinical Development Perspective on Adult Adherent Stem Cells

Robert Deans, Athersys Inc

CIRM Webinar

September 28, 2010

Forward-Looking Statements

The statements and discussions contained in this presentation that are not historical facts constitute forward-looking statements, which can be identified by the use of forward-looking words such as "believes," "expects," "may," "intends," "anticipates," "plans," "estimates" and analogous or similar expressions intended to identify forward-looking statements. These forward-looking statements and estimates as to future performance, estimates as to future valuations and other statements contained herein regarding matters that are not historical facts, are only predictions, and that actual events or results may differ materially. We cannot assure or guarantee you that any future results described in this presentation will be achieved, and actual results could vary materially from those reflected in such forward-looking statements.

Information contained in this presentation has been compiled from sources believed to be credible and reliable. However, we cannot guarantee such credibility and reliability. The forecasts and projections of events contained herein are based upon subjective valuations, analyses and personal opinions.

This presentation shall not constitute an offer to sell or the solicitation of an offer to buy any securities. Such an offer or solicitation, if made, will only be made pursuant to an offering memorandum and definitive subscription documents.

Discussion Agenda

- Toxicity and Safety Testing Objectives
  - Acute toxicity profiles
  - Immunological sensitization
  - Ectopic tissue and tumorigenicity risk
  - Exposure of cell to concomitant medications

- Pre-Clinical Efficacy Objectives
  - Dose ranging and regimen
  - Potency assay development and correlation with in vivo response
  - Route of delivery and compatibility with delivery device

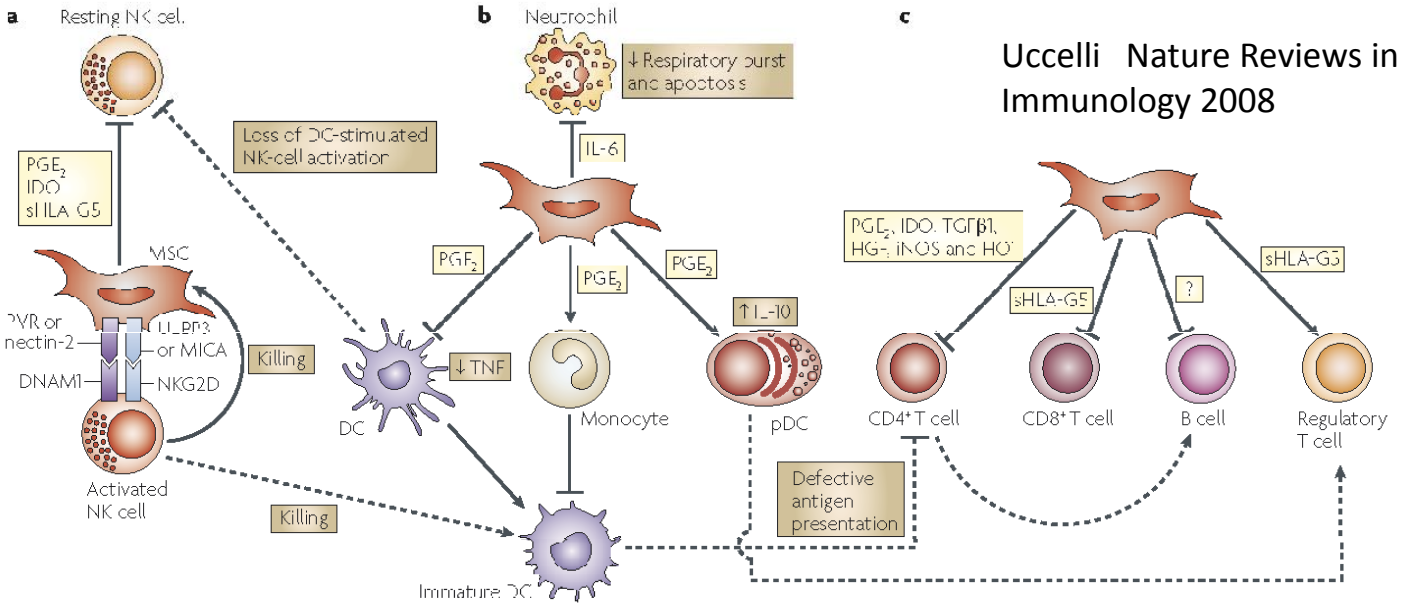
- Developing a PK/PD Profile for a Cell Therapeutic
  - Need for sensitive models of in vivo cell persistence
  - Biomarker development to correlate duration of therapeutic response

Principles of Adherent Stem Cell Therapy

- Adherent stem cells (ASC) can be isolated from a wide variety of tissues
  - typified by mesenchymal stromal cell or MSC (*Dominici, M., et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells Cytotherapy 8:315-317*)
  - Common properties, although epigenetic differences can be ascribed to tissue source,
    - biological implications are unclear
  - Culture conditions and cell type can enable extensive replicative capacity and large scale production
- Biological impact is primarily trophic, emphasizing impact on inflammatory and immunomodulatory pathways
- Persistence in vivo is very limited, and direct role in regenerative tissue repair is minor or ne͟li͟ible

Universal Donor Properties

- Adherent stem cell culture with low immunogenicity, active immunomodulatory properties with no patient matching



- Ability to produce sufficient products from single or limited donors to meet large commercialization requirements

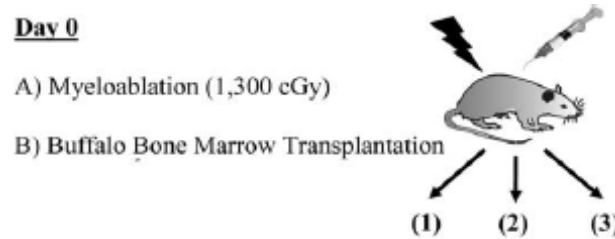
Adult Stem Cell Therapy Transitions from Transplant Product to Biologic/Drug Paradigm

- Hematopoietic stem cell therapy practiced as a trans¸ lant¸ with emphasis on testing the process rather than individual product.

- With acceptance of adherent stem cell universal donor approach, ¸ ers¸ ective shifts towards drug or biologic type profiling

With Transition to Drug-like Paradigm, Expectations for Acute Toxicity Testing Evolve

- It has become routine to test an infused cell product for impact on :
  - Clinical chemistry
  - Hematology
  - Respiratory rate
  - Immune response
  - Clinical observations
  - GLP full tissue histopathology



C) MultiStem infusion	(1) PBS - Day 2, 9, 16, 23, 30 (2) MultiStem - Day 2, 9, 16, 23, 30 (3) MultiStem - Day 2
Clinical Evaluation	Day 0, 6, 8, 10, 13, 15, 17, 20, 24, 27 and 30
Respiration Rates (infusion days)	Day 2, 9, 16, 23 and 30
Hematology	Day -1, 6, 14, 20, 27 and 35
Chemistry	Day -1, 3, 17 and 31
MLR	Day -1, 37
Alloantibody	Day -1, 37
Gross Necropsy, Histopathology	Day 37

(Kovacsovics, M, et al Cytotherapy 2008)

Confirming Immunogenicity Profile of Adherent Stem Cells

Immune sensitization risk in using allogeneic cell product, particularly delivered in repeated dosing scheme

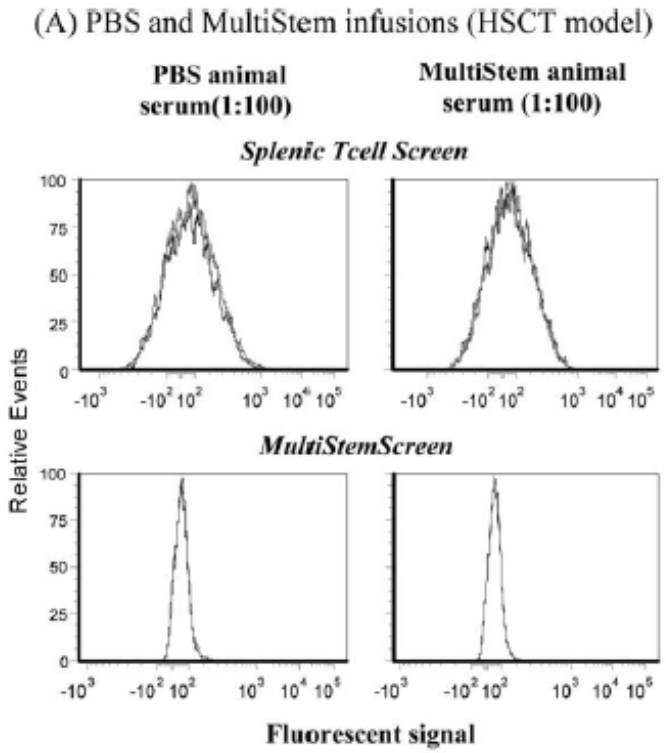
Immune Sensitization

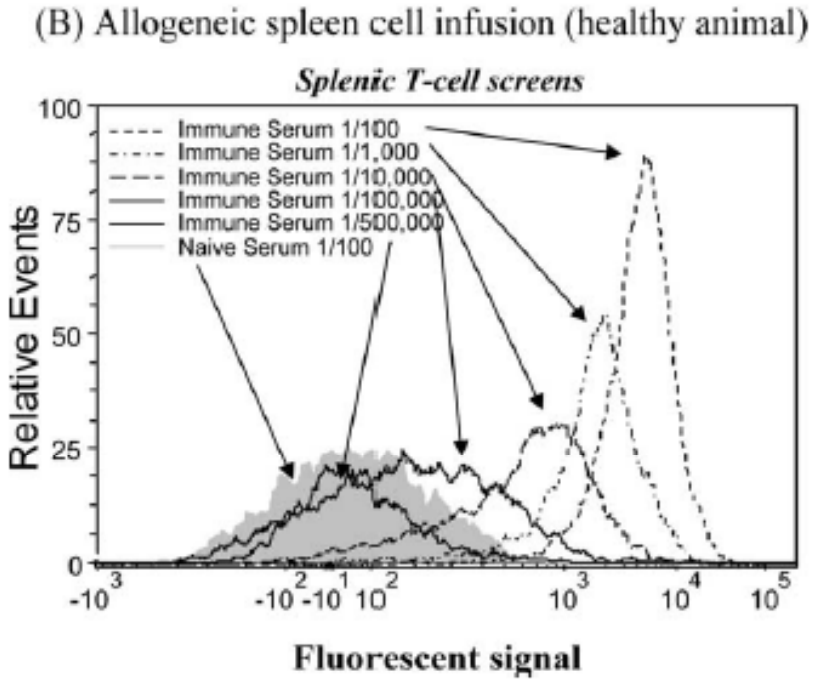
- Repeat administration carries risk of immune sensitization and hypersensitivity reaction
  - Close attention paid to respiratory distress on infusion

- Pre-clinical toxicity studies can evaluate T and B cell sensitization
  - Control using allogeneic splenocytes
  - Comparison of allogeneic cell use +/- immunosuppression

Flow Cytometric Testing for Allogeneic Stem Cell AB Following Repeat Infusion

No evidence for allogeneic B cell generation following repeat administration of adherent stem cells, whereas splenocytes induce a vigorous repsonse

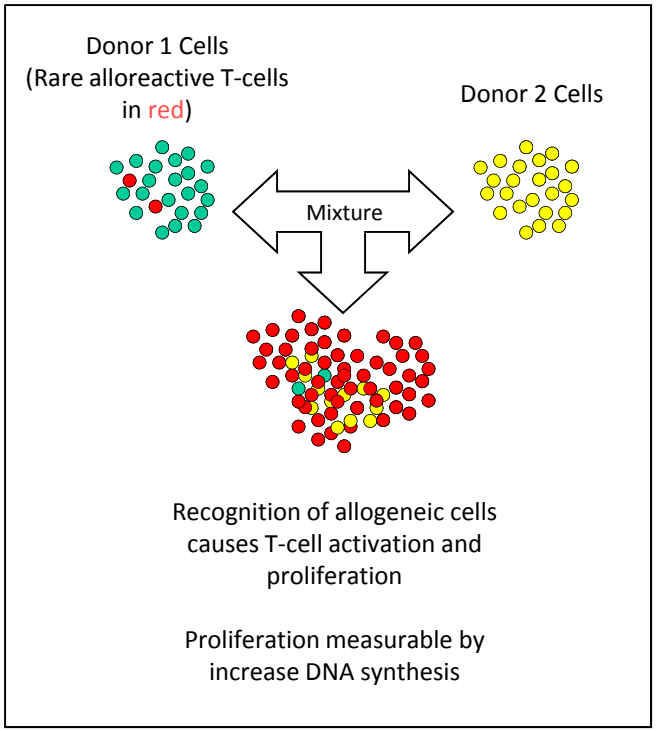




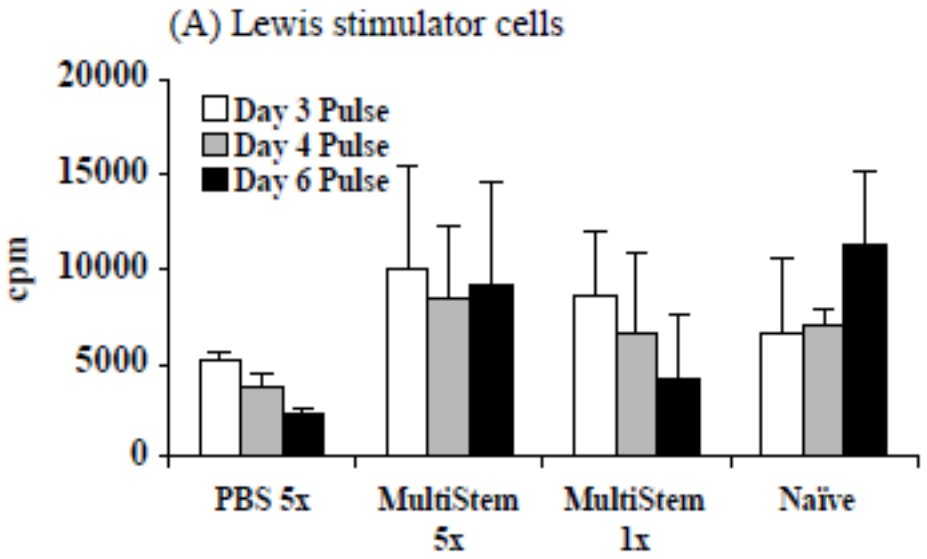
Kovacsovics, M Cytotherapy 2008

Repeat Infusion of Adherent Stem Cells Does Not Induce T Cell Sensitization

Mixed Lymphocyte Reaction



ASC infusion is equivalent to PBS treated or naïve animals in inducing T cell sensitization



Kovacsovics, M Cytotherapy 2008

Safety and Concomitant Medication

- In vitro testing of cell product exposed to clinical medications recommended
  - Test at ranges significantly above PK exposure
  - Assay for interference with potency, replication of stem cells
  - Assay for cytogenetic stability
- Disease model testing should include use of concomitant medications where possible
  - Eg, cyclosporineA, methotrexate in allogeneic bone marrow transplant models

Pre-Clinical Efficacy

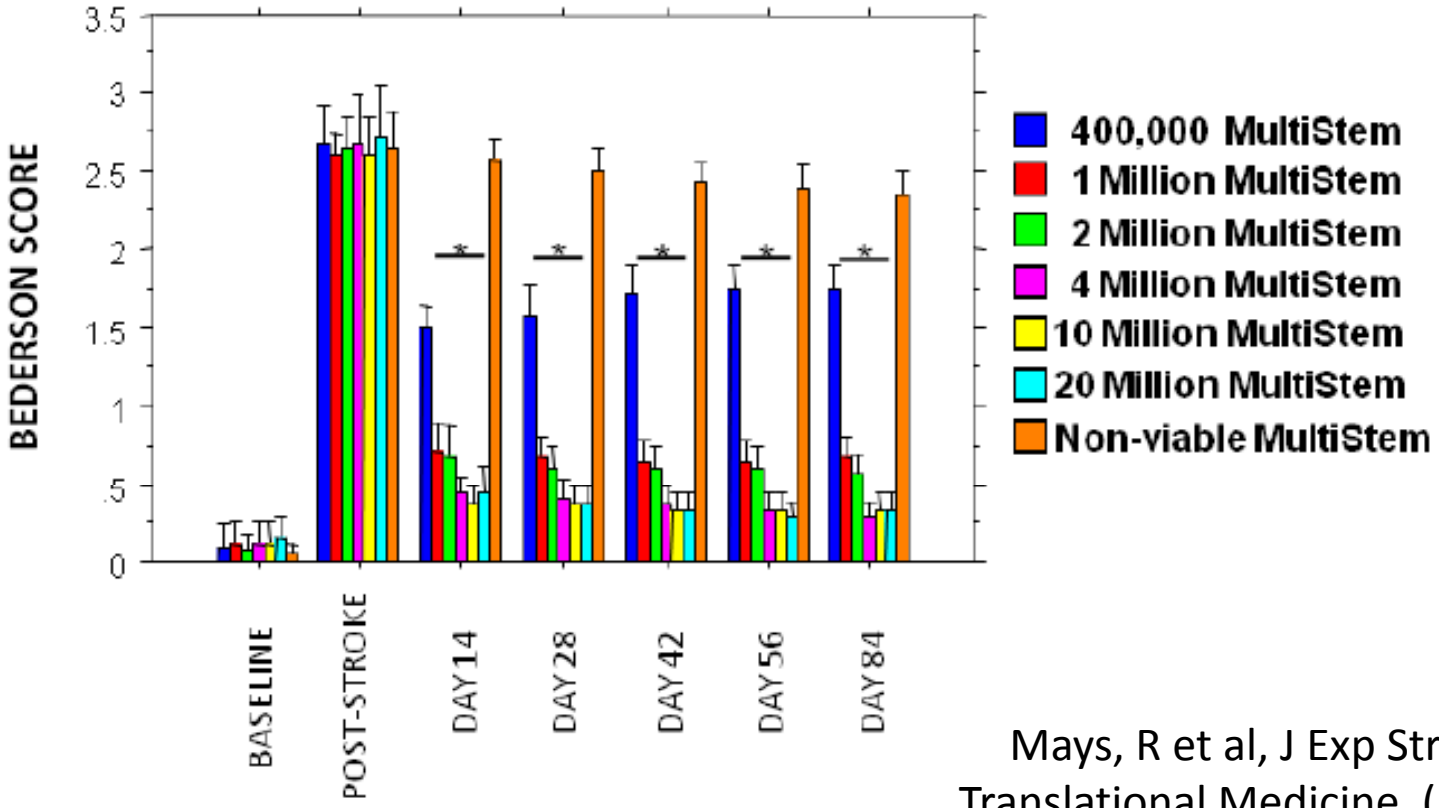
Pharmacodynamics

Pre-Clinical Efficacy Objectives

- Dose ranging and regimen
  - Important to establish dose response for informed design of human dose strategy
  - Studies frequently fail to identity top or bottom end dose effects
- Pre-clinical models inform but are not decisive in correlating animal to human dose
- Balanced decision must be made between use of human product vs analogous animal
  - Growing acceptance for use of human product without immune suppression
  - Significant science associated with validating analogous animal cell product

Rat Adult Stroke Model: Dose Response to Stem Cell Infusion

A critical threshold is determined for sustained long term functional recovery



Mays, R et al, J Exp Stroke and Translational Medicine (2010) 3:34

Allogeneic Rat or Xenogeneic Human Stem Cells Function Equivalently With or Without Immune Suppression

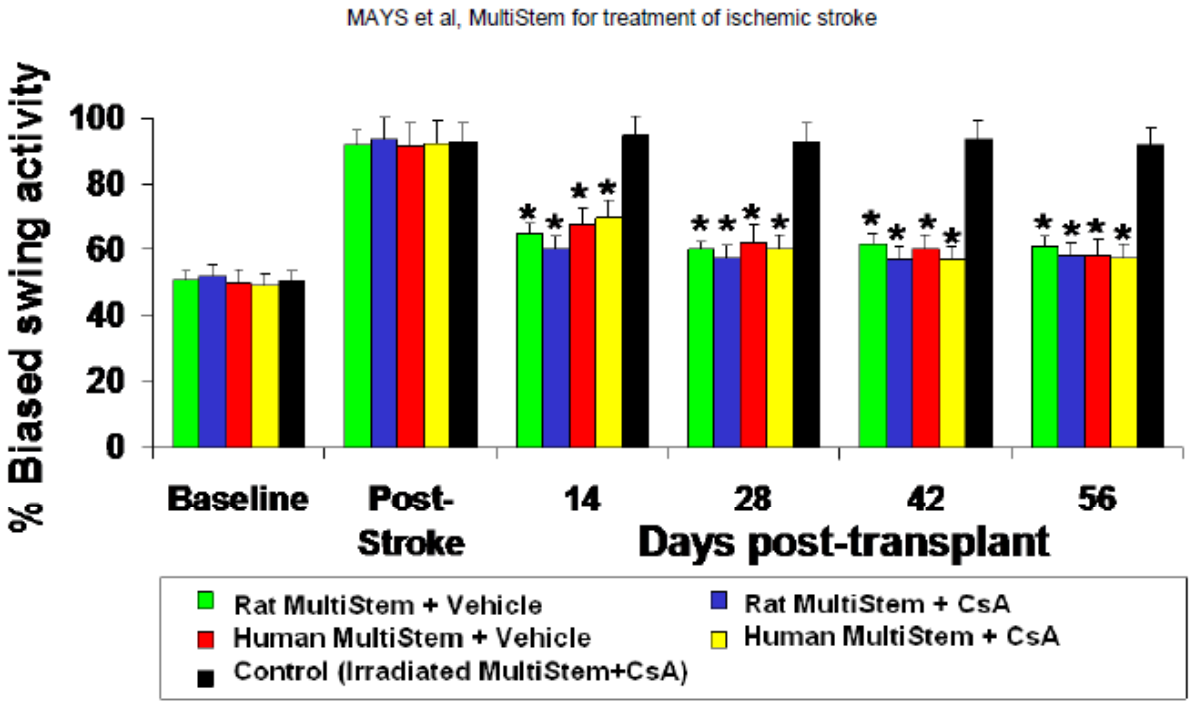


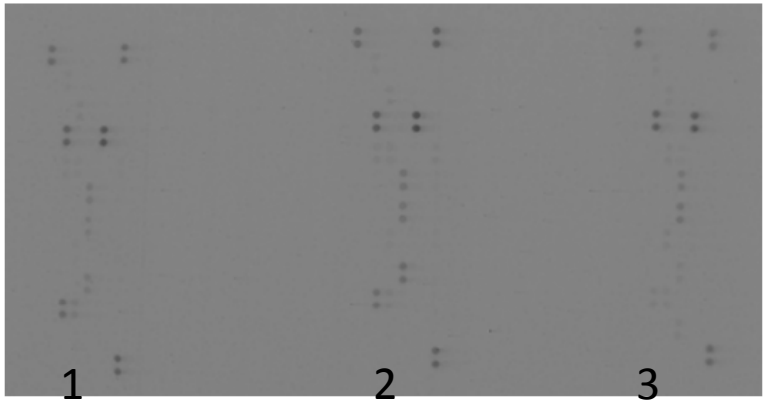
Figure 1: Xenogeneic and allogeneic MultiStem promote sustained and statistically significant locomotor recovery following ischemic stroke in rats. Rats underwent a distal middle cerebral artery ligation surgery to induce a focal ischemic stroke. 7 days after induction of the stroke injury, rats were randomly placed into one of 5 treatment groups. Eight animals per group received either 400,000 allogeneic rat MultiStem via intracranial injection with or without Cyclosporine A treatment (CsA) or 400,000 xenogeneic human MultiStem cells with or without Cyclosporine A treatment. 400,000 irradiated non-viable human MultiStem were transplanted into the negative control animals. The Elevated Body Swing Test (EBST) was performed to demonstrate locomotor outcomes every 14 days post-transplantation for 8 weeks. The asterisks indicate a significant difference between the negative control treatment group and the MultiStem experimental groups (One-Way ANOVA, $p < 0.05$; Fisher's PLSD, $p < 0.05$).

Mays, R 2010

Pre-Clinical Potency Assay Development

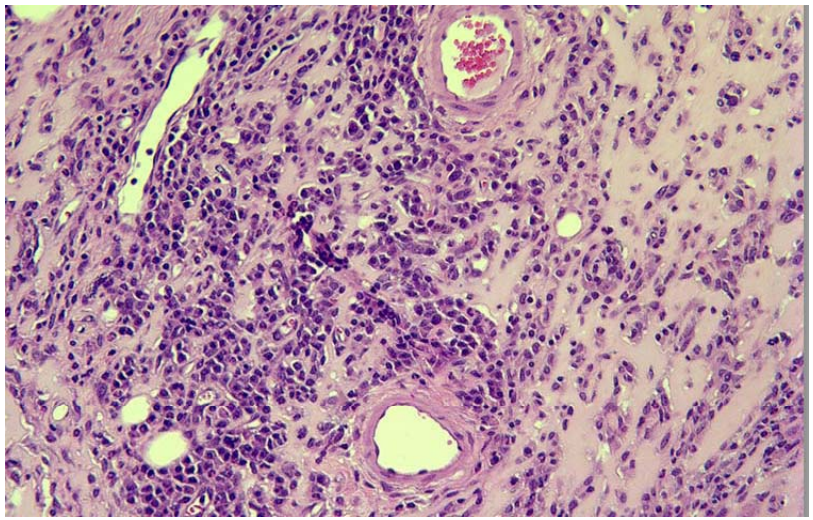
- Potency assays are intended to define key mechanistic pathway(s) integral to therapeutic benefit
- Potency assays are required to confirm consistency in manufacturing product relative to therapeutic use
- Optimal potency assay is an in vitro surrogate for performance in a pre-clinical model
  - Can be run rapidly
  - Allows quantitative pass/fail criteria
- Developing surrogate assay can be complex
  - Therapeutic response to injury is multi-modal
  - Redundancy exists in many pathways (eg., angiogenesis), and knockdown experiments may not be informative

Levels of Angiogenic Factors Correlate with Ability to Form New Vessels with Integrity

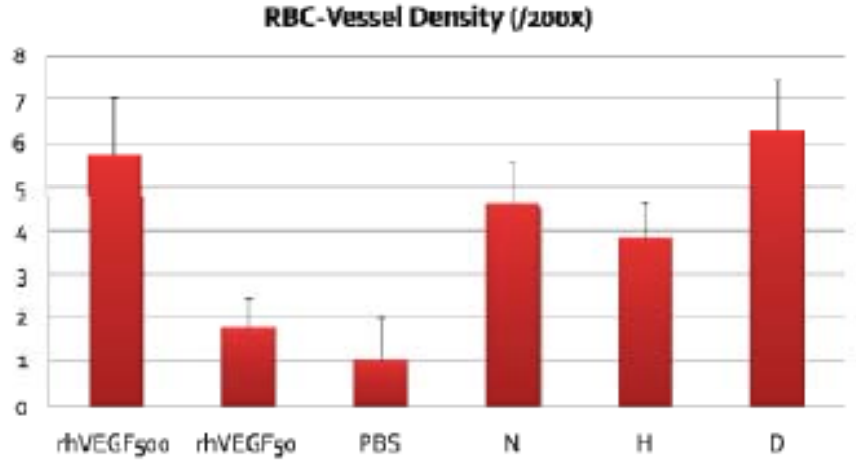


An_io_enic screening of conditioned media can identify consistent angiogenic factor expression, which can be correlated with biological activity

Matrigel subcutaneous plug assay



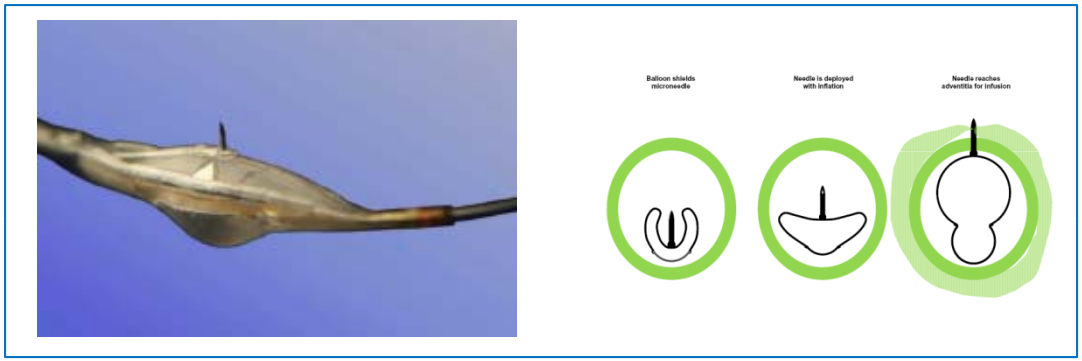
Quantifying RBC Containing Vessels

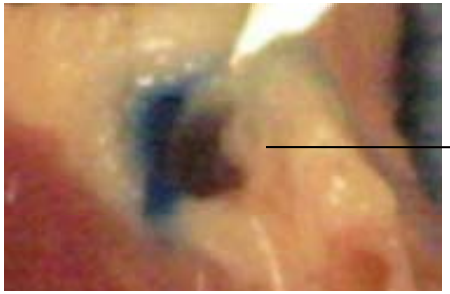


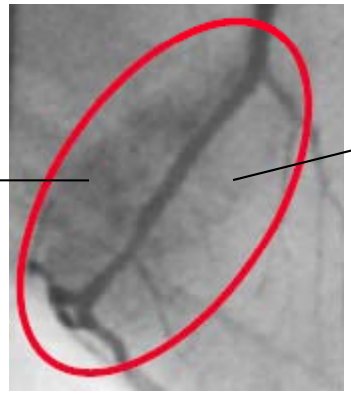
Biocompatibility and Delivery Devices

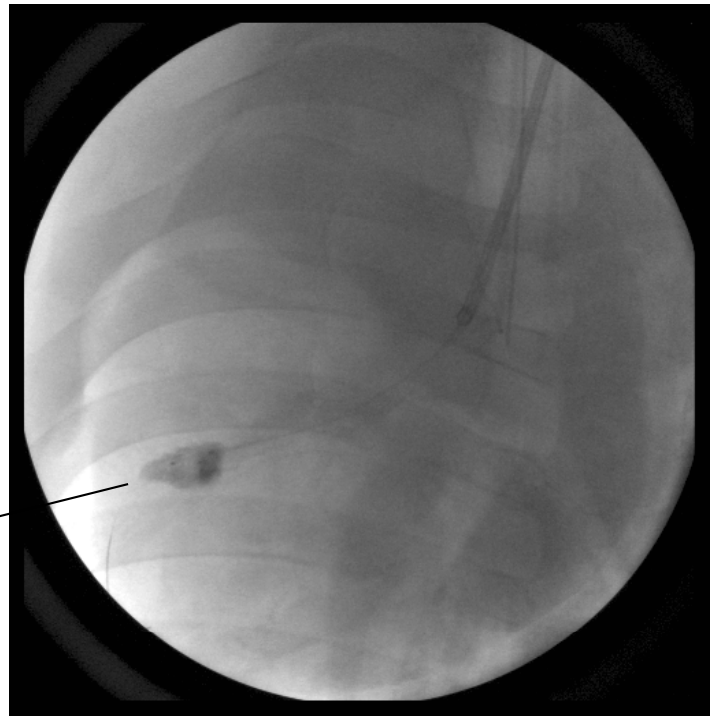
- IV infusion pathway finds adherent stem cells initially associated with lung and RES
  - Cells are subsequently detected at site of injury
  - Cell size (impacted by culture) can significantly change biodistribution patterns
- Regional delivery of cells to target organ by catheter (heart, brain) carries additional pre-clinical testing requirements
  - Use of animal model with significant homology to human organ geometry and vessel dimension to provide adequate correlatation

Cells Delivered Effectively using Transarterial Catheter





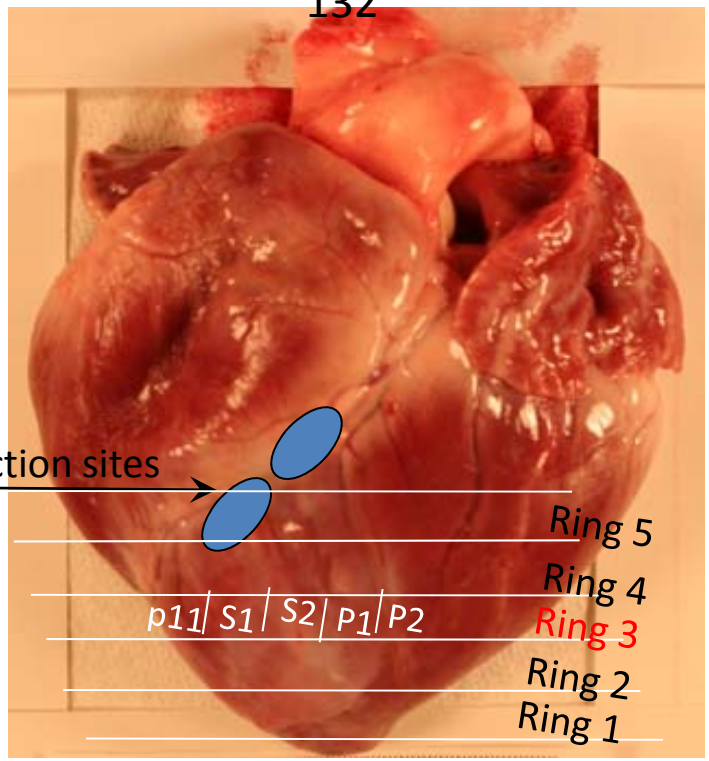


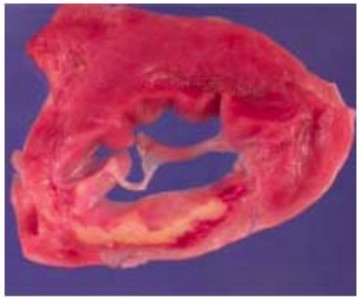


Medicitty, S et al in press

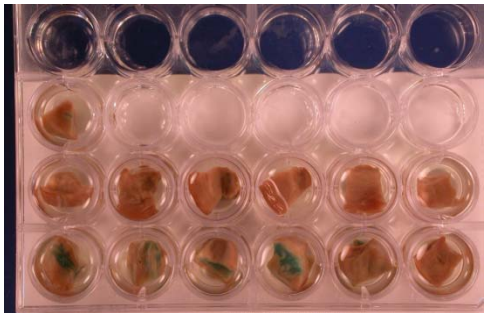
Biodistribution Following Transarterial Delivery in Pig Ischemia Model

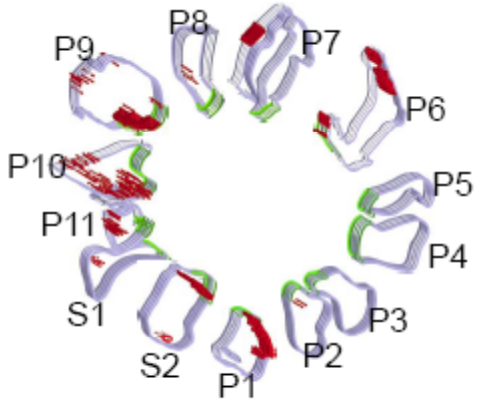
Transarterial catheter delivery of pig MultiStem® cells, 2 weeks, animal 132





Distribution of b-gal cells within Tissue Block, Ring #3



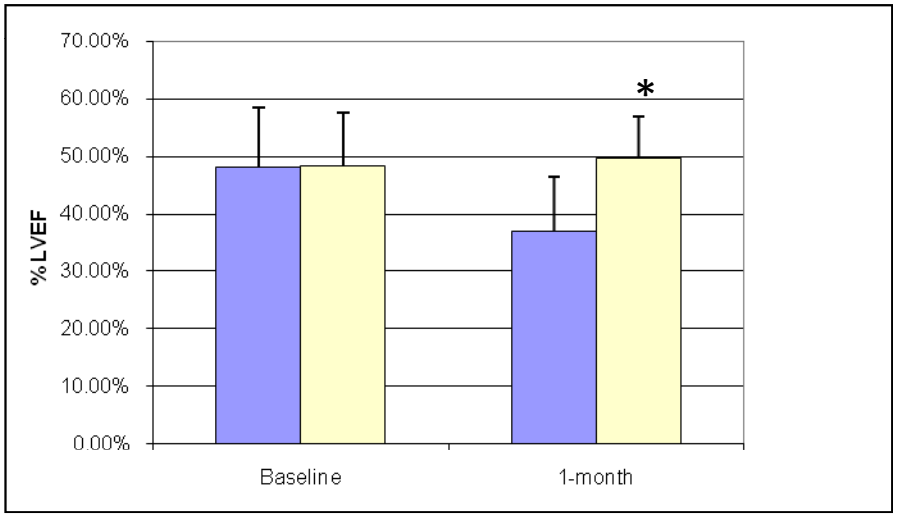


Medicitty, S et al in press

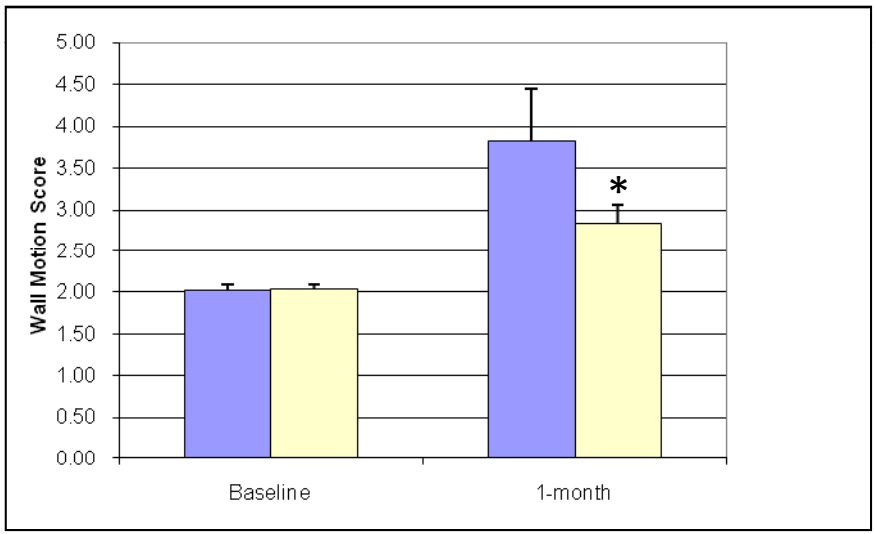
Improvements in Functional Performance Observed in Reperfusion Model

- Long-term safety study in AMI pigs
- Delivery of MultiStem with the transarterial catheter 2 days after transient ischemia

Left Ventricular Ejection Fraction



Wall Motion Score (Echo)



Medicitty, S et al in press

Pre-Clinical Models for Comparability Testing

- When is com. arability needed?
  - Changes to media formulation (serum free)
  - Comparison between donors (master cell banks)
  - Changes in expansion limits
  - Changes to increase cell potency, biodistribution
- Do pre-clinical models meet these requirements?
  - Are biomarkers sufficiently correlated to recovery to provide accurate
  - Can biomarkers or animal response be statistically quantified and provide good decisions

Developing a PK/PD Profile for Cellular Therapeutics

Transplant to Drug Paradigm and Impact on Pre-Clinical Approach

- Typical biologic or drug development would focus on
  - Anatomical distribution of drug within body
  - Exposure and duration of drug to system
  - Duration of response with respect to drug concentration

- What are the implications for cell based therapy?
  - Requirement to monitor distribution of cell within body with high sensitivity – creating a mass balance accounting for product
  - Determination of cell persistence
  - Biomarkers with sufficient correlation to primary action of cell product to measure potency and duration of effect

Thanks

- Athersys scientists and staff (Drs. Mays, Ting, and van't Hof)

- Dr. David Wilson and laboratory (Case Western Reserve Univ.)
- Dr. Marc Penn and laboratory (Cleveland Clinic Foundation)
- Dr. David Hess and laboratory (Medical College of Georgia)
- Dr. Richard Maziarz and laboratory (Oregon Health Sciences Univ.)