WAISMAN BIOMANUFACTURING

THE UNIVERSITY of WISCONSIN MADISON

Cell Therapy for Lung Indications Choosing the appropriate IND Enabling Animal Model

Marlowe Eldridge MD
Department of Pediatrics and Biomedical Engineering
University of Wisconsin School of Medicine and Public Health

National Heart Lung and Blood Institute
People Science Health

PACT Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

Key Steps for Pre-clinical animal studies for IND applications

- Design studies that match the protocol for the human safety study.
- Discuss the initial design with the FDA prior to pre-clinical study initiation.
- Select the most appropriate animal model(s) that best reflects the human disease, stage of disease, or specific question.
- Discuss the potential models with the FDA before initiating the study (pre-IND meeting)

Factors to consider for selecting the appropriate animal model

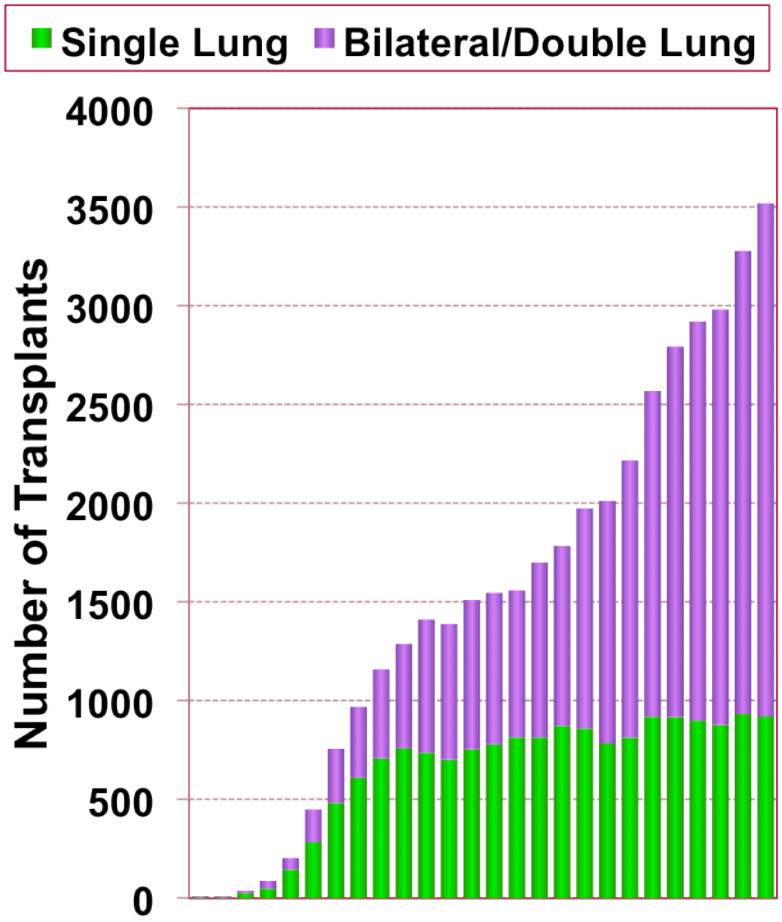
- What is the primary question? Safety? Efficacy?
- How closely does the animal model translate to the human disease and stage of disease?
- What are the technical challenges of the model development?
- Does the model allow measurement of relevant physiologic information?
- Does the model allow mimicking of the planned human dosing schedule?

Proposed Clinical Trial

- Lung transplant recipients with treatment refractory bronchiolitis obliterans (BO/BOS) that are not eligible for re-transplant
- Allogeneic, bone marrow-derived MSCs
- Two dose groups –
  - Low dose – 2 x 10^6 MSC/kg, 3x (day 0, 2, 4)
  - High dose – 4 x 10^6 MSC/kg, 3x (day 0, 2, 4)
  - 3 patients per dose group
- MSC administration -
  - 2 x 10^8 MSC/20 mL in 2.5% DMSO, 5% HSA, 70% Plasma-Lyte
  - Ship cryopreserved product to Mayo Clinic in LN2 vapor shipper
  - Thaw product and dilute to 200 mL in Plasmalyte
  - IV infusion at 2-4 mL/min

Lung Transplantation and Rejection

- The first attempted lung transplant was performed on a prisoner in 1963 who died eighteen days later.
- Successful long term survival was not achieved until the mid-1980s
- Today lung transplantation is the definite treatment option for end-stage lung disease with well over one-thousand transplants performed annually.



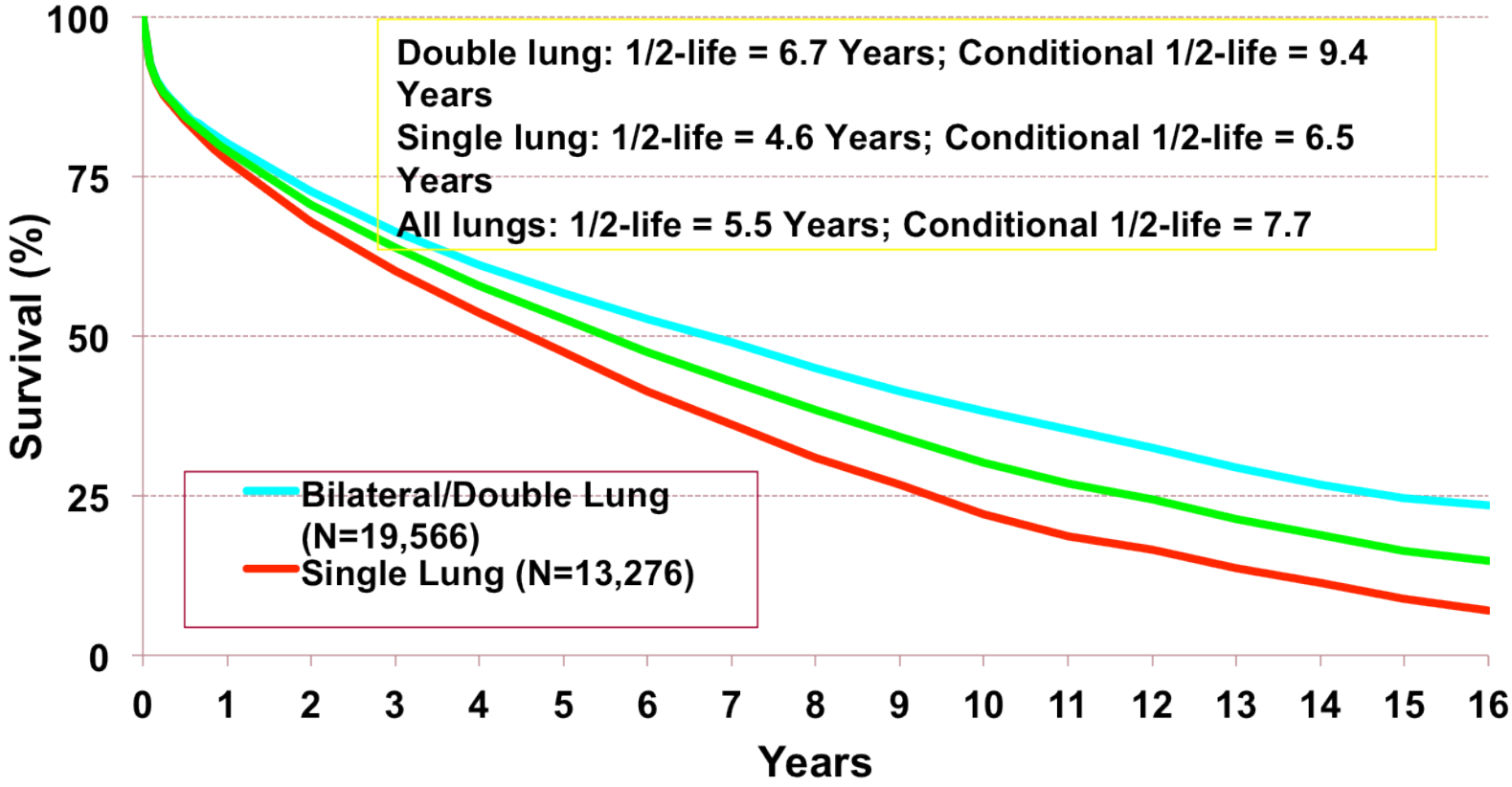
J Heart Lung Transplant. 2012 Oct; 31(10): 1045-1095

PACT
Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

ADULT LUNG TRANSPLANTS

Kaplan-Meier Survival

(Transplants: January 1994 - June 2010)

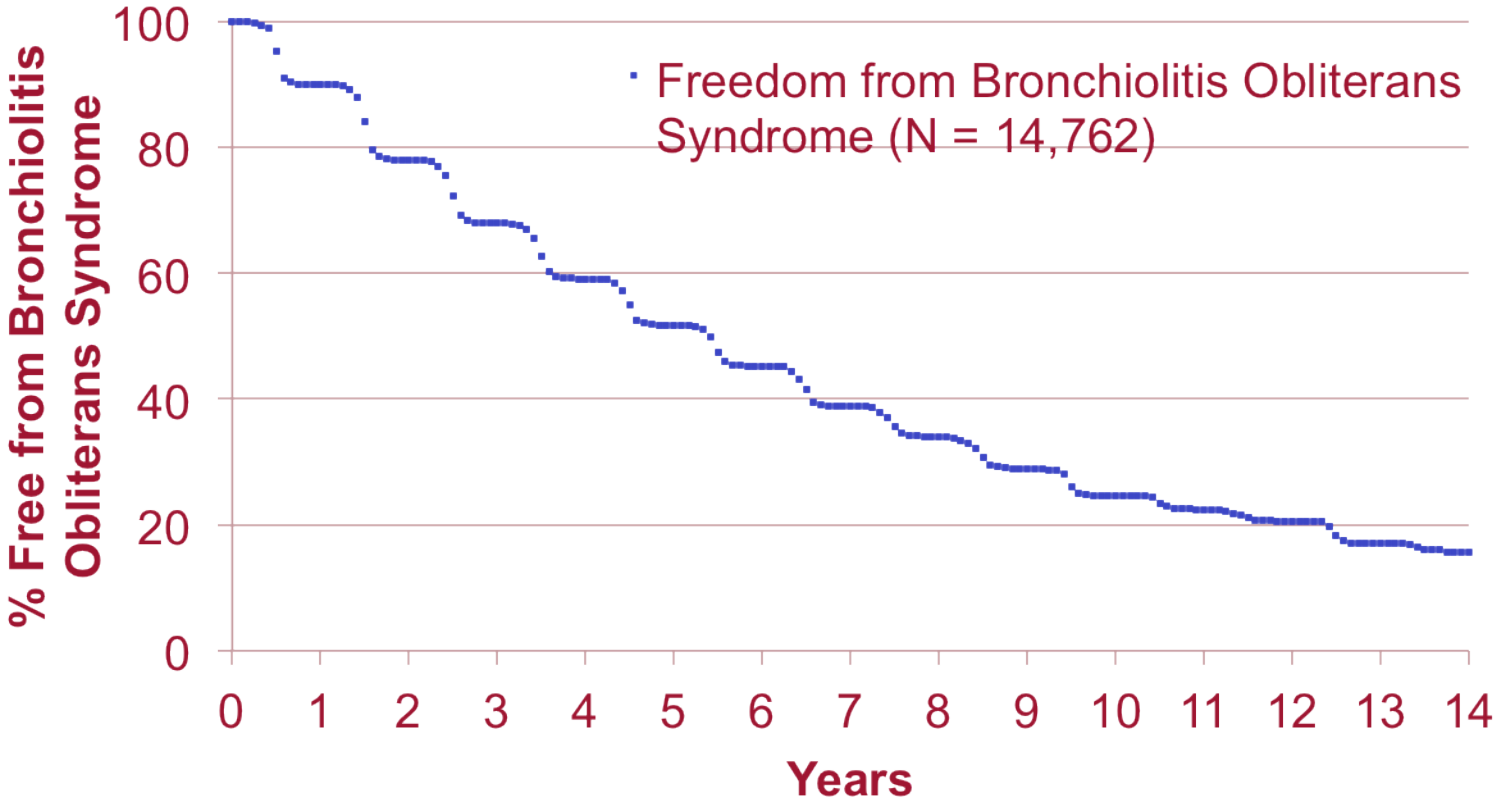


5-year survival rates for lung transplant recipients remains around 50% which is the worst of all solid organ transplants

J Heart Lung Transplant. 2012 Oct; 31(10): 1045-1095

PACT Production Assistance for Cellular Therapies
National Heart Lung and Blood Program

Bronchiolitis Obliterans Syndrome



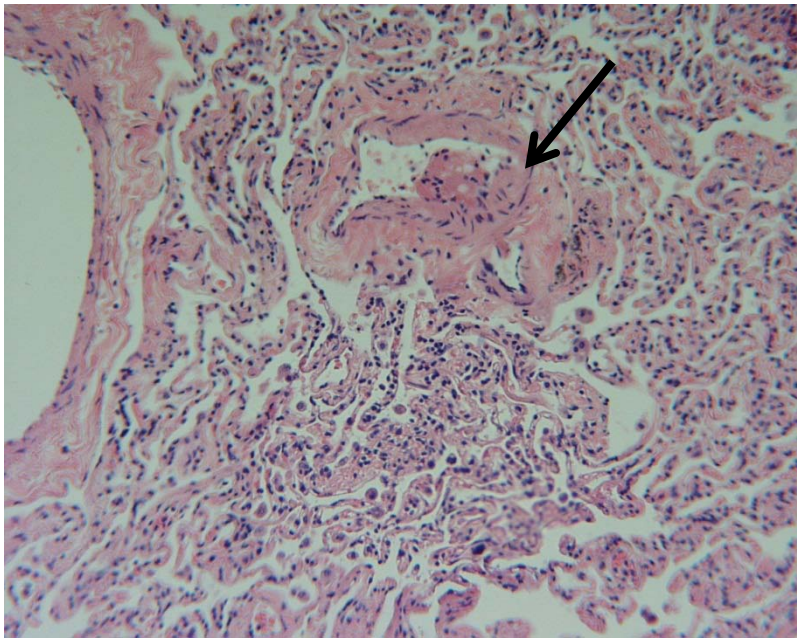
These survival rates are attributed to the prevalence of chronic rejection or bronchiolitis obliterans syndrome (BOS).

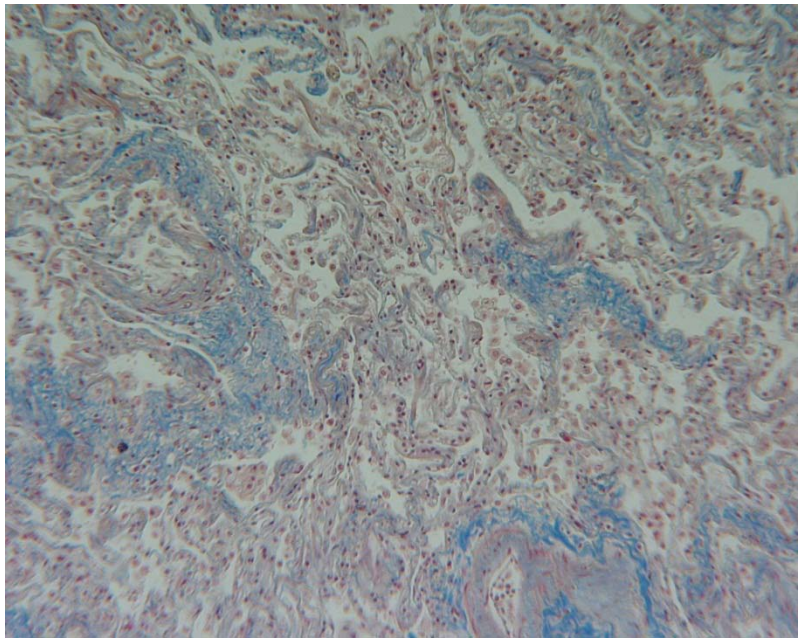
J Heart Lung Transplant. 2012 Oct; 31(10): 1045-1095

Bronchiolitis Obliterans Syndrome (BOS)

- BOS is a form of chronic lung allograft dysfunction that affects a majority of lung transplant recipients
- Principal factor limiting long-term transplant survival.
- Characterized by progressive airflow obstruction unexplained by acute rejection, infection, or other coexistent condition.
- Primary pathologic correlate of BOS is obliterative bronchiolitis, a condition of intraluminal airway fibrosis.

Obliterative Bronchiolitis: Histology





OB affects small airways of the transplanted lung and is histologically characterized by a patchy submucosal fibrosing process leading to a slowly narrowing of the bronchioles (arrow)

Factors to consider for selecting the appropriate animal model

- What is the primary question? Safety? Efficacy?
- How closely does the animal model translate to the human disease and stage of disease?
- What are the technical challenges of the model development?
- Does the model allow measurement of relevant physiologic information?
- Does the model allow mimicking of the planned human dosing schedule?

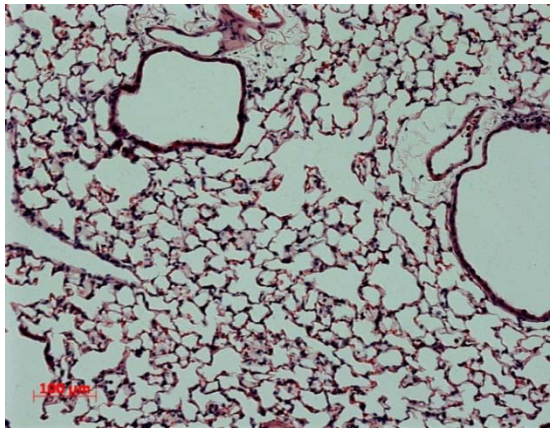
Potential Animal Models discussed with FDA

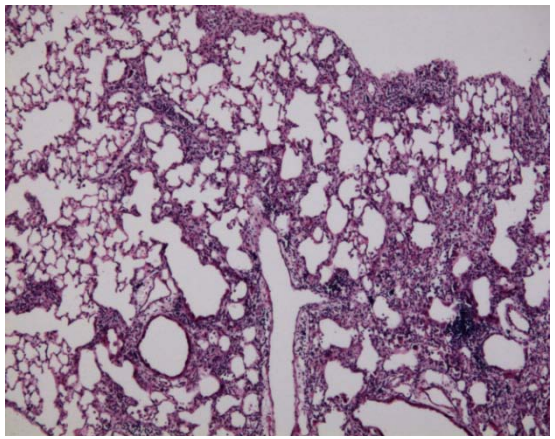
Model	OB Pathology	Technical Challenges	Physiologic Data	Mimic Human Dosing
Swine Lung Tx	very good	Costly	Very relevant easy to obtain	yes
Rat Lung Tx	good	Difficult surgery	Healthy lung compensates	yes
Mouse Lung Tx	fair	Very difficult surgery	Difficult to obtain and healthy lung compensates	Yes but more challenging

Animal Model Development

- FDA – use animal lung injury model to demonstrate safety of MSC IV infusion in compromised lung.
- Rat bleomycin-induced lung fibrosis model - closely represents moderate to severe BOS (40-50% reduction in pulmonary function), allows for the ability to monitor both cardiac/pulmonary function and mimicking of dosing schedule.

Bleomycin induced lung fibrosis





- Intratracheal instillation induces strong neutrophilic inflammation (similar to acute rejection)
- Is followed by severe fibrosis, interstitial, peribronchial (dominant) and perivascular (minor).
- Affects the whole lung

Preclinical Animal Studies

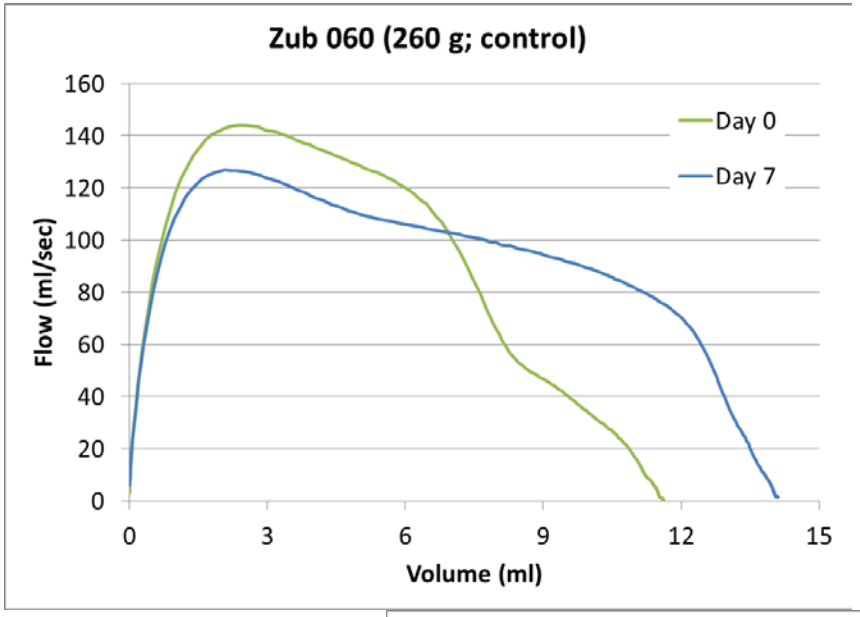
- Rats – male, Sprague Dawley, 6-8 weeks, 200-350g
- Bleomycin – intratracheal administration days 1, 4
- MSCs/control – femoral vein infusion, 30 minutes, days 7, 9, 11
- Acute Study

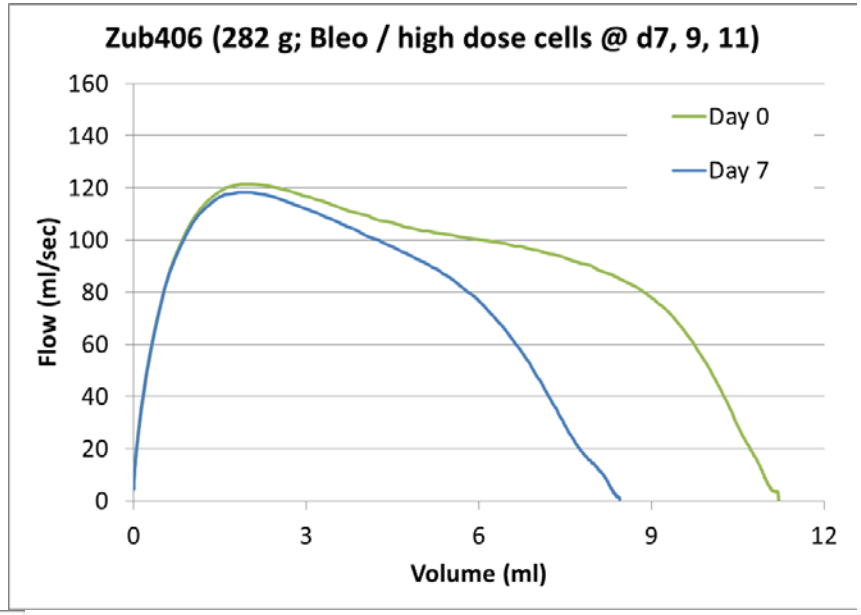
- Monitor 1 hour post injection
- 3 Groups (n=5/group) - Freezing medium, low dose, high dose
- Endpoint measurements - respiration and cardiac function, arterial blood gas, arterial and right ventricular blood pressure

- Chronic Study

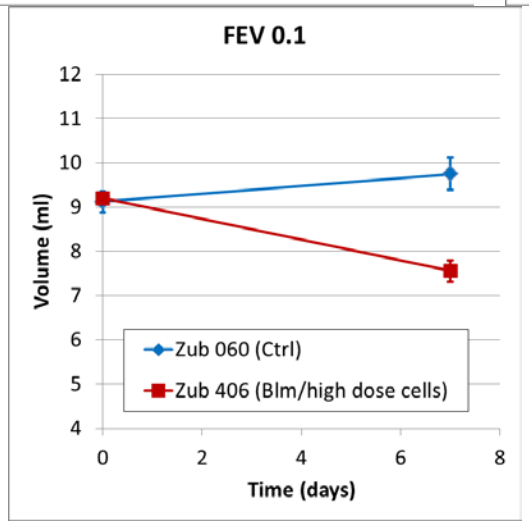
- Monitor 3 months post injection
- 3 Bleo Groups (n=5/group) - Freezing medium, low dose, high dose
- 2 Control groups (saline IT) – Freezing medium, high dose MSCs
- Study measurements – survival, body weight, pulmonary function, SpO_2, hematology
- Endpoint measurements - lung/organ histology, qPCR for cell tracking

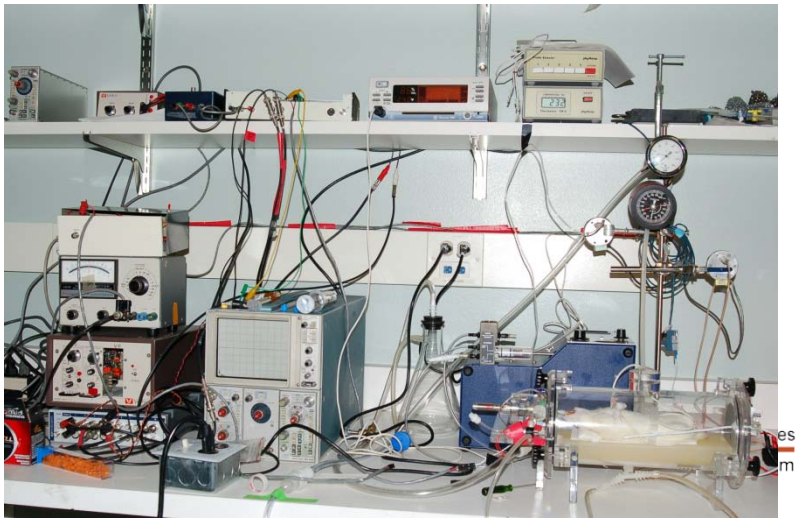
Lung Function Measurements





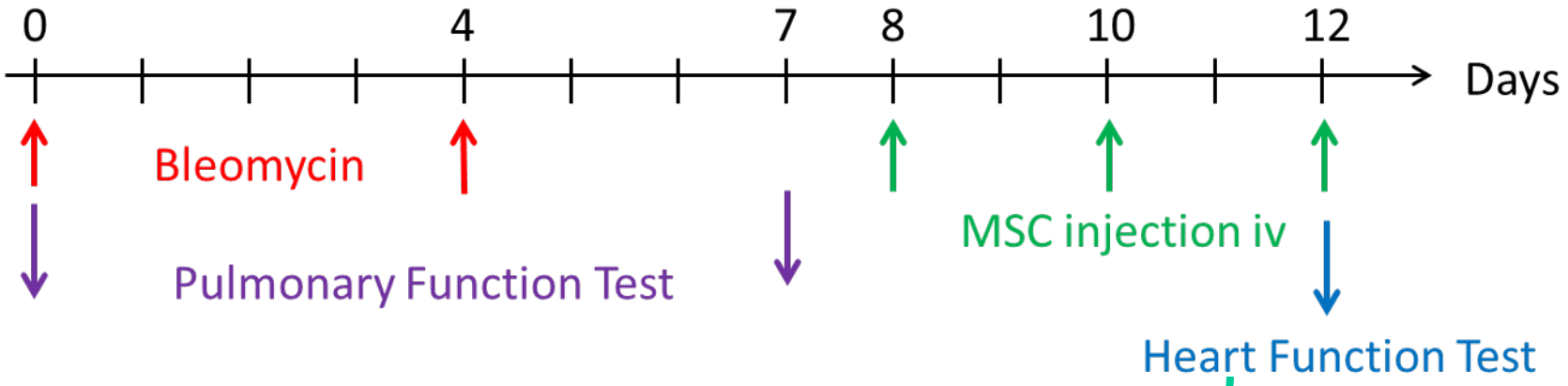
Reduced $FEV_{0.1}$
25 to 40% compared to control





Acute Study Design

Short term effect of MSC injection



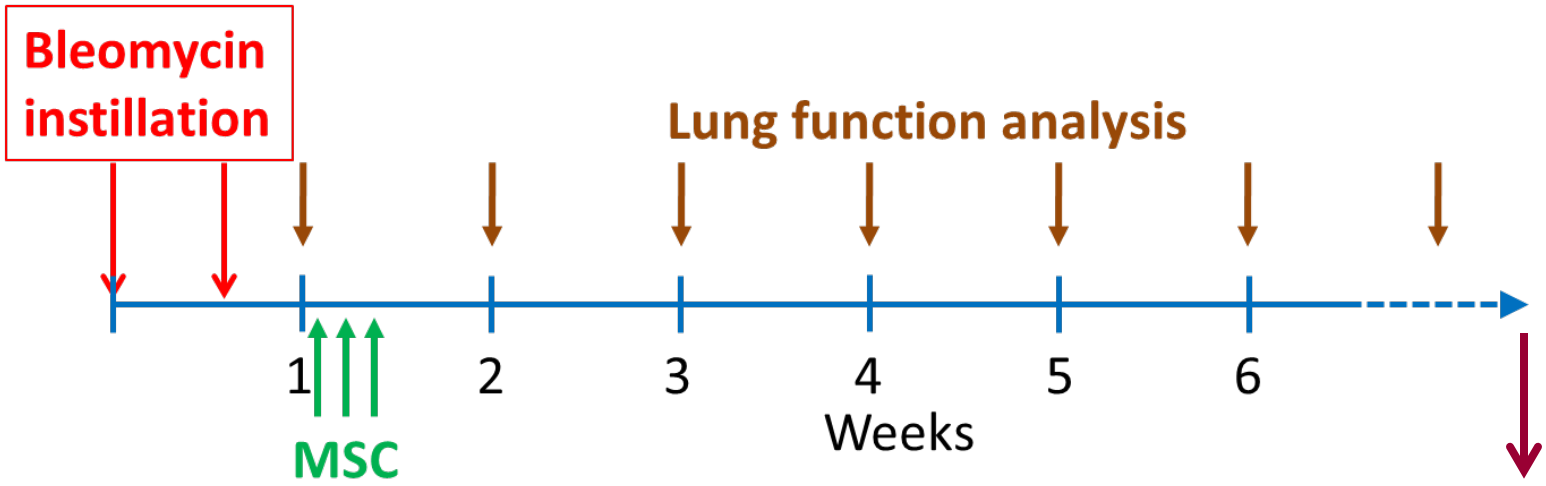
Left and Right heart catheterization

2.8×10^6 cells/kg

5.6×10^6 cells/kg

Chronic Study Design

Long term effect of MSC injection



2.8 x 10^6 cells/kg

5.6 x 10^6 cells/kg

Endpoint at 12 weeks

Terminal readout:

Pathology, Histology, blood chemistry

Animal Model Challenges

- Cell administration more difficult to mimic human dosing than expected –
  - IV infusion over extended period
  - Cell settling during administration
  - Dilution issues
- Rats with surgically implanted catheters for cell administration and blood sampling – trading one challenge for many others
- Chronic study requires long-term monitoring of animals

Next Steps

- Complete chronic and acute studies, analyze and compile data
- Re-design or repeat studies if necessary
- Update IND to include revised clinical protocol, CMC section, nonclinical safety data
- Continue to develop potency assay
- Generate clinical-grade MSCs to support human clinical studies
- IND filing target for Q1 2013