



# Comparative Biosciences

## Model of Monocrotaline-Induced Pulmonary Hypertension in Rats

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COMPARATIVE BIOSCIENCES, INC.  
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

# Comparative Biosciences Inc.

## Premier Preclinical Contract Research Organization

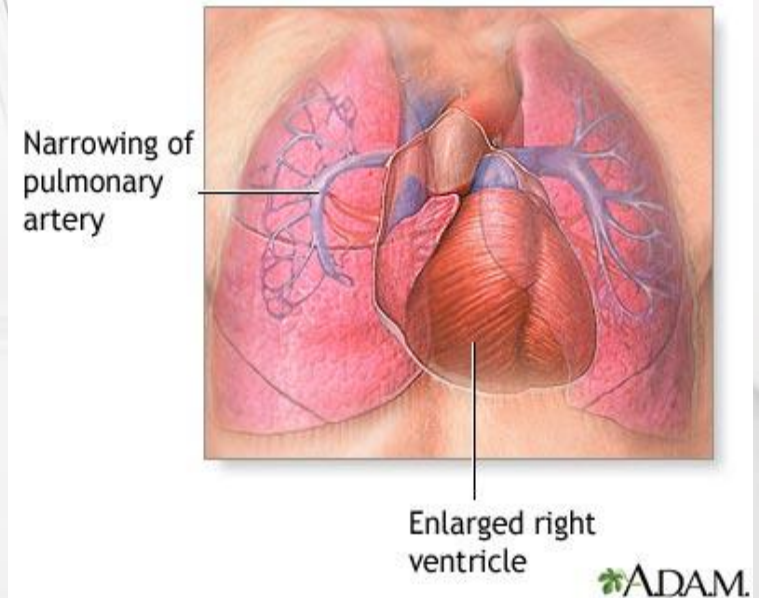
- **20 years** of experience
- Conveniently **located in the heart of Silicon Valley**, amidst many biotech companies
- **State of the art, purpose-built facility**
- Approximately **35 employees**
- **Highly experienced staff**
- **GLP, OECD, FDA, USDA, OLAW**
- **AAALAC Accreditation**



# Pulmonary Arterial Hypertension (PAH)

Pulmonary hypertension is the narrowing of the pulmonary arterioles within the lung. The narrowing of the arteries creates resistance and an increased work load for the heart. The heart becomes enlarged from pumping blood against the resistance. Some symptoms include chest pain, weakness, shortness of breath, and fatigue.

There is no known cure. The goal of treatment is to control symptoms



# Monocrotaline Induction

- Monocrotaline is a toxin extracted from a plant name "*Crotalaria spectabilis*"
- Monocrotaline causes endothelial injury leading to medial hypertrophy in the pulmonary arterioles
- A single subcutaneous dose of about 60 mg/kg will induce pulmonary changes



# MODEL OF MONOCROTALINE-INDUCED PULMONARY HYPERTENSION

- Induction of PHA
  - Subcutaneous injection of monocrotaline (60 mg/kg)  
PAH will be induced in 4 weeks
- Dosing with control and test article
  - Dosing after induction, number of injections or route of injection are varied
- Hemodynamic measurements
- Histology and histomorphometry



## TYPICAL STUDY DESIGN

- Wistar Rats (220-250g males)
- Acclimate and randomize by body weights
- Administered MCT 60 mg/kg SC
- Treat with two doses of Test Article as per sponsor
- Sildenafil (12.5 mg/kg/day) positive control
- Daily clinical observations
- Weekly body weights
- Blood for PK as desired by sponsor



# TYPICAL STUDY PROCEDURES

- In Life Procedures
  - Clinical Observation
    - Once daily
  - Body weight:
    - Daily, after 1<sup>st</sup> dose.
  - Dosing:
    - MCT: once on Day 0
    - Test and Control Articles: Dosing schedule to be determined by sponsor
  - Pharmacokinetics
    - Blood for plasma will be collected as needed



# TYPICAL STUDY PROCEDURES

- Terminal Procedures
  - Hemodynamic Determinations
    - Carotid arterial blood pressure
    - Pulmonary artery pressure
    - Right ventricular pressure
  - Necropsy
    - Macroscopic examination of heart and lungs
    - Dissection and weighing of lungs, heart,
    - Weigh: right ventricle (RV) and the left ventricle including the septum (LV+S)
  - Histopathology
    - Assessment of heart and lungs
  - Histomorphometry
    - >40 arteries/ rat
    - For each artery, external (P1) and internal (P2) perimeter measured.
    - Areas (S1) bounded by P1 and the area (S2) bounded by P2 calculated by the imaging software
    - For each artery, medial wall thickness expressed according to following formula:
      - % wall thickness (%W) =  $(S1-S2) / S1 \times 100$





# HEMODYNAMIC MEASUREMENTS

- Terminal procedure followed by euthanasia and necropsy
- Isoflurane (induction chamber), intubate and mechanically ventilate, maintain at 2%
- Cannulate carotid artery, pulmonary artery and right ventricle and collect pressure determinants



# HEMODYNAMIC MEASUREMENTS, SYSTEMIC ARTERIAL PRESSURE

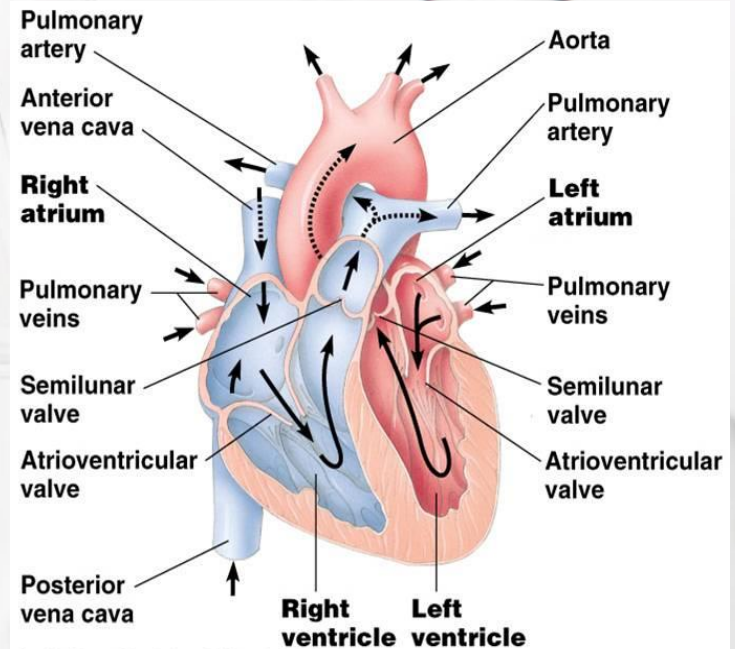
- Expose left carotid artery and cannulate with catheter
- Connect the catheter to a transducer and blood pressure analyzer (BPA)
- Make recordings for 2 minutes



# HEMODYNAMIC MEASUREMENTS;

## RIGHT VENTRICULAR AND PULMONARY ARTERY PRESSURE

- Expose heart by thoracotomy through the sternum
- Dissect pectoral muscles, and carefully remove pericardium
- Insert 3.5 Fr. Catheter through the right ventricular outflow tract.
- Connect catheter to a pressure recording system (LPA 400) through a saline-filled system.
- Two minutes of continuous recording will be performed at:
  - Right ventricle
  - Pulmonary artery
- Animal euthanized by IV injection of KCl without recovery from anesthesia.

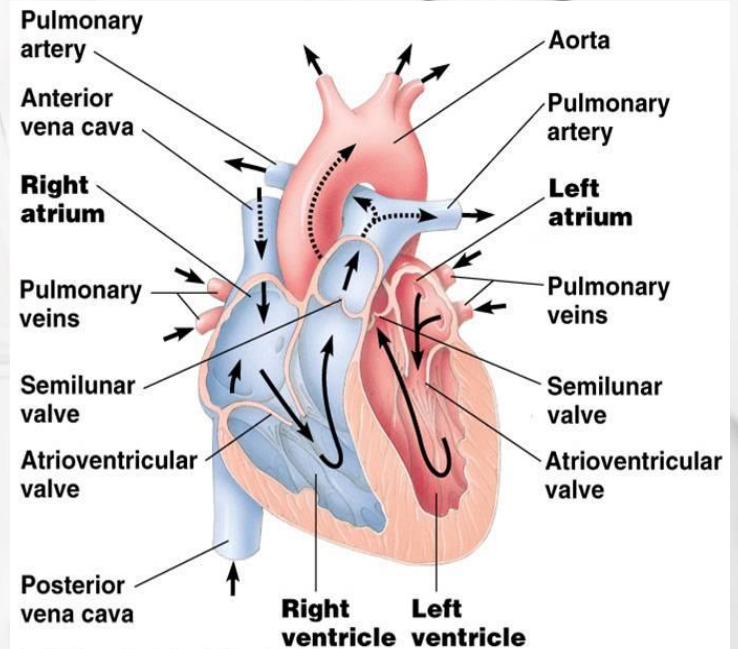


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# CARDIOPULMONARY NECROPSY

- Chambers opened and blood flushed
- Weigh entire heart
- Dissect away atria, and large blood vessels
- Dissect heart into right ventricle (RV) and left ventricle including the septum (LV+S)
- Weigh each of the sections
- Lungs-carefully dissect and weigh
- Inflate with saline then drop fix



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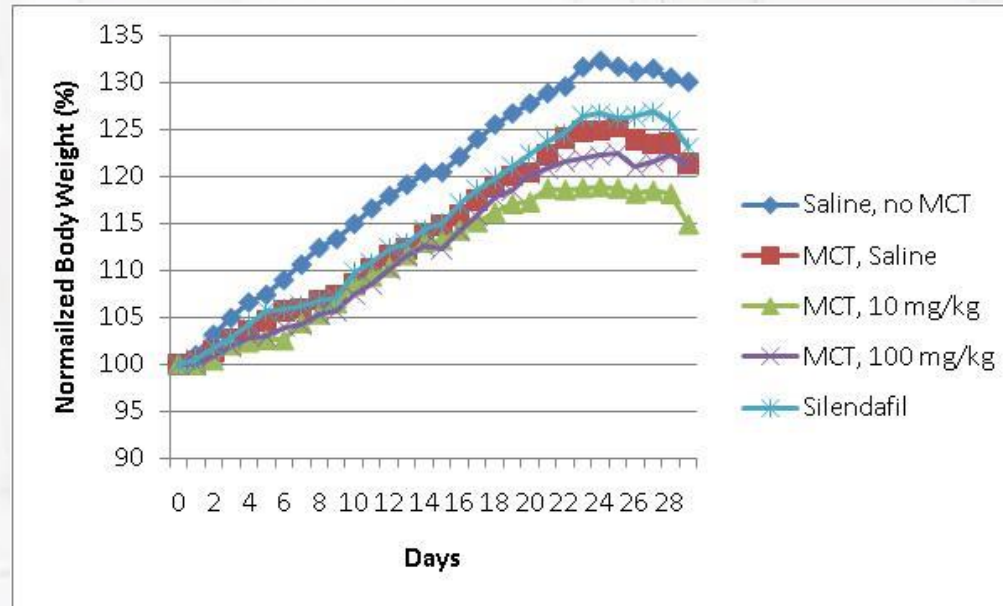
## SUMMARY OF STUDY FINDINGS

- Decreased body weights in all treated groups
- MCT associated with
  - increased PV
  - right ventricular size and weight
  - hypertrophy of small muscular arteries
  - Sildenafil – decreases in right ventricular size, right ventricular pressure, pulmonary inflammation, vessel size
  - test articles presented-no real effects



# SUMMARY OF STUDY FINDINGS

## Body Weights

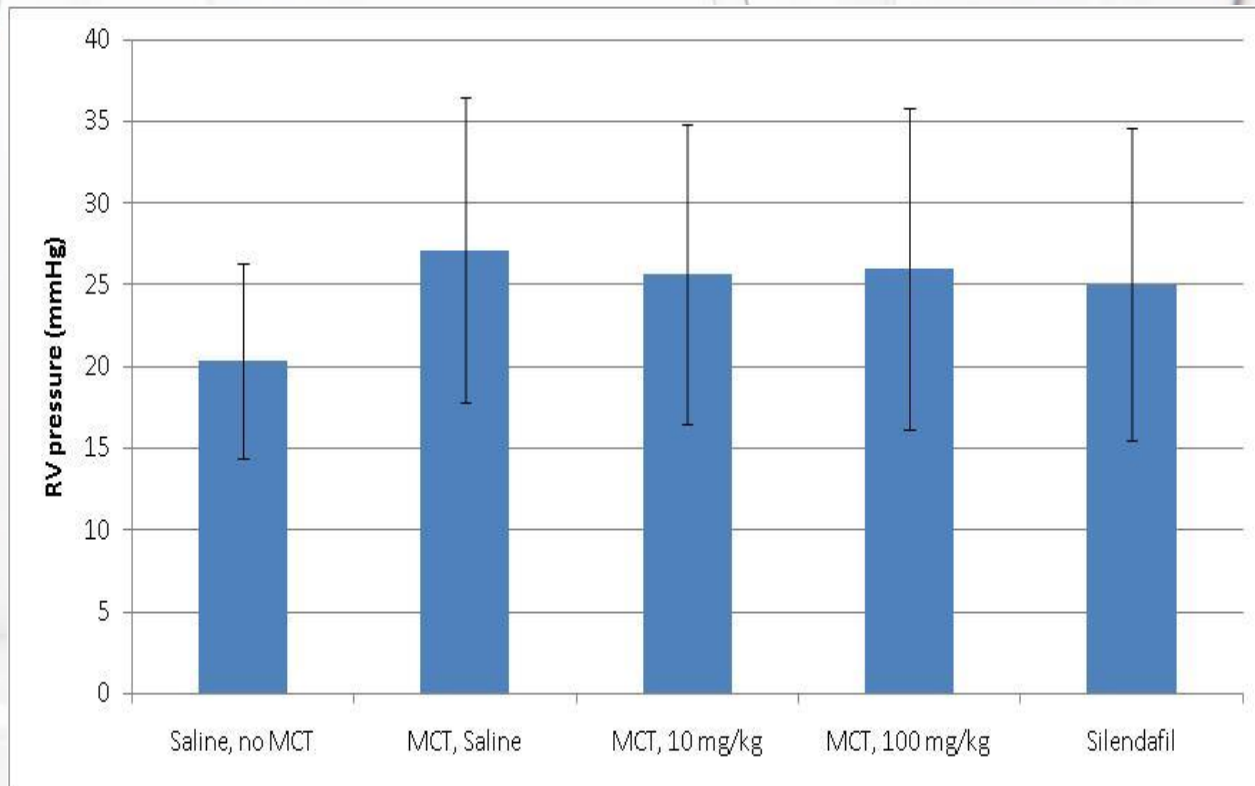


MCT: 60 mg/kg, Test Article at 10 and 100 mg/kg, Sildenafil 12.5 mg/kg

There is body weight loss in all MCT-treated groups



# RIGHT VENTRICLE PRESSURE MEASUREMENTS IN MCT-TREATED RATS

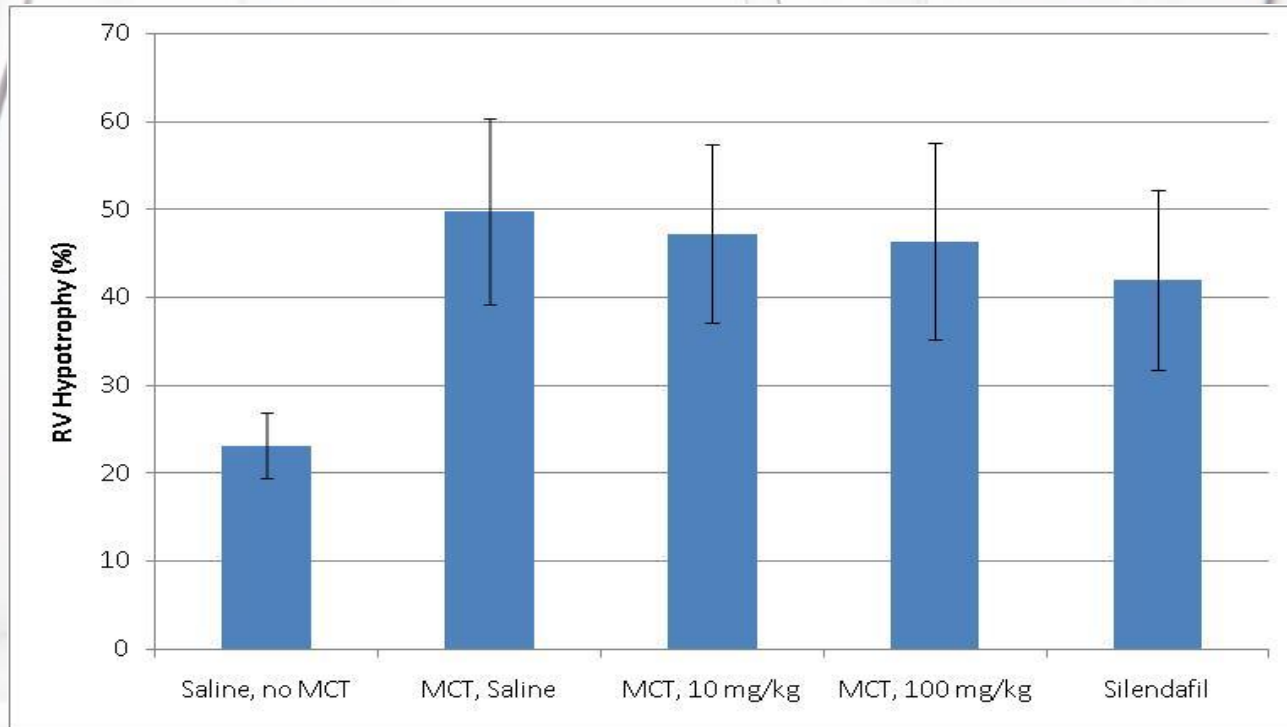


MCT: 60 mg/kg, Test Article at 10 and 100 mg/kg, Sildenafil 12.5 mg/kg

There are increases in right ventricular pressure in all MCT-treated groups.



# RIGHT VENTRICLE HYPERTROPHY



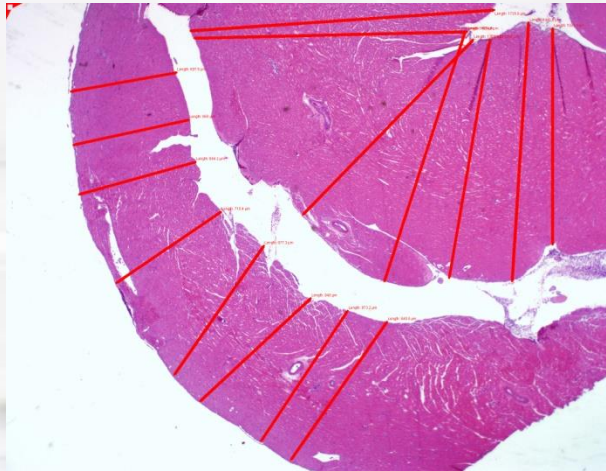
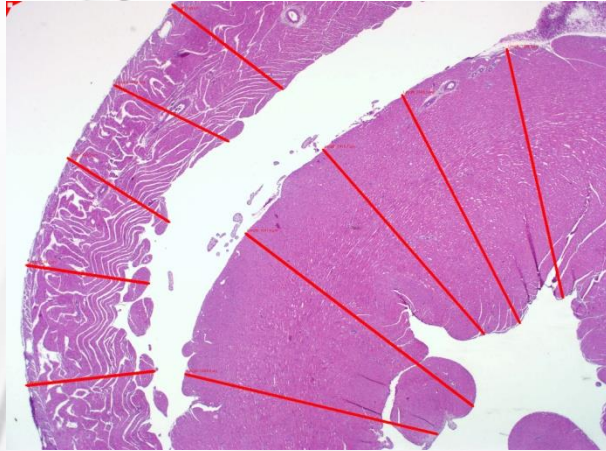
MCT: 60 mg/kg, Test Article at 10 and 100 mg/kg, Sildenafil 12.5 mg/kg

There is right ventricular hypertrophy in all MCT-treated groups with a downward trend in Sildenafil and test article treated groups.





## RIGHT VENTRICLE HYPERTROPHY

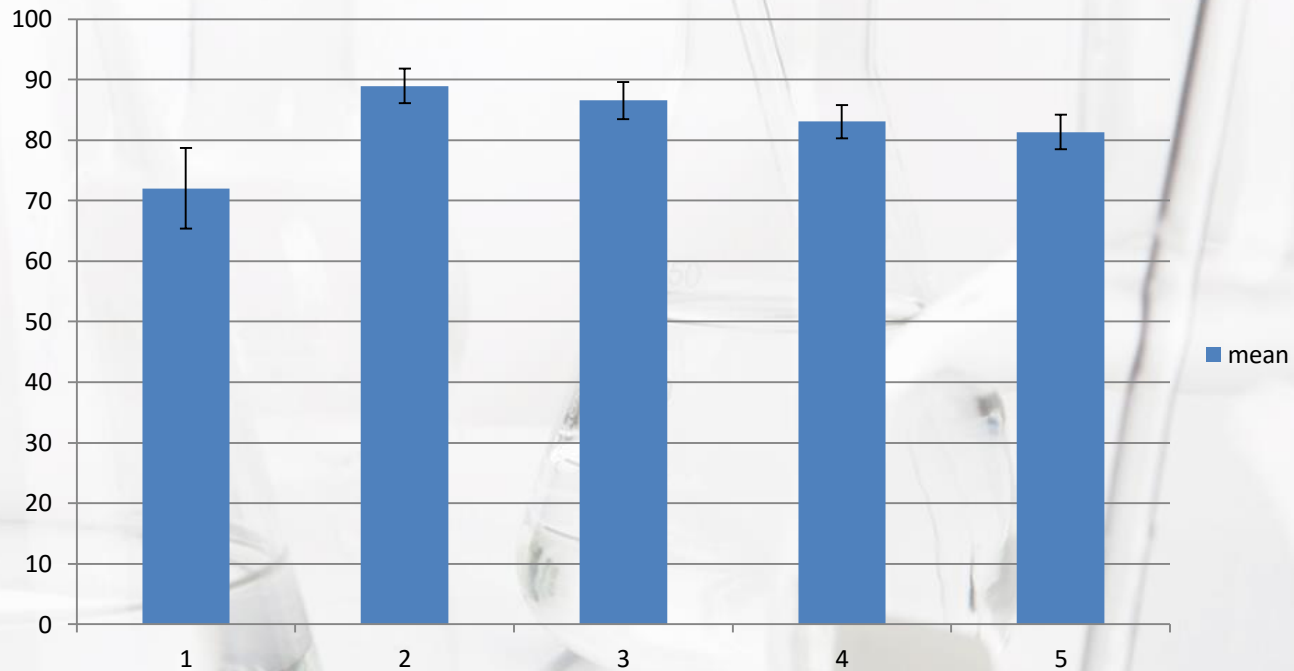


- MCT administration is associated with increased thickness of the right ventricular free wall and septum
- Top: Normal right ventricle from normal mouse
- Bottom: Increased thickness of right ventricular free wall and septum of MCT-treated rat at 4 weeks.



# HISTOMORPHOMETRY: ARTERIAL WALL THICKNESS

Mean Arterial Wall Thickness (um)



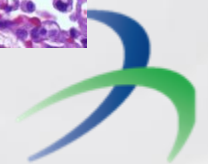
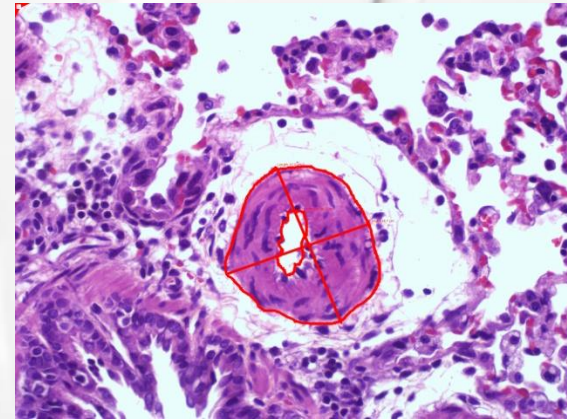
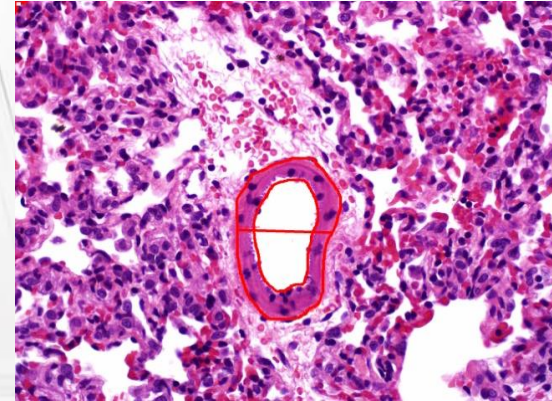
Vehicle, MCT: 60 mg/kg, Test Article at 10 and 100 mg/kg, Sildenafil 12.5 mg/kg

There is increased arterial wall thickness in all MCT-treated groups with a downward trend in Sildenafil and test article treated groups.

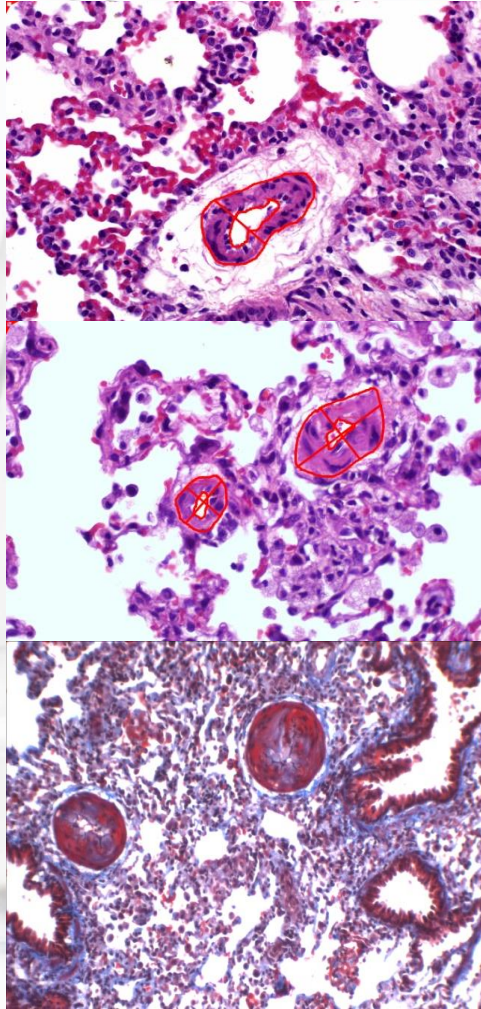


# PULMONARY VESSEL MEASUREMENTS

- Paraffin sections of a cross-section of the full lung field at about the mid-level will be made so that both lungs can be examined, allowing for examination of cross-sections of the vessels.
- For each artery, the external (P1) and the internal (P2) perimeter measured.
- The areas (S1) bounded by P1 and the area (S2) bounded by P2 calculated by the imaging software
- For each artery, the medial wall thickness will be expressed according to the following formula:
  - $\% \text{ wall thickness } (\%W) = (S1 - S2) / S1 \times 100$



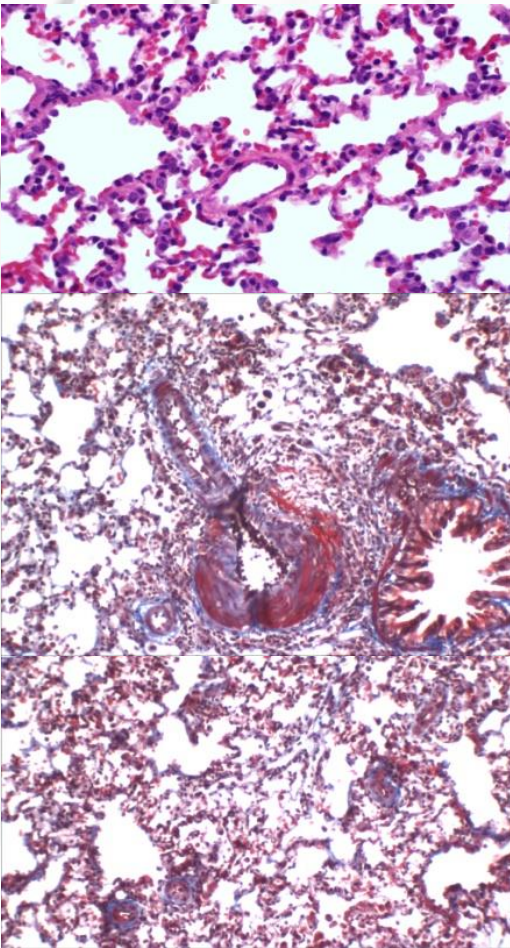
# PULMONARY VESSEL MORPHOLOGY



- Top: Normal small artery from vehicle treated mouse
- Middle: Increased thickness and hypertrophy of small muscular artery in MCT-treated rat at 4 weeks
- Bottom: Trichrome-stained section of MCT-treated rat at 4 weeks.



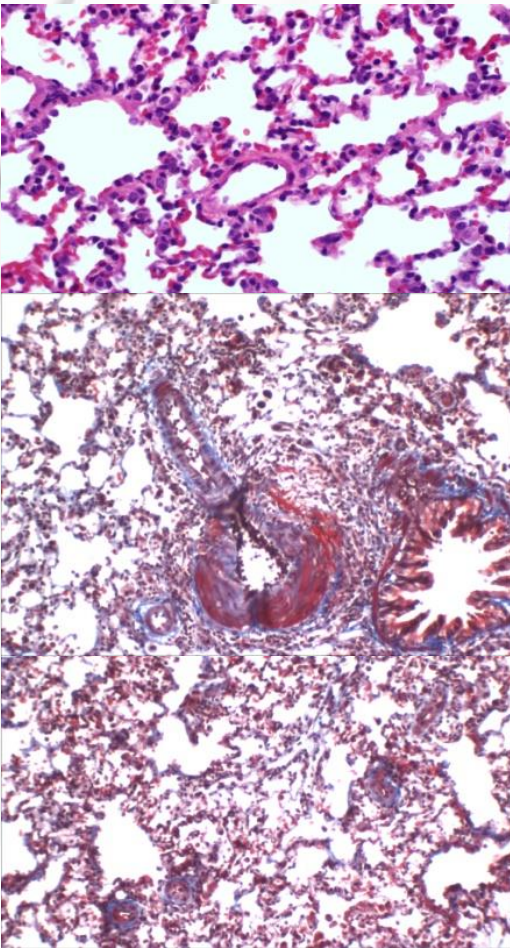
## PULMONARY INFLAMMATION



- MCT administration is associated with a moderate to severe, multifocal, chronic active inflammation.
- Sildenafil is associated with a somewhat milder to moderate reduction in inflammation
- There was no effect on inflammation with the test articles.
  - Top-normal mouse-no inflammation
  - Middle: MCT treated-marked inflammation
  - Bottom: Sildenafil treated-decreased inflammation



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## Service and Quality

- ***Thoroughness in planning and execution is key to a successful study.*** All protocols are vetted and approved by multiple personnel. Our QAU has a rigorous training program. All non-GLP studies are conducted in the spirit of GLP.
- ***We believe in sound science.*** Our ratio of scientists to non-scientists is one of the highest in the industry. Every study director is a PhD-level scientist.
- ***We believe in communication.*** Timely responses to your inquiries and frequent updates on your study are mandatory.
- ***We welcome visitors.*** You are always welcome at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.

