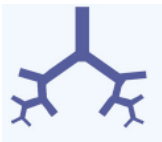




Sheep models for testing respiratory delivery technologies and drug formulations

Robert Bischof, PhD

Allergenix Pty Ltd

Airway characteristics and pulmonary physiology

Parameter	Human	Sheep	Rat
body mass	~ 80 kg	~ 45 kg	~ 0.3 kg
nose and/or mouth breathers	mouth/nose	mouth/nose	nose
branching system of trachea-bronchial airways	 dichotomous	 dichotomous	 monopodial
tidal volume (ml)	400 - 616	180 - 405	0.87 – 2.08
respiratory rate (breaths/min)	12 - 20	15 - 30	85

Human respiratory vs *sheep* and *small rodent* models

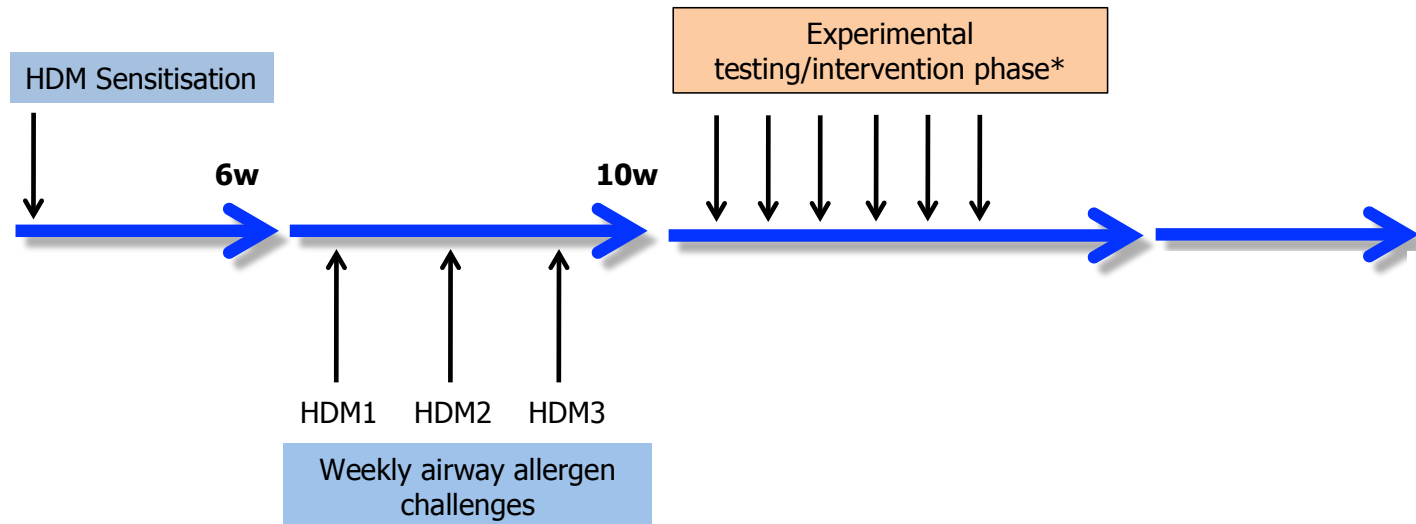
	Human	Sheep	Mouse
Th-2 biased immune responses	✓	✓	✓
Allergen induced airway constriction	✓	✓	✓
Airway hyperresponsiveness	✓	✓	✓
Comparative physiology	✓	✓	✗
Airway branching	✓	✓	✗
Sensory nerves	✓	✓	✗
Bronchial glands	✓	✓	✗
Blood vessels associated with smaller airways	✓	✓	✗
Mast cells around small airways	✓	✓	✗
Histamine effect on airway smooth muscle	✓	✓	✗
Cough reflex/wheeze	✓	✓	✗

Allergenix sheep respiratory models – *in vivo* capabilities

Disease	Induction stimulus	Features
Acute lung injury (ALI); Acute respiratory distress syndrome (ARDS)	Saline lavage; Intravenous LPS; Oleic acid; Cotton smoke inhalation injury with/without instillation of <i>Pseudomonas aeruginosa</i>	Severe hypoxemia; progressive decrease in air volume; hyperkinetic cardiovascular response
Asthma	<i>Ascaris suum</i> airway challenges in naturally sensitised sheep; House dust mite (HDM) extract sensitisation and airway challenges	IgE; early- and late- phase bronchoconstriction (EAR, LAR) and AHR; airway inflammation; elevated Th2 cytokines; airway wall remodelling and decline in lung function in chronic condition
Bronchoalveolar carcinoma (BAC)	Jaagsiekte sheep retrovirus (JSRV) or JSRV envelope	Natural and fatal disease of sheep; progressive respiratory distress; impaired alveolar function
Bronchopulmonary dysplasia (BPD) or chronic lung disease of early infancy	Ventilator-induced lung injury (VILI) of premature lambs; Live ureoplasma or endotoxin-induced chorioamnionitis	Abnormal collagen and elastin deposition; myofibroblast differentiation; early lung maturation
Chronic bronchitis	Tobacco smoke; Sulphur dioxide	Glandular hypertrophy or hyperplasia
Chronic pulmonary hypertension	Sephadex beads injected into the pulmonary circulation	Development of right ventricular hypertrophy
COPD/emphysema	Chronic instillation of LPS or elastase into the lung	Microscopic emphysema and reduction in TGF β ; increased collateral ventilation
Pulmonary fibrosis (IPF)	Bleomycin administered to the lungs	Increased collagen and elastin deposition; elevated TGF β ; lung fibrosis correlates with decline in lung function
Respiratory syncytial virus (RSV) infection	Infection of neonatal lambs with RSV	Mild peribronchiolar interstitial pneumonia; induction of surfactant proteins A and D

HDM sheep model of allergic asthma

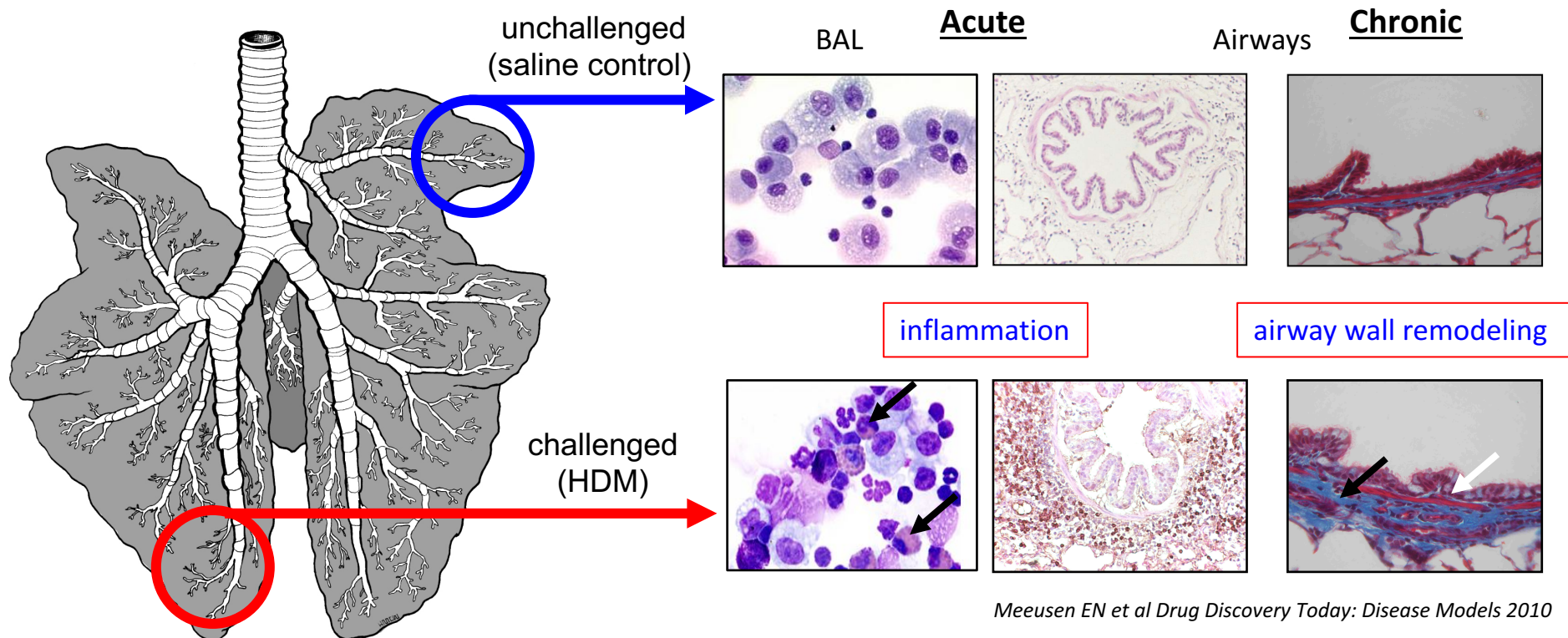
- induced via (1) HDM sensitisation (sc injections) and (2) weekly HDM airway challenges
- capacity for 'acute' and 'chronic' models of disease



* Experimental testing phase may include allergen challenge(s) before/during/after drug administration



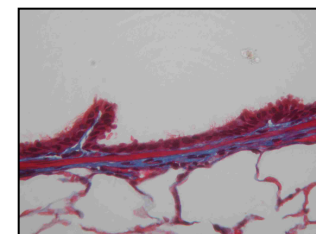
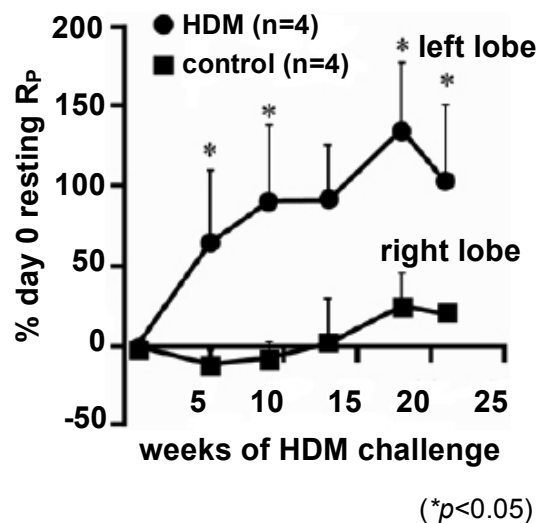
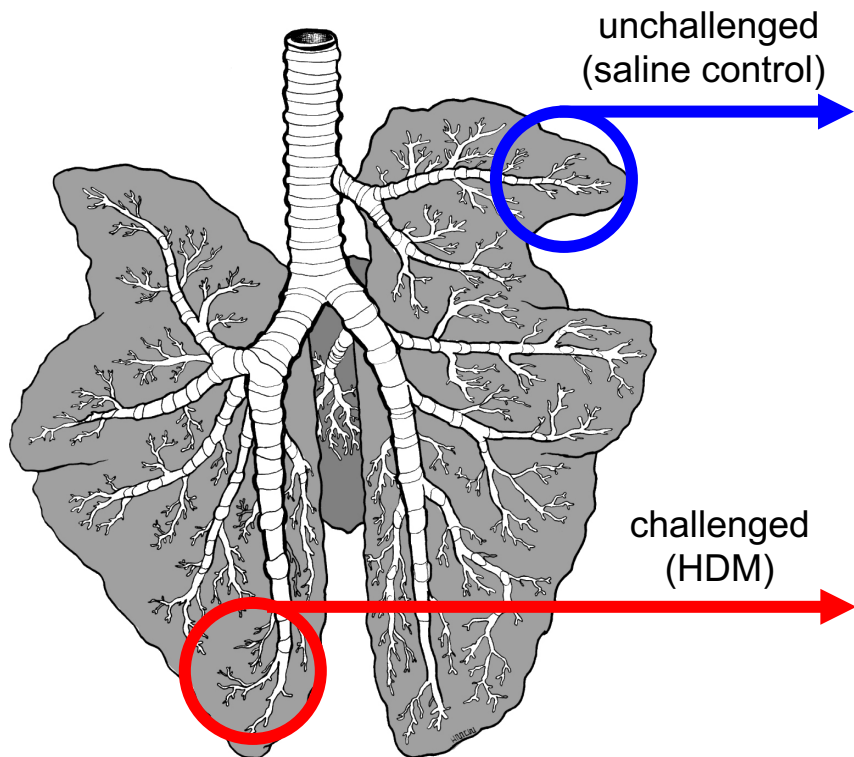
HDM sheep model of allergic asthma



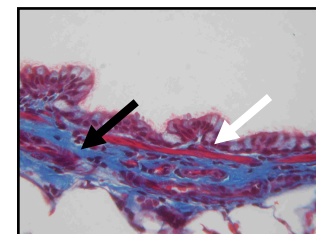
- 'acute' and 'chronic' phases display pathophysiology of human asthma
- customised model system - whole lung or targeted segmental lobe studies
- potential for investigations of disease pathogenesis; efficacy of asthma therapies



HDM sheep model of allergic asthma



airway wall remodeling



Meeusen EN et al Drug Discovery Today: Disease Models 2010

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Anti-asthma therapies are effective in sheep

Drug class	Drug details	Delivery mode	Dose regime	Outcome measures	
				Lung function	Inflammation
Beta-agonist	Salbutamol (Ventolin TM)	Airway (liquid, nebulised)	2.5 mg; once following allergen challenge	↓ EAR	ND
Steroid anti-inflammatory	Budesonide (Pulmicort TM)	Airway (liquid, nebulised)	0.5 mg; twice daily from -48h to +24h after allergen challenge	↓ EAR	↓ BAL eosinophils ↓ HDM-specific IgE
Leukotriene receptor antagonist	Zafirlukast (Accolate TM)	Oral	40 mg; once, 24h before allergen challenge	ND	↓ blood eosinophils ↓ BAL eosinophils

Notes:

↓ diminished response versus vehicle alone;

EAR, early airway response (effects on LAR not determined); ND, not determined

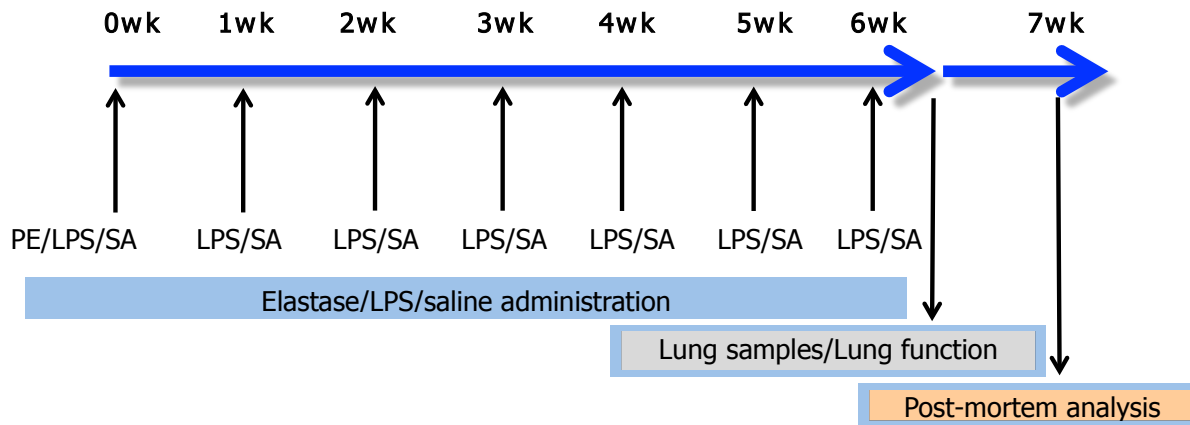
- common asthma therapies shown to resolve or inhibit disease symptoms
- proprietary compounds tested in acute and chronic disease outcomes

HDM (Allergenix) vs *Ascaris* – relevance to human airways?

	Human	HDM sheep model	<i>Ascaris</i> sheep model
Human-relevant allergen/IgE responses	✓	✓	✗
Allergen induced airway constriction	✓	✓	✓
Early/late airway responses	✓	✓	✓
Airway hyperresponsiveness	✓	✓	✓
Mucus hypersecretion	✓	✓	✓
Airway inflammation	✓	✓	✓
Airway eosinophils	✓	✓	✗
Airway mast cells	✓	✓	✗
Th2 driven cytokines/mechanisms	✓	✓	✗
Chronic decline in lung function	✓	✓	✗
Chronic tissue changes - remodelling	✓	✓	✗

Sheep model of COPD/emphysema

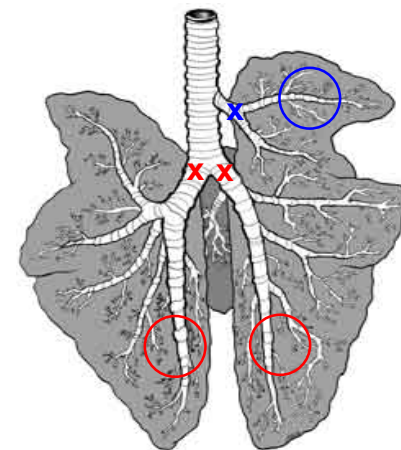
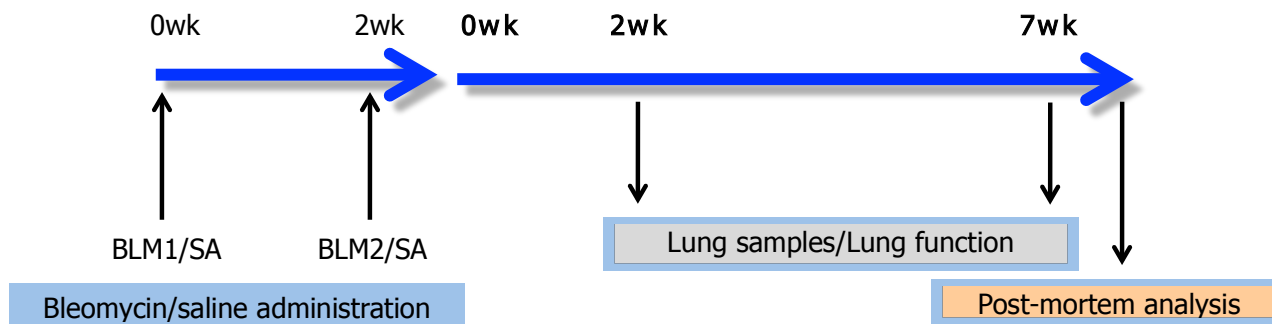
- induced by elastase (PE, 600 U/lung segment) +/- LPS (450 µg/segment) administrations



- inflammation: neutrophils and macrophages (tissue, BAL)
- tissue damage consistent with emphysema: enlargement of air spaces & airway wall (alveolar) destruction
- potential for therapeutic intervention studies to alter disease-related airway inflammation, tissue destruction, and lung function decline

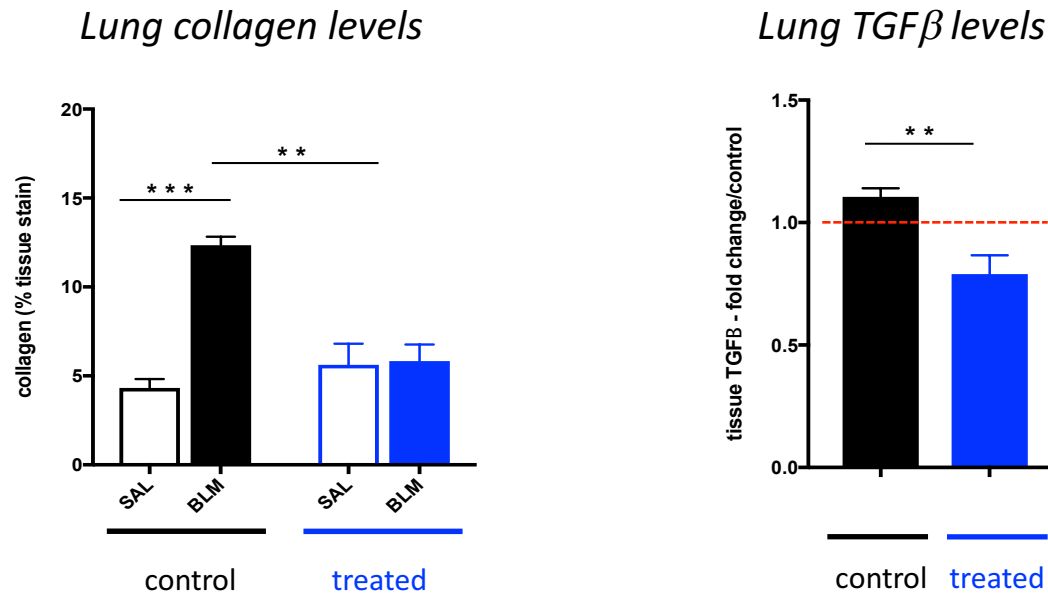
Sheep model of pulmonary fibrosis (IPF)

- induced by bleomycin (BLM) administrations (x2 @ 3U/lung segment)



- inflammation, fibrosis and collagen deposition developing from 2 wks following BLM2, persisting to at least 7 wks
- fibrosis correlates with decline in lung function at 7 wks
- potential for therapeutic intervention studies to change the course of fibrosis and rate of lung function decline

Antibody treatment reduces fibrosis in a sheep model of IPF



Antibody treatment (targeting fibrosis pathways) →

- reduced lung tissue fibrosis and collagen staining
- reduced lung tissue TGF-β levels
- improved lung function

Sheep models – drug delivery

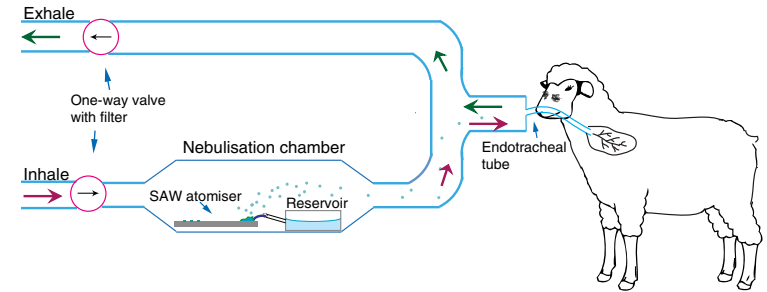
- **Pulmonary delivery systems:**

- testing of new delivery platforms
- instillation; liquid aerosolisation; dry powder

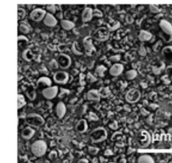
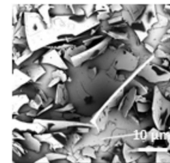
- **Pulmonary drug/compound delivery:**

- testing of new formulations
- soluble/liquid; dry powder formulations
- drugs for local and systemic targets
- effective delivery of aerosolised DNA vaccine
- lipid nanoparticles; siRNA; antibodies ie ‘nanobodies’

- PK and PD assessments of drug efficacy
- sampling to determine kinetics; in-life monitoring
- surgical interventions to monitor drug clearance



Rajapaksa A et al, Resp Res 2014



Prankerd R et al, PlosOne 2013

Allergenix sheep models – *in vivo* capabilities

- **Administration routes:**

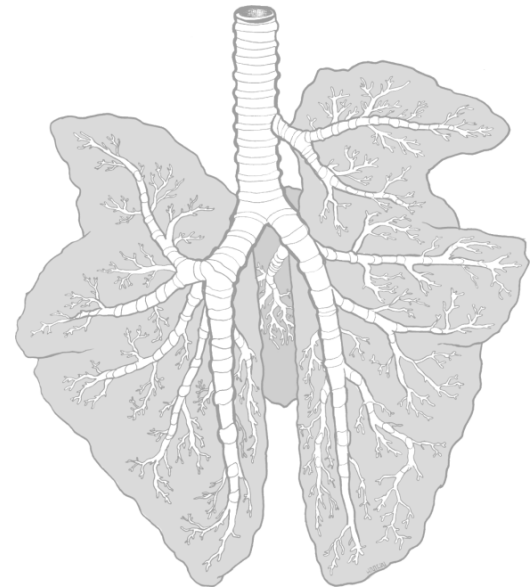
- systemic – IV, IM, ID, SC, peritoneal
- local/mucosal – intranasal, oral, pulmonary (bolus/nebulised/aerosolised or dry powder)
- whole lung or targeted to individual lung lobes/segments
- cross-over design; sequential testing of different treatments/drugs/doses in one animal

- **In-life monitoring:**

- body weight, body temperature, blood pressure, heart rate, O₂/CO₂ blood gases

- **Immunology/pharmacology:**

- repeated sampling throughout disease/treatment
- BAL fluid, blood, lung lymph (cannulation) collections
- endobronchial sampling (epithelial brushings, tissue biopsies) comprehensive immune cell/mediator analyses
- cellular, biochemical and molecular analyses



Allergenix sheep models – *in vivo* capabilities

- **Lung function:**
 - lung resistance (R_L), compliance, volumes, flow, EAR, LAR, AHR
 - continuous measures over time
 - lung function in fully conscious (unsedated) animals
- **Tissue damage/changes/repair:**
 - long-term analysis of tissue inflammation, remodelling, fibrosis
 - endobronchial sampling throughout disease/treatment
 - whole lung analysis and detailed histology/molecular analyses
- **Measurements/biomarkers:**
 - tissue structure, cell distribution/structure (surface, intracellular, tissue elements)
 - cell phenotype, activation, function
 - proteins, cytokines, biomarker detection
 - gene expression analysis
 - lung imaging
 - access to platform technologies – Hudson Institute of Medical Research, Monash University

Allergenix sheep models – *in vitro* & *ex vivo* capabilities

- **Isolated cells:**

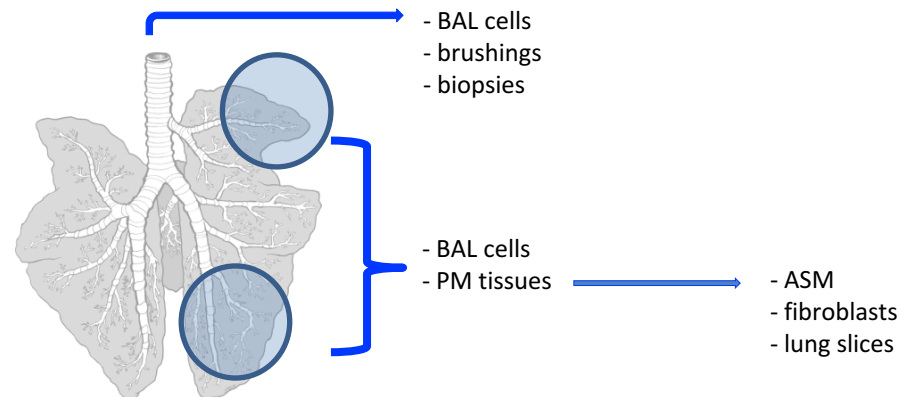
- immune cells – blood/lymph node/BAL macrophages, lymphocytes, dendritic cells;
- airway cells – epithelial cells, fibroblasts, airway smooth muscle
- monolayers, cell co-cultures

- **Tissue explants:**

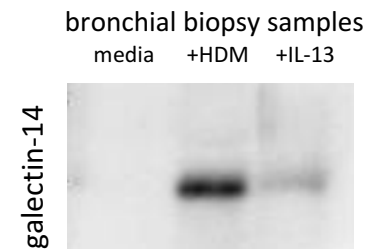
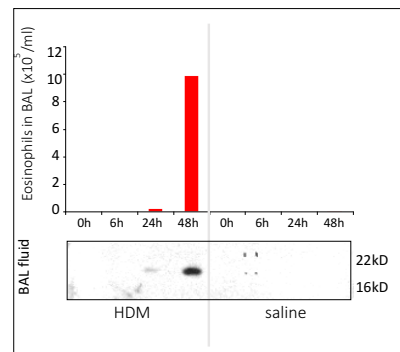
- endobronchial biopsy samples
- tracheal explants

- **Precision-cut lung slices (PCLS):**

- airway contractility
- active/passive treatments



Galectin-14 in asthmatic airways



Allergenix sheep models – *in vitro* & *ex vivo* capabilities

- **Isolated cells:**

- immune cells – blood/lymph node/BAL macrophages, lymphocytes, dendritic cells;
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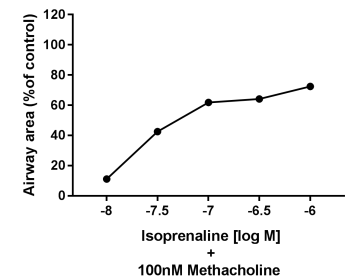
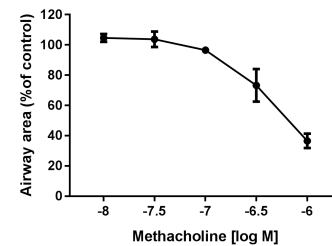
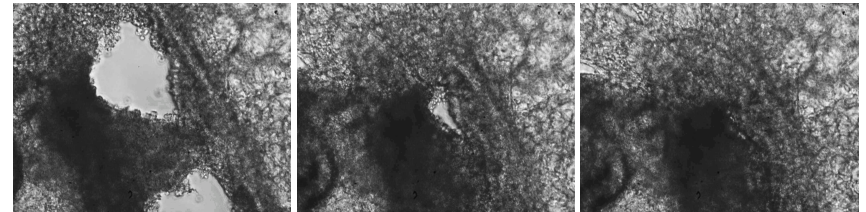
- endobronchial biopsy samples
- tracheal explants

- **Precision-cut lung slices (PCLS):**

- airway contractility
- active/passive treatments

- cellular, biochemical, immune and molecular analyses
- disease vs healthy airway cells and tissues
- relevant *in vitro/ex vivo* screening platforms

sheep PCLS: response to methacholine



Thank-you for your attention