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Challenge 14 - Inhalation Translation

Launch Meeting
5 September 2013

The Challenge

Efficacy

- **Can you measure the resolution of induced inflammation in the lung longitudinally and non-invasively?**

✓ Establish background cellular (macrophage) levels
✓ Demonstrate increased cellular infiltration in response to a challenge (e.g. LPS, HDM)
✓ Monitor resolution of inflammatory response following exposure to drug

Safety

- **Can you measure the lung response to chronic dosing of drug (as dry powder) longitudinally and non-invasively?**

✓ Demonstration of an adaptive, reversible (physiological) response to chronic exposure
✓ Discriminate from an adverse (pathological) response to chronic dry powder inhalation
✓ Demonstrate a difference between the two states that can be used to describe the safety profile of a new drug being developed for treatment of respiratory diseases
✓ Can such approaches be used to monitor patients? (i.e. is translatable to the clinic)

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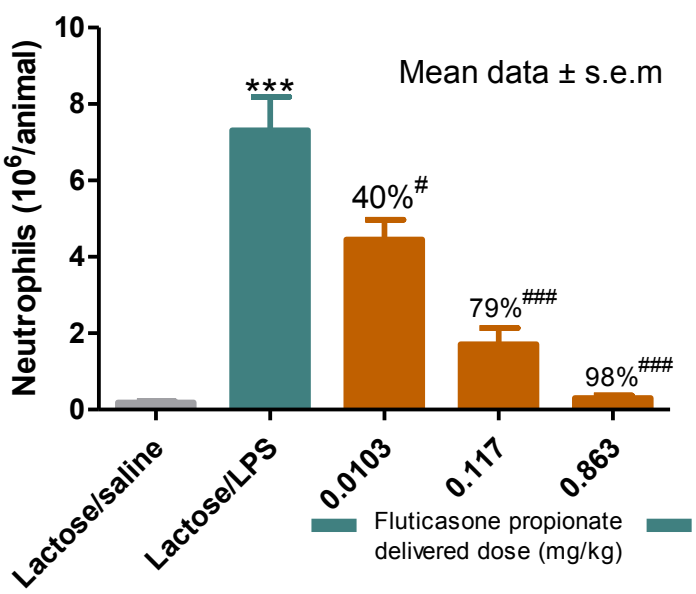
Efficacy – current practice

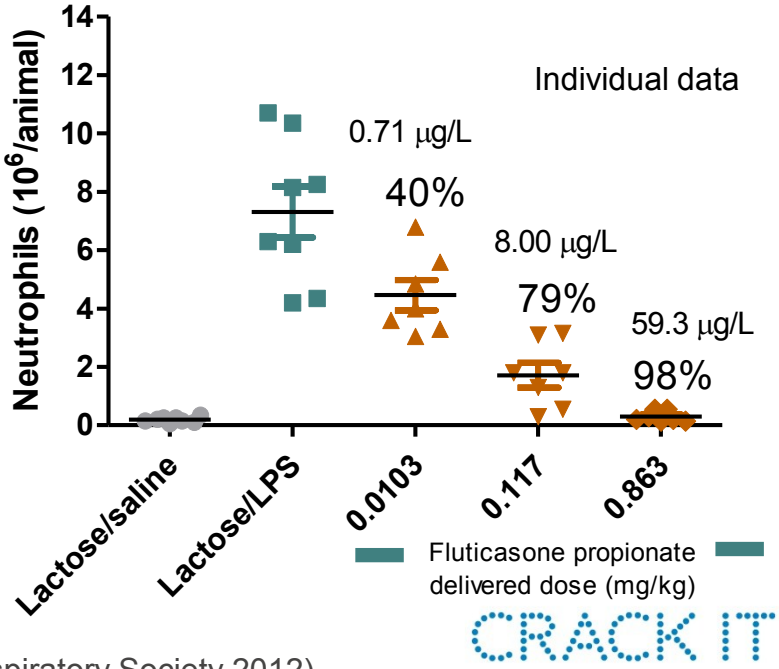
- **Rodent model of lung inflammation induced by a stimulus (LPS, HDM, OA)**

✓ Exposure to test drug (IT, inhalation) 30 min prior to treatment

✓ Exposure to stimulus (e.g. LPS), followed by a 4 hour incubation period

✓ Animals humanely killed and cellular infiltrate by BAL sampling undertaken





(HLS presentation at the European Respiratory Society 2012)

Confounding factors in interpretation of efficacy data

- Single time-point examined to evaluate a dynamic process – assessment at multiple time points requires new cohorts of animals at each point.
- No understanding of phenotypic cellular changes taking place
- Background / pre-existing low level inflammation in some animals
- Low number of animals/dose group, may in some cases increase variability
- Inconsistent recovery of BAL cells increasing heterogeneity
- May require repeat studies, increasing animal numbers

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Safety assessment of dry powder drugs by inhalation - current practice

- Rodents exposed to increasing doses of micronised drug for periods ranging from 7 to 180 days (commonly 0.5 – 2 hours exposure/day)
- At the end of the inhalation period animals are humanely killed and the organs are removed for evaluation to assess pathological response and define the no adverse effect level
- Histology is reviewed at the light microscopy level and the degree of pathological change is scored by an experienced pathologist (categorised as minimal, mild, moderate, severe)
- A NOAEL is established for the novel agent under investigation and a therapeutic index (TI) is established between the estimated clinical dose and the pathology (the TI must be at least 10 fold, or 100 fold for a non-monitorable finding)
- The data is submitted for review by regulatory authorities at the time of submission

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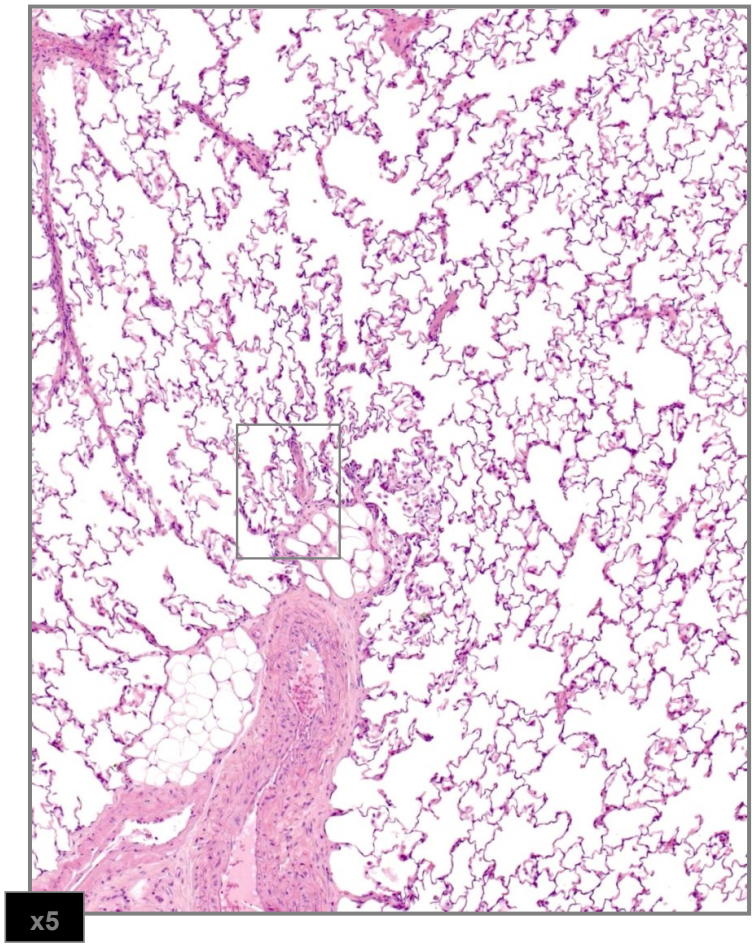
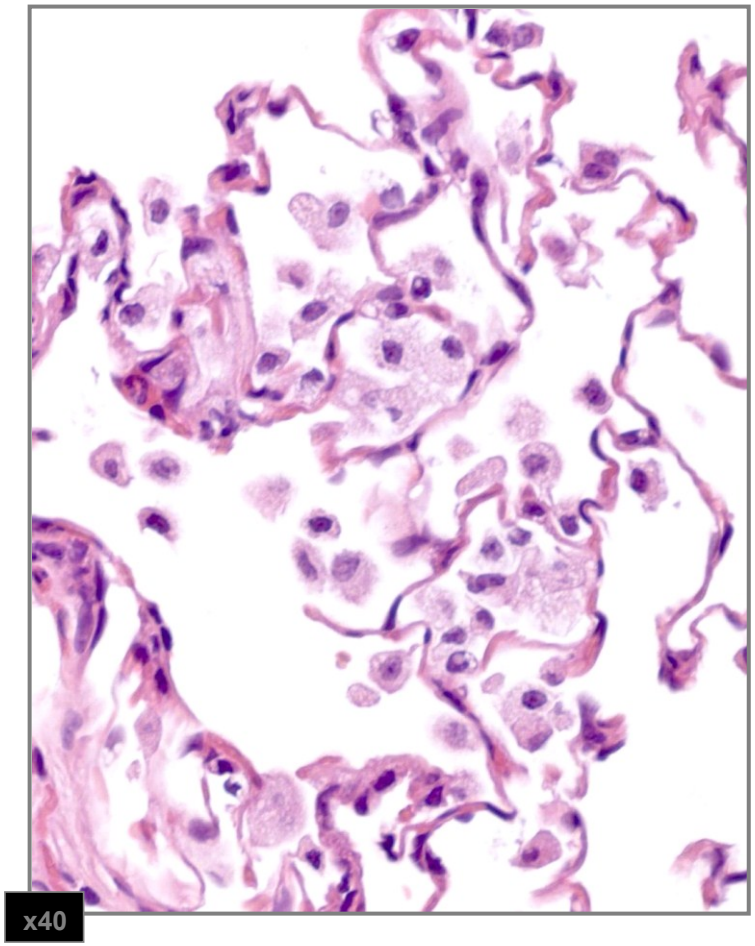
Alveolar macrophage changes (quantitative and / or qualititative)

- Increased macrophage numbers (normal morphology)
- Aggregation of macrophages
- Foamy appearance of macrophages
- Other cytoplasmic alterations (eg pigment/granules)
- Granulomatous (with / without foreign bodies)
- Increased macrophages in interstitium
- With / without macrophage degeneration
- With / without secondary inflammatory changes – acute / chronic
- With / without secondary epithelial changes – hypertrophy / hyperplasia / metaplasia
- With / without interstitial changes – fibrosis / oedema

Rat 4 Wk Tox Study





Control at 28d showing small accumulations of macrophages

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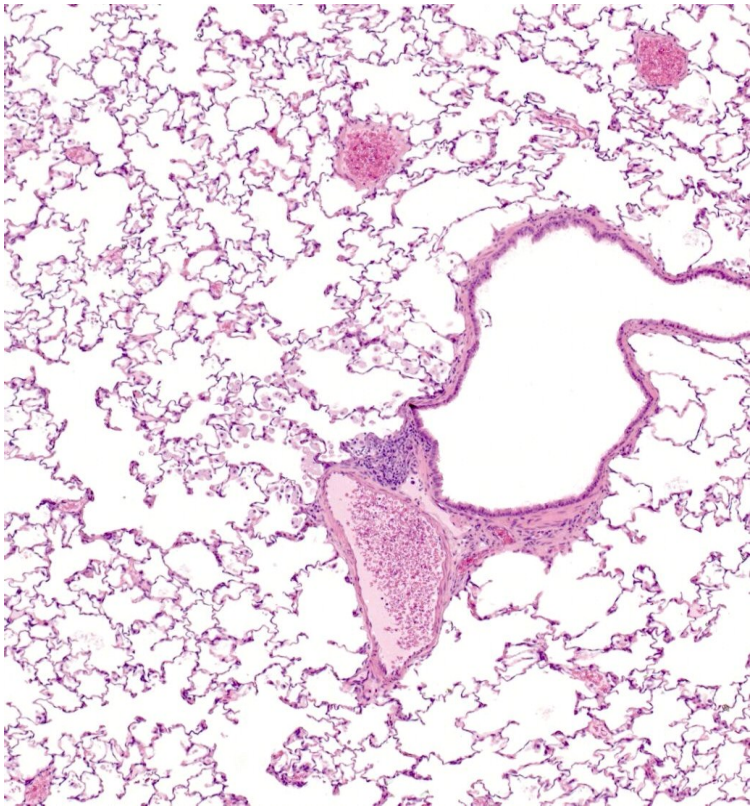
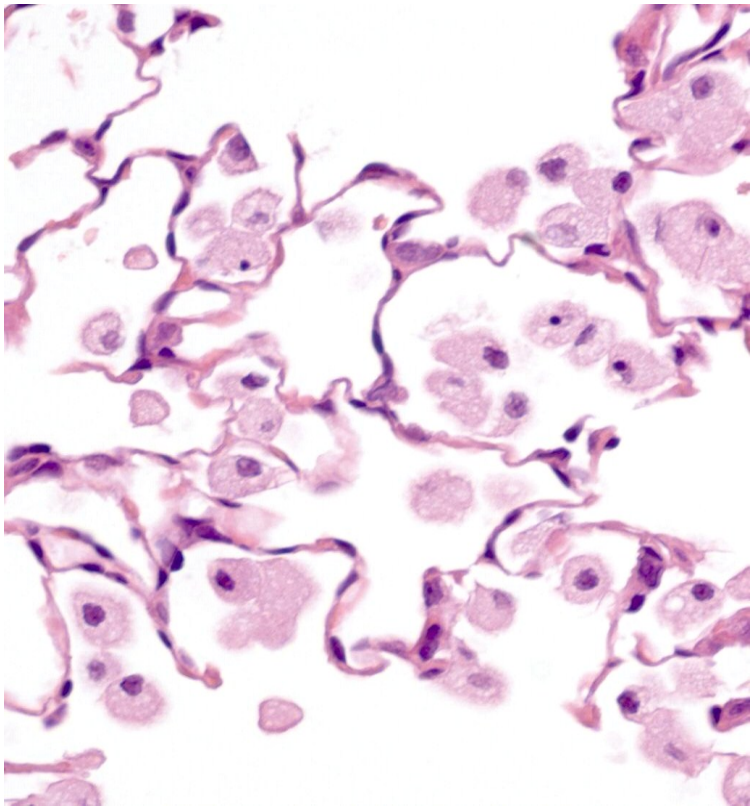
Rat 4 Wk Tox Study





High Dose at 28d showing small accumulations of macrophages is this test article related?

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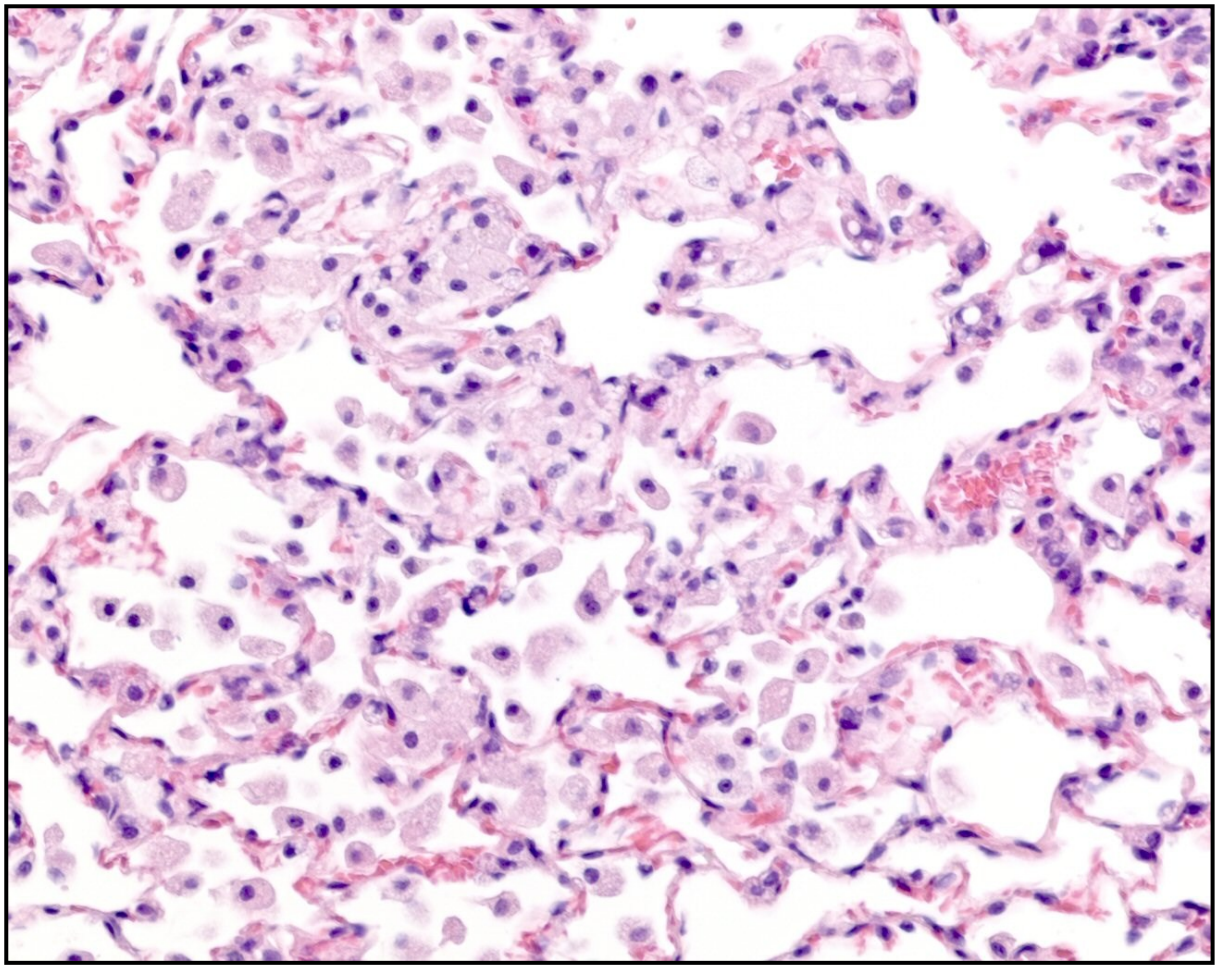
Rat 4 Wk Tox Study



28d showing larger accumulations of macrophages - is this adverse?

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Confounding factors in pathology interpretation

- Background / pre-existing / spontaneous pathology
- Inter-animal variability limits interpretation
- Single time-point examined to evaluate a dynamic process
- Small pathology sample to represent large volume of tissue
- Primary target / lesion not always clear after repeat dosing – many secondary changes / sequelae

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3Rs benefits

Successfully solving this challenge would have the following 3Rs benefits:

- Ability to evaluate toxicity and efficacy longitudinally in the same animal, potentially reducing animal use by up to 90% at certain stages of drug discovery and development.
- More informative and less variable data resulting in fewer animals being used (fewer repeat studies).
- Earlier go/no-go decisions can be made on drug candidates therefore reducing the number of regulatory toxicity studies to candidates that have the greatest chance of success
- Potential for similar 3Rs benefits to translate to other therapeutic areas where inhaled therapy could be used (e.g. diabetes, heart failure, oncology).
- Potential to be applied to environmental particulate and nanomaterial exposure studies as well

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What can be done?

- The response of the lung to challenge (be that inflammatory or dry powder) is a dynamic process, yet evaluation for decision making purposes tends to be an assessment at the end of the treatment phase.
- Can more sensitive ways of evaluating a macrophage response be developed which is specific and more quantitative, providing more informative data?
- Is it possible to monitor this response over a long period of time without the need for serially sampling and using a minimal number of animals?
- The data would need to be robust enough to support a regulatory filing

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Phase 1: The means to assess changes

- The development/application of novel in vitro, ex vivo and/or in vivo approaches to better understand FM biology. In vitro/ex vivo approaches must go beyond those already available and incorporate the latest advances in microfluidic and tissue engineering technologies. In vivo approaches must use the absolute minimum number of animals monitored over time. Applicants must demonstrate:
  - Identification of potential markers of FM presence and their functional state.
  - Development of novel biomarkers that demonstrate a change in the activation state of FM but which do not induce any cellular activation in the lung in their own right.
  - Demonstration that changes in functional state of FM are recapitulated in healthy lung macrophages and proof that the changes translate across species.
  - Use of the identified biomarkers to understand and characterise the occurrence of FM following particulate dry powder delivery in rodents.
- Demonstration of a relationship or collaboration with the expertise needed for Phase 2

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Phase 2: The means to monitor longitudinally

Essential

- System to simultaneously monitor disease progression/efficacy of compound, drug deposition and retention and FM presence longitudinally and non-invasively in the same animal without impacting on animal welfare (e.g. use of gel, requirement for shaving, etc.). Alternatively it could include biomarkers amenable to microsampling that clearly define an adverse response. The system needs to be independent of drug delivery devices.

Desirable

- Development of non-invasive approaches to quantitate increased influx of mononuclear cells into the rodent lung following administration of a pro-inflammatory stimulus (e.g. LPS) and ultimately the resolution of such a response.
  - ✓ Define the FM phenotype that indicates an adverse response.
  - ✓ Measure of inflammation that is transferable across a range of inflammatory stimuli.
  - ✓ Non-invasively define the rodent lung response to inhaled pharmaceutical agents that have known detrimental effects upon lung pathology compared to negative controls.
  - ✓ Improve understanding of:
    - ✓ The timeframe over which FM responses occur;
    - ✓ The influence of compounds with different pharmacology on FM biology (vs. particulates).

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In-kind contributions

Phase 1

- ✓ Expert guidance and advice on industry requirements

Phase 2*

- ✓ Access to inhalation delivery systems
- ✓ In vivo validation of novel biomarkers
- ✓ In vivo validation of any human relevant functional markers
- ✓ Provision of suitable inhaled particulate materials
- ✓ Assessment of novel technology/approach (e.g. specific contrast agents)
- ✓ Expertise in respiratory disease and relevant efficacy and toxicity readouts

* All animal studies will be ethically reviewed and carried out in accordance with national regulatory and legal requirements and company policies on the care, welfare and treatment of animals.

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Thank you

The sponsor s are happy to discuss the challenge and potential applications with people in the run up to the submission deadline

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