In vitro macrophage assays for selection of drug candidates for preclinical development.

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GSK
Role of macrophages in the lung

- First line of host defence to inhaled matter.
- Regulate immune responses and inflammation.
- Phagocytose and destroy invading microorganisms.

- Uptake of environmental particulate material.
  - Clearance.
  - Inflammatory response
    - E.g. induction of TNF.
  - Toxic response.
  - Particle overload and loss of function.

Macrophage uptake of titanium dioxide
Alveolar macrophages

- Unique alveolar macrophage phenotype in the lung microenvironment.
- Alveolar macrophages show remarkable plasticity.
- Responses can be diverse.
- Key lung findings *in vivo* include inflammatory infiltration and macrophage "foamyness".
What data do we need to demonstrate whether a macrophage response to inhaled drugs is adverse or non-adverse?

A photomicrograph of a bronchoalveolar-lavage specimen shows an alveolar macrophage with a segmented nucleus (Giemsa stain, ×400).

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Reduce Attrition of Inhaled Drugs

- We are withdrawing molecules in development due to unforeseen inflammatory responses of a variable nature.
  - macrophages appear to play a major role.
Aims

- Define the likely biological responses *in vitro* to drug substance selected for the inhaled route.
- Increase scientific knowledge around lung macrophage responses.
- Introduce early assessments of potential hazard prior to candidate selection.
- Enhance toxicology studies to maximise output.
- Seek to develop inhaled drugs with a greater chance of success.
Objectives

- To implement a set of *in vitro* assays to assess the macrophage response to drug.
- Evaluate compounds pre candidate selection.
- Recommendations on compounds with improved probability of success.
- Advise on *in vivo* studies
  - End points
  - Observations.
- Combine *in vitro* and *in vivo* data to identify key decision making parameters.
In vitro advantages

- Control throughput, screen and rank compounds.
- Expensive time consuming *in vivo* studies.

Many cellular models available.

- Cell lines.
  - Nasal, tracheal, bronchial, alveolar epithelium, alveolar macrophages.
- Primary cells.
  - Tracheal, bronchial, alveolar epithelium.
- Co-culture.
  - Epithelium + macrophages + dendritic cells.
- 3D culture.
  - Polarised, air-liquid interface, secreting mucous.
- Lung on a chip...
Lung on a chip (Wyss Institute, Harvard)

- 3D culture mounted on a flexible porous membrane.
- Air-liquid interface.
- Mechanical ventilation.
- Mimic the steady state lung & reproduce stress and strain.
- Transparent device allows real time fluorescence microscopy measurements.

In vitro macrophage screen

- Assays to assess
  - Cell health
  - Cell function
  - Cell activation
  - Cell morphology
Cell health

- Viability test
  - Trypan blue
  - Neutral red

- Plate based toxicity assays.
  - LDH release
  - Cell titer glo (ATP)
  - MTT

- Flow cytometry live/dead discrimination.
  - Nuclear dye of membrane compromised cells
    - DAPI, DRAQ7, PI, 7-AAD etc
Macrophage Function

- **Phagocytosis.**
  - Functional capacity in terms of phagocytic ability may be compromised following drug treatment.
  - Flow cytometry to quantitate phagocytosis of apoptotic neutrophils by macrophages.
Phagocytic ability reflects viability

- GSK5 was terminated in development due to the generation of foamy alveolar macrophages in rodents and lung inflammatory infiltrates in dogs.
- GSK6 successfully progressed into an inhaled medicine.
Oxidative Stress

- Reactive oxygen species (ROS) production.
- Glutathione (GSH:GSSG).
- Lipid peroxidation.
- Heme oxygenase-1 (HO-1) expression.

Macrophage Activation

  - ELISA, MSD etc

- It is a challenge to determine which cytokines/chemokines are important and how to interpret the response.

  - Which cytokines & chemokines are chosen?
  - What does the cytokine/chemokine profile mean?
  - Does the profile relate \textit{in vivo}?
  - Is the profile driving pathology?
Macrophage Activation

- Phenotypic changes of macrophages.
  - Activation marker expression; MHCII, CD40 etc
  - M1/M2 polarisation

Is macrophage phenotype important and would this aid the understanding of adverse/non adverse responses to inhaled drugs?
Macrophage Morphology

- Brightfield / phase contrast microscopy.
  - Initial indication of cell health and particle uptake.
  - “Foamy” cells.
Basic microscopy

“Foamy” cells

Toxicity
Macrophage Morphology

- Brightfield / phase contrast microscopy.
  - Initial indication of cell health and particle uptake.
  - “Foamy” cells.

- Confocal microscopy.
  - Particle uptake.
  - Physical changes.
Confocal microscopy

More detail of “foamyness”.

UV fluorescence associated with cells indicating compound presence.
Macrophage Morphology

- Brightfield / phase contrast microscopy.
  - Initial indication of cell health and particle uptake.
  - “Foamy” cells.

- Confocal microscopy.
  - Particle uptake.
  - Physical changes.

- Cellular granularity by flow cytometry.
Compare granularity as % of control (untreated cells) for compounds.
Alveolar macrophage *in vitro* risk assessment strategy

- Chemistry candidates

  - *In vitro* Macrophage assessment
    - Toxicity.
    - Inflammatory.
    - Physical changes.
    - Compound specific.
    - Nature of target.

  - *In vivo* Candidate selection
    - More informative selection of compound to take forward.
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