BACKGROUND
Foamy macrophage response are often observed in histological lung slices of rats from pre-clinical studies in vivo, which are typically characterised by a high vacuolated appearance and larger cell size. The mechanism of induction of the foamy alveolar macrophage phenotype is not well known and it has not been explained if these observations are truly an adverse response or not.

AIMS
- Develop in vitro cell culture assay to better characterise alveolar macrophage responses
- Characterise interspecies differences between human and rat macrophage models in vitro

METHODOLOGY

1. CELL CULTURE
Rat macrophages (NR8383) and human monocyte cells (U937) were seeded into 96-well plates at a density of 3 x 10⁴ cells/well. U937 cells were differentiated to a macrophage phenotype using 4nM PMA in complete culture media for 96h followed by a 24h rest period in complete culture media.

2. INDUCTION AND CHARACTERISATION OF MACROPHAGE RESPONSES
Cells were incubated with amiodarone (0.003 – 100 μM) or staurosporine (0.0003 – 10 μM) in complete cell culture medium.

FOR CELL HEALTH AND MORPHOLOGY ASSESSMENT, cells were stained with Hoechst 33342 (10 µg/ml), MitoTracker Red (300 nM), Image-It Dead Green (25 nM) for 30 min followed by fixation with 3.7 %w/v paraformaldehyde for 20 min. Fixed cells were then stained overnight with Cell Mask Deep Red (diluted 1:1000).

FOR THE DETERMINATION OF LIPID CONTENT, cells were incubated with HCS LipidTox Phospholipid Red (diluted 1:1000) for 24 h and fixed with 3.7 %w/v paraformaldehyde containing Hoechst 33342 (10 µg/ml) for 20 min. Cells were then incubated with HCS LipidTox Green (diluted 1:1000) for 30 min for detection of neutral lipids.

Cells from both assays were imaged using the In Cell Analyser 6000 (GE Healthcare, Little Chalfont, Bucks, UK) with a 40x objective.

RESULTS

1. BASELINE PROFILE OF UNTREATED MACROPHAGES IN VITRO

CONCLUSION
- Similar baseline profile of untreated lung macrophages in vitro between rat and human models.
- Observed differentiated in macrophage responses to drug challenges:
  A typical profile for phospholipidosis (increased phospholipids content) was observed for both cell lines after amiodarone treatment.
  A profile for apoptosis was observed (reduced viability, elevated cell permeability, increased cell area) was observed for both cell lines after staurosporine treatment.
- Characterising macrophages in this way may provide a better understanding of the pathophysiology of airway immune responses to inhaled medicines.

REFERENCES