CROSS-INDUSTRY ORGANIZATIONS

Research activities following a Drugs in the Lungs Network workshop on induced alveolar macrophage responses

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On behalf of the Drugs in the Lungs Network

This report provides an update on research activities succeeding a workshop by the Academy of Pharmaceutical Scientists of Great Britain (APSGB) Drugs in the Lungs Network on "Induced alveolar macrophage responses."

Workshop on induced alveolar macrophage responses

Motivated by the problem that a proportion of drug development programs fail to progress to the clinic on the basis of "difficult-to-interpret" pre-clinical findings associated with induced macrophage responses, a two-day workshop on this topic was co-organized by the APSGB with the Health and Environmental Science Institute in 2012.

A record of the workshop, including the presentations, a summary of the breakout sessions and panel discussion, is available for free at the Drugs in the Lungs website: http://www.apsgb.org/drugsinthelungs. The outcomes were also published as a review article (Forbes B, et al., Challenges for inhaled drug discovery and development: Induced alveolar macrophage responses, Advanced Drug Delivery Reviews 71: 15-33, 2014).

Research needs

Consensus at the workshop indicated a clear imperative to improve understanding of alveolar macrophage responses to inhaled materials, thereby providing better evidence upon which to evaluate the risk of taking inhaled investigational products into human trials when primary adaptive lung responses (i.e., non-adverse, reversible responses) are observed pre-clinically. Recommendations were made for (i) better industry/academia/regulatory cooperation, (ii) sharing of existing pre-competitive data, and (iii) priority topics for collaborative research to address gaps in knowledge. Research priorities included recommendations to:

Improve scientific understanding by:

- Macrophage phenotyping—defining responses as adaptive or adverse, through morphometric, biochemical and functional markers;
- Mechanisms of response—understanding the underpinning cellular mechanisms that drive macrophage responses and may indicate an adverse response;
- Longitudinal studies—to address the question of whether macrophage responses resolve, stabilize or progress over time.

Improve candidate selection and lead optimization by:

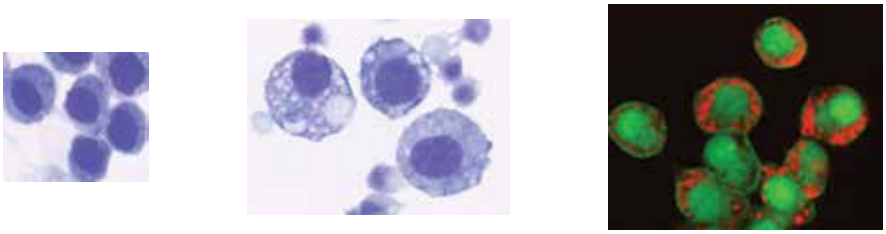
- Discriminatory assays—discriminatory screens that provide an early assessment of whether compounds will induce macrophage responses;
- Predictive science—developing algorithms for predicting adversity/safety based on dose, material and macrophage responses.

NC3R CRACK-IT Challenge

The National Centre for the Replacement, Refinement and Reduction of Animal Research (NC3R) picked up these issues as a CRACK-IT challenge in 2014. Four 6-month pilot projects (phase 1) led to the formation of an academic/industry consortium to deliver a 3-year program, "Differentiating macrophage responses to inhaled medicines" (phase 2) to address the challenge described on the NC3R's CRACK-IT website: https://crackit.org.uk/challenge-14-inhalation-translation. The core consortium included King's College London, the National Physical Laboratory, the University of Hertfordshire, GlaxoSmithKline, Envigo and Pfizer, with additional contributions from GE Healthcare, the University of Cranfield, the Open University and Stevenage BioScience Catalyst. Progress on the project is described below.

Predictive in vitro model

In vitro assays are being developed to predict whether a compound will induce macrophage responses, using high content analysis to evaluate macrophage morphology, cell health and lipid content (Hoffman E, et al., An *in vitro* multi-parameter assay development strategy towards differentiating macrophage responses to inhaled medicines, Molecular Pharmaceutics 12: 2675-2687, 2015; and Hoffman E, et al., Morphometric comparisons of primary rat alveolar macrophages with rat and human cell lines, Pharmaceutical Research 34: 2466-2476, 2017). Using advanced bioinformatics available through the National Physical Laboratory, algorithms are being developed with the aim of predicting the likelihood of induced macrophage responses to drug compounds based on molecular properties and *in vitro* responses.



Light microscopy images of healthy macrophages (left) and vacuolated "foamy" macrophages (middle), and macrophages in the high content assay, showing phospholipid in red and neutral lipid in green, associated with the vacuoles (right).

Understanding macrophage responses
Complimentary approaches to understanding the responses of macrophages include transcriptomic analysis of cells under the conditions in the predictive *in vitro* assay and mass spectrometry imaging to identify drugs, metabolites and lipid changes in lung cells and tissues. Additionally, to monitor responses longitudinally in pre-clinical studies and provide the prospect of non-invasive clinical monitoring, methods for incorporating exhaled breath analysis into inhalation toxicology studies are being investigated.

Reduction of animals in research
Regular updates have been presented at Association of Inhalation Toxicologists annual conferences in 2015 to 2017 and are being shared with the MHRA and FDA. The aim of the project is to help translate safe inhaled therapies to the clinic for the benefit of patients and to reduce the use of animals in inhalation toxicology studies by providing:

- Earlier go/no-go decisions on drug candidates, therefore requiring fewer regulatory toxicity studies in candidates that may fail later in development;
- More reliable and less variable data, resulting in fewer animals being used; and
- The 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.

Additional information, including detailed experimental methodologies, are available through the project website linked to the CRACK-IT Challenge (above).

Drugs in the Lungs Network: Future meetings

The Drugs in the Lungs Network is happy to receive any suggestions, collaborate in the organization of symposia or facilitate initiatives for open innovation in any area of inhalation biopharmaceutics. Please contact Ben Forbes for further information.

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