Challenge 14: Inhalation Translation

Q. What is the incidence of macrophages in control animals?

A. The incidence is quite low and it is difficult to show whether their appearance is adverse or not. If macrophages are present, regulators will require reassurance, – e.g. how do you know their occurrence won't progress to something worse and isn't benign? This is one of the biggest hurdles to overcome in this Challenge.

Q. We need better understanding of pathophysiology of disease processes – is the Challenge aimed at this too, because animals have limited physiological relevance to asthma, COPD etc.? Is there a place for biomimetic human models as part of this Challenge?

A. We have a variety of approaches to understanding disease and we are already using patients to understand what is happening phenotypically. The problem is processing drugs from discovery into the clinic because of the small subtle changes observed in the lung. It is difficult to reassure regulators that these are benign. Animal models do not entirely replicate human disease, but they are still used to determine whether physiological changes are taking place (e.g. to establish PK/PD relationships early).

Q. Does the call include human tissue based approaches?

A. Yes, if we can be confident that molecules have an impact on physiological process *in vitro*, then that itself would enable better selection of which drug candidate to take forward. Industry already uses primary cells and commercially available cell lines. To date, we cannot rely on these for predictivity.

Q. Is there any quantitative measurement of histological changes at present?

A. Some, yes, but a wider approach would be useful.

Q. You have a lot of knowledge of the rat model, but have you looked at how your biomarkers benchmark against other *in vitro*/*in vivo* systems?

A. Yes, we have looked to see how macrophages are changing and in what way, but often there is just one person doing this in the organisation. There is a need to broaden this and know which biomarkers to use. A fundamental understanding of the response is needed and how to choose an appropriate biomarker.

Q. You need some means of quantification of lung slices, and of gathering of longitudinal data. Can you get analysis of macrophages from taking a swab?

A. We normally terminate the animal to remove the cells and assess them. There is too much inherent variability even in this process, to try an alternative. Variability in sampling procedures is about 30%. If we could tag the macrophages so they're illuminated that

would be extremely valuable to assist with quantification. However, imaging agents don't last long, and can be expensive and time consuming.

Q. Do you take biopsies in humans; could that be done?

A. Biopsies in humans can only go down to bronchial branch six, so we are limited to some degree by remaining in the upper airway. This hasn't developed well in the clinic as it is not pleasant for patients.

Q. What about downstream effects? If it is adverse, would you know what the downstream effects look like?

A. Downstream effects include cytokine release; a trained pathologist would be able to identify an adverse effect. The problem is that the changes we see are often subtle.

Q. Do you have a human or computational algorithm to interpret the data?

A. There is no computational algorithm, just a candidate selection dossier pulling together all that is known about the compound and its effects. We look at histology, the number of macrophages and the nature of them, and expert pathologists interpret the data in order to determine a safe dose. However, if this dose is low, it may not be efficacious in the clinic.

Q. Is the histology quantitative, or is it only qualitative?

A. There are basic quantifications that categorise the macrophage infiltration as severe, moderate, slight or minimal. It is important to relate these to the consequences. Routine quantification and characterisation of each and every macrophage is not carried out. The area samples are very small (approximately 4μm) with about four slices of the lung at each time point, therefore the sampling error associated with this could be substantial.

Q. Is the human literature (and controls) a useful data set?

A. There is minimal publically available information to use in this area.

Q. If you stop dosing, do the changes reverse?

A. Sometimes, but we don't know if they're the same macrophages, or even if it's the same lung region. What is seen, may not be drug effects, just particulate effects. A fundamental issue is how to discriminate adaptive versus adverse effects.

Q. Any healthy individual is full of macrophages. Have you tried to align pathological changes in the animal model with any other biomarkers?

A. No. The fundamental problem is that we don't understand the biology well enough, so this is difficult to align and to make objective decisions.

Q. Have you tried to use whole body imaging systems?

A. We do this for efficacy studies, but we want to probe and understand consequences of insults at a cellular level for toxicology studies.

Q. Could you apply optical approaches to your particular question, e.g. by using imaging modalities in positive controls, can you detect effects?

A. Potentially, but we don't currently have the expertise.

Q. Are co-culture models with macrophages a possible method?

A. Yes. However, we would need to develop a body of data to allow confidence in using such an option to convince regulators.

Q. Some imaging modalities use mice. If there was a proposal to use mice rather than rats, would that be acceptable?

A. Potentially, if the applicant could demonstrate the safe monitoring of macrophages in a meaningful way.

Q. Are you looking for damage to lung tissue, or that macrophages are grouped where the compound is absorbed into the lung?

A. We don't know if the macrophages we see are drug related as the fundamental biology is not well known.

Q. What about breath analysis?

A. We have done some work in this area but it is complex in rodents and we have no evidence this has been used in a GLP safety environment. In a clinical context, there is monitoring of nitrous oxide. This area has developed and it might be possible to identify a marker in breath.

Q. **What do you mean by the response should be independent?**

A. It should not matter how the material is delivered into the lung as the response is biological and not due to the device.

Q. Phase 1 is over a six month period; do you want to see proof-of-concept in this timescale?

A. We would like to see the formation of good ideas and clear steps towards future progress.

For further information and to be put in contact with the sponsors, please email

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