Increased focus on non-animal models for COVID-19 and non-COVID lung research

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To the Editor:

Horizon Europe is a major stakeholder in the immediate future of lung research. As such, we believe it is worthwhile drawing the attention of the *European Respiratory Journal* readership to the recent publication by Hynes *et al.* [1] on *Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases*. This report from the European Commission's Joint Research Centre EU Reference Laboratory (EURL ECVAM), and its accompanying dataset [2], identifies well-established current and emerging *in vitro*, *in silico* and *ex vivo* non-animal models for respiratory disease modelling, and reinforces the concept of "reduce, refine, or replace" animals used for scientific purposes. The 3R approach is not only embraced by the European Commission, but also the respiratory research community, as evidenced by many European Respiratory Society publications as well as frequent ERS Congress sessions and research seminars on the topic.

The EURL ECVAM study, prepared with the partnership of external experts in the field, involved a systematic review of peer-reviewed scientific literature. Over 21 000 abstracts (11 636 non-cancer and 9421 cancer) were scanned for relevant non-animal models of respiratory disease culminating in a knowledge base containing detailed descriptions of 284 non-animal models currently or recently being used for respiratory disease research. Briefly, the review shows that while simple models are still prominent and have their uses, in the past 5 years research focus has been shifting towards increasingly sophisticated bioengineering approaches that recapitulate human lung development, anatomy and physiology *in vitro*. Such novel approaches hold promise as blueprints for modelling human-relevant diseases and can be used not only to elucidate mechanism of disease, but also to aid in the development of new therapies and responses thereto. The findings from the study are freely available to download from the EURL ECVAM Collection in the JRC Data Catalogue, together with a JRC Technical Report that describes the review methodology and presents the main findings [1, 2]. Ideally, this knowledge base will serve as a means to explore the strengths and limitations of both animal and non-animal models used in biomedical research; to stimulate healthy scientific debate; to challenge mindsets; and ultimately to pave the way for doing better science. Key conclusions of the work are summarised in table 1. Its overarching aim is to bridge methods and disciplines in order to improve biomedical science for the benefit of patients and society.

Respiratory diseases, including asthma, COPD, cystic fibrosis, pulmonary fibrosis and lung cancer represent a significant health burden to society. However, the majority of preclinical studies of new therapeutics for chronic lung diseases fail to identify effective treatments that are ultimately translated to the clinic, with over 90% of new drug programmes not progressing to market due to lack of efficacy or unexplained toxicity. This failure may be due in part to the use of preclinical models that do not fully recapitulate the complexity of human disease, are inherently hindered by interspecies differences, employ cells which are not specifically affected by the target disease in patients, or do not represent the genetic background of affected patients. Emerging non-animal preclinical models, such as lung-on-a-chip models, three-dimensional spheroid and organoid systems derived from primary and stem cell origins, and human tissue cultures such as precision cut lung slices, offer the opportunity to simulate human disease *in vitro* and *ex vivo* in a more robust and patient-specific manner [3–5]. Thereby, these novel investigative platforms provide important mechanistic information about respiratory diseases and identify targets for novel treatments.

TABLE 1 Key summary points related to uptake, development and standardisation of non-animal models for respiratory disease research

Standard operating procedures for model generation and testing are essential
The specific purpose of any model must be clearly defined
Realistic lung-on-a-chip models will become more widespread, especially for biomarker studies
Plastic substrates in any model are not representative of human tissue and should be replaced
To improve organoid models more effective cross-talk between oncology and respiratory fields is needed
Lessons learned from lung cancer organoid studies could be equally valid for the wider respiratory disease field
Pioneering functional organoid studies in cystic fibrosis have relevance for other inflammatory and infectious lung diseases
Precision cut lung slice (PCLS) is the most representative human model in terms of cell types, tissue architecture and physiological response, notwithstanding difficulties in obtaining sample material

Adapted from [1].

The current coronavirus disease 2019 (COVID-19) crisis also highlights the need for better patient- and disease-relevant lung models. In the relatively short period since the identification of SARS-CoV-2 as the causative virus of COVID-19, important progress has been made in advancing our knowledge about the disease using *in vitro* culture methods and *in silico* approaches. A variety of approaches have been used for a range of purposes, including the study of epithelial cell tropism of the virus [6], effectivity of antiviral drugs [7], and application of organoids [8] and lung-on-chip models for COVID-19 modelling [9]. In addition to using primary lung epithelial cells isolated from lung tissue, human induced pluripotent stem cell-derived alveolar type 2 cells have been used to obtain insight into the transcriptional response of these cells to infection, and their potential use for drug testing [10]. Whereas these examples used epithelial culture models, other studies have used cells from other organs to delineate their involvement in COVID-19. Careful matching of the research question to the culture model used and an awareness of the limitations intrinsic to any model remains essential.

In conclusion, this report from the European Commission highlights research strategies for the development of safe and efficacious new therapies for well-known and emerging respiratory diseases especially those that exploit non-animal methods and recapitulate the mechanistic basis of human disease. Such approaches are widely welcomed and in due course are likely to outnumber and outperform animal studies.

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