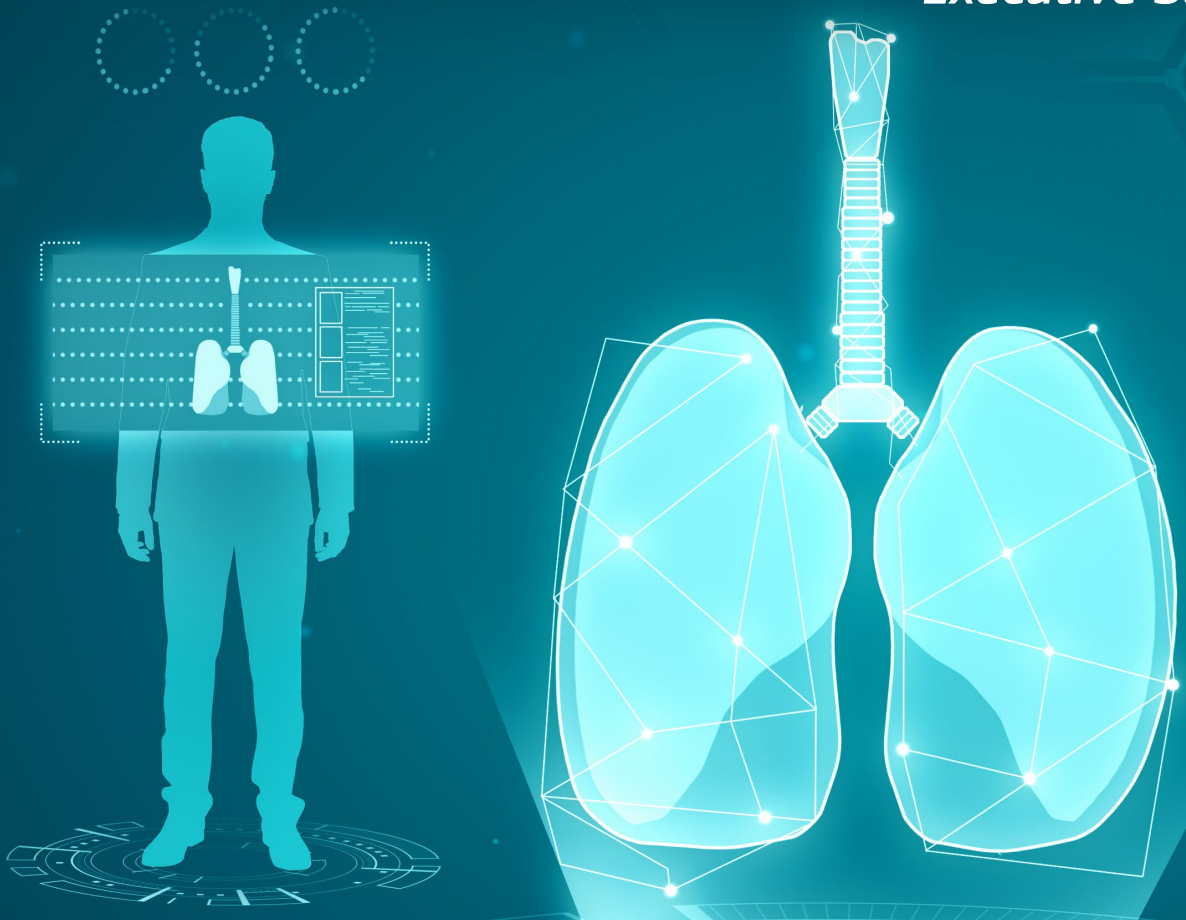
European Commission

Advanced Non-animal Models in Biomedical Research

Respiratory Tract Diseases

Executive Summary



Joint Research Centre

EUR 30334 EN

This Executive Summary describes a study conducted by the JRC's EU Reference Laboratory for alternatives to animal testing (*EURL ECVAM*) to identify current and emerging non-animal models and methods being used for biomedical research related to respiratory diseases.

The resulting collection of non-animal models are analysed in a JRC Technical report (Hynes, J. *et al.*, *Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases*, EUR 30334 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-21380-2, doi:*10.2760/725821*, JRC118161) and publicly available from the *JRC Data Catalogue*.

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EXECUTIVE SUMMARY

Respiratory diseases including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, pulmonary fibrosis and lung cancer represent a significant health burden to society. Chronic respiratory diseases are among the most common of all diseases and causes of death globally. Over 235 million people suffer from asthma and around 65 million live with moderate to severe COPD[1].

Failure of the current paradigm for drug development

Over 90% of new drug programmes fail to progress to market due largely to a lack of efficacy or unexplained toxicity[2]. This is particularly evident for drugs targeting respiratory disease where the rate of successful translation (29%) from small to larger scale clinical trials[3] is lower than the average for other diseases (29%)[4]. Although there are several factors underpinning the failure of biomedical research to develop safe and effective treatments, the use of animals in basic and applied research to model human biology and disease is coming under increasing scrutiny.

Whilst animal models may recapitulate some of the features of a disease, the lack of effective new therapies for serious respiratory conditions like asthma indicate that reliance on animal models is failing to identify pathways to novel treatments. In addition, significant differences in lung size, structure and physiology limit the degree to which airway dynamics measured in animals, particularly in small rodents, can be compared to humans.

Research strategies for the development of safe and efficacious therapies for respiratory diseases are therefore beginning to exploit non-animal methods that recapitulate the mechanistic basis of human disease rather than continuing to use animal models which poorly reflect the human clinical situation.

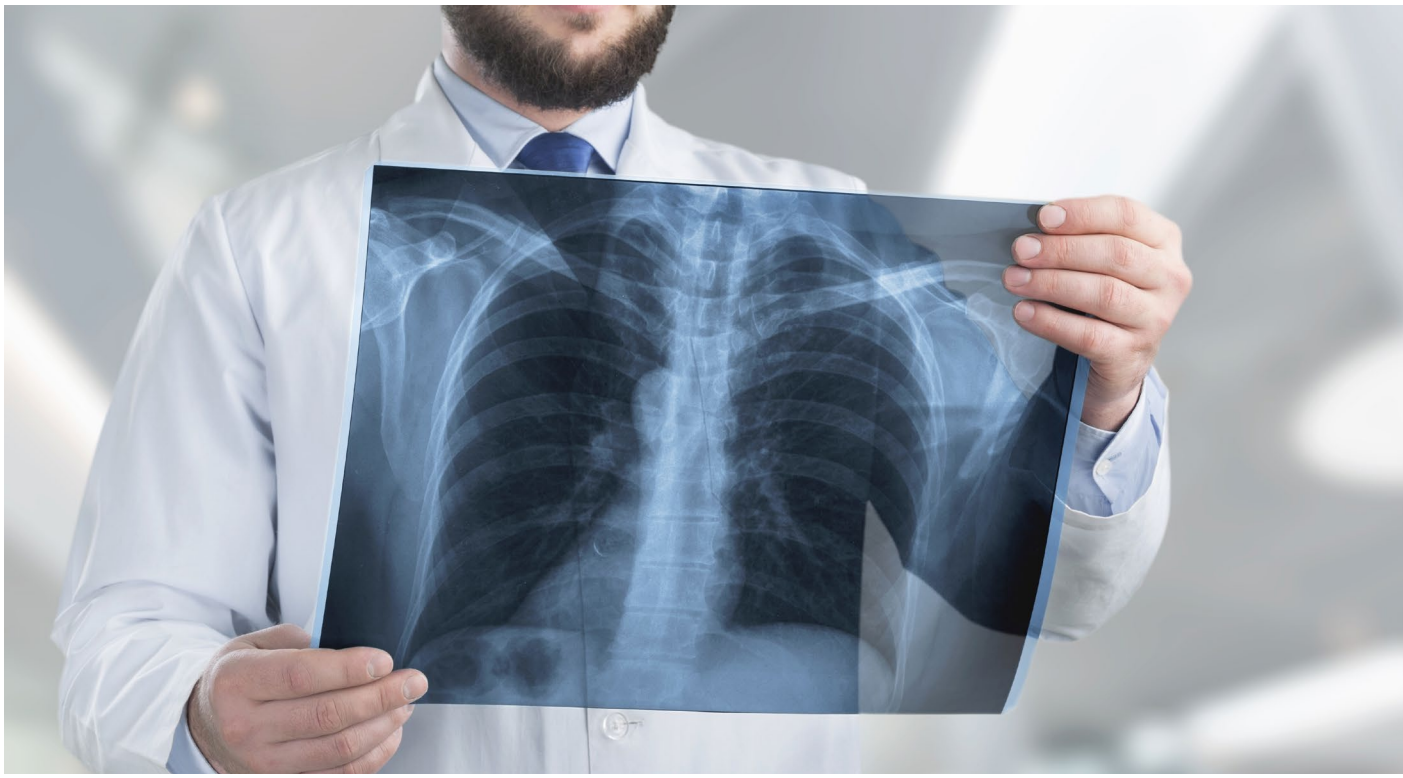
Within the biomedical research domain, there is currently little international standardisation, with most research bodies and laboratories establishing their own methods for conducting novel research across a wide array of disciplines. Therefore the JRC's EU Reference Laboratory for alternatives to animal testing

1 Forum of Respiratory Societies (2013), Respiratory Diseases in the World, Sheffield UK

2 Thomas, DW, Burns, J, Audette, J, Carroll, A, Dow-Hygelund, C, Hay, M. *Clinical development success rates 2006–2015*, San Diego: Biomedtracker/Washington, DC: BIO/Bend: Amplion, 2016. Available at: *https://journals.sagepub.com/doi/full/10.1177/0141076818812783*

3 Phase II to Phase III

4 Waring, M. J., Arrowsmith, J., Leach, A. R., Leeson, P. D., Mandrell, S., Owen, R. M., Pairaudeau, G., Pennie, W. D., Pickett, S. D., Wang, J., Wallace, O. and Weir, A. (2015), An analysis of the attrition of drug candidates from four major pharmaceutical companies, *Nat Rev Drug Discov*, 14(7), pp. 475-86, doi:*10.1038/nrd4609*

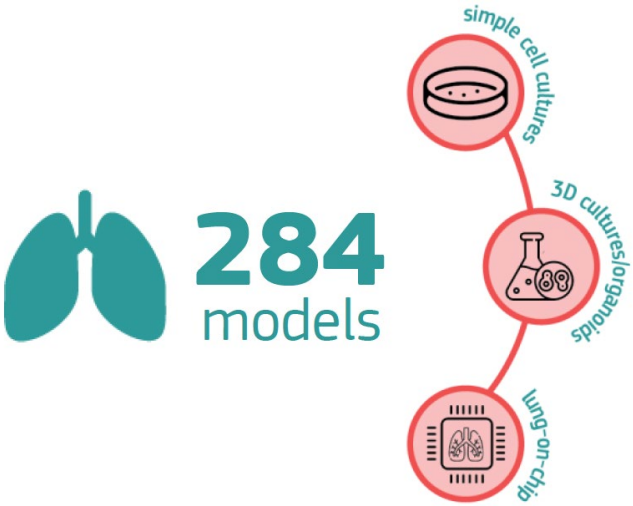


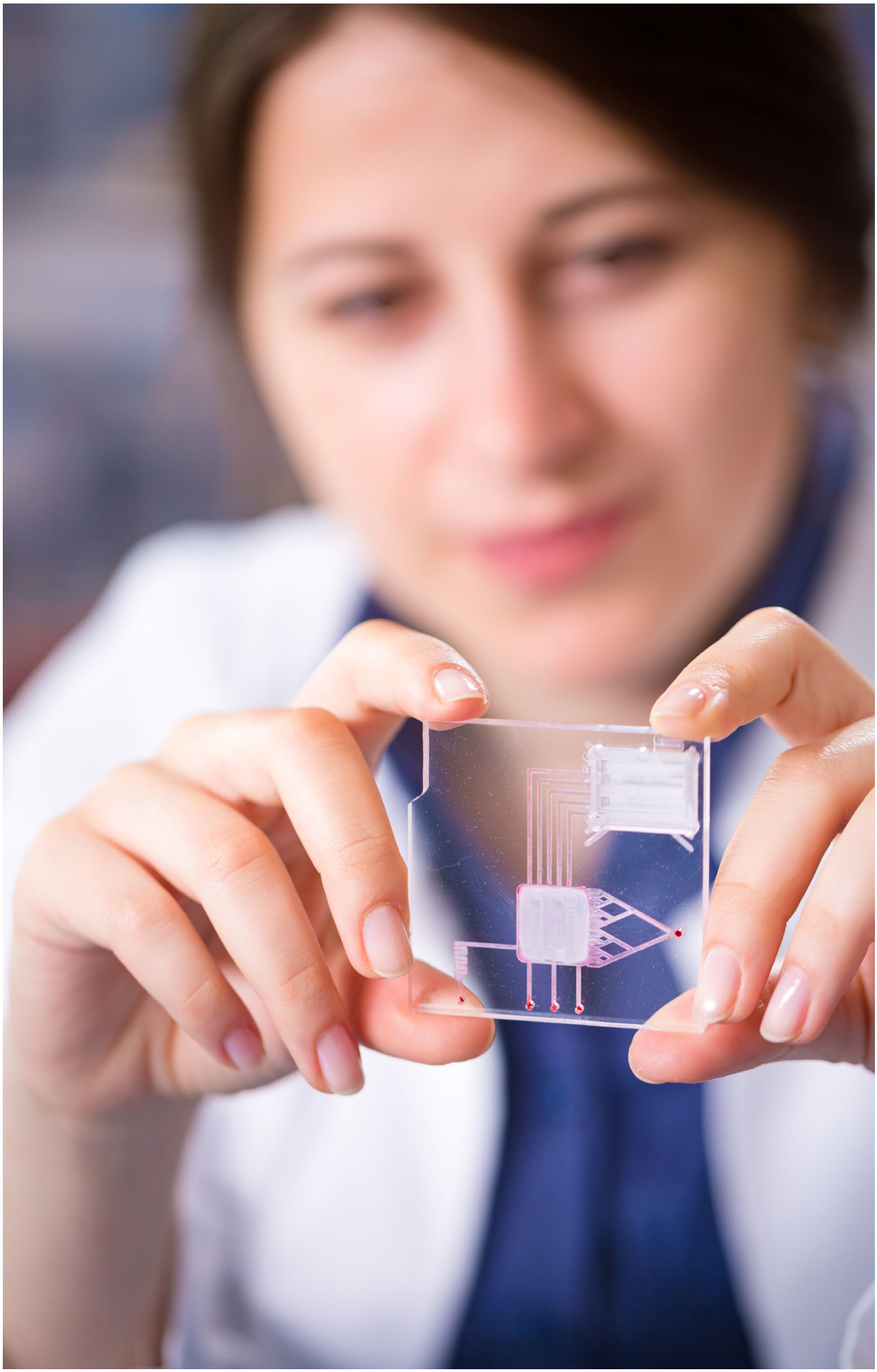
(EURL ECVAM) launched a study to identify current and emerging non-animal models and methods being used in the biomedical realm, including for respiratory disease research. The review describes both well-established non-animal approaches to respiratory disease modelling and many under development, the majority of which are based on techniques that use cells and tissues cultured in the laboratory (*in vitro* methods), computer modelling and simulation (*in silico* methods) or cells and tissues explanted from an organism (*ex vivo* methods).

Models and methods

The EURL ECVAM study was based on a comprehensive review of peer-reviewed scientific literature. Over 21,000 abstracts (11,636 non-cancer and 9,421 cancer) were scanned for relevant non-animal models of respiratory disease. From this, a total of 284 publications were identified as being promising candidate methods according to defined criteria.

The review found that historically, the predominant non-animal models used for respiratory disease research have been based on simple *in vitro* cell cultures. Such models are still prominent and have their uses, particularly because they are inexpensive and easy to work with. However in the past five years or so, research focus has been shifting towards increasingly sophisticated bioengineering approaches that recapitulate lung development, form and physiologic functions *in vitro*. Many of these are based on 3D human tissue cultures, spheroids, organoids, and microfluidic or 'lung-on-chip' systems (see Box 1).





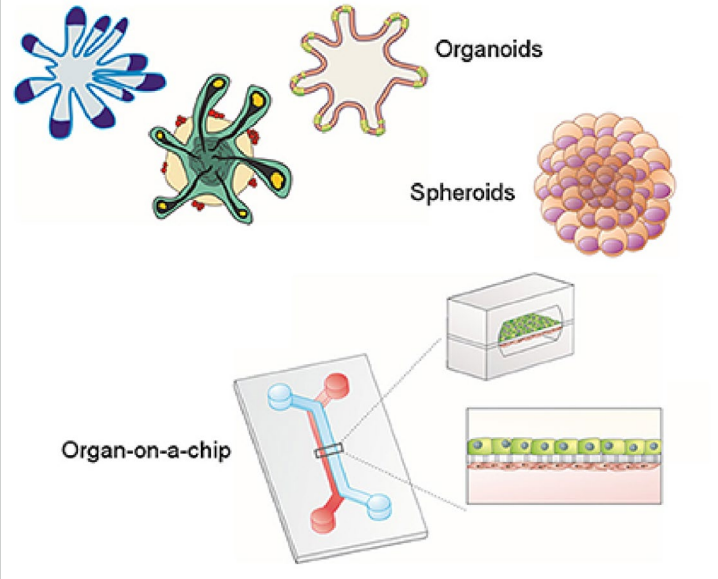
Box 1. **Advanced *in vitro* models**

3D human tissue cultures: environment in which cells grow and interact with their surroundings in all three dimensions.

Spheroids: simple 3D models generated by spontaneous cell aggregation that do not require any scaffolding system.

Organoids: miniaturised 3D representations of an organ that reflect histological and functional aspects of in vivo tissue.

Microfluidic or lung-on-chip systems: micro-fabricated platforms engineered to recapitulate the physiology of human organs by capturing critical aspects of living organs such as cell-tissue interface and biochemical and physical stimuli, such as flow and pressure.



From ©Torra, N., García-Díaz, M., Fernández-Majada, V. and Martínez, E. Mimicking Epithelial Tissues in Three-Dimensional Cell Culture Models (2018), 6, p. 197, doi:10.3389/fbioe.2018.00197 under CC BY 4.0.

It was found that models and methods were generally inseparable from one another. The vast majority of publications describe both a (disease) model and a specific method for utilising that model.

For example, in cystic fibrosis research, much importance is attached to the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). Therefore, cystic fibrosis models are usually designed specifically to investigate the mechanisms behind CFTR dysfunction (see Box 2). Such models have limited application in other disease areas such as asthma or pulmonary fibrosis where very different disease mechanisms are at play.

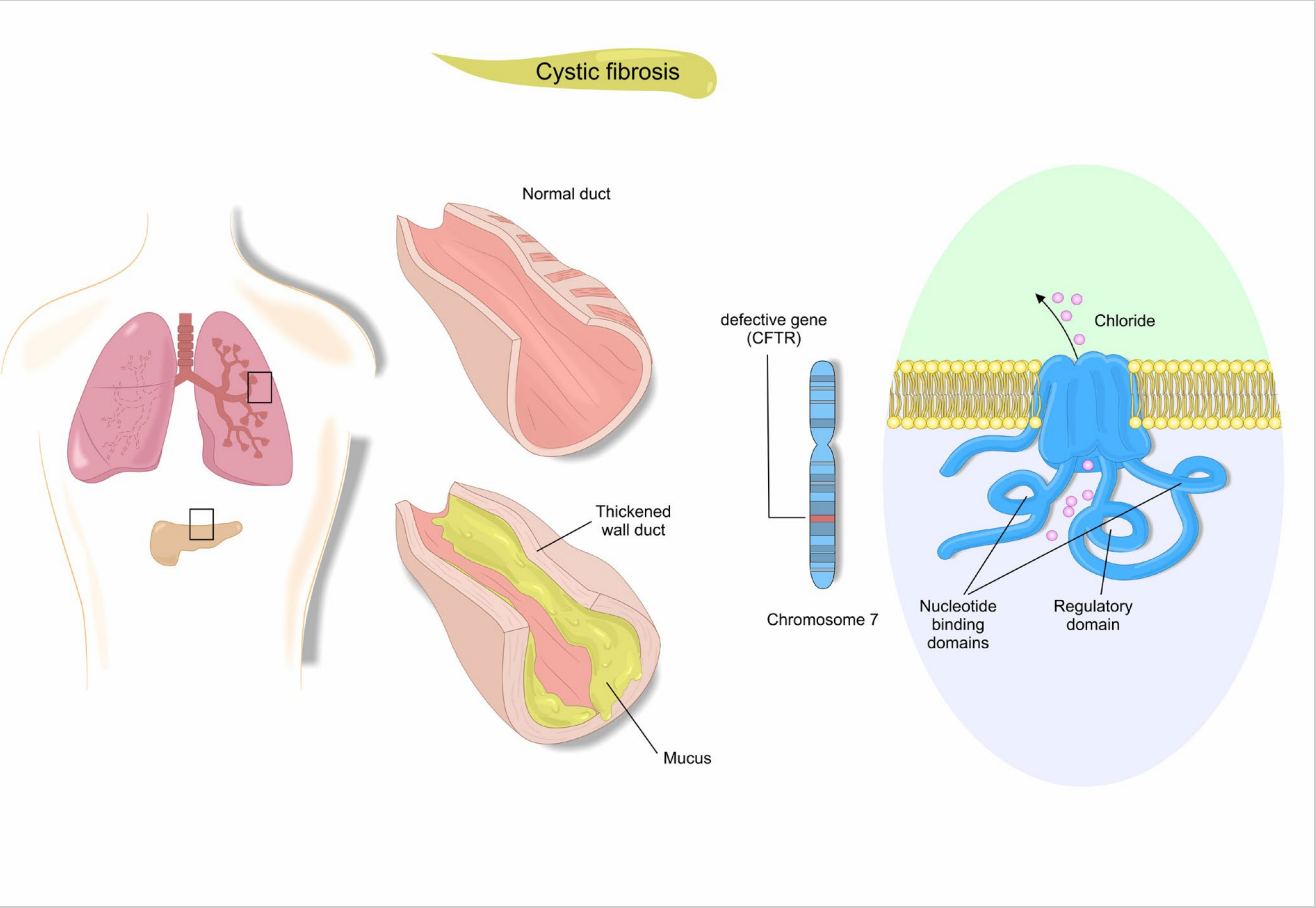
Methods looking at cancer are generally not informed by advances in non-cancer methods. It is noteworthy that air-liquid-interface cultures are used far less frequently than spheroids in cancer research, and that spheroids have not become widespread tools in non-cancer disease areas. However, a large number of publications describe general models and methods, many in the early stages of development, with the aim of demonstrating reproducibility and potential application in multiple disease areas. These include: renewable sources of human epithelial cells; lung models based on human pluripotent stem cells (hPSCs); novel microfluidic devices using organoid models that mimic the lung microenvironment during homeostasis and disease states; and methods for studying respiratory absorption.

Of the 51 biological biomarkers or 'endpoints' identified in the review, those based on the detection of key proteins or measurement of gene expression are the most commonly used across all methods. Cell viability, migration, gene expression and metabolism are key endpoints used for *in vitro* lung cancer.

Box 2. Models and methods used in cystic fibrosis research

Cystic fibrosis stems from mutations in the CTFR gene responsible for the protein that regulates a chloride channel on the surface of cells in the body. Normally, this channel transports chloride ions into and out of cells mediating the movement of water in tissues thereby leading to the production of thin, freely flowing mucus.

Defects in the CFTR channel prevent the transport of chloride ions and thus the humidification of the respiratory tract. As a result, the airways of affected individuals get covered by a thick, viscous mucus that leads to airway obstruction.



The knowledge base

This study has produced a unique highly curated knowledge base that contains detailed descriptions of 284 non-animal models being used for respiratory disease research. It is freely available to download from the EURL ECVAM Collection in the JRC Data Catalogue[5], together with a JRC Technical report[6] that describes the review methodology and presents the main findings (see Box 3).

This unique knowledge base can serve the needs of multiple stakeholders:

- **researchers** can identify models and methods that can be adapted and applied to tackle their own research questions;
- **educators** can provide the latest information on the state-of-the-art to their students;
- **funding bodies** can consider trends, identify impactful research avenues and target promising areas for investment;
- **project evaluation committees** can ensure that project proposers have properly considered the use of non-animal models and methods in their research proposals;
- **National Contact Points** and **National Committees**[7] can ensure proper knowledge sharing on non-animal methods within Member State networks and organisations involved in biomedical research using animals.

5 https://europa.eu/!BM64Yw

6 Hynes, J., Marshall, L., Adcock, I., Novotny, T., Nic, M., Dibusz, K. and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research: Respiratory Tract Diseases*, EUR 30334 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-21380-2, doi:*10.2760/725821*, JRC118161

7 As referred to in Directive 2010/63/EU for the protection of animals used for scientific purposes.

Findings of this study can also inform aspects of **policy making** regarding the protection of animals used for scientific purposes, setting of research priorities to progress the development and uptake of non-animal methods, and the promotion of modern human-relevant scientific approaches to combat diseases such as cancer.

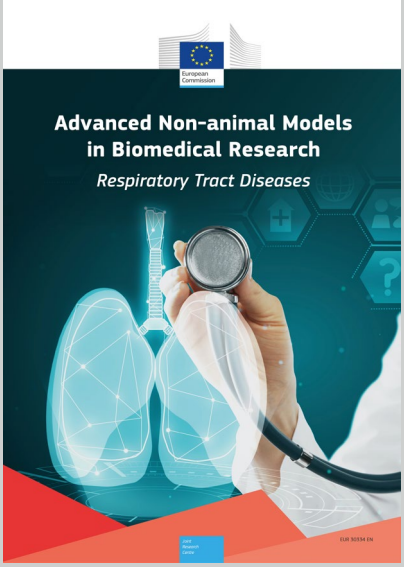
Finally, this knowledge base can serve as a means to explore the strengths and limitations of both animal and non-animal models used in biomedical research, to stimulate healthy scientific debate, to challenge mind-sets, and to pave the way for doing better and more predictive science. Thus the knowledge base can act as a bridge across methods and disciplines in the biosciences[8] to improve biomedical research for the ultimate benefit of patients and society.

8 Carusi A., Whelan M. and Wittwehr C., *Bridging Across Methods in the Biosciences – BeAMS*, EUR 29852 EN, Publications Office of the European Union, Luxembourg, 2019, ISBN 978-92-76-11181-8, doi:*10.2760/190697*, JRC116305.

Box 3. Knowledge base of advanced non-animal models

This study has produced a unique knowledge base that contains detailed descriptions of nearly 300 non-animal models being used for respiratory disease research.

The knowledge base is in an easy-to-use spreadsheet format and is freely available to download from the EURL ECVAM Collection in the *JRC Data Catalogue*.



In building the knowledge base, over 21,000 abstracts from the scientific literature were retrieved and screened and from these, a total of 284 publications were selected that were considered the most representative and innovative models.

To complement the knowledge base, the JRC also publishes a *report* analysing the models and methods identified to describe the state-of-the-art from different perspectives, including the most prevalent model types, typical contexts of use and emerging trends. A description of the review methodology used is also provided.

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