European Commission

Advanced Non-animal Models in Biomedical Research

Breast Cancer

Executive Summary

Joint Research Centre

EUR 30334/1 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 breast cancer.

The resulting collection of non-animal models are analysed in a JRC Technical Report (Folgiero, V., Romania, P., Rossi, F., Caforio, M., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Straccia, M. and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research: Breast Cancer*, EUR 30334/1 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-24689-3, doi:10.2760/618741, JRC122309) and publicly available from the *JRC Data Catalogue*.

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Breast cancer is the most common cancer among women in the European Union and worldwide. The European Cancer Information System (ECIS) indicates that in the EU over 355,000 women were diagnosed with breast cancer in 2020 (13.3% of all cancer diagnoses).

Breast cancer is characterised by a considerable degree of morphological and molecular heterogeneity (see Box 1). It can vary greatly between different patients and from one tumour site to another.

Despite advances in early detection and understanding of the molecular basis of breast cancer biology, approximately 30% of patients with early-stage breast cancer have recurrent disease, which is metastatic in most cases[1]. To offer more effective treatments with fewer and less severe side effects, selecting therapies best suited to the individual patient and the clinical and molecular characteristics of the tumour is a necessity.

Human-based models can better address heterogeneity of human breast cancer

Preclinical breast cancer research currently relies heavily on animal models, mostly rodents. However, animal models only capture limited aspects of human breast cancer disease.

For this reason, research is gradually moving towards the development of advanced non-animal systems that can recreate the heterogeneous environment peculiar to human breast cancer.

In this context, the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) carried out a study to survey and characterise the state-of-the-art of non-animal models being used in biomedical research.

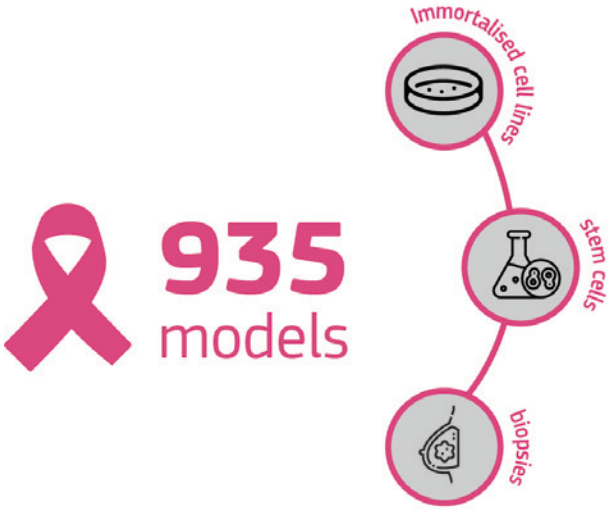
The review identified promising non-animal approaches being used for breast cancer. These are based mainly on techniques that use cells and tissues cultured in the laboratory

1 Waks, A. G., Winer, E. P. (2019) Breast Cancer Treatment: A Review, JAMA, 321(3), 288-300, doi:10.1001/jama.2018.19323

(*in vitro* methods), computer modelling and simulation (*in silico* methods) or cells and tissues explanted from a patient (*ex vivo* methods).

Categories of advanced models and their applications

The EURL ECVAM study involved an extensive review of scientific literature published from January 2014 to March 2019 that identified 935 papers describing relevant non-animal models for breast cancer.



In vitro models based on a variety of immortalised cell lines were the most popular approach used for breast cancer research (see Box 2). These cell lines are mostly commercially available and already qualified.

While 2D cell lines are convenient research models to study breast cancer, they are relatively simplistic. Thus the use of 3D culture conditions has increased over time reaching in 2018 the publication level of 2D models. 3D models can better mimic the complexity and heterogeneity that characterise human breast tumours.

The use of a scaffolding system emerged as the main technique employed to generate 3D models, followed by organoids and spheroids, known as mammospheres. Different culture conditions were also observed in combination with microfluidic systems.

It was found that *in silico* methods are sometimes used in combination with *in vitro* models. This still represents a minor niche, but it is worth highlighting their applications

Publications that use 3D models

278 Spheroids

63 Scaffolds

15 Organoids

7 Scaffold/Spheroids

Spheroids: simple 3D models that do not require any scaffolding system

Scaffolds: polymeric biomaterials that provide structural support for cell attachment and tissue development

Organoids: miniaturised 3D representations of an organ

to most of the breast cancer disease features. The use of machine learning in predictive modelling is a very interesting approach, especially in drug development and for testing new therapeutic strategies.

Although 5% to 10% of breast cancer can be hereditary, the high incidence of the disease highlights the lack of knowledge about the events triggering breast cancer initiation and whether it skips immunological recognition. This review found that non-animal models are employed mainly to study cancer initiation and development in order to fill this knowledge gap and discover the molecular basis of the driving events.

Box 1. What is tumour heterogeneity?

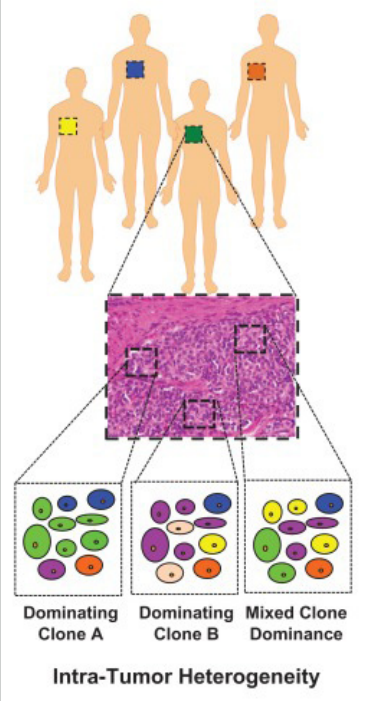
Heterogeneity is a word of Greek origin meaning “different kinds” and indicates a composition of different constituents rather than a uniform mass.

Tumours develop as a clonal process beginning with a single transformed cell. Therefore, heterogeneity in tumours is observed between different patients. Not all malignant cells within a tumour are the same and heterogeneity occurs also within a single patient.

Some tumours such as breast cancer are characterised by a high degree of heterogeneity. These variations have serious implications for patients ranging from the initial diagnosis to the treatment of metastatic disease.

Diagnosis often relies on needle biopsy, a procedure in which small samples of cells are extracted from a tumour and analysed. This method of sampling may not be representative of the entire tumour mass, leading leading for example to uncertainty in selecting the right therapy..

With metastatic progression, heterogeneity can be even more pronounced, raising the question of which lesion to sample in order to identify the most useful treatment targets.

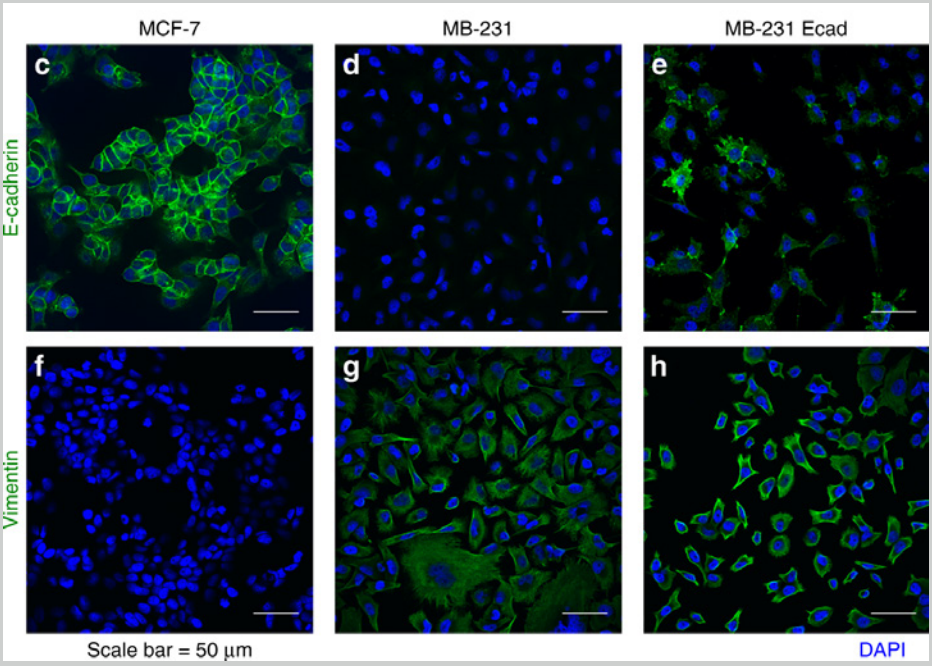


From ©Zhang, J., Späth, S. S., Marjani, S., *et al.*, (2018), Characterization of cancer genomic heterogeneity by next-generation sequencing advances precision medicine in cancer treatment, *Precision Clinical Medicine*, 1(1), p. 29-48, doi:10.1093/pcmedi/pby007 under CC BY 4.0.

Box 2. Immortalised cell lines

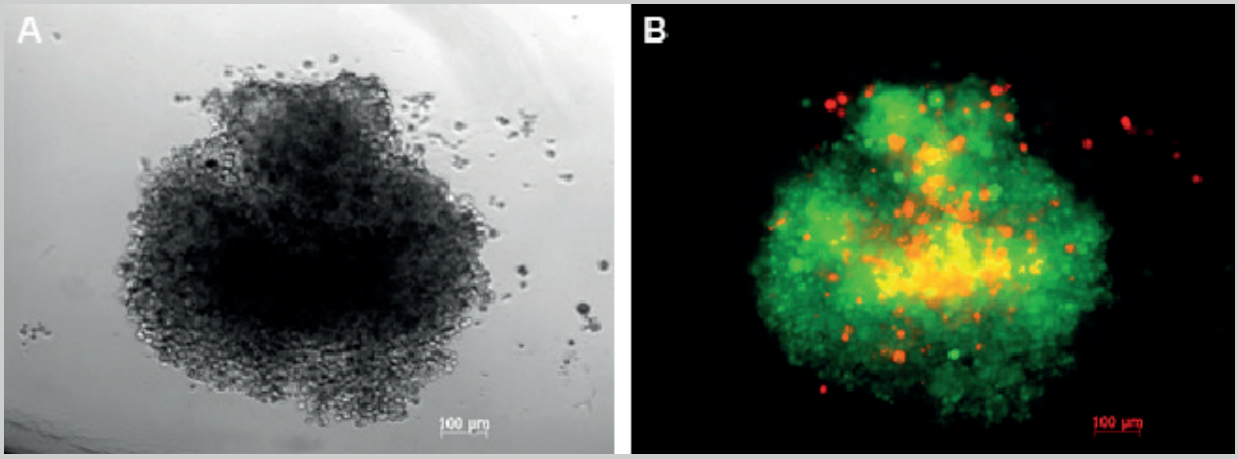
Immortalised cell lines have been the workhorse in biomedical research labs for decades. They are cells capable of renewing themselves indefinitely in artificial environments due to mutations, such as those found in tumours, or introduced through genetic manipulation.

In breast cancer studies the most commonly used cell line is MCF-7, which was established in 1973 at the Michigan Cancer Foundation. It was isolated from an invasive ductal breast cancer which developed in a Caucasian nun, Frances Mallon. Since then, a number of different breast cancer cell lines have been developed and used for *in vitro* cell culture or in animal experiments using xenografts.



Three immortalised breast cancer cell lines: MCF-7, MB-231 and MB-231 Ecad. ©Beckwitt, C. H., Clark, A. M., Ma, B. *et al.* (2018), Statins attenuate outgrowth of breast cancer metastases. *Br J Cancer*, 119, 1094–1105, doi:10.1038/s41416-018-0267-7 under CC BY 4.0.

Immortalised cell lines can be grown in 3D structures to better simulate the tumour microenvironment that it is well known to play an active role in tumour growth and progression. 3D systems, which can also be derived from tumour biopsies, stem cells or primary cell cultures, are developed through a wide range of technologies, such as scaffolds, spheroids - also called mammospheres - and organoids.



Structure of a spheroid derived from human breast cancel stem cells. ©Trinh, N., Dang, N., Tran, D., and Pham, P. (2016), Taraxacum officinale dandelion extract efficiently inhibited the breast cancer stem cell proliferation. *Biomedical Research and Therapy*, 3(07), 733-741 under CC BY 4.0.

The knowledge base

This study has produced a unique and highly curated knowledge base that contains detailed descriptions of 935 non-animal models being used for breast cancer research. It is freely available to download from the EURL ECVAM Collection in the JRC Data Catalogue[2], together with a JRC Technical report[3] 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**[4] can ensure proper knowledge sharing on non-animal methods within Member State networks and organisations involved in biomedical research using animals.

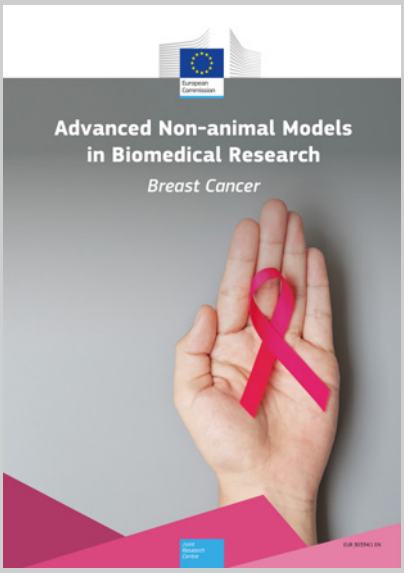
2 *https://europa.eu/!bM83pv*

3 Folgiero, V., Romania, P., Rossi, F., Caforio, M., Nic, M., Dibusz, K., Novotny, T., Busquet, F., Straccia, M. and Gribaldo, L., *Advanced Non-animal Models in Biomedical Research: Breast Cancer*, EUR 30334/1 EN, Publications Office of the European Union, Luxembourg, 2020, ISBN 978-92-76-24689-3, doi:*10.2760/618741*, JRC122309

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

Box 3. Knowledge base of advanced non-animal models

This study is a part of a series that EURL ECVAM is carrying out to review available and emerging non-animal models being used for research in seven disease areas. Details on the published studies are available on the *EURL ECVAM website*.



In this study around 120,000 peer-reviewed publications on breast cancer were initially retrieved and screened for representative papers describing innovative and promising advanced non-animal models.

An important outcome of this study is a highly curated knowledge base containing detailed descriptions of 935 non-animal models being used for breast cancer. It is easily downloadable as a spreadsheet file from the EURL ECVAM collection in the *JRC Data Catalogue*.

This knowledge base is complemented with a *Technical Report* that provides an in-depth analysis of the models identified and of the review methodology used.

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[5] to improve biomedical research for the ultimate benefit of patients and society.

5 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.

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