Advanced Non-animal Models in Biomedical Research:

Breast Cancer|

*Breast cancer is the most common cancer among women in the European Union and worldwide. Preclinical breast cancer research currently relies on animal models, mostly rodents. However, animal models mimic **limited aspects** of human breast cancer. The European Commission's Joint Research Centre (JRC) has carried out an extensive review of the state-of-the-art of advanced non-animal models used for basic and applied research on breast cancer. Researchers characterised and catalogued about **900 models** to make them more accessible for human relevant studies that avoid the use of animals.*

"Before reaching the age of 75, 1 in 22 women will be diagnosed with breast cancer and 1 in 73 women will die from breast cancer, worldwide"

IARC Handbooks of Cancer Prevention Volume 15

BREAST CANCER AND ITS HETEROGENEITY

Breast cancer is the **most commonly occurring cancer in women** in the European Union and worldwide. The European Cancer Information System (ECIS) estimates that in 2020 over 355,000 women were diagnosed with breast cancer in the EU, accounting for 13.3% of all cancers diagnosed.

Despite advances in early detection and understanding of breast cancer biology, relapse and subsequent metastasis often occurs in bone, lung, liver and brain.

Human breast cancer is **highly heterogeneous**, even within the same tumour. To offer better treatment with increased efficacy, it is necessary to use therapies that match patient profiles and the clinical and molecular characteristics of the tumour.

Breast cancer research currently relies heavily on animal models, which, however, have limitations in capturing important cancer traits.

For this reason, research is gradually moving towards the use of advanced non-animal models that more faithfully represent the characteristic heterogeneity peculiar to human breast cancer.

LEGISLATIVE FRAMEWORK

Directive 2010/63/EU on the protection of animals used for scientific purposes sets out clear legal requirements for the implementation of the 'Three Rs' principles of **Replacement**, Reduction and Refinement of animal procedures. The final goal is the phasing out of animal testing when scientifically valid non-animal alternatives are available.

To aid this transition, the JRC's EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) produced a unique **knowledge base** of detailed descriptions of non-animal models used for breast cancer research.
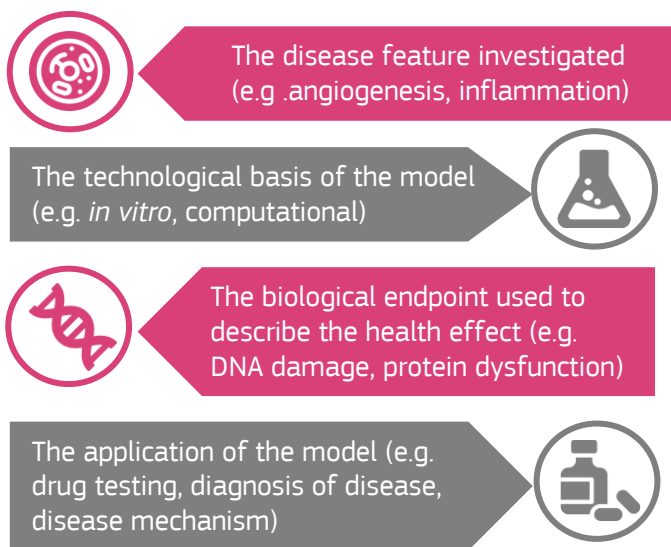
KNOWLEDGE BASE OF NON-ANIMAL MODELS

About 120,000 scientific papers were reviewed to identify relevant human-based models of breast cancer. From those, a total of **935 models** were selected as being the most representative and promising.
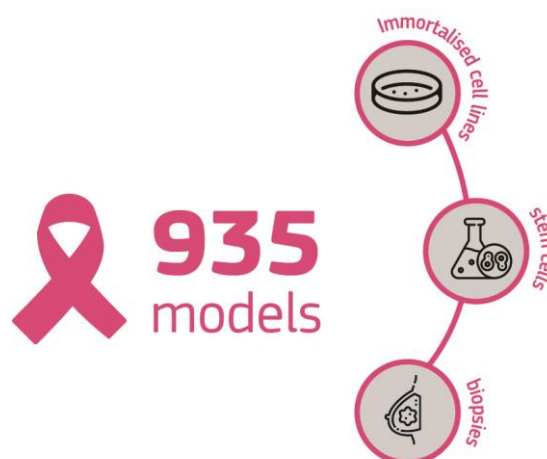
This collection of models is freely available for download from the **JRC Data Catalogue** in an easy-to-use spreadsheet format. This knowledge base is complemented by a **Technical Report**, which provides an in-depth meta-analysis of the approaches being used and a separately published **Executive Summary** intended for the general reader.

ANALYSYS OF THE MODELS

The selected models were characterised according to many different features including:

The disease feature investigated (e.g .angiogenesis, inflammation)

The technological basis of the model (e.g. *in vitro*, computational)

The biological endpoint used to describe the health effect (e.g. DNA damage, protein dysfunction)

The application of the model (e.g. drug testing, diagnosis of disease, disease mechanism)

The collection shows that *in vitro* models based on a variety of immortalised cell lines are the most representative approach used for breast cancer research.

Although monolayer cultures based on cancer-like cell lines are convenient to study aspects of breast cancer, their simplicity limits their relevance. Attention has been shifting therefore to the development and application of three-dimensional tissue models since they can better capture the complexity and heterogeneity that characterise human breast tumours.

Three-dimensional models include spheroids, known also as 'mammospheres', engineered tissues grown on biocompatible polymer scaffolds, and organoids which reflect more complex aspects of tissue composition and micro-architecture.

WHO IS THIS KNOWLEDGE FOR?

This review and model catalogue represent a valuable resource for **scientists** and **funding bodies,** as well as various actors involved in the application of **Directive 2010/63**, such as Animal Welfare Bodies, Competent Authorities, National Committees and National Contact Points.
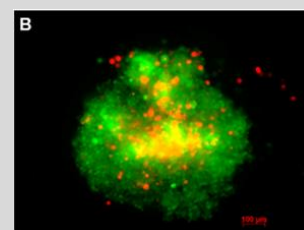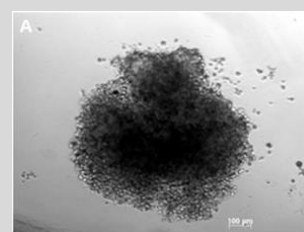
The knowledge gathered can also be introduced into **education and training programmes** to inform a new generation of researchers about the non-animal models currently available and to stimulate innovative approaches in biomedical research.

Non-animal models in breast cancer research: an example

In 2016, Trinh and colleagues developed a three-dimensional (3D) model using breast cancer stem cells (BCSCs), which are able to self-renew and differentiate while keeping the heterogeneity trait of breast cancer. BCSCs are also important due to the role they play in the development of resistance to treatment and cancer relapse.

This 3D model was grown as spheroid mammospheres and was shown to possess many properties of actual micro-tumours such as dead cells in the inner spheroid layer. It can be used for screening anti-cancer drugs in an automated high-throughput process where hundreds of experiments are performed in a fully automated manner.

To be used for screening however, the tissue must not adhere to the bottom of the multi-well plates in which they are contained. The solution was the use of biological 'non-stick' coatings that can be applied in a very practical and economical way.