BREAST CANCER TREATMENTS - DEVELOPMENTS OVER TIME

OVERVIEW OF BREAST CANCER

Breast cancer is the second most common type of cancer to be diagnosed in Australia[1] and is the leading cause of cancer-related female death worldwide[2].

In 2021, cancer research received $153.7 million in funding from the Australian Government through the National Health and Medical Research Fund, just behind funding for neurological diseases (such as Multiple Sclerosis) and infectious diseases (such as Covid-19)[3].

WHAT TREATMENTS ARE AVAILABLE NOW FOR BREAST CANCER?

There are approximately 80 approved brands of individual and combination drug treatments approved for breast cancer (as of Dec 2022), including chemotherapy and hormone therapy drugs[4], 30 of which were approved during 2010-2020. Treatment also includes surgery and radiation.

There have been more drug approvals for breast cancer than any other type of solid tumor[5].

HOW EFFECTIVE ARE THESE TREATMENTS?

Drug effectiveness depends on the subtype and stage of breast cancer and whether or not resistance to treatment occurs. Many hormone therapy drugs work initially and then stop working when the tumor develops resistance to treatment[9].

Some do not work initially – up to 30% of tumors will be initially resistant to the drug tamoxifen, which is often the first-line treatment for the most common type of breast cancer[6, 7, 8].

New human-relevant methods have been developed that can help recreate the original tumor in vitro, and accurately predict response to treatment within 10 days of obtaining the tumor sample[9].

Resistance can mean other cancers develop instead, like uterine cancer[8].

WHY ARE TREATMENTS SO LIMITED?

Cancer drugs have a 2-4 times higher rate of drug failure than other types of drugs[10], for example;

- Between 1979 and 2014, 411 breast cancer drugs went into clinical trials and the attrition (failure) rate was roughly 345 trials (or 83.9%)[10].
- Of all the drugs developed to treat cancer only 5% of molecules that show safety and efficacy in preclinical animal trials proceed to phase III human clinical trials[11]. This is compounded by many drugs being discontinued, suspended, or withdrawn from the market[11].

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HOW ARE TREATMENTS FOR BREAST CANCER DEVELOPED?

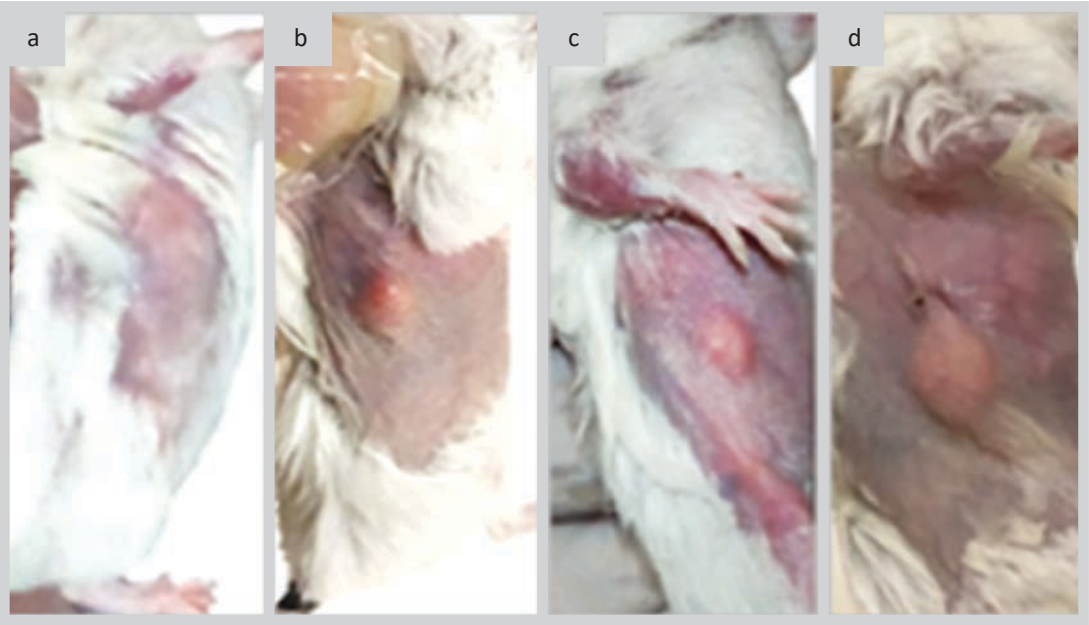
Treatments are developed primarily in mouse models. According to the Fund for Replacement of Animals in Medical Experiments (FRAME), mice are predominantly used because[12]:

- They are small and relatively easy and inexpensive to keep in a laboratory setting.
- They have a short lifespan so results can be seen quickly.
- They can be easily bred to see side effects on offspring.
- Modern technology allows scientists to manipulate or alter mouse genetics to create new research strains quickly and easily.
- Mice are mammals and therefore share similar genes and physiology to us

Mouse models include CDX, PDX, CDX/PDX humanized, and GEMM mice[13,14]:

An example of a humanized mouse is a mouse that has had human bone pieces implanted, which helps to make the tumor microenvironment more similar humans[14].

- CDX = cell-line derived xenograft. This means that cancer cells are grown and transplanted into mice.
- PDX = patient-derived xenograft. This means that a cancer growth from a patient is removed and transplanted into mice.
- Humanized PDX and CDX mice. A CDX or PDX mouse, made more "human". For example, by implanting human bone pieces into mice to make the tumor microenvironment more similar to humans.[14]
- GEMMs = genetically engineered mouse models. This means that the mouse DNA blueprint is changed so that cancer growths will form.



Mammary tumor at days 10 (a), 20 (b), 30 (c), and 40 (d). Image reprinted under creative commons license (To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.) from: Khodayari, H., Khodayari, S., Khalighfard, S. et al. Gamma-radiated immunosuppressed tumor xenograft mice can be a new ideal model in cancer research. Sci Rep 11, 256 (2021). https://doi.org/10.1038/s41598-020-80428-5

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WHAT ARE THE LIMITATIONS OF USING ANIMAL MODELS TO DEVELOP TREATMENTS FOR BREAST CANCER[13]?

Based on drug failure rates, animal models are very limited in predicting human response to drugs accurately.

More specifically, no animal model can accurately recreate the spread (metastasis) of breast cancer to other organs and tissues[13].

In humans, when cancer spreads to a different part of the body from where it was first formed, this is usually to the brain and bones, whereas in mice this is the lymph nodes and lungs[13].

The accurate study of metastasis is important however, as 30% of patients with early-stage breast cancer will have metastasis[15].

Other limitations of animal models which could lead to inaccurate prediction of human response are:

• The cells and blood supply (stroma) that support the tumor in any animal model is not human[13].

• Tumors in CDX mice grow more rapidly than the tumor from the human donor, and are more responsive to growth-suppressing drugs than the primary tumor[13].

• Cell lines obtained and used to generate CDX are taken from a secondary tumor site (such as a lung metastasis) and are therefore not suitable for studying early-stage growth and spread from the primary tumor site[13].

• PDX mice do not have an immune system and so drugs that help increase the immune system cannot be tested in these mice[13].

• GEMM mice do not have a human tumor, they are grown specially to develop mice tumors[13].

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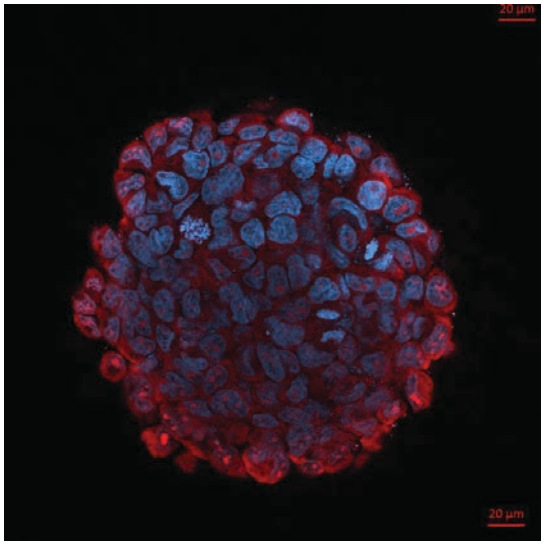
BREAST CANCER TREATMENTS - DEVELOPMENTS OVER TIME

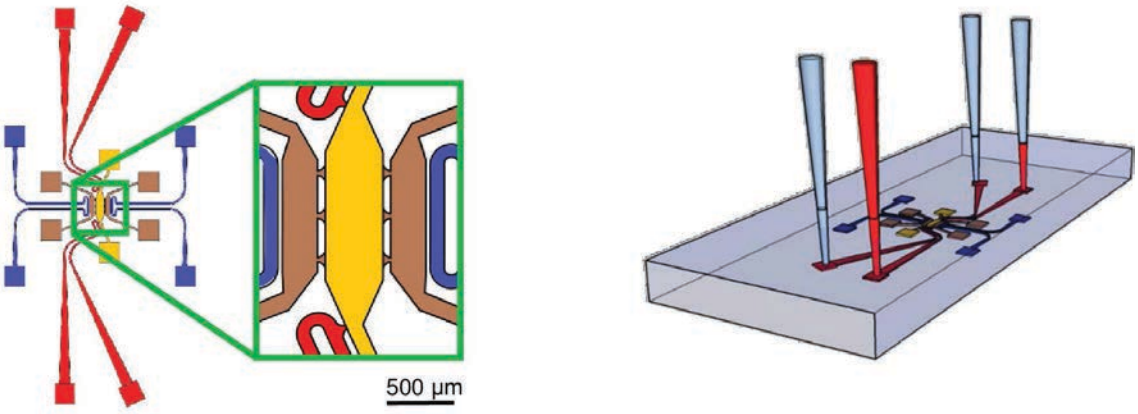
WHAT ARE THE ALTERNATIVES TO USING ANIMALS IN BREAST CANCER RESEARCH?

The 2021 European Union Joint Research Center report found 935 non-animal models were used to study breast cancer between 2014 and 2019[16].

- *In vitro* models were used in 91% of studies, 5% were *in silico* (computational) and 4% were a combination of both.
- The majority (84 of 91%) of *in vitro* studies used human cells lines, which are cultured with or without use of a 3D model (those without are 2D).
- Less than 40% of the 935 studies used 3D models. The most used 3D models to grow human cancer cells were (tumor) spheroids, followed by scaffolds and organoids.
- The use of scaffold and spheroid 3D models were also applied in microphysiological systems, which accounted for 4% of the 935 studies.



3D Tumor Spheroid Model Showing Medulloblastoma Stem Cells. Image Credit: Translational Genomics Laboratory, IB-USP. Sourced from www.eurekalert.org/multimedia/551719/



MICROFLUIDIC DEVICE (microphysiological system)
IMAGE RETREIVED FROM: Microfluidic device to attain high spatial and temporal control of oxygen. Lam SF, Shirure VS, Chu YE, Soetikno AG, George SC (2018). PLOS ONE 13(12): e0209574. https://doi.org/10.1371/journal.pone.0209574. Reprinted under creative commons license. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

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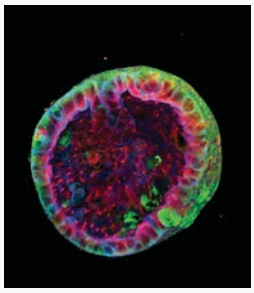
3D CELL MODELS: DEFINITIONS

ORGANOID



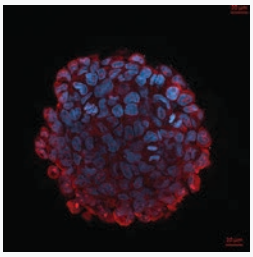
"A tiny, 3-dimensional mass of tissue that is made by growing stem cells (cells from which other types of cells develop) in the laboratory. Organoids that are similar to human tissues and organs, or to a specific type of tumor, can be grown. Organoids are used in the laboratory to study how normal tissues or cancers form and to test new drugs and other types of treatment before they are given to people."[35]

A kidney organoid. Image credit: Anne Rios (Princess Maxima Centre) in Nature Biotechnology. Source from: https://www.eurekalert.org/multimedia/677718

MICROFLUIDIC DEVICE

"An instrument that uses very small amounts of fluid on a microchip to do certain laboratory tests. A microfluidic device may use body fluids or solutions containing cells or cell parts to diagnose diseases. Also called lab-on-a-chip."[35]

TUMOR SPHEROID



"Spherical, heterogeneous aggregates of proliferating, quiescent, and necrotic cells in culture that retain three-dimensional architecture and tissue-specific functions. They represent an in-vitro model for studies of the biology of both normal and malignant cells. Generally the ability to form spheroids is a characteristic trait of malignant cells derived from solid tumors, though cells from normal tissues can also form spheroids."[36] *3D Tumor Spheroid Model Showing Medulloblastoma Stem Cells. Image Credit: Translational Genomics Laboratory, IB-USP. Sourced from: https:// www.eurekalert.org/multimedia/551719.*

SCAFFOLD

"Polymeric biomaterials that provide structural support for cell attachment and tissue development."[37]

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KEY ADVANCEMENTS OF NON-ANIMAL METHODS AND MODELS IN BREAST CANCER RESEARCH OVER TIME[14,17].

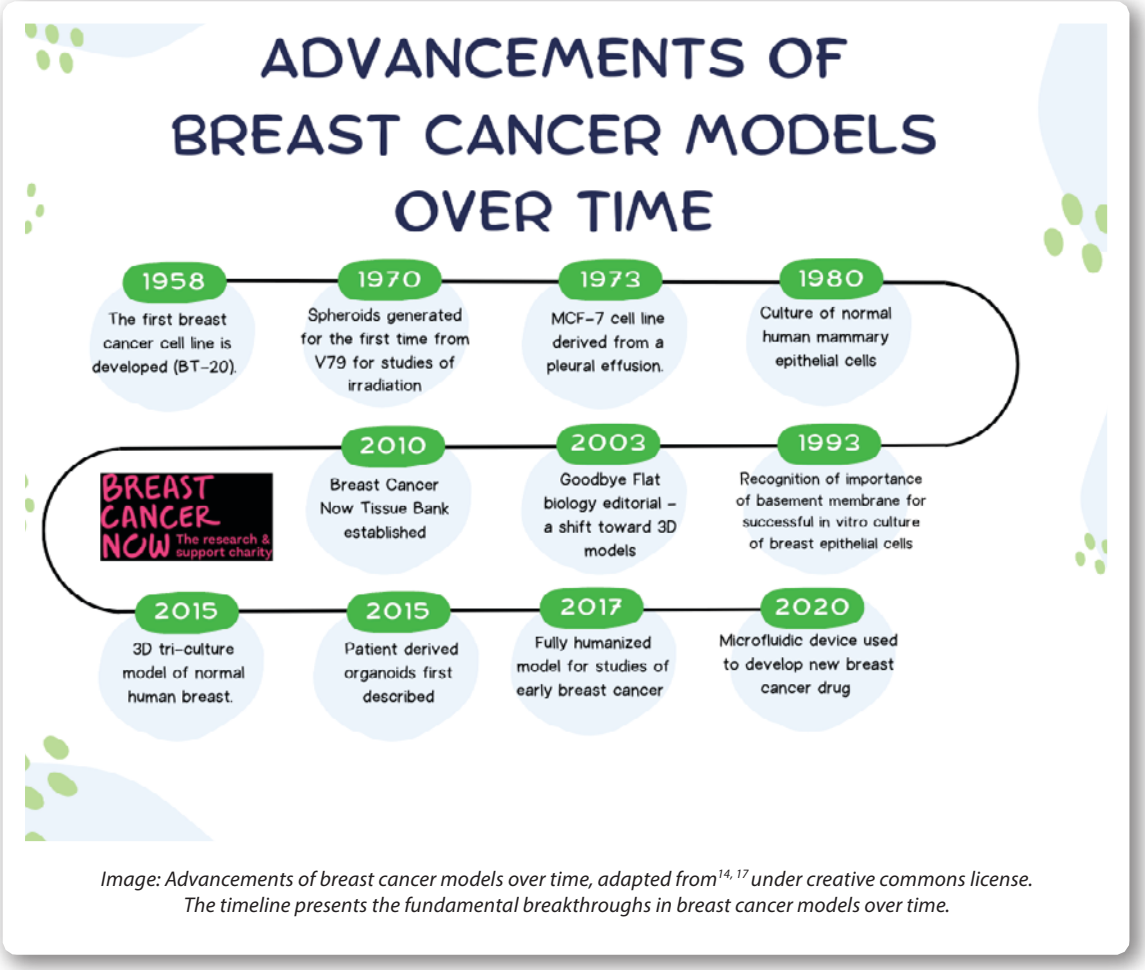

Image: Advancements of breast cancer models over time, adapted from[14, 17] under creative commons license. The timeline presents the fundamental breakthroughs in breast cancer models over time.

HOW RELEVANT ARE NON-ANIMAL MODELS AT TESTING OR DEVELOPING DRUGS FOR BREAST CANCER?

"HUMAN BASED MODELS CAN BETTER ADDRESS THE HETEROGENEITY OF BREAST CANCER" – JRC REPORT[16]

Whilst no alternative model can mimic breast cancer entirely[16], many non-animal models can more accurately represent the human condition than animal models[18]. For example, liver chips have 87% accuracy to assess toxicity in humans[19].

THE TOXICITY OF THE BREAST CANCER DRUG TAMOXIFEN HAS BEEN ACCURATELY MIMICKED IN ORGAN CHIPS[20].

LIMITATIONS OF NON-ANIMAL MODELS INCLUDE[17]:

- Different cell types have varying abilities to form spheroids and spheroid growth is difficult to control, effecting reproducibility.
- Cell lines are simplistic (especially 2D) and lack the heterogeneity and complexity that breast cancer has.
- Limited access to tissue banks can be a limitation to developing primary cell culture models, although this has improved since the Breast Cancer Now Tissue bank was established in 2010.

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LOOKING TO THE FUTURE

The United States Food and Drug Administration (US FDA) have recently accepted a drug application using non-animal *in vitro* and *in silico* data only[21]. Based on this and other submissions, the alternative model most likely to replace animals is the "human-on-a-chip" – multiple organ-on-chips combined – as they can more accurately mimic physical and chemical aspects of human biology, and are three dimensional – which are all important for toxicity testing of new drug molecules[18]. Breast cancer has already been replicated in multiple organ chip types at the blood vessel and mammary gland level, and the liver + heart + breast + bone marrow combination of organ-chips has already been used successfully to study off-target effects of breast cancer drugs [18,20,21].

Better understanding of the mechanism of action of drug molecules gained from organ chip and human-relevant research methods can help avoid early-stage drug failure.

LANDMARK STUDY – drug application accepted with no animal data! The mechanism of kidney toxicity of the cancer chemotherapeutic drug, cisplatin – used to treat breast cancer – was identified with a kidney (spheroid) chip using microsensor technology[21]. The study also identified an appropriate treatment for this toxicity, and their submission was accepted by the US FDA. They stated that the discovery would not have been possible in animal models.

TO ENSURE BETTER OUTCOMES FOR CANCER PATIENTS, WE NEED INVESTMENT IN HUMAN-RELEVANT RESEARCH METHODS.

Whilst breast cancer continues to impact countless lives, you can support charities providing services for breast cancer patients. For more information, see humanecharities.org.au.

Humane Charities
Donate with confidence

TAKE ACTION

Ask Our Government to fund Human-Relevant Research:
www.humaneresearch.org.au/i_am_not_a_lab_tool

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