Preclinical mouse cancer models for drug discovery and development

Virtual THERACAT meeting

Sep 2020

Tel Aviv University

Evolution of preclinical mouse models

Preclinical mouse models have co-evolved with cancer therapy development. This evolution was highly dependent on technical advances, resulting in waves of activity.

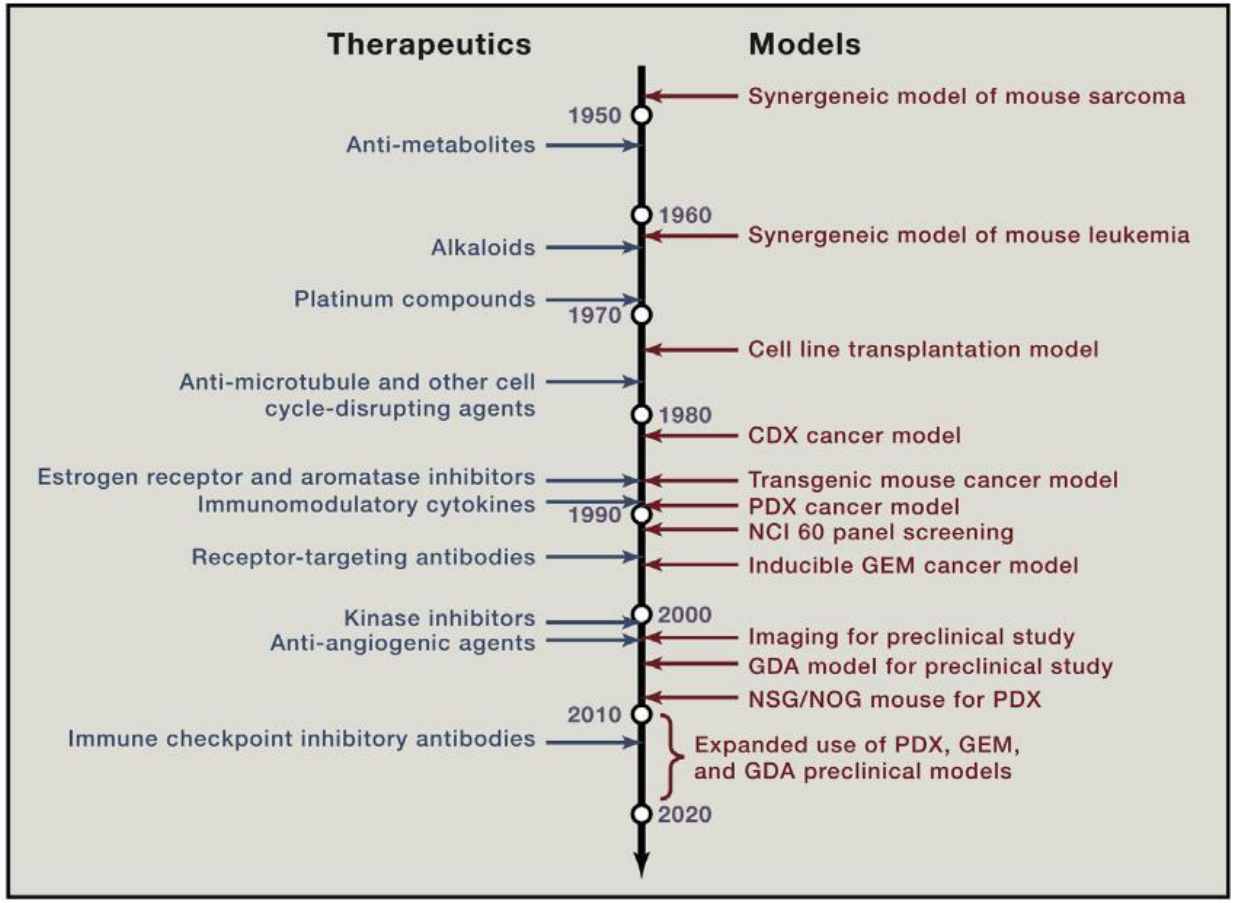
Murine syngeneic models (1950s) –
The earliest models; Built through transplantation of murine tumors into immunocompetent host mice.

CDX - Cell line-derived xenografts (early 1980s) –
Subcutaneous injection of *in vitro*-established human cancer cells into immunocompromised mice.

PDX - Patient-derived xenografts (late 1980s) –
Subcutaneous implantation of surgically derived clinical tumor samples into immunocompromised mice.

Inducible GEM – Genetically-Engineered Mouse models (1990s) –
Generated through the introduction of and induction genetic mutations associated with particular human malignancies.

- **GDA – GEM-derived allografts**

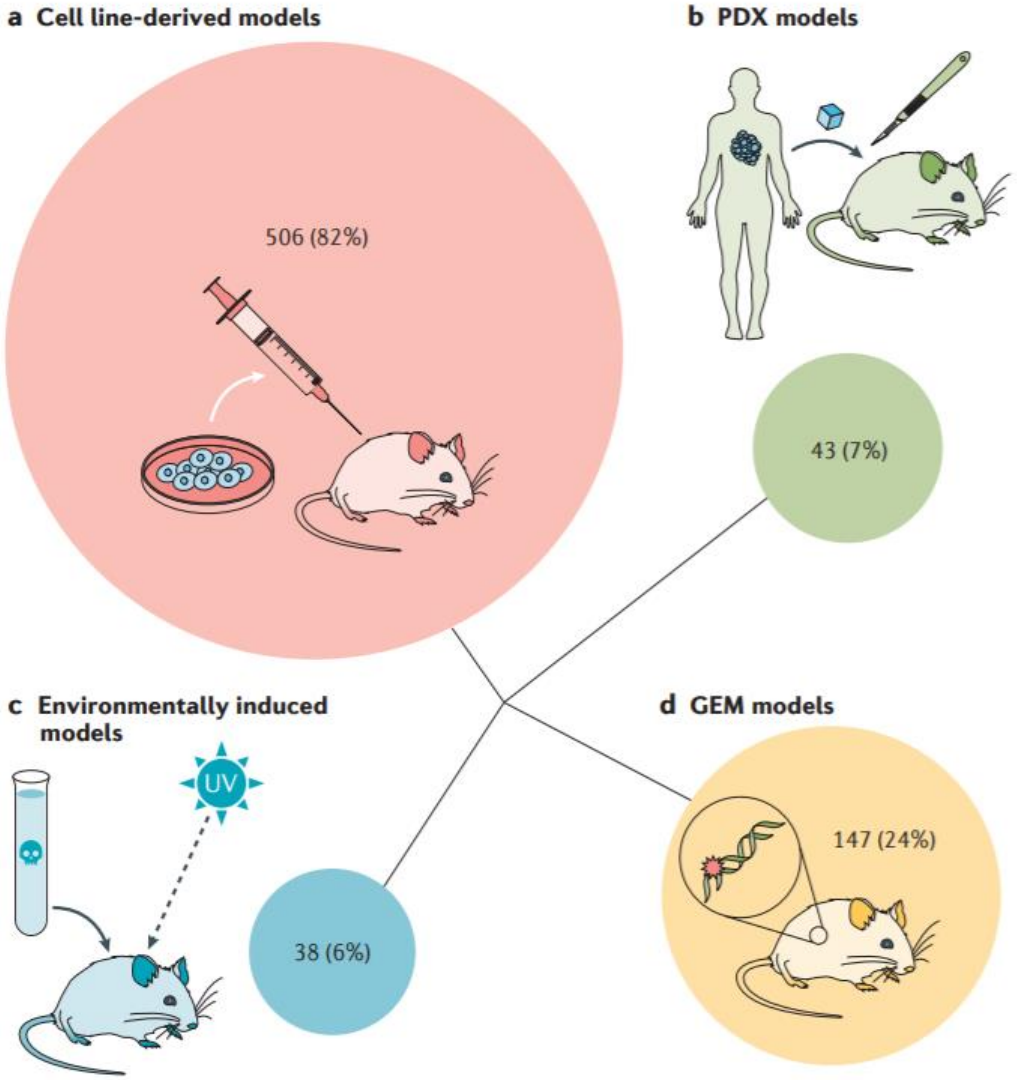


Preclinical Mouse Cancer Models: A Maze of Opportunities and Challenges, Day C.P. et al., Cell 2015

Frequency distribution of Preclinical mouse models

Categories of pre-clinical mouse tumor models:

a. Cell line-derived models
   i. Syngeneic (murine)
   ii. Xenogeneic (human) – CDX
b. Patient-Derived Xenografts (PDX)
c. Environmentally/chemically induced models
d. Genetically engineered Mouse (GEM) models



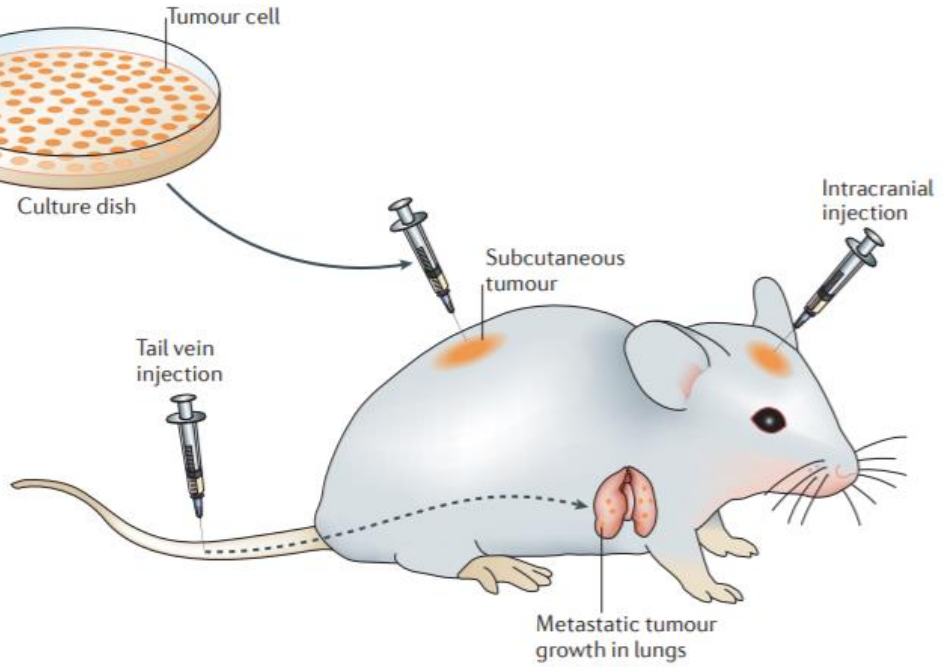
Preclinical mouse solid tumour models: status quo, challenges and perspectives. Nicolas Gengenbacher et al. NATURE REVIEWS CANCER, 2017

Cell line-derived models

Most of our current understanding of cancer and its hallmarks is based on the establishment of long-term *in vitro* cultured tumor cell lines and their *in vivo* inoculation in mice.

Advantages:

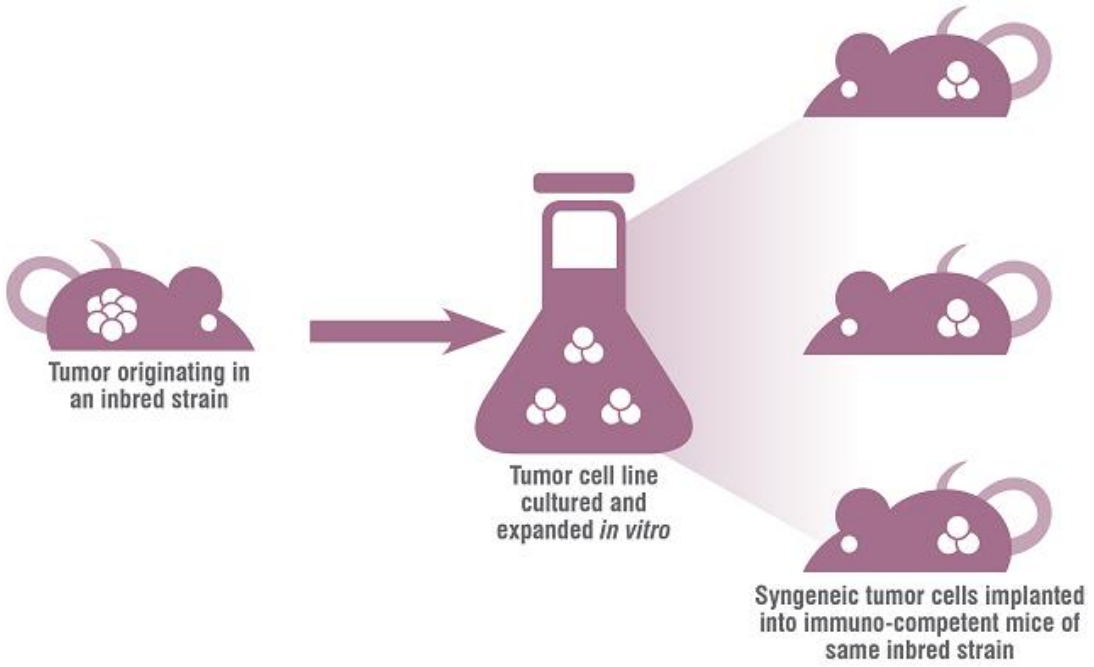
- Low cost
- Synchronous tumor growth
- Easy technical manipulability
- Tumor cells can be injected:
  - Ectopically (mostly subcutaneously)
  - Orthotopically, to mimic tumor growth in its organ of origin
  - Systemically (mostly intraperitoneally, intravenously or intracardially) to study metastatic spread



*Syngeneic murine cell line-derived models

***Syngeneic** - genetically similar or identical and hence immunologically compatible

Murine cells-line derived models are established by injection of tumor cells isolated from **spontaneously-arising mouse** tumors into **immunocompentent** mice from **a similar genetic background**.



https://www.criver.com/products-services/discovery-services/pharmacology-studies/oncology-immuno-oncology-studies/oncology-models/syngeneic-mouse-models?region=3696

Syngeneic murine cell line-derived models

Mouse strains commonly used for syngeneic models:

C57BL/6 mice - the most used inbred strain in research

- High degree of uniformity (appearance, behavior, response to treatments)
- Easy breeding
- Robustness

Balb/c mice (Bagg Albino) - an albino, laboratory-bred strain of the house mouse, developed by H.J. Bagg in 1913.

- General multipurpose model
- Hybridoma development
- Monoclonal antibody production
- Infectious disease

Human cell line-derived xenograft (CDX) models

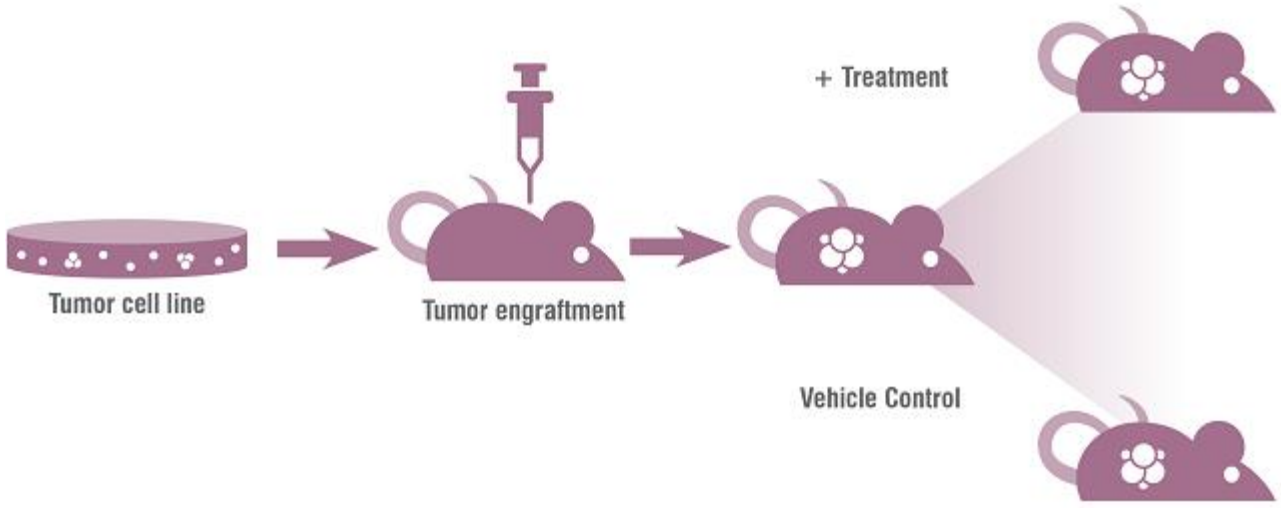
***Xenogeneic** - Derived from a different species and therefore genetically and immunologically incompatible.

Human tumor CDX models involve implantation of **commercially available** tumor cell lines or **patient-derived xenograft-derived cell lines** into **immunodeficient** mice

The cell lines have been **selected over many passages for rapid 2D growth** on plastic in serum-containing media

The NCI60 cell line panel:

A group of 60 human cancer cell lines used by the National Cancer Institute (NCI) for the screening of compounds to detect potential anticancer activity.



Human cell line-derived xenograft (CDX) models

Mouse strains commonly used for xenogeneic models:

Nude mice – the first immunocompromised mouse strain to be used in cancer research.

- Lack a normal immune system and thymus gland
- **Cannot generate mature T lymphocytes**
- Ideal for tumor and tissue studies
- **Hairless** – easy to identify and monitor tumor growth

Genetic basis for nude mice:

- The gene responsible for the mutation was identified in 2000
- Mice have a spontaneous deletion in the **FOXN1 gene**
- Humans with mutations in FOXN1 also are athymic and immune deficient

Life span

- Normally - 6 months to a year
- In controlled, germ free environments and with antibiotic treatments - 18 months to two years

Human cell line-derived xenograft (CDX) models

Mouse strains commonly used for xenogeneic models:

Severe combined immunodeficiency (SCID) mice – have a genetic immune deficiency that affects their **B and T cells**.

- Unable to reject non-self tissues - ideal for xenoengraftment of human cells and tissue
- Commonly used to study the biology of the immune system

Genetic basis for SCID in mice:

- Discovered in 1983 in the **CB/17** mouse line.
- SCIDs occurs in these mice due to **a mutation in the PRKDC gene**, which plays a role in **repairing double-stranded DNA breaks**.
- The mutation has implications for B and T cell receptor development.

Next-generation in vivo modeling of human cancers

CDXs have failed to predict human efficacy for most therapies targeted to cancer-driving proteins, as evidenced by the low FDA approval rate of 5%–7% for targeted therapeutics

Current standard preclinical practice inadequately addresses:

- Host immune responses
- Cancer heterogeneity
- Drug resistance

➢ **Need to design mouse models that better represent cancer patients**

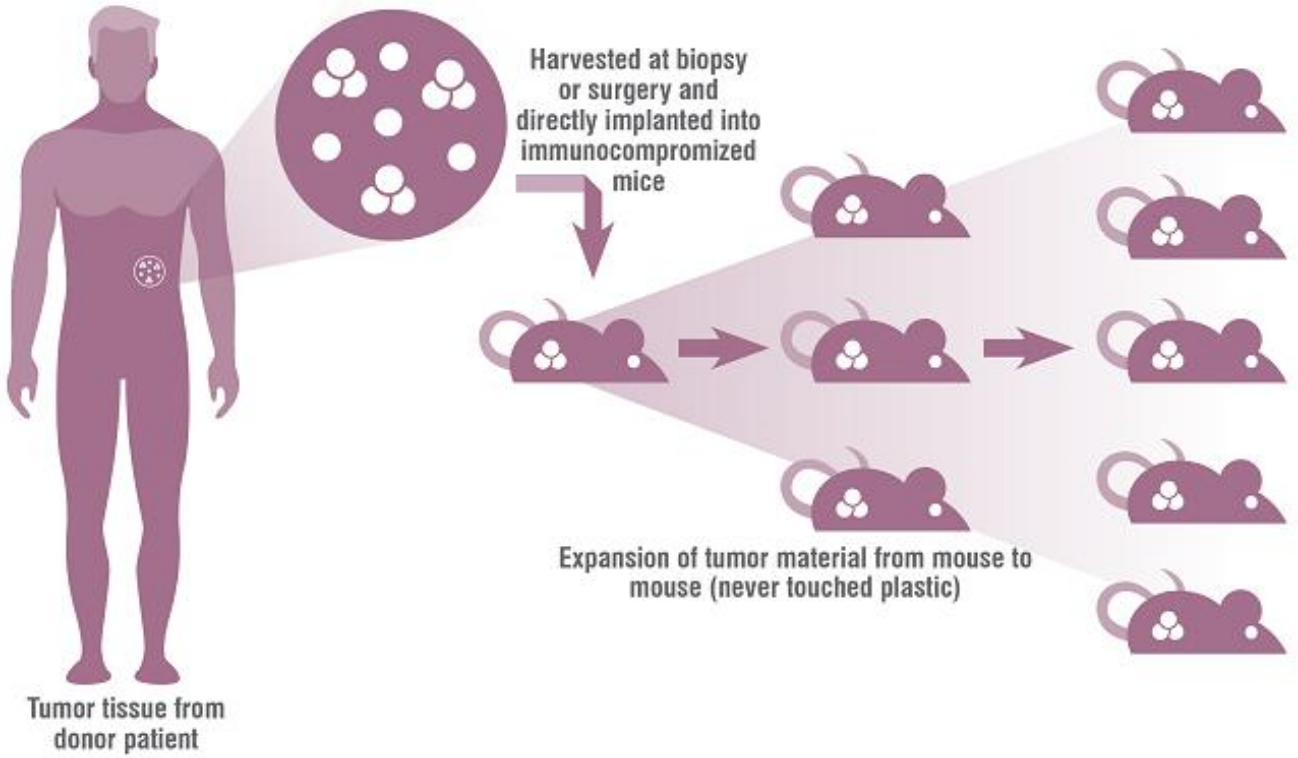
Patient-derived xenograft (PDX) models

PDX models are generated by **subcutaneous implantation of surgically-derived clinical tumor samples**.

Better aligned with human disease:
- Biological phenotype
- Tumor architecture
- Stromal properties

PDX Models were first developed in 1984 by Fiebig:
- Showed that chemotherapeutic agents elicit similar response in mice and in patients
- Low predictive value in NCI60-based CDX
- Limited studies due to insufficient clinical samples

Resurgence of PDX models in the 2000s
- Better clinical sample access
- Improved transplantation technology



Patient-derived xenograft (PDX) models

Challenges in PDX models:

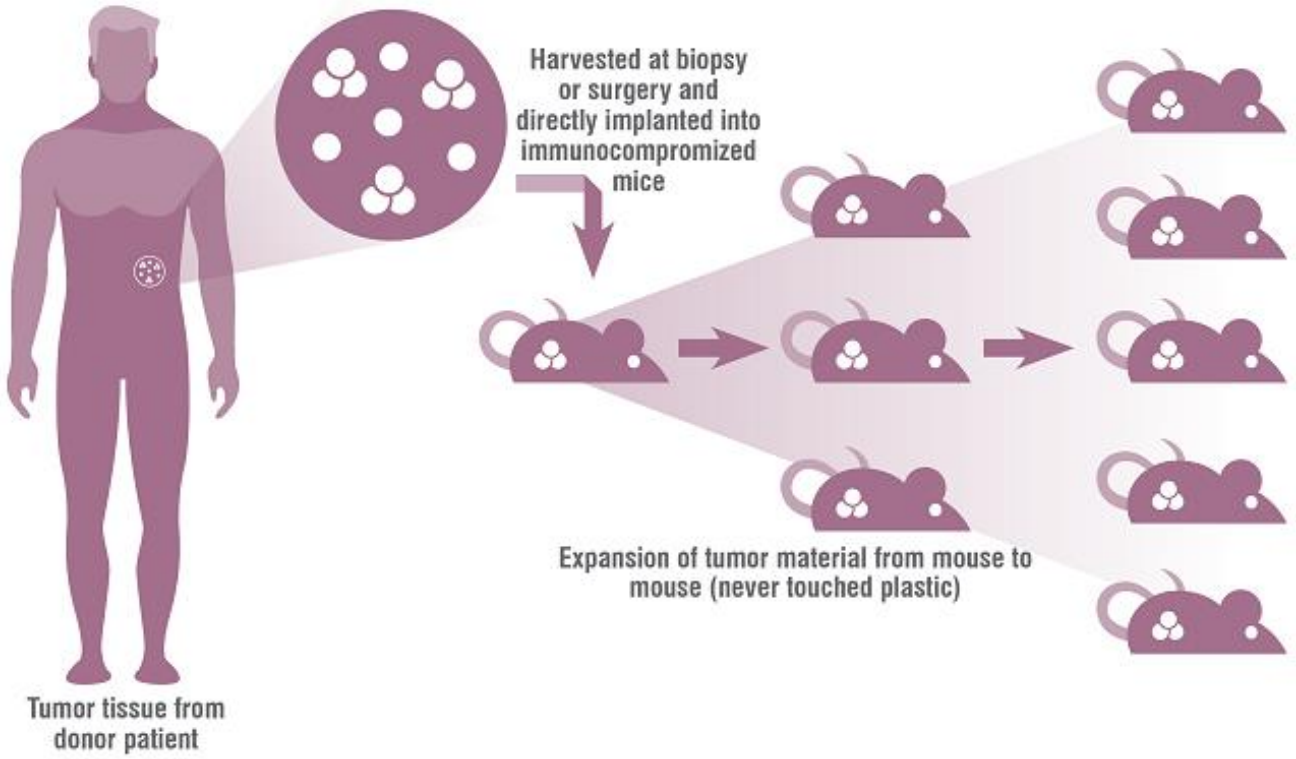
Laborious and expensive process

Rate of transplantation take
- Depends on the aggressiveness of the cancer
- Lower in some cancer types

Time required for tumor growth
- 2-4 months

Success depends on sample type and amount
- Fresh tissue biopsy
- Fine needle aspirate
- Circulating tumor cells

Orthotopic PDX production is technically challenging
- The majority of PDX models use **subcutaneous** models



Limitation of PDX cohorts produces by serial tumor transplantation
- human stroma and vascular tissue in PDX models are **gradually replaced by murine equivalents** with time
- Therapeutic studies are most representative in **low-passage models**

Patient-derived xenograft (PDX) models

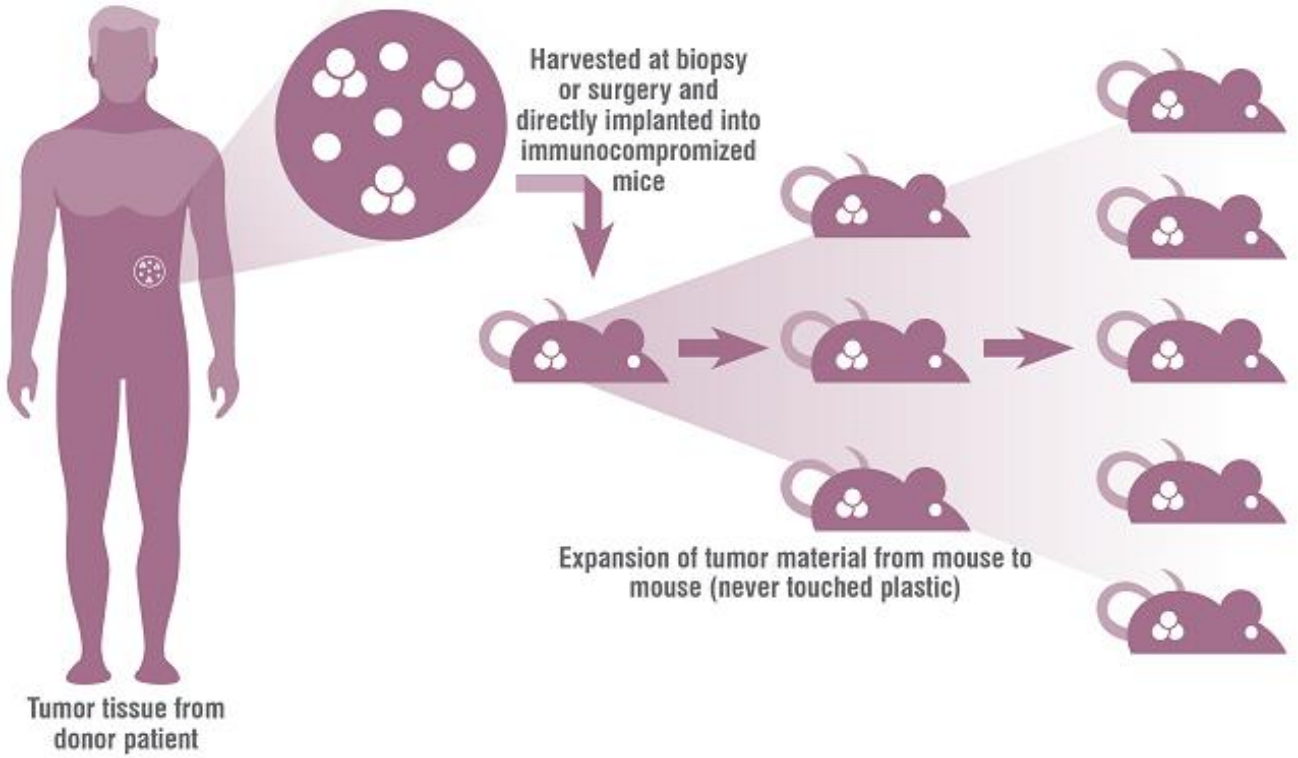
Challenges in PDX models:

Implantation into immunodeficeint mice

- limited predictive value
- Do not take into account cancer-dependent immune phenotypes and immune responses following therapy
- Precludes assessment of arising therapies designed to **modulate immune function** (*e.g.*, immune checkpoint inhibitors α -CTLA-4, α-PD-1, α -PD-L1).

Despite the challenges to routine preclinical application, several PDX studies have proven effective in:

- recapitulating human outcomes
- exploring drug resistance mechanisms
- identifying targets for second-line treatment



Genetically engineered Mouse (GEM) models

Increasing understanding of the genetic alterations underlying tumorigenesis facilitated the generation of diverse genetically engineered cancer models

Generated through the introduction of **genetic mutations associated with particular human malignancies**

- gain-of-function oncogenes
- loss-of-function tumor suppressors

Flexibility to create models with precise molecular specificity

GEMs provide **the most complete representation of cancer development** (out of all murine cancer models)

- cancers develop from initiation through progression
- intrinsic stroma
- intact immune system

Cancers evolve within their **natural microenvironment**; therefore GEMs better capture:

- Overall disease properties
- Inter and intra-tumoral heterogeneity

Mice can be ‘‘humanized’’ by engineering the expression of human specific drug targets

Genetically engineered Mouse (GEM) models

Examples of GEMs

(i) The first GEM tumor models

- Established in the mid-1980s
- Mice harboring randomly integrated oncogenes under the control of a tissue-specific minimal promoter

(ii) Global tumor suppressor gene (TSG) knockout animals

(iii) Conditional models based on site-specific recombinase (SSR) systems

- Allow a spatially and temporally controlled introduction of human-cancer relevant mutations into mice
- Examples: Cre–loxP and Flp–FRT
- Inducibility is achieved via:
    - exogenous SSR delivery (*e.g.*, adenoviral Cre) to an accessible somatic tissue
    - tissue-specific expression of an SSR fused with a hormone-responsive nuclear receptor domain
    - use of the doxycycline-inducible Tet system

Genetically engineered Mouse (GEM) models

Limitations of GEMs

Reduced clonal heterogeneity compared to human tumors
Overexpression or inactivation of potent oncogenes/tumor suppressors bypasses major bottlenecks to malignant transformation

Preclinical evaluation of metastasis is challenging
Most GEM models must be sacrificed before developing metastatic disease due to heavy tumor load

The generation of GEM models is very expensive and time consuming

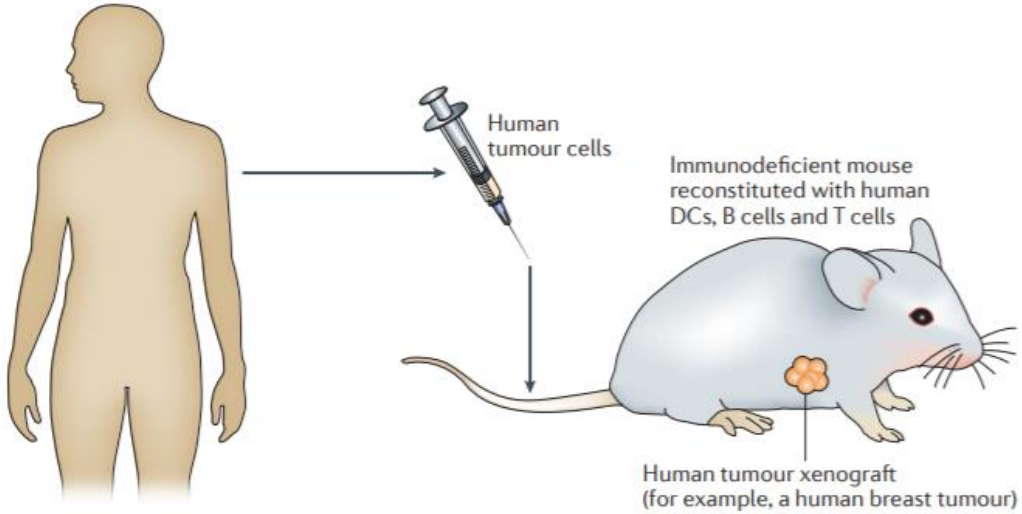
Humanized mouse models

Human tumor xenografts that are implanted in immunodeficient mice do not allow to perform a meaningful analysis of immunotherapies due to impaired/absent adaptive immune response in these mice

Solution:
Reconstituting of immunodificeint mice with a human immune system:

- Dendritic cells (DCs)
- B cells
- T cells

This system can be used to characterize the human immune response that is directed against a human cancer.



Potential applications:

- Optimization of immunotherapy
- Identification of crucial immune pathways that promote tumor growth.

Limitations

- Incomplete reconstitution of human immune response
- Impact of mouse-derived factors on human immune cells not fully defined
- Expensive

Ex vivo 3D models – Tumor spheroids

Model the 3D architecture of tissues that is absent in conventional culture formats

- Multicellular arrangement
- Extracellular matrix

Closely simulate biological and molecular properties of the original tumor tissue

Enable a more accurate evaluation of nanomedicines

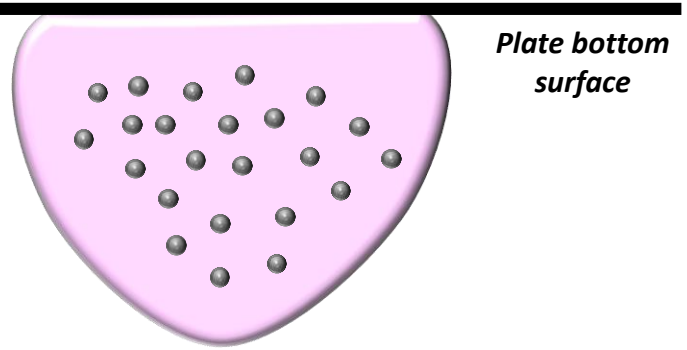
Experimental mouse tumour models: what can be learnt about human cancer immunology?
Dranoff G. et al., NATURE REVIEWS IMMUNOLOGY, 2012

Ex vivo 3D models – Tumor spheroids

1. Dispensing cells droplet onto a petri dish

2. Flipping the plate upside down

3. Spheroid formation



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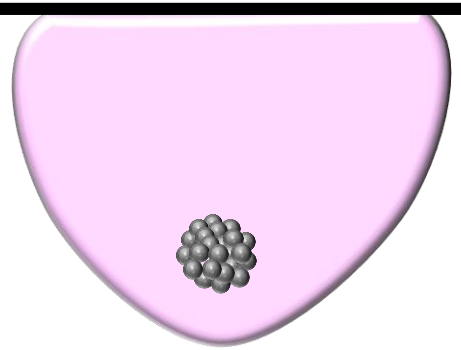
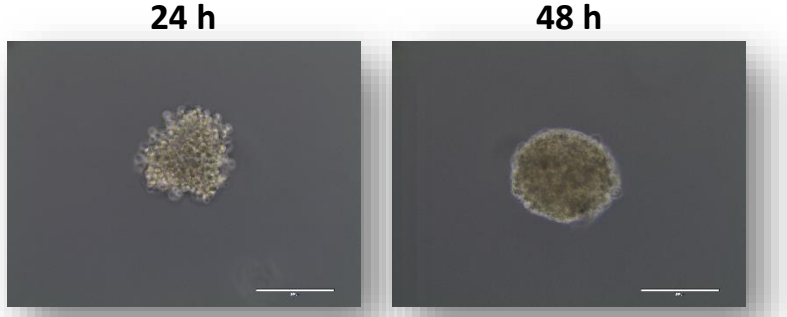
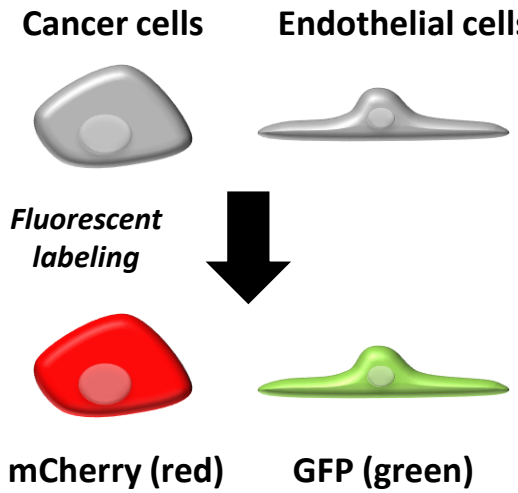


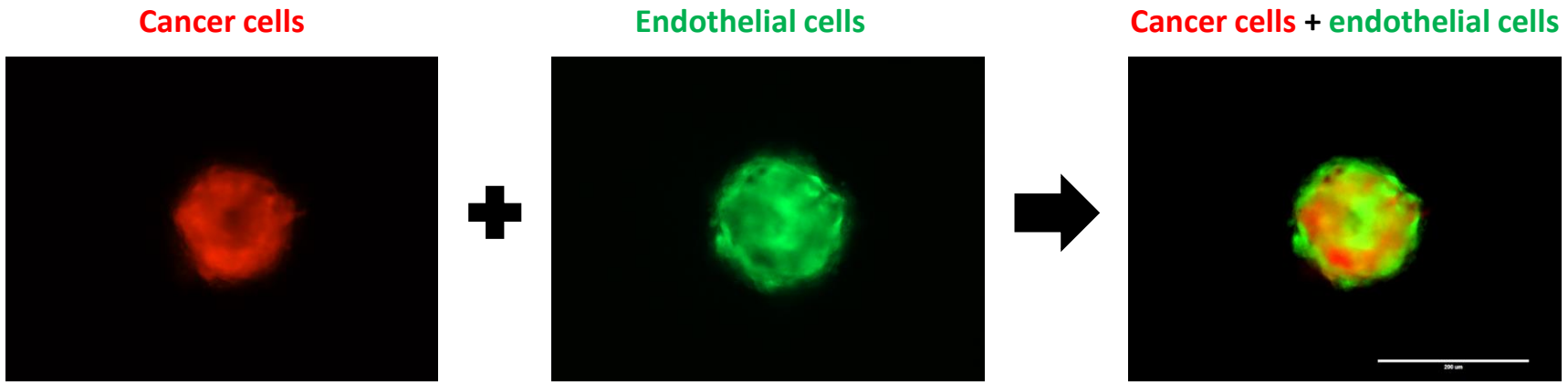
4. Follow up by microscopy



Ex vivo 3D models – Tumor spheroids

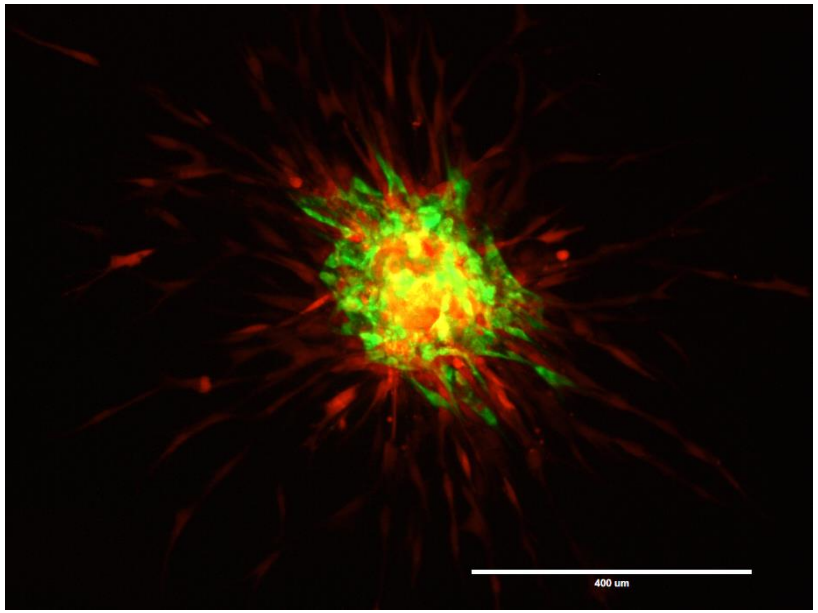
Co-culture of cancer cells with cells from the microenvironment





Ex vivo 3D models – Tumor spheroids

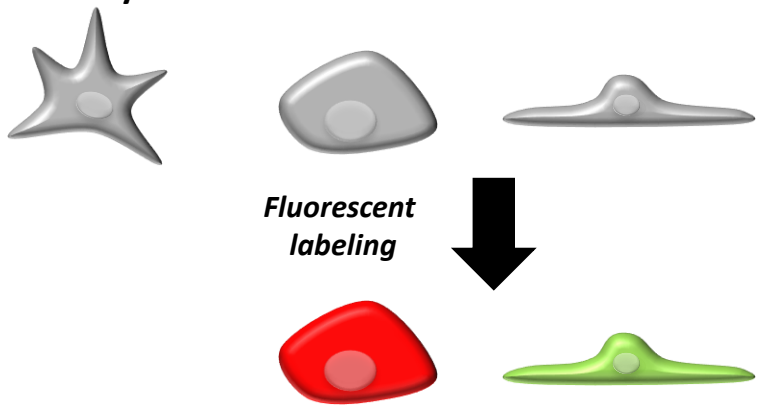
Embedding spheres into Matrigel to evaluate tumor properties



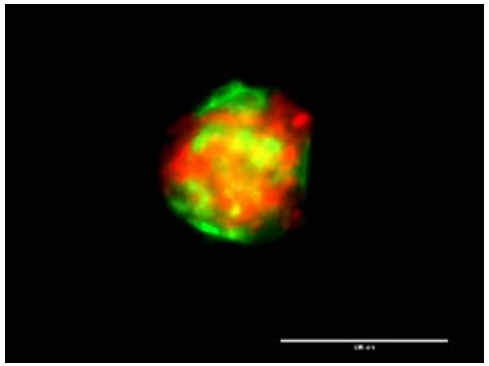
Tumor cells invasion
Endothelial cells sprouting

Ex vivo 3D models – Tumor spheroids

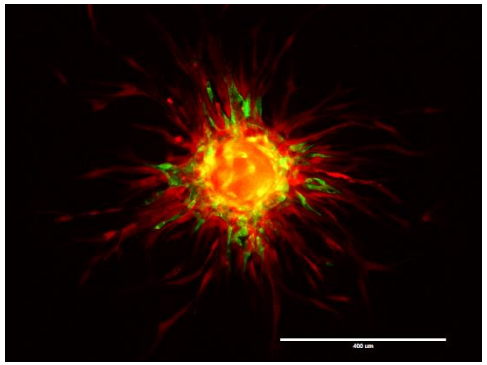
Co-culture of cancer cells with cells from the microenvironment



Cancer cells + endothelial cells + Astrocytes

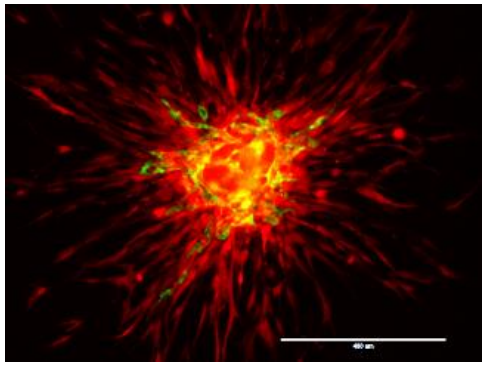


No astrocytes



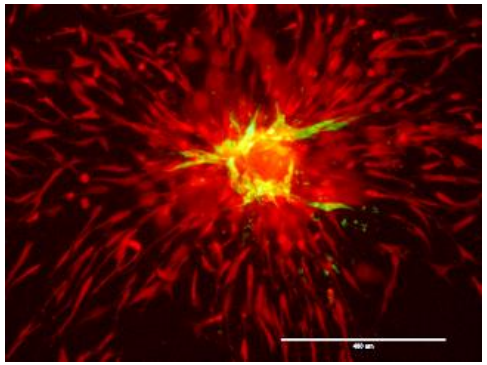
(1:2)

With astrocytes (x1)



(1:2:1)

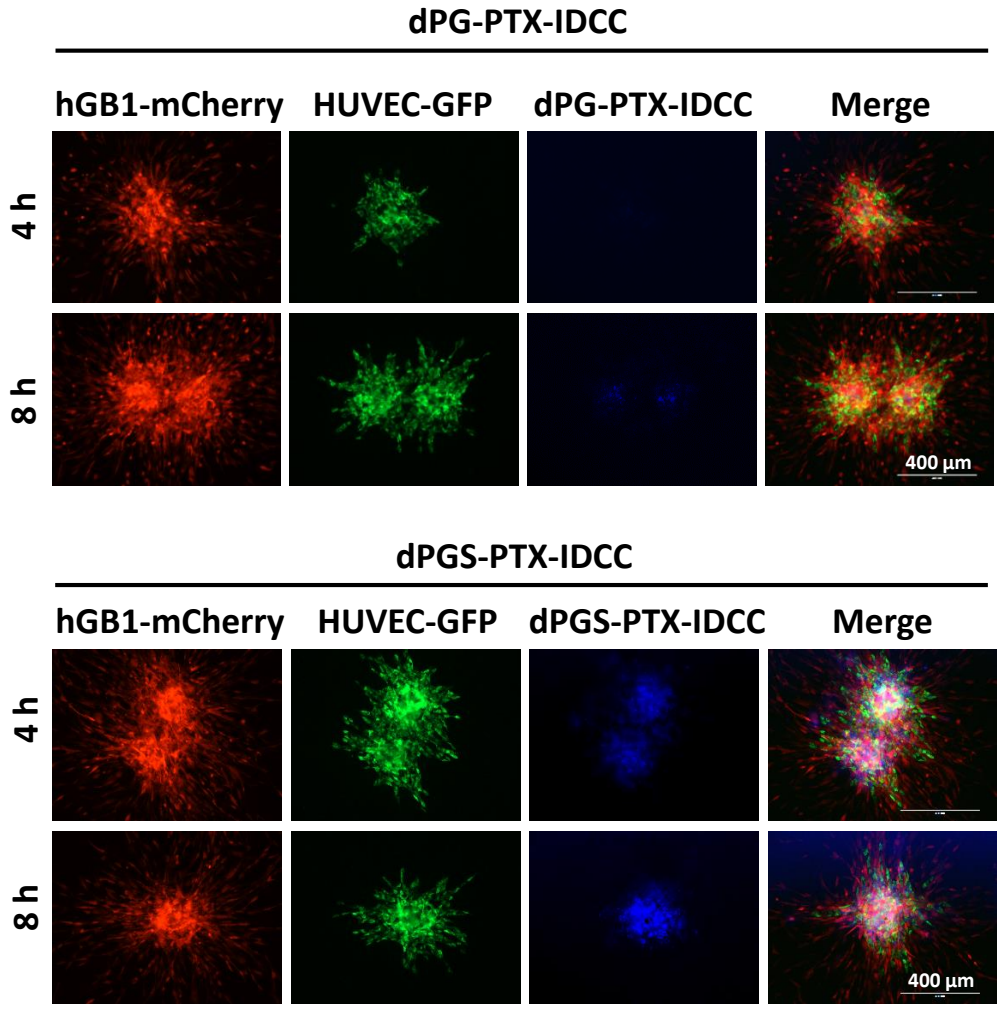
With astrocytes (X2)



(1:2:2)

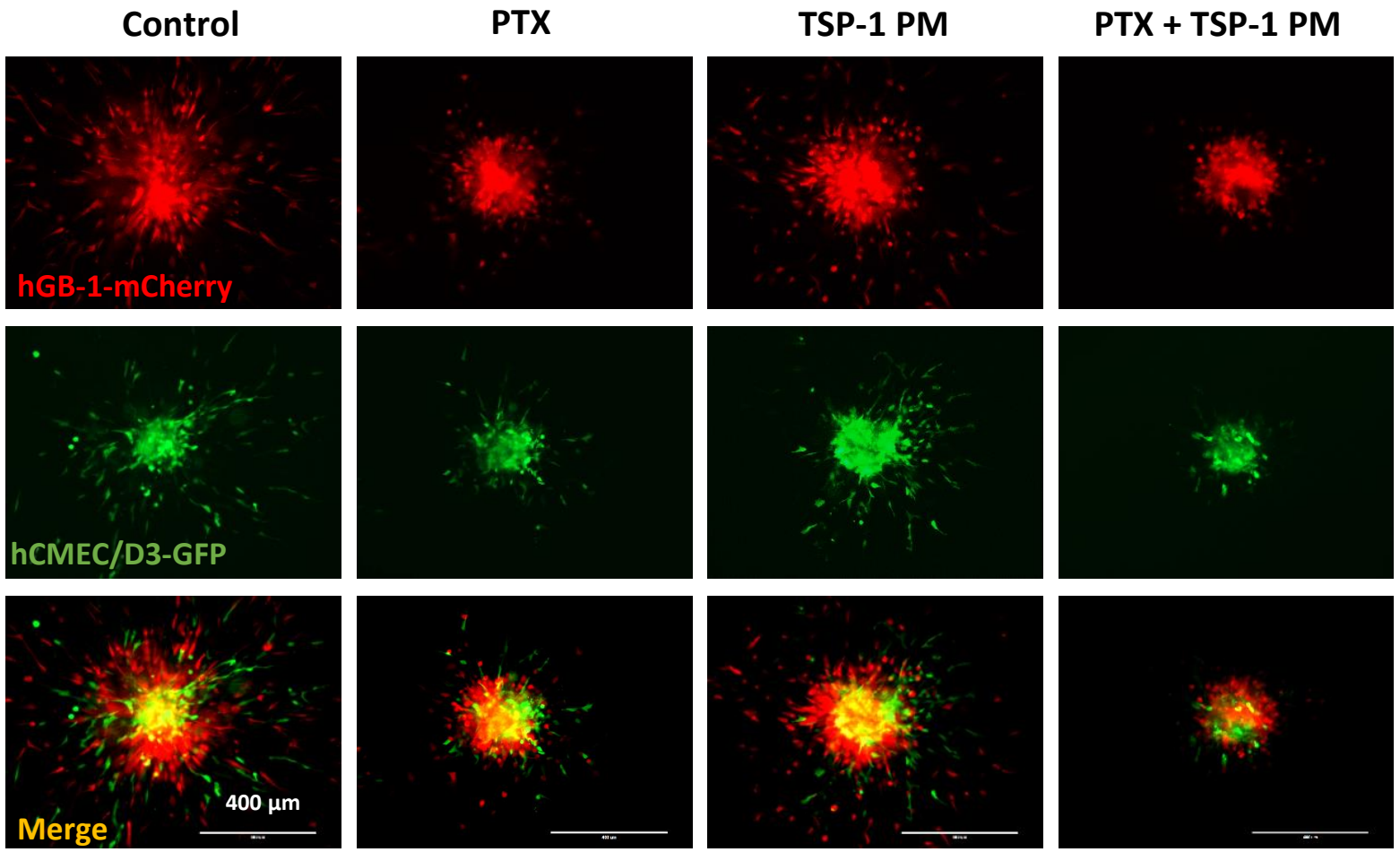
Ex vivo 3D models – Tumor spheroids

Harnessing the tumor spheroids model for nanomedicine research - Internalization kinetics of nanomedicines



Ex vivo 3D models – Tumor spheroids

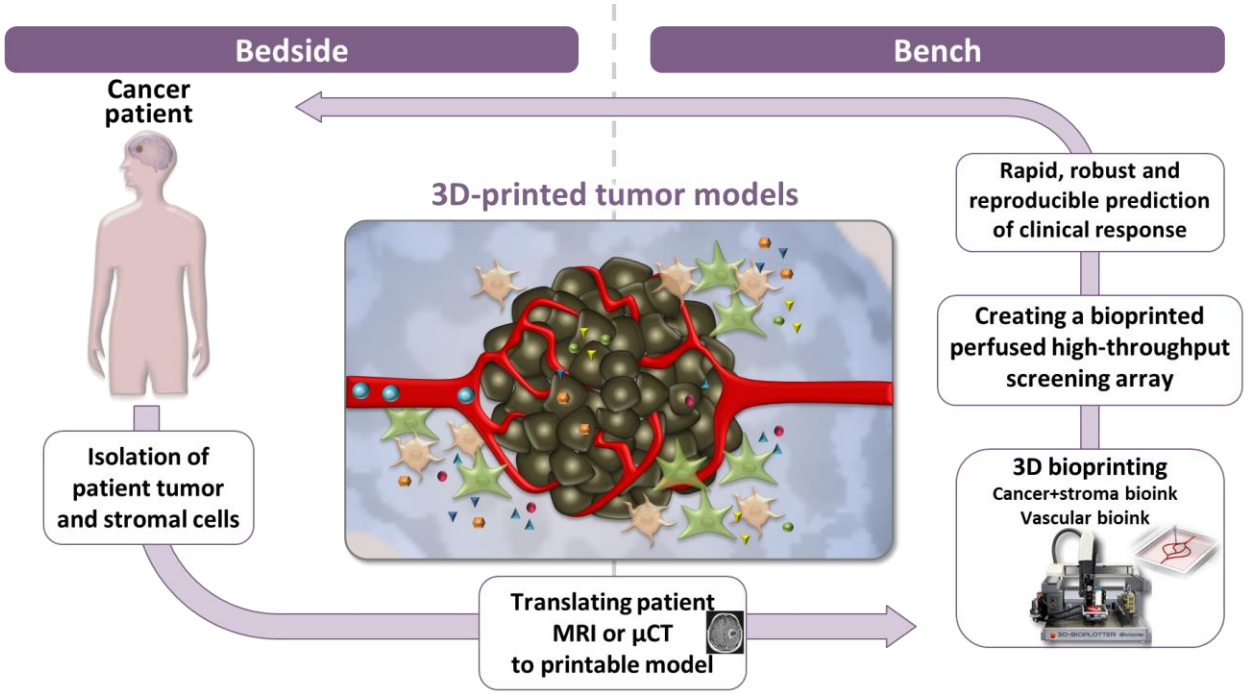
Harnessing the tumor spheroids model for nanomedicine research - Antitumor efficacy of combination therapy



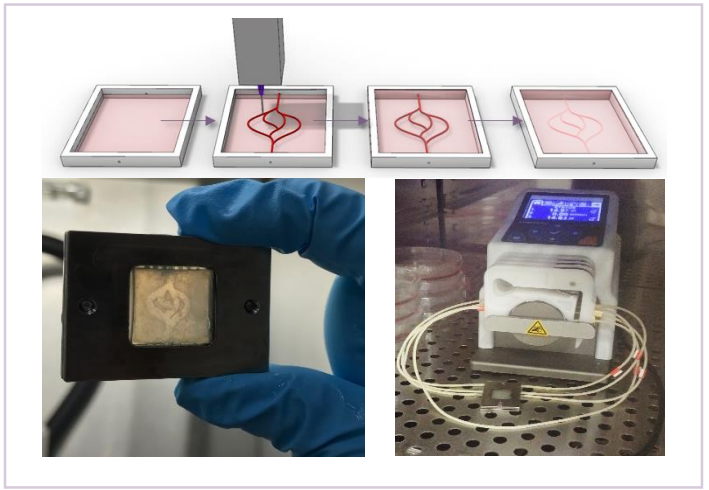
Ex vivo 3D models – 3D-bioprinting

Simulations of research, surgery and treatment

- 3D-bioprinted tumor model based on patient MRI or µCT scan
- Containing several types of cells, originated from the patient's biopsy
- The **4th dimension** – microfluidic channels with circulating drugs or immune system cells

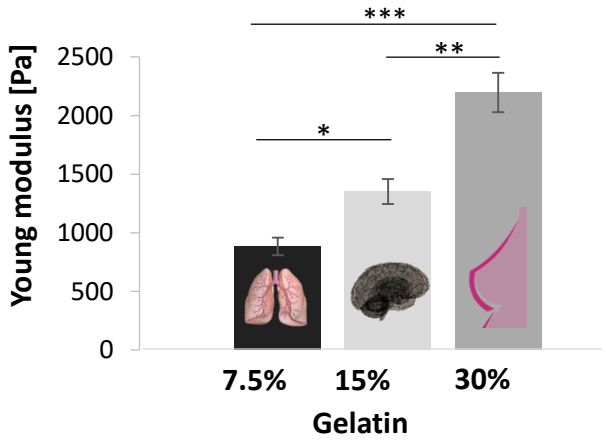


3D-printed microvascular network

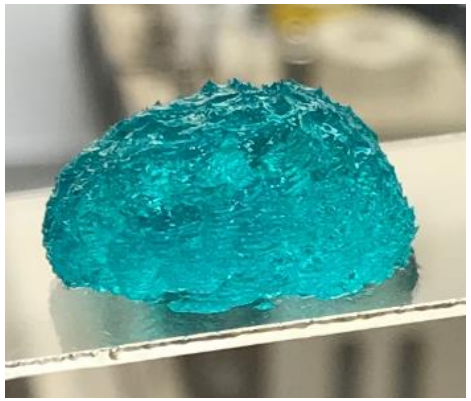


Ex vivo 3D models – 3D-bioprinting

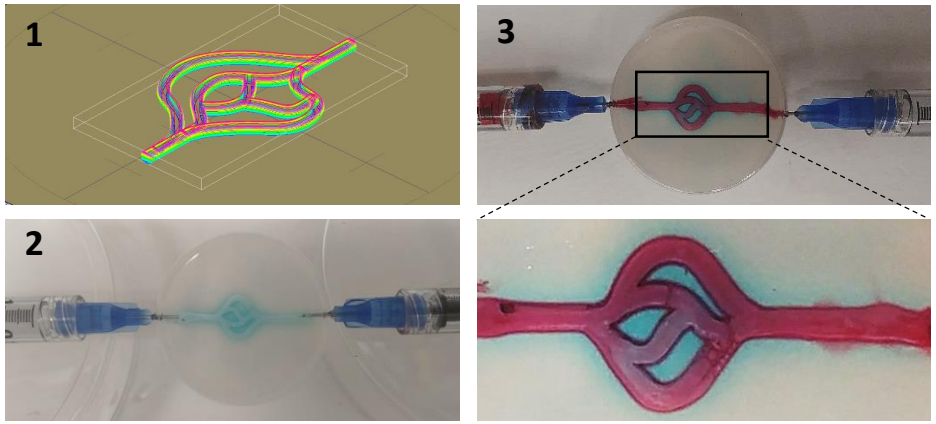
A. **Rheological characterization of fibrin hydrogels**



B. **3D-printed brain**

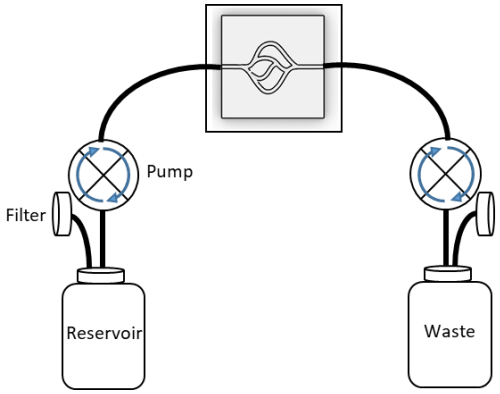


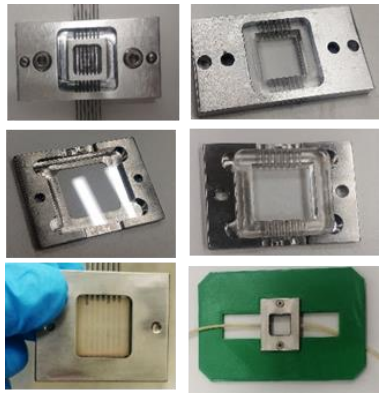
C. **3D-printed vascular network**

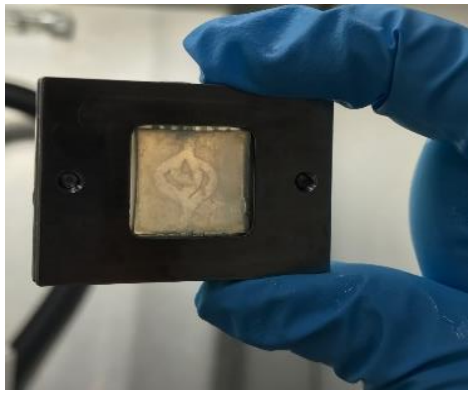


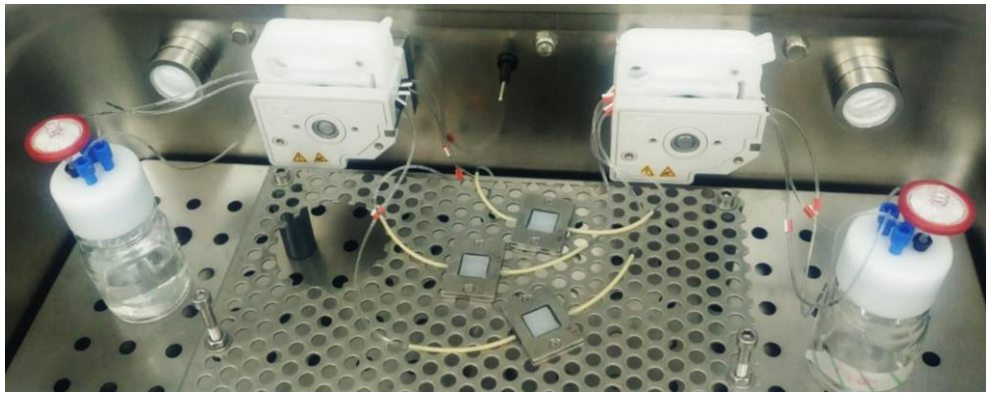
Ex vivo 3D models – 3D-bioprinting

Connecting the printed model to a perfusion system



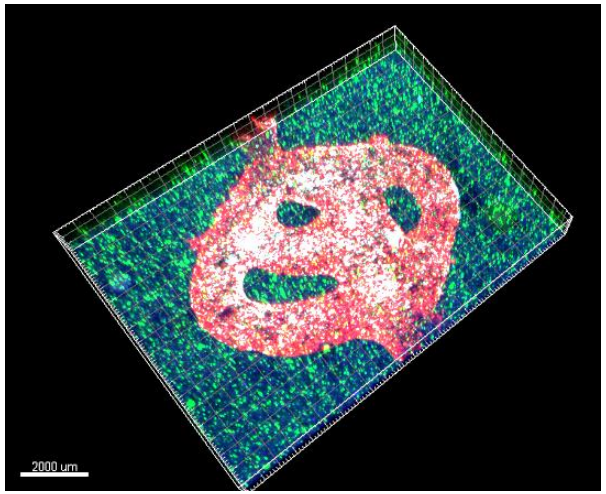




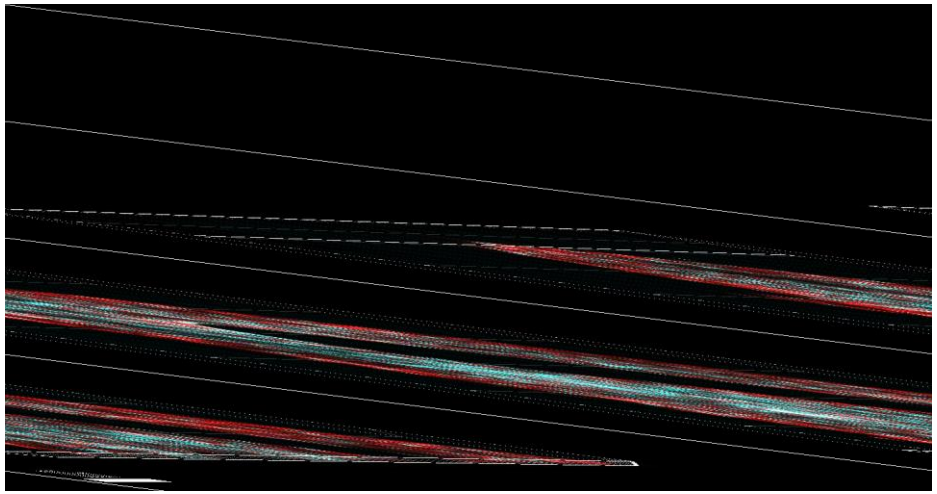


Chip engineering: 3D printing of the model directly into the chip> Connection to perfusion system> Monitoring via microscopy

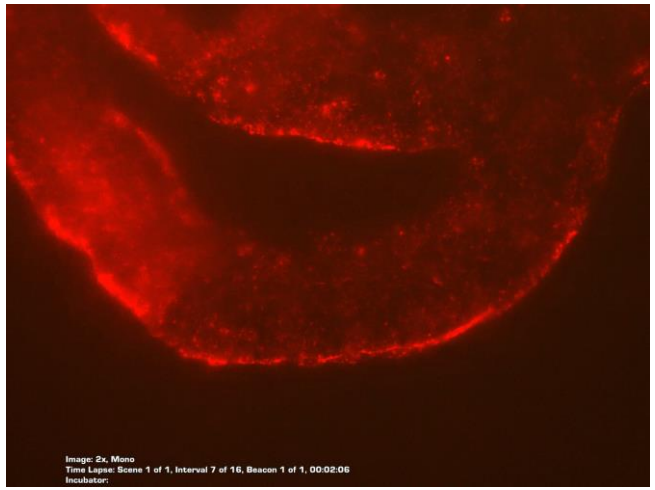
Under the microscope:



Endothelial cells hPericytes Cancer cells hAstrocytes hMicroglia



Perfusable vascular lumens



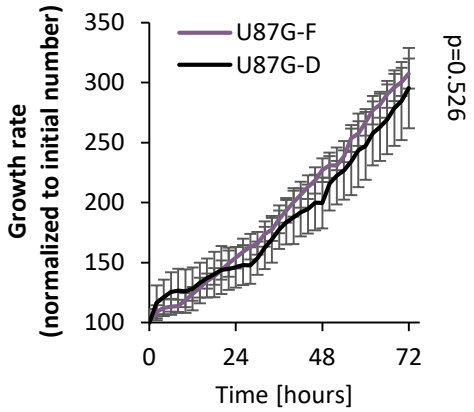
Perfusion of dextran FITC

Ex vivo 3D models – 3D-bioprinting

3D-bioprinted models recapitulate the *in vivo* setting

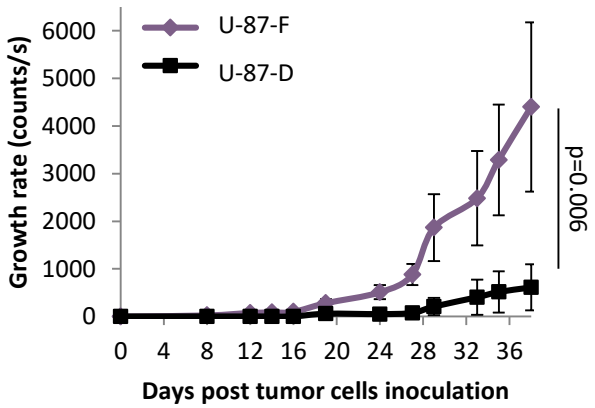
2D culture

No difference

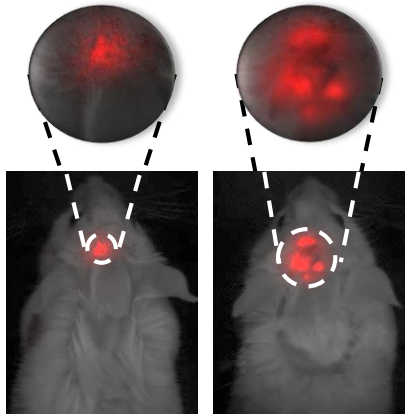


in vivo (orthotopic tumors)

Fast growing > Dormant



Fluorescence imaging (Maestro CRi™)



U-87-D U-87-F

3D-bioprinting

Fast growing > Dormant