In vivo models for cancer research - strategies for selecting the right model

Virtual THERACAT meeting

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Tel Aviv University

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Preclinical melanoma models

Chemically Induced Melanoma Models

Spontaneous melanoma is extremely rare in laboratory animal

Chemical carcinogens inducing melanoma

- 7,12-dimethylbenz(a)anthracene (DMBA) - immuno-suppressing, polycyclic aromatic hydrocarbon
- 12-O-tetradecanoylphobol-13-acetate (TPA) - phorbol ester, acts as a tumor promoter by activating protein kinase C

Chemical carcinogens are often used to accelerate melanoma development in combination with other modeling techniques, including:

- ultraviolet (UV) radiation
- Xenotransplantation
- genetic engineering

Advantages

- can be used to test immunotherapeutic strategies
- DMBA alone can induce nevi in pigmented mice - can be used to study mechanism(s) of malignant transformation

Limitations

- lack of clinical relevance to the human disease

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4013311/

Cell line-derived melanoma models

Site of primary tumor cell transplantation:

- Subcutaneous injection (ectopic)
- Intradermal injection (orthotopic)

Metastatic melanoma

Common site for melanoma metastases:

- Brain
- Lymph nodes
- Lungs
- Liver
- Abdomen

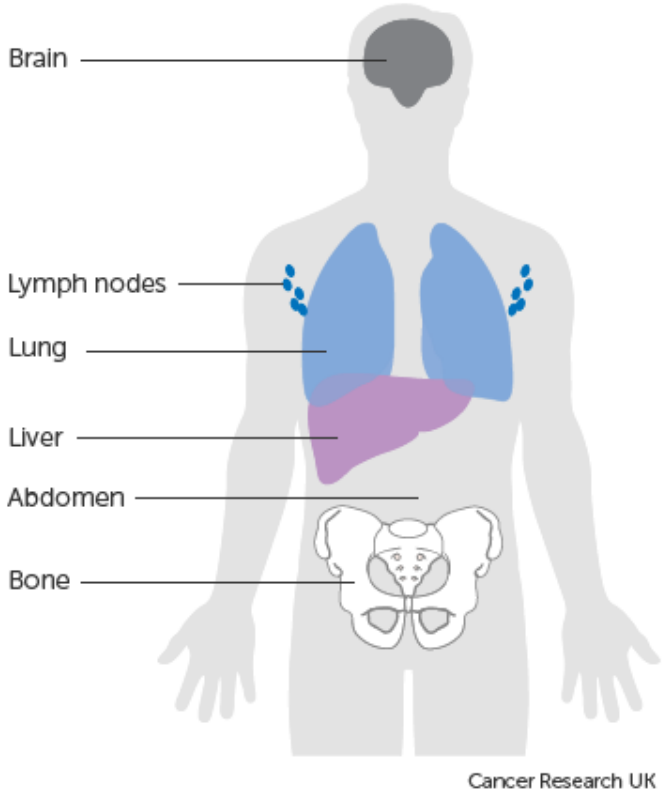
Establishing melanoma metastases in cells with low metastatic potential:

Intradermal injection followed by resection and monitoring metastatic spread

Direct injection of melanoma cells into ectopic sites*:

- Intravenously (experimental lung metastasis model)
- Femur or tibia
- Brain

Intracardiac injection (brain metastases)*



**Cells do not go through critical steps in the classical metastatic cascade (i.e., intravasation, survival in circulation, extravasation*

Models of melanoma metastases

Establishment of Melanoma Brain Metastases

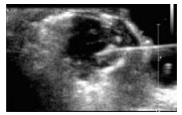
Orthotopic injection + resection

Orthotopic intradermal inoculation

Tumor resection

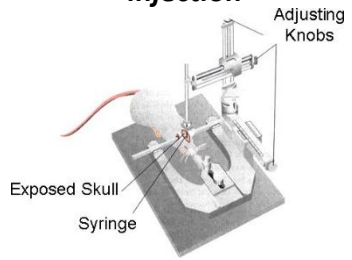
Intracardiac injection

Ultrasound-guided Intracardiac inoculation



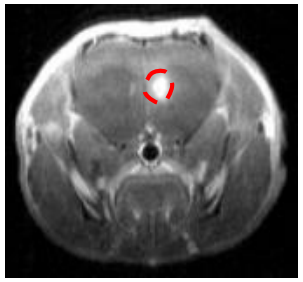
Direct injection to the metastatic site

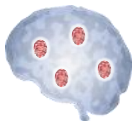
Stereotactic injection



4 weeks

MRI



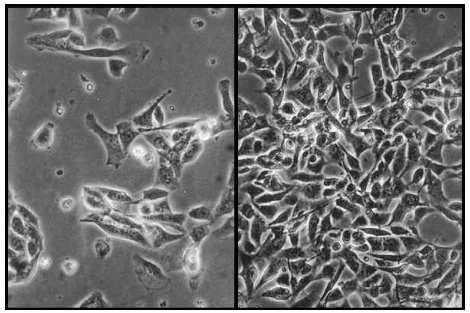


Presence of brain metastases

Syngeneic cell line-derived melanoma models

The most widely used syngeneic transplantation melanoma model is B16

- Derived from a chemically induced melanoma arising in a C57BL/6J mouse
- Express low levels of major histocompatibility complex class I (MHC I)
- Express high levels of melanoma-associated antigens (Gp100, tyrosinase related protein 2 (TRP2))
- **$BRAF^{wt}$**
- Form **spontaneous metastases** following primary tumor inoculation (primarily to the lungs)



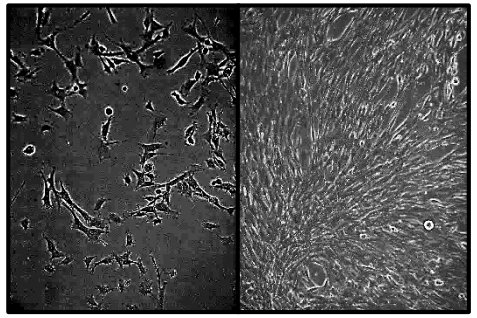
Syngeneic murine cell line GEM-derived xenograft (GDX) melanoma models

Murine cell lines commonly used for primary and metastatic syngeneic melanoma models:

D4M.3A

- Established from the conditional mouse model of metastatic melanoma: ***Tyr::CreER;Braf^{CA};Pten$^{lox/lox}$*, which recapitulates human disease**
- Express high constitutive **pERK**
- D4M cell lines **recapitulate human BRAF^{V600E} melanoma** *in vitro*.
- Transplantable into syngeneic host mice, thus **allowing immunological studies**
- Formation of metastases following primary tumor resection/intracardiac injection?

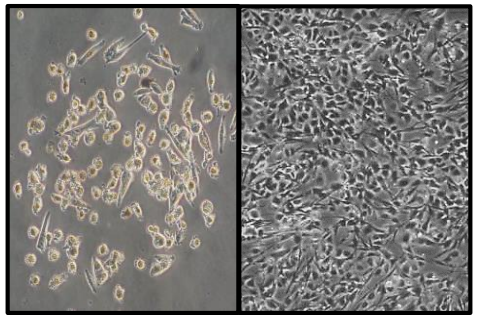
BRAF^{V600E}/PTEN



RET

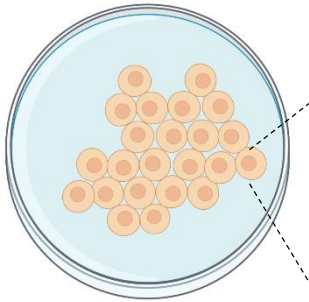
- Isolated from **Ret** transgenic mouse model of skin malignant **melanoma**
- Ret transgenic mice models is characterized by overexpression of the human transgene in melanin-containing cells
- Establish **spontaneous metastases** following orthotopic inoculation
- Form metastases following primary tumor resection/intracardiac injection (primarily to the brain)

MT1:RET



Human cell line-derived xenograft (CDX) melanoma models

Human cell lines

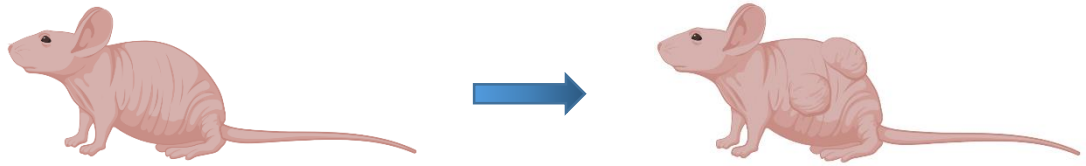


- A375
- A431
- B2905
- HCmel1274
- IGR-1
- MEL114433
- MEL-CLS
- MEWO
- MML-1
- WM115

Immuno-compromised Mouse Strains

Melanoma orthotopic xenograft: primary tumor

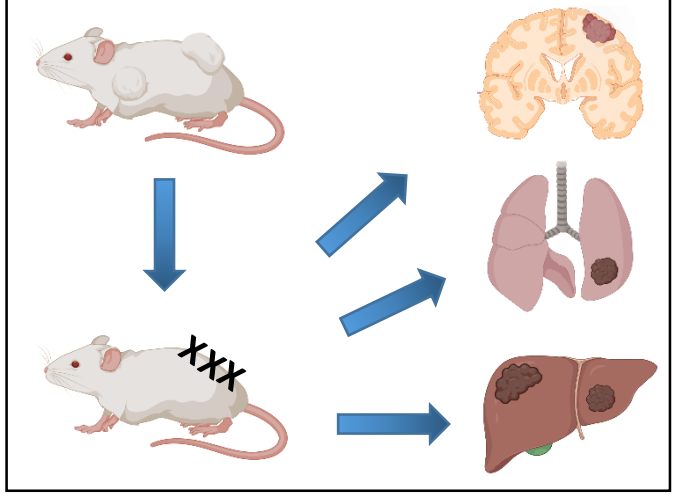
Nude



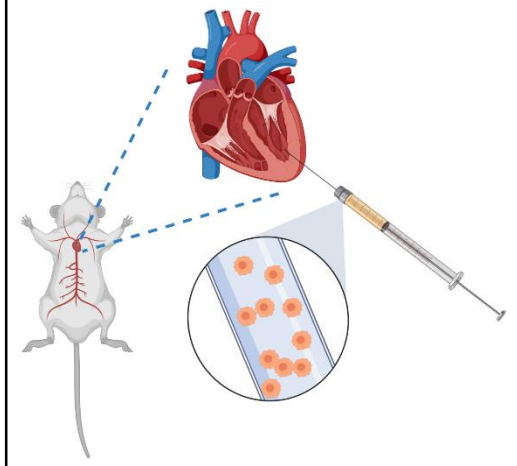
NOD/SCID

Melanoma Metastasis

Primary resection	Distant mets



Intracardiac or tail vein injection



Human cell line-derived xenograft (CDX) models

Human cell lines commonly used for melanoma xynogeneic models:

A375

- Human **melanoma cell line** initiated through explant culture of a solid tumor from a 54-year-old female
- **BRAF**^{V600E} mutant
- Generate rapidly-growing tumors following inoculation into athymic **Nude mice**

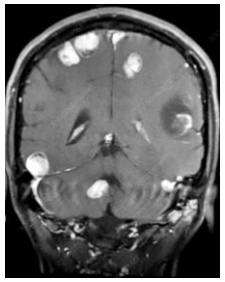
WM115

- Established from a metastatic site (right anterior leg) in a 55-year-old female with superficial spreading melanoma.
- **BRAF**^{V600D} mutant
- **PTEN loss of function** mutation
- **Wild type for N-RAS, c-KIT, and CDK4 genes**
- Produce xenograft tumors when injected into immunocompromised (**SCID**) mice
- Has **metastatic capabilities**

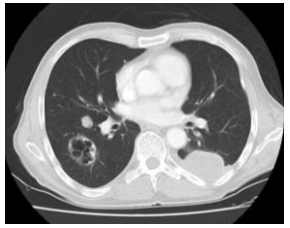
Patient-derived xenograft (PDX) melanoma models

Diagnosed Patient

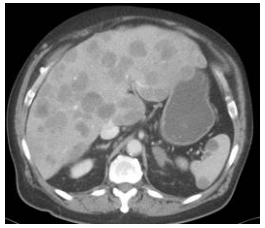
Metastasis of Melanoma



Brain



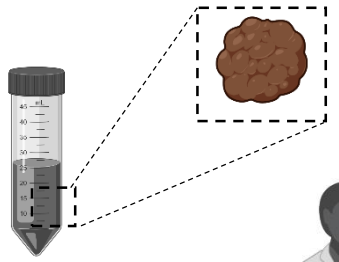
Lung



Liver

Surgeon

Resected mets chunk



Researcher

In vitro assays

2D/3D models

In vivo studies

Immuno-compromised mouse model

Genetically Engineered Mouse (GEM) melanoma models

Melanomas rarely develop spontaneously in mice

Those generated in GEMs are mainly dermal and share limited histologic similarities with human melanomas

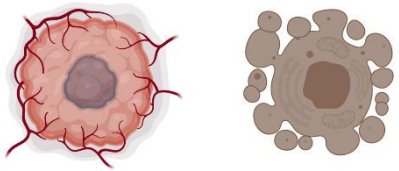
Melanoma Promoter → **Deletion/Activation of oncosuppressorgene/oncogene** → **Genetic manipulation (deletion/insertion)**

MT1
Try
Dct

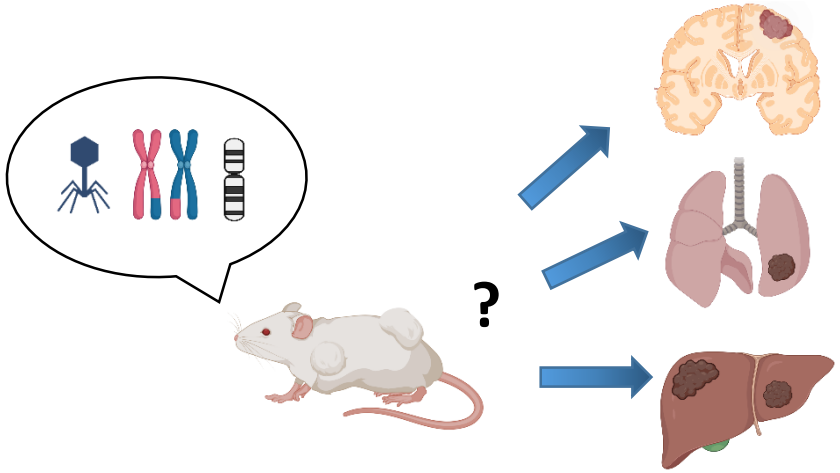
NRAS
BRAF
PTEN
P53
Cdkn2a
P19
RET

Infection (SV40)
TetON/TetOFF
Cre-ER^{T2}

Specific promotor activation



Genes for cell fate



GEM mouse

Preclinical breast cancer models

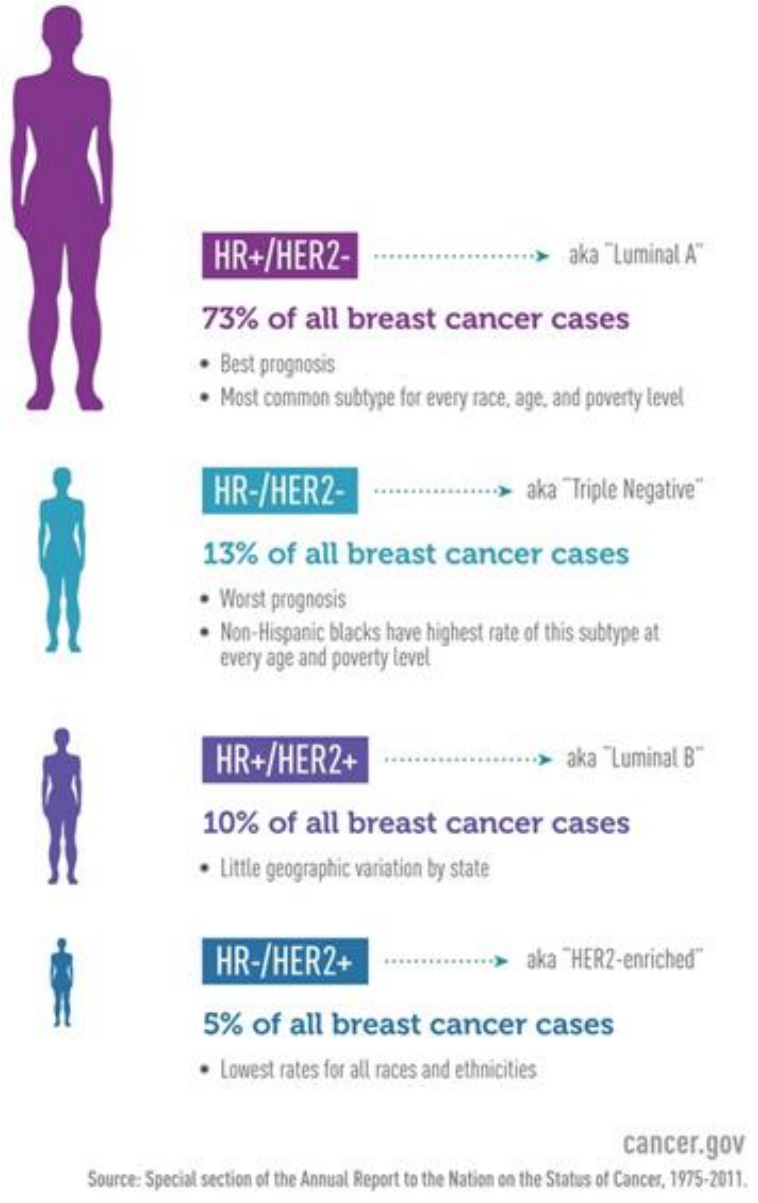
Breast cancer models

Breast cancer is treated based on the receptor status of the tumor:

- estrogen receptor (ER)
- progesterone receptor (PR)
- human epidermal growth factor receptor-2 (HER2)

The main molecular subtypes are termed

- Luminal A (ER/PR-positive)
- Luminal B (ER/PR-positive, higher histological grade than Luminal A)
- HER2-positive
- Triple-negative (ER/PR/HER2-negative)



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5399571/

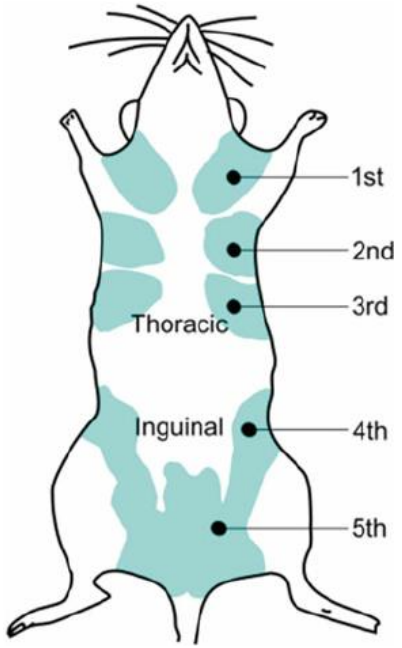
Cell-line-derived breast cancer models

Well-characterized cell lines representing the common clinical subtypes:

- luminal A (*e.g.* MCF-7, T47D)
- luminal B (*e.g.* BT474, MDA-MB-361)
- $HER2^+$ (*e.g.* SKBR3, HCC202)
- Triple negative (*e.g.* 4T1, BT20, MDA-MB-231, MDA-MB-468)

Site of transplantation:

- Subcutaneous injection (ectopic)
- Implanting cells in the mouse mammary gland (orthotopic) – considered to be more complicated than subcutaneous injection, but easier compared to orthotopic site injections of other cancer types.



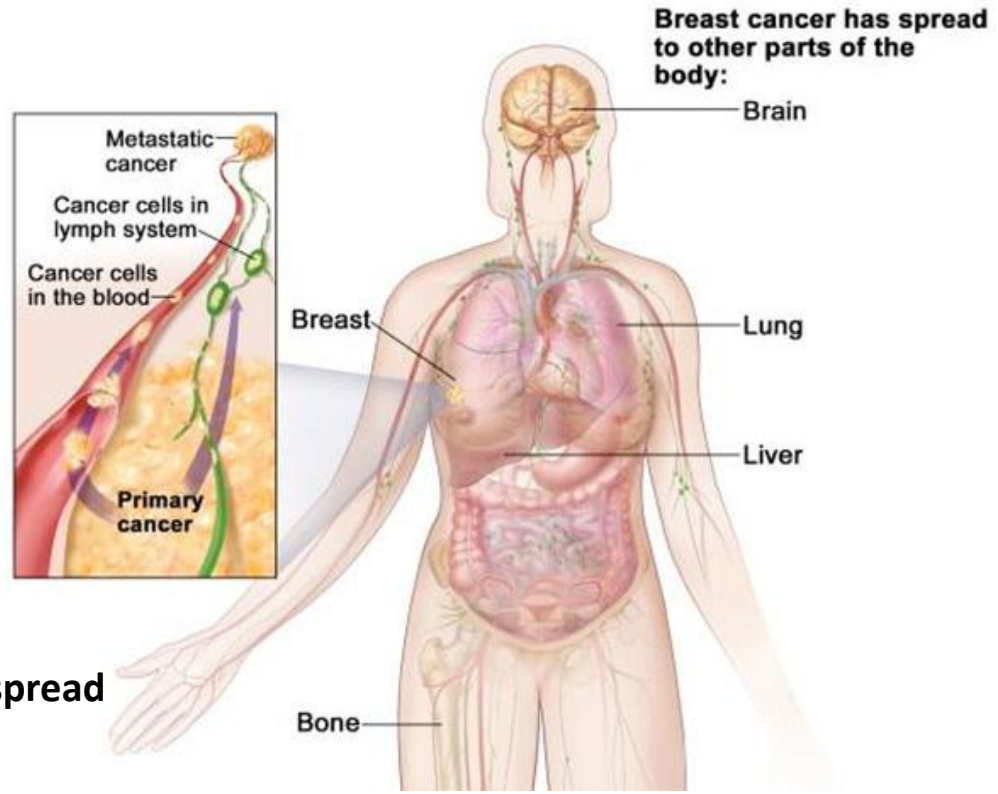
Mouse mammary fat pad

Metastatic breast cancer models

Metastatic breast cancer

Common site for breast cancer metastases:

- Brain
- Lung
- Liver
- Bone



Modelling metastases

- Spontaneous metastasis from CDX models is rare
- Few murine cell lines (*e.g.* 4T1) do metastasize in syngeneic models

Alternatives to spontaneous metastases models

Intramammary injection followed by resection and monitoring metastatic spread

Direct injection of breast cancer cells into ectopic sites*:

- Intravenously (experimental lung metastasis model)
- Femur or tibia
- Brain

Intracardiac injection (brain metastases)*

**Cells do not gothrough critical steps in the classical metastatic cascade (i.e., intravasation, survival in circulation, extravasation*

Metastatic breast cancer models

Establishment of Breast Cancer Brain Metastases

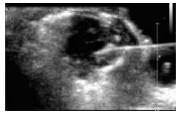
Orthotopic injection + resection

Orthotopic intramammary inoculation

Tumor resection

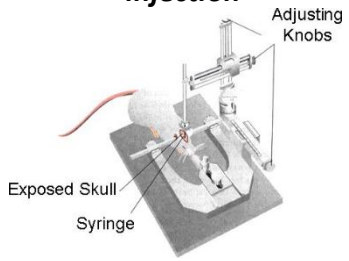
Intracardiac injection

Ultrasound-guided Intracardiac inoculation



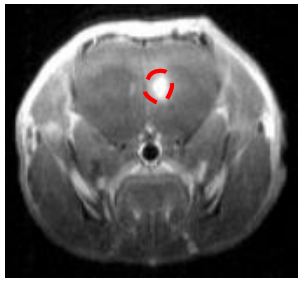
Direct injection to the metastatic site

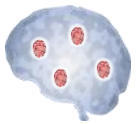
Stereotactic injection



4 weeks

MRI





Presence of brain metastases

Syngeneic murine cell line-derived breast cancer models

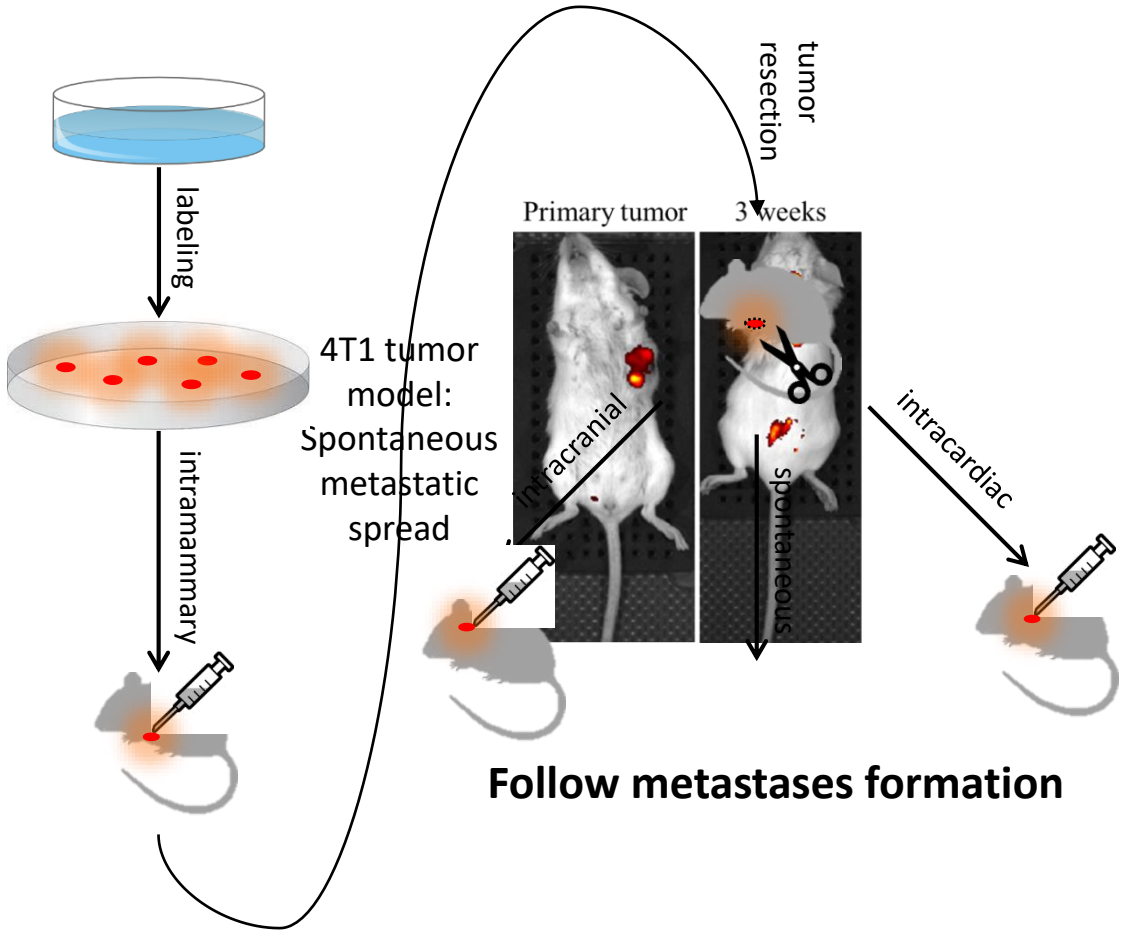
Murine cell lines commonly used for breast cancer brain metastases syngeneic models:

4T1- one of the most widely used breast cancer models.

- Triple negative (ER^-, PR^-, $HER2^-$).
- 6-thioguanine resistant cell line selected from the 410.4 tumor without mutagen treatment.
- When injected to BALB/c mice, forms tumors and spontaneously metastasizes to lung, liver, lymph nodes and brain.
- TP53 mutant.

EMT6

- A clonal isolate of EMT (the 25th animal passage of KHJJ, which was established from a BALB/cCRGL mouse after implantation of a hyperplastic mammary alveolar nodule).
- Forms solid tumors in some sublines of BALB/c mice.
- BRCA mutated.



Xenogeneic human cell line-derived xenograft (CDX) breast cancer models

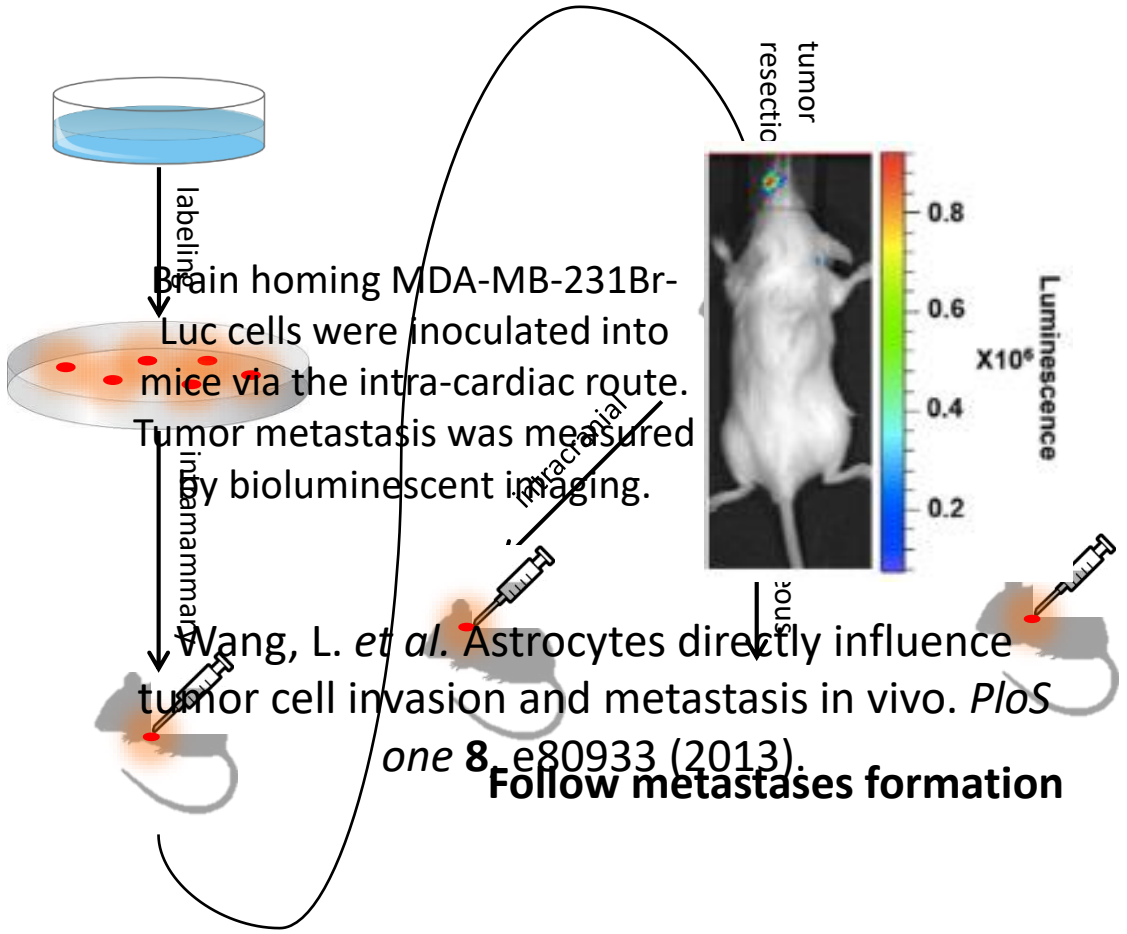
Human cell lines commonly used for breast cancer brain metastases xenogeneic models:

MDA-MB-231
- Triple negative (ER^-, PR^-, $HER2^-$).
- Derived from pleural effusion of a 51-year-old breast cancer patient.
- Express the WNT7B oncogene.
- Forms poorly differentiated adenocarcinoma (grade III) in nude mice.
- HER2 overexpressing mutant was generated.

MCF7
- Luminal A (ER^+, PR^+).
- Derived from pleural effusion of a 69-year-old breast cancer patient.
- Express the WNT7B oncogene.
- Grown in mice with slow release estrogen pellet.

CAL51
- Triple negative (ER^-, PR^-, $HER2^-$).
- Derived from pleural effusion of a 45-year-old breast cancer patient.

HCC1954
- HER2 positive (ER^-, PR^-, $HER2^+$).
- Derived from mammary gland of a 61-year-old breast cancer patient.



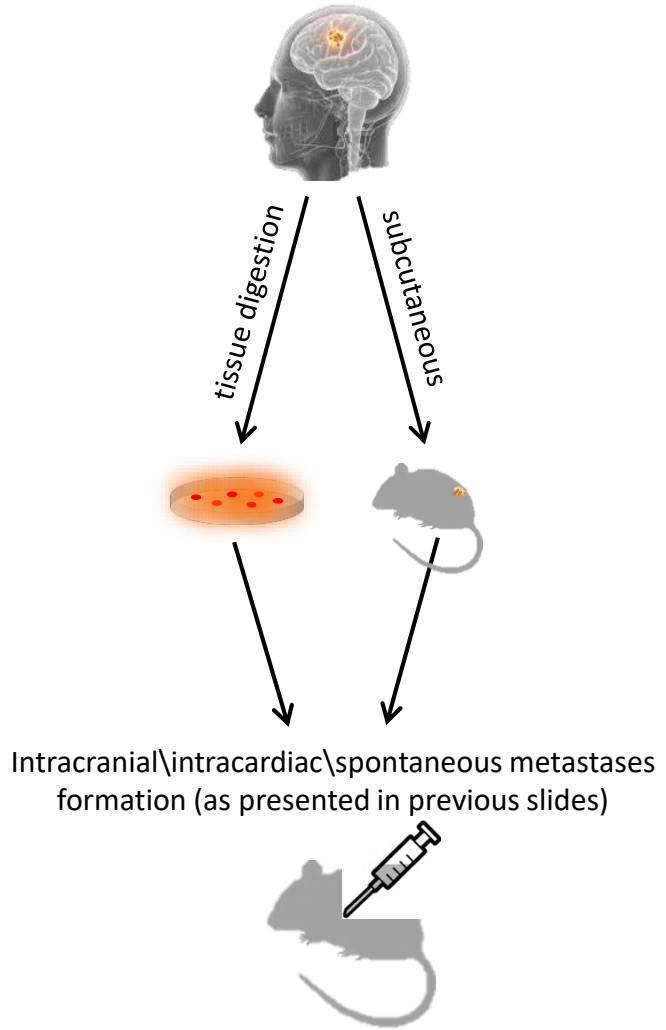
Brain homing MDA-MB-231Br-Luc cells were inoculated into mice via the intra-cardiac route. Tumor metastasis was measured by bioluminescent imaging.

Wang, L. *et al.* Astrocytes directly influence tumor cell invasion and metastasis in vivo. *PloS one* **8**, e80933 (2013).

Follow metastases formation

Patient-derived xenograft (PDX) breast cancer models

- Isolated directly from brain metastases of breast cancer patients
- Can be grown subcutaneously in immunocompromised mice for many generations
- Can be used to generate new brain tropic breast cancer cell lines
- Reflects the molecular and genetic characteristic of the patient

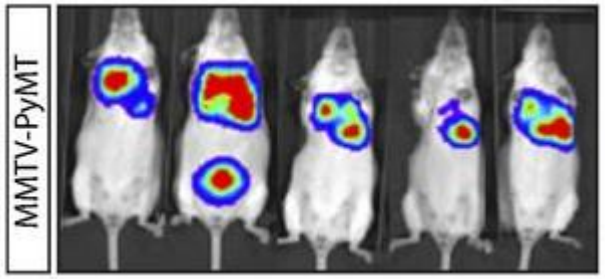


Genetically engineered Mouse (GEM) breast cancer models

Transgenic mice commonly used for breast cancer brain metastases models:

MMTV-PyMT

- MMTV-PyMT transgenic mice express the Polyoma Virus middle T antigen under the direction of the mouse mammary tumor virus promoter/enhancer.
- Hemizygous MMTV-PyMT females develop palpable mammary tumors which metastasize to the lung. These mice have high penetrance of early onset of mammary cancer compared to other mammary tumor models.
- This strain can be used as a platform to alter the tumor microenvironment.



bioluminescence imaging of MMTV-PyMT mice 4 wk after i.v. injection of VO-PyMT-Luc-GFP probing cells.

Conditional GEM - Blg-Cre;Brca1$^{fl/fl}$;p53$^{fl/fl}$

- **Cre/*loxP* system** - tissue-specific promoter drives expression of Cre recombinase (*e.g. Blg-Cre*) within the mammary gland
- Elicit recombination of DNA between *loxP* sites
- Introduced into the coding region of tumor suppressors such as *p53* and *Brca1*

Owyong, M. *et al.* MMP9 modulates the metastatic cascade and immune landscape for breast cancer anti-metastatic therapy. *Life Science Alliance* **2**, e201800226, doi:10.26508/lsa.201800226 (2019).

Preclinical glioblastoma models

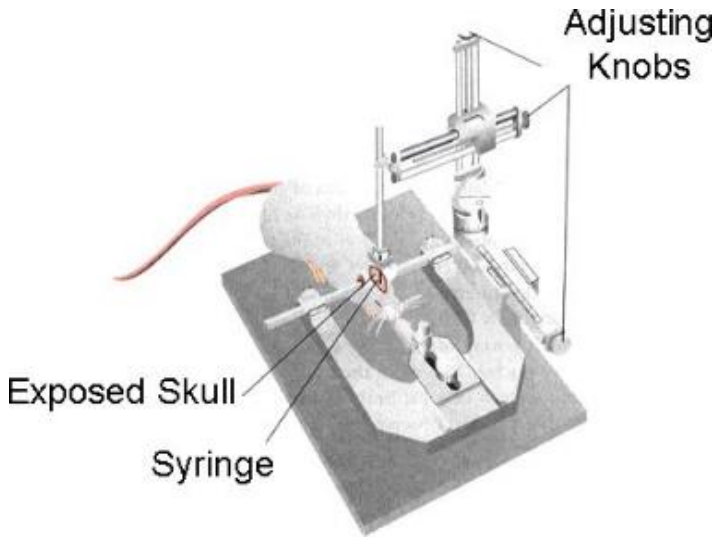
Cell line-derived glioblastoma models

Site of primary tumor cell transplantation:

- Subcutaneous injection (ectopic)
- Intracranial injection (orthotopic)

Intracranial inoculation of tumor cells

- Direct injection into the brains of mice using stereotactic devices
- Injection into precise anatomic locations
- Monitoring tumor growth intravitally by MRI (preferably), CT or using bioluminescence/fluorescence imaging devices
- Neurological symptoms, specifically- abnormalities of gross motor function, are often used as an endpoint for tumor latency.
- Time consuming and complex process



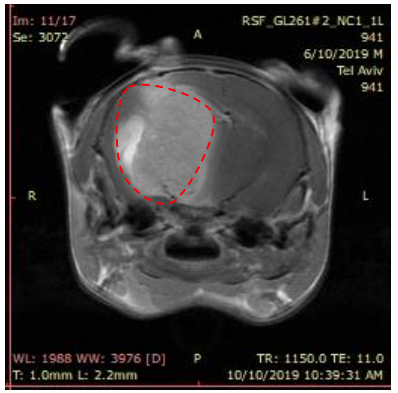
Syngeneic cell line-derived glioblastoma models

Murine cells line commonly used for syngeneic glioblastoma models:

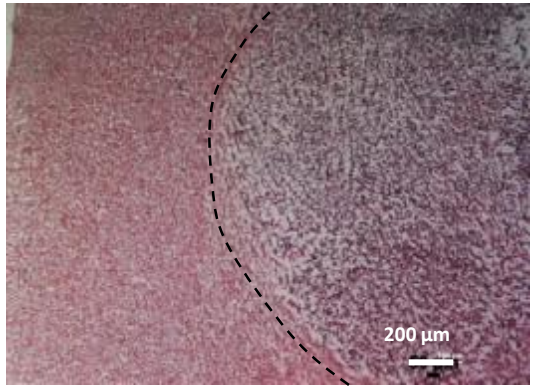
GL261

- Was generated by injection of the alkylating agents 3-methylcholantrene into C57/BL6 mice
- Establish rapidly-growing tumors in C57/BL6 mice
- Partially immunogenic
- Carry KRAS and TP53 mutations, which resemble the clinical settings

MRI



H&E



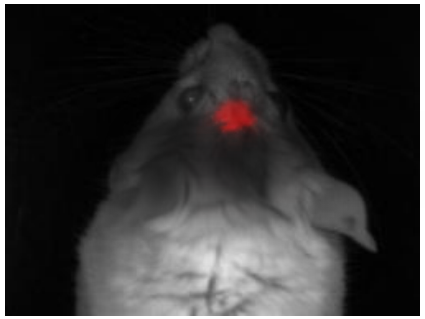
Human cell line-derived xenograft (CDX) glioblastoma models

Human cells line commonly used for syngeneic glioblastoma models:

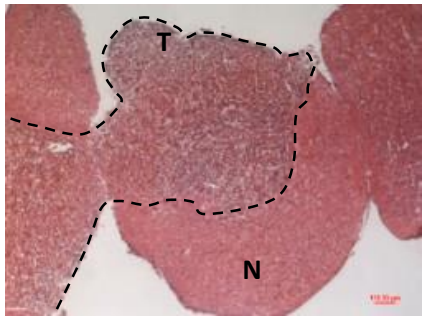
U87-MG

- Was generated from glioblastoma patients and is commercially available
- Human cell line which develops glioblastoma-like tumors when intracranially injected into immunocompromised mice
- The most studied glioblastoma cell line in the past few decades
- Exhibits less infiltrative phenotype with a disrupted BBB
- Considered to be sub-cloned due to a genetic drift

Fluorescence signal (maestro)



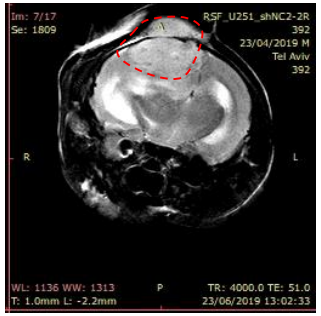
H&E



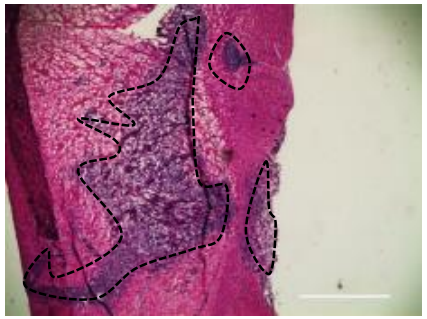
U251

- Was generated from glioblastoma patients and is commercially available
- Develops glioblastoma-like tumors when intracranially injected into immunocompromised mice
- U-251 MG and U-373 MG were found to have the same origin but present different drug-sensitivity
- Was used to **evaluate BCNU and rapamycin** treatments
- Orthotopic U251 mice xenografts show **infiltrative tumors with high similarity to the human disease**

MRI

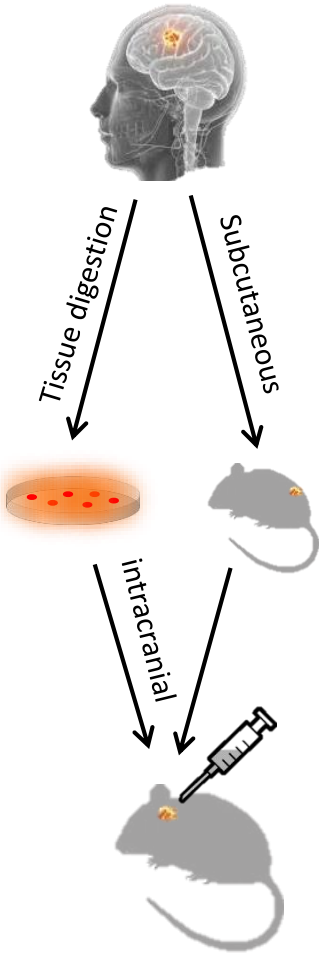


H&E

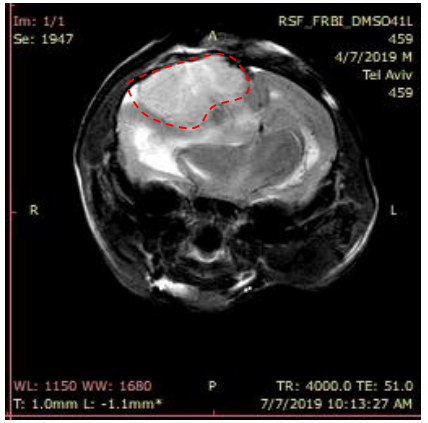


Patient-derived xenograft (PDX) glioblastoma models

- Isolated directly from glioblastoma patient
- Can be grown subcutaneously in immunocompromised mice for many generations
- Can be used to generate new glioblastoma cell line
- Reflects the molecular and genetic characteristic of the patient



MRI



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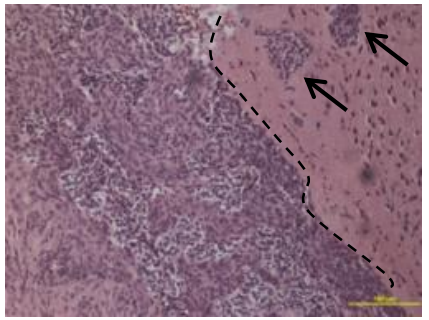
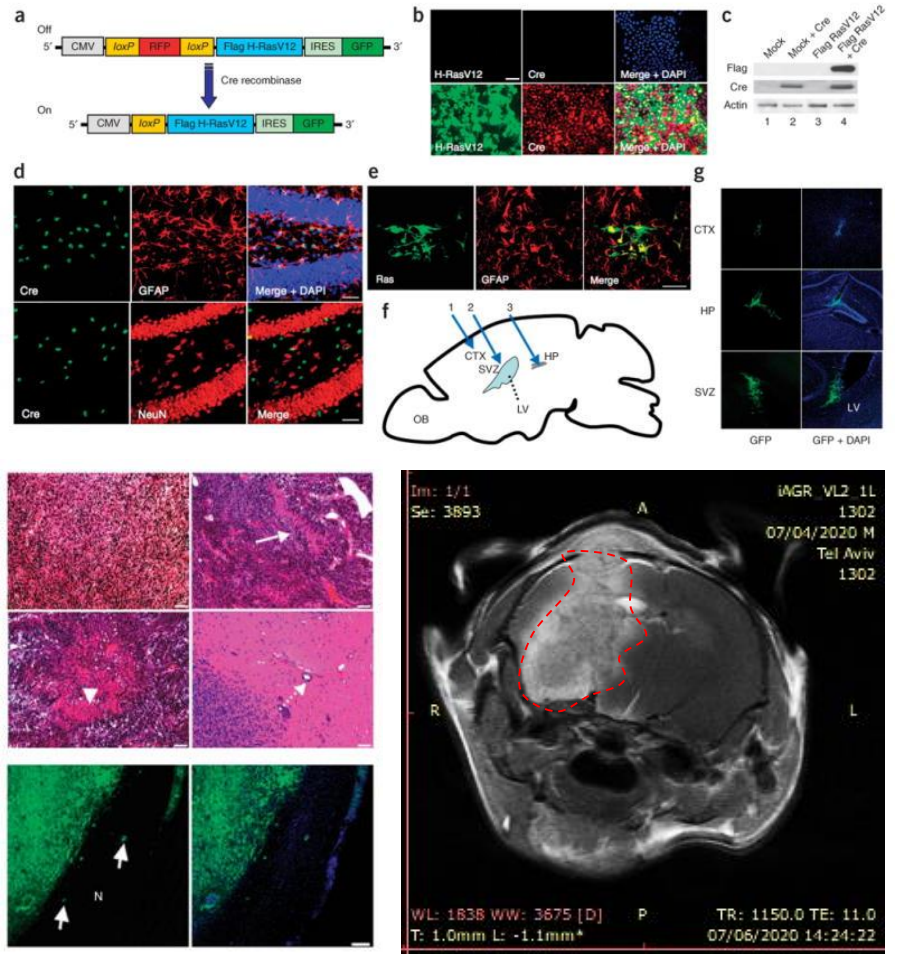


Lenti-viral vector induced murine glioblastoma models

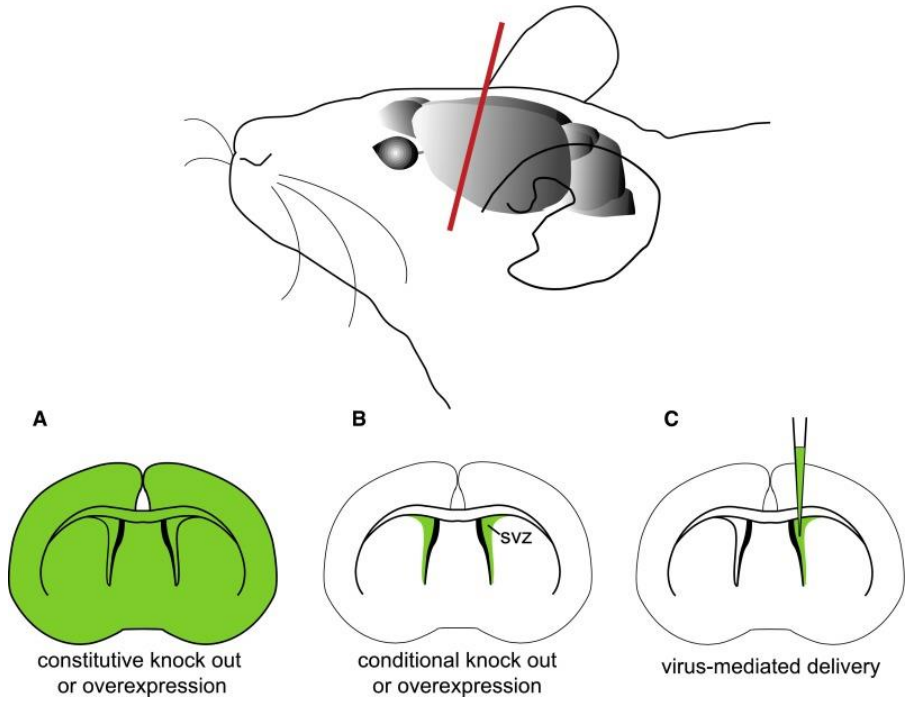
- Generated by injecting Cre-*loxP*–controlled lentiviral vectors expressing oncogenes
- Cell type- or region-specific
- Can be used to generate specific glioblastoma molecular subtype cell lines (mesenchymal, proneural and classical)
- Following genetic manipulations can be studied in culture or injected intracranially into immunocompetent mice
- Transplantation of brain tumor cells into naive recipient mouse brain resulted in the formation of glioblastoma–like tumors which contained glioma cancer stem cells



Marumoto, T., et al., Development of a novel mouse glioma model using lentiviral vectors. Nature medicine, 2009. 15(1): p. 110-116.

Genetically-modified mouse (GEM) glioblastoma models

- Suitable for cell-origin studies and to investigate tumor-initiating oncogenic processes
- Development of de novo tumors, which may offer more reliable model for tumor-host interaction studies
- Can prove to be valuable tools for testing targeted therapies
- Can be used to generate specific glioblastoma molecular subtype mouse models (mesenchymal, proneural and classical)
- Have the advantage of using defined genetic alterations to induce tumor development de novo, in an immunocompetent host



Simeonova, I.; Huillard, E., In vivo models of brain tumors: roles of genetically engineered mouse models in understanding tumor biology and use in preclinical studies. Cellular and Molecular Life Sciences 2014, 71 (20), 4007-4026.

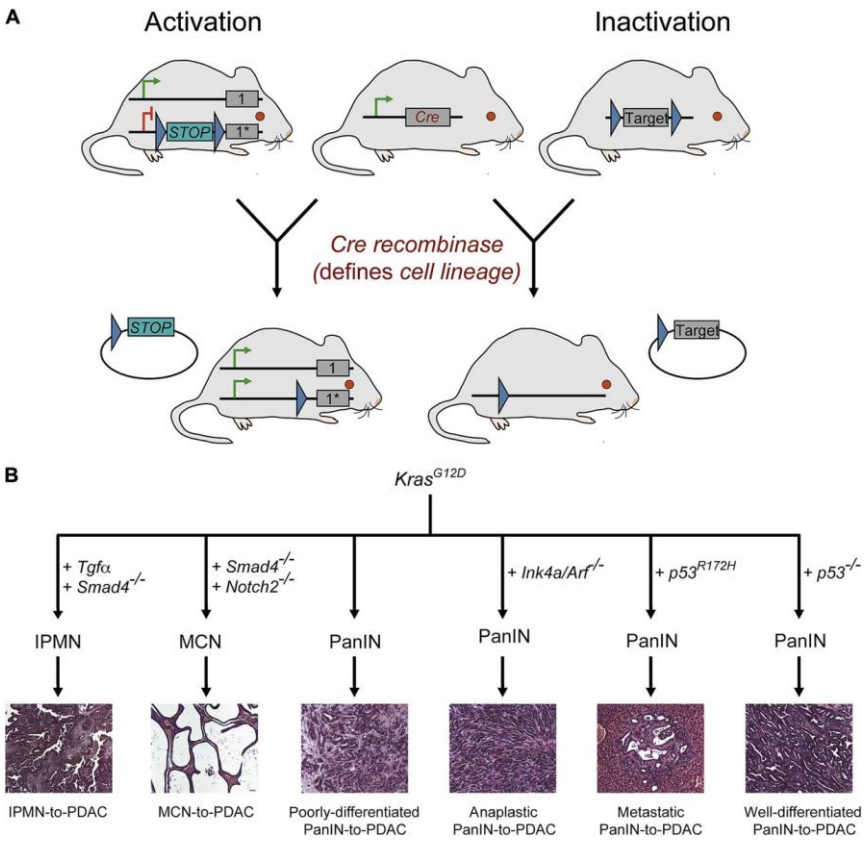
Preclinical pancreatic cancer models

Genetically engineered Mouse models (GEMM)

Transgenic KPC* mice

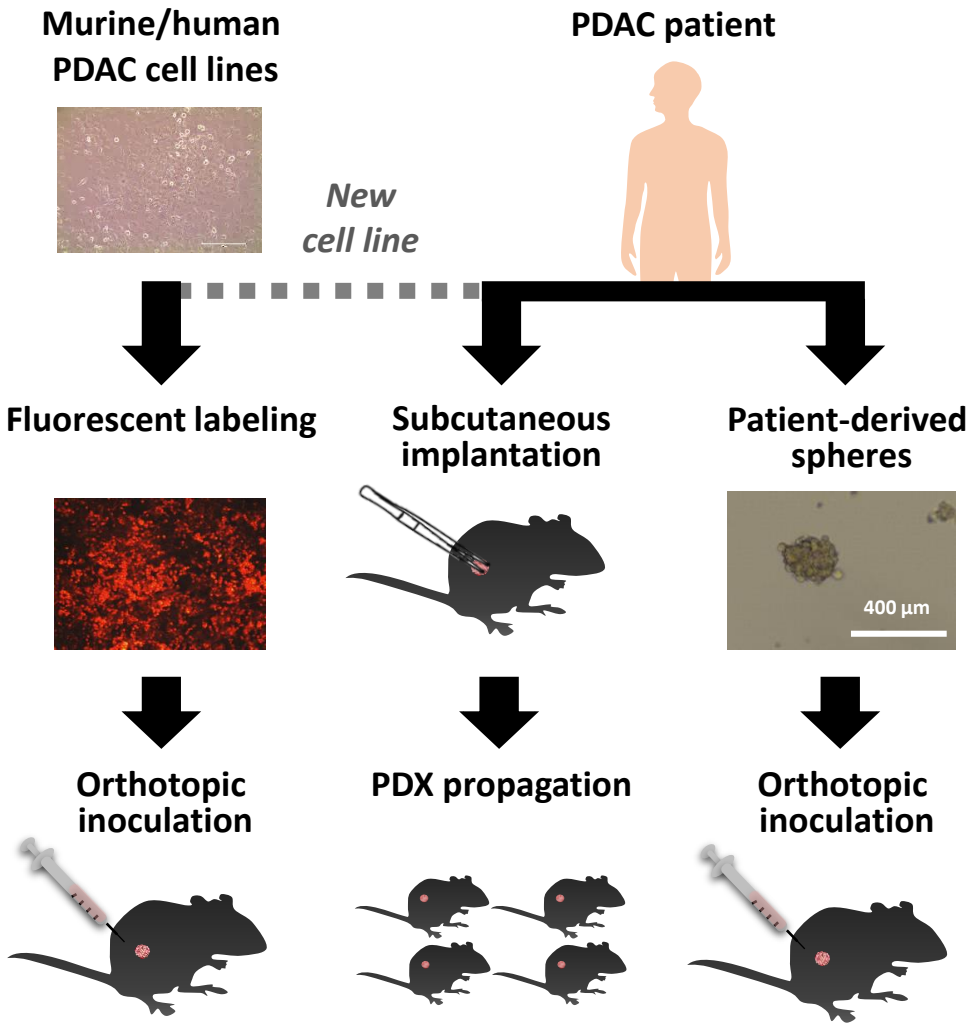
* K-ras$^{LSL.G12D/+}$; p53$^{R172H/+}$; PdxCre

- The most well-studied GEMM of PDAC
- Contains mutations in KRAS (KRASG12D) and TP53 (TP53R172H) genes, both driven by the pdx1-Cre transgene which specifically expressed the Cre recombinase in all cells of pancreas starting from an early phase of embryonic development.
- The mutations in both KRAS and TP53 genes are found in around 80% and 70% of all human PDAs respectively and generate non-functional proteins.
- In KPC model, tumors develop spontaneously with a dense desmoplasia and poor vasculature, similarly to human PDAC, thereby preserving the dynamics of tumor microenvironment.
- Metastases are observed in around 80% of KPC animals located primarily in the liver and lungs.
- Tumours present in KPC mice display many immune-histological markers of PDAC as well as possessing complex genomic rearrangements – a key sign of genomic instability.
- The GEMM intact immune system allows the study the immune response in PDAC and investigation of novel immune-therapies.
- The co-morbidities, cachexia, jaundice and ascites, associated with human PDCA are also observed in this model and most pancreatic tumours are resistant to chemotherapy.



Mazur PK, Siveke JT. Genetically engineered mouse models of pancreatic cancer: unravelling tumour biology and progressing translational oncology *Gut* 2012;**61:**1488-1500.

Pancreatic ductal adenocarcinoma (PDAC) preclinical mouse models



Syngeneic murine cell line-derived models

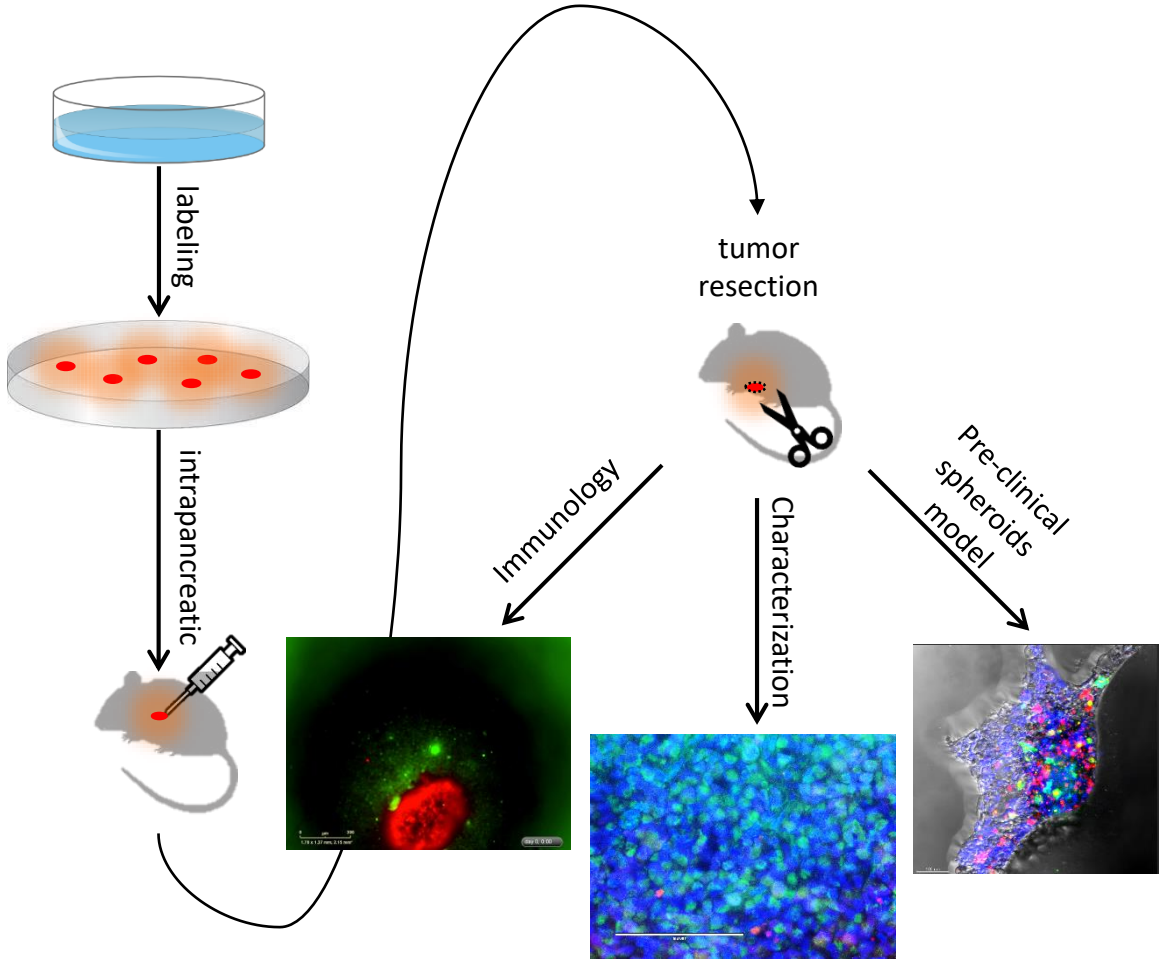
Murine cell lines commonly used for PDAC syngeneic models:

KPC-

- one of the most widely used PDAC models.
- Derived from autochthonous KPC mouse (C57BL/6) model of pancreatic ductal adenocarcinoma (PDA) - Recapitulates major features of the human disease, including mutated Kras and p53 (as mention before).
- Expresses the ductal marker CK19,the epithelial-mesenchymal markers E-cadherin and N-cadherin, and, Muc1 and Muc4 mucins.

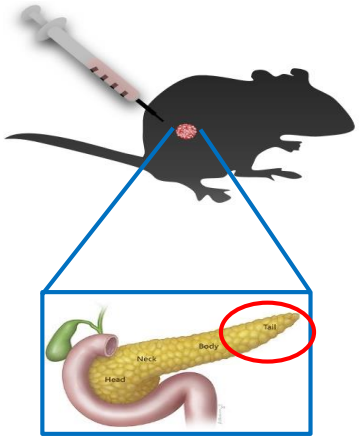
Panc 02-

- Derived from PDAC tumors induced by implanting 3-methyl cholanthrene (3-MCA)-saturated threads of cotton in the pancreas of C57BL/6 mice.
- Pane 02 originate as a well-differentiated histological appearance tumor (Grade III).
- Panc 02 is one of the most meta-static solid tumors (gross metastases were seen in the lungs of >70% of all tumor deaths
- Panc02 cells lack strong clinical significance for PC due to absence of mutational spectrum when compared to human disease (lack of KRAS and P53 nutation).

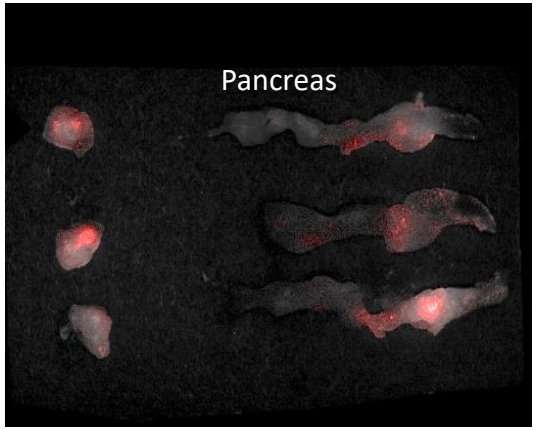


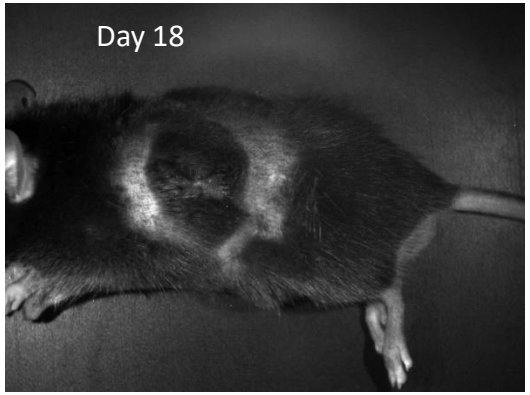
Imaging of syngeneic models for tumor growth monitoring

KPC cells

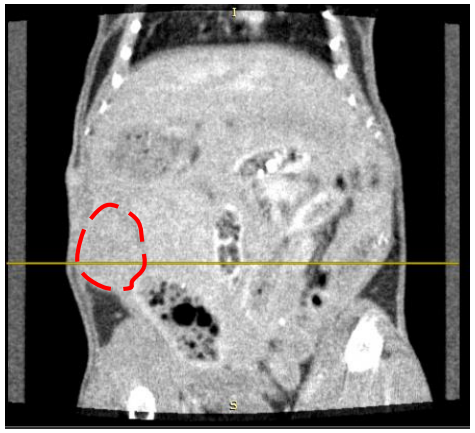


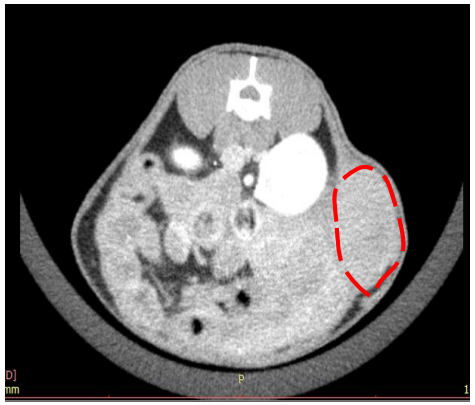
C57BL/6J mice



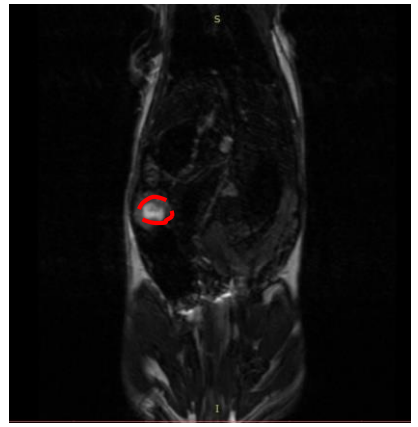


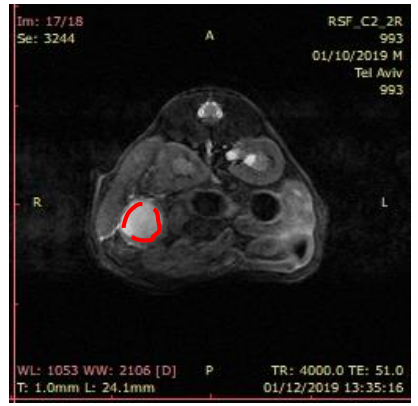
Fluorescent imaging
(**Maestro CRi™**)





CT





MRI

Xenogeneic human cell line-derived xenograft (CDX) models

The first human pancreatic cancer cell line was generated in 1963

Human cell lines commonly used for PDAC xenogeneic models:

BxPC-3

- was cultured from a 61-year-old woman's adenocarcinoma of the body of the pancreas.
- The patient died 6 months later despite radiation and chemotherapy. No evidence of metastasis was found.
- BxPC-3 cells produce mucin and the tumor produced in a nude mouse is moderately well to poorly differentiated like the primary adenocarcinoma.

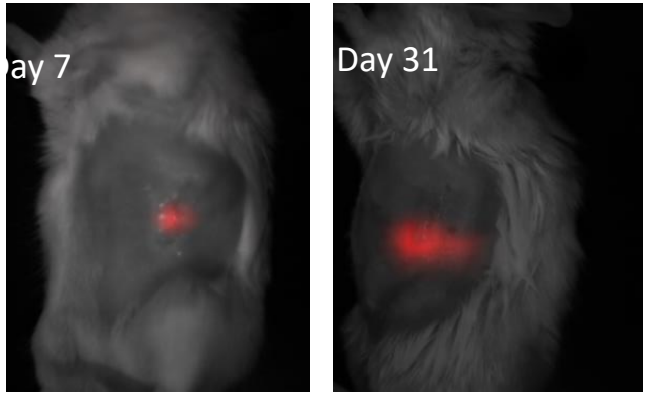
Mia PaCa-2

- Derived from the pancreas adenocarcinoma of a 65-year-old man who presented with abdominal pain for 6 months and a palpable upper abdominal mass.
- The tumor involved the body and tail of the pancreas and had infiltrated the periaortic area.
- MIA Paca-2 cells are KRAS mutant, have a p53 mutation, contain a p16 (CDKN2A) homozygous deletion and are wild type for Smad4. MIA Paca-2 cells are epithelial as they express CK5.

PANC-1

- was cultured from a pancreatic carcinoma of ductal origin of a 56-year-old Caucasian male.
- The cells possess the type B phenotype for G6PD.
- The Y chromosome could not be detected in this cell line (It is a known phenomenon that due to the increased genetic instability of cancer cell lines the Y chromosome can be rearranged or lost resulting in lack of detection).
- PANC-1 cells are are KRAS mutant, have a p53 mutation, contain a p16 (CDKN2A) homozygous deletion and are wild type for Smad4.

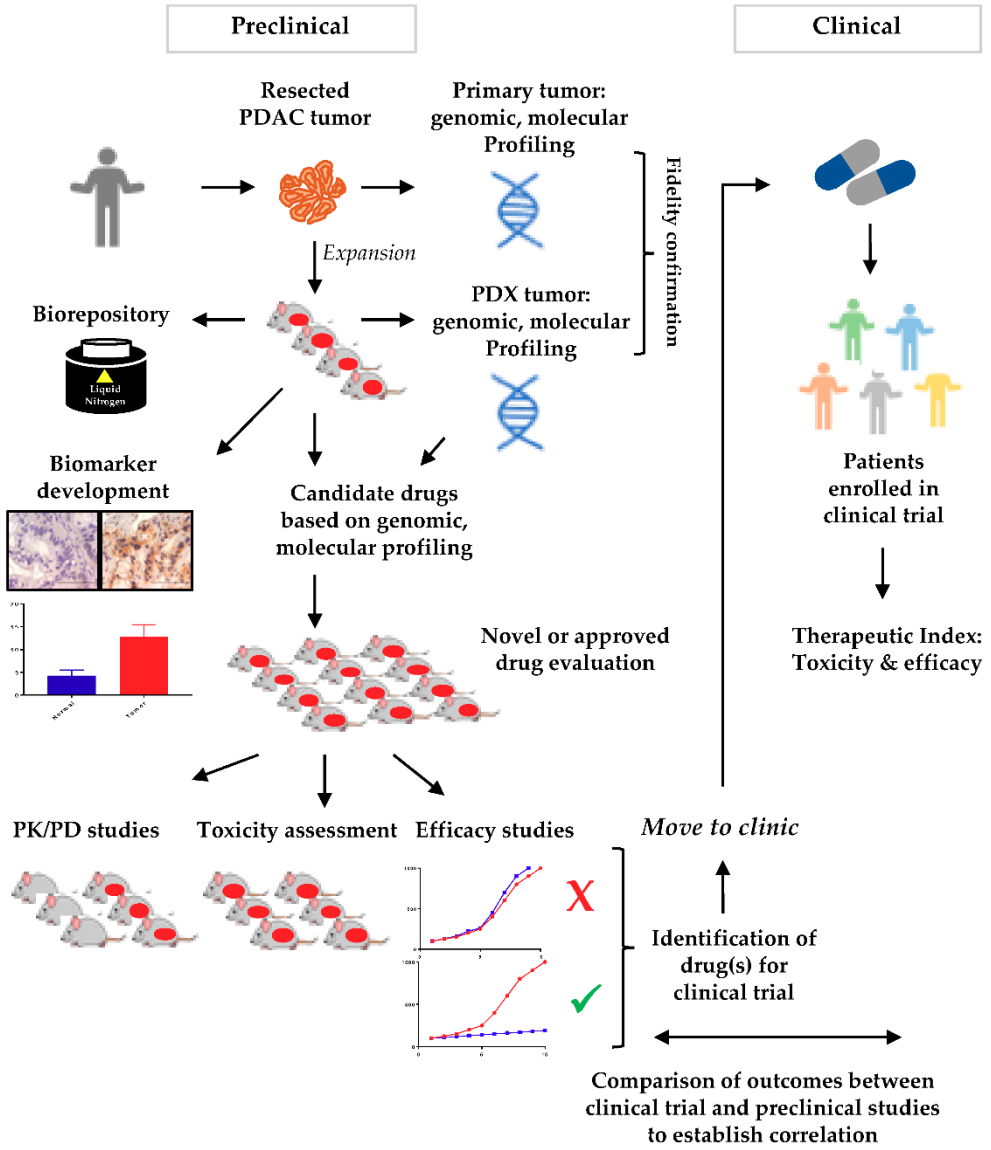
Human xenografts in SCID mice:
mCherry-labeled tumor cells allowing for intravital non-invasive imaging and follow up of the tumor progression and co-localization with the nanomedicine



Fluorescent imaging
(**Maestro CRi™**)

Patient-derived xenograft (PDX) models

- PDXs are made by transplanting a piece of patient's tumor tissue derived from surgical resection or from tumor biopsies in immune-deficient mice.
- The PDXs retain the morphological characteristics of the primary tumor as well as its metastatic potential.
- Can be used to generate new pancreatic cancer cell lines.
- Despite the promising potential of PDXs as preclinical drug testing platforms, several concerns need to be addressed such as:
  - PDXs don't fully replicate stromal compartment of PDAC or host immune system.
  - The use of immune-compromised mice limits the ability of using PDTX to examine responses to new immune-therapies.
  - Tissues for engraftment are limited, as only ~20% of the patients diagnosed each year will be eligible for surgical resection.



Garcia, Patrick L et al. "Patient-Derived Xenograft Models of Pancreatic Cancer: Overview and Comparison with Other Types of Models." *Cancers* vol. 12,5 1327. 22 May.