Scientific project design in translational oncology

Michele De Palma – Freddy Radtke

Transgenic mice and cancer mouse models

Alan Huguenin
Benjamin Louis
Alexy Mann
Kazuma Miyai

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Introduction

Really need a genetically modified mouse (high financial and time cost) ?

- In vitro or ex vivo alternative (faster and cheaper)
- Non-germline mouse model alternative (recombinant viral vector, shRNA, CRISPR/Cas9)

Mouse advantage:

- same organ and tissue systems as man
- short generation time (3 months)
- Mouse germline can be genetically modified by engineering

Search if the transgenic mouse already exists

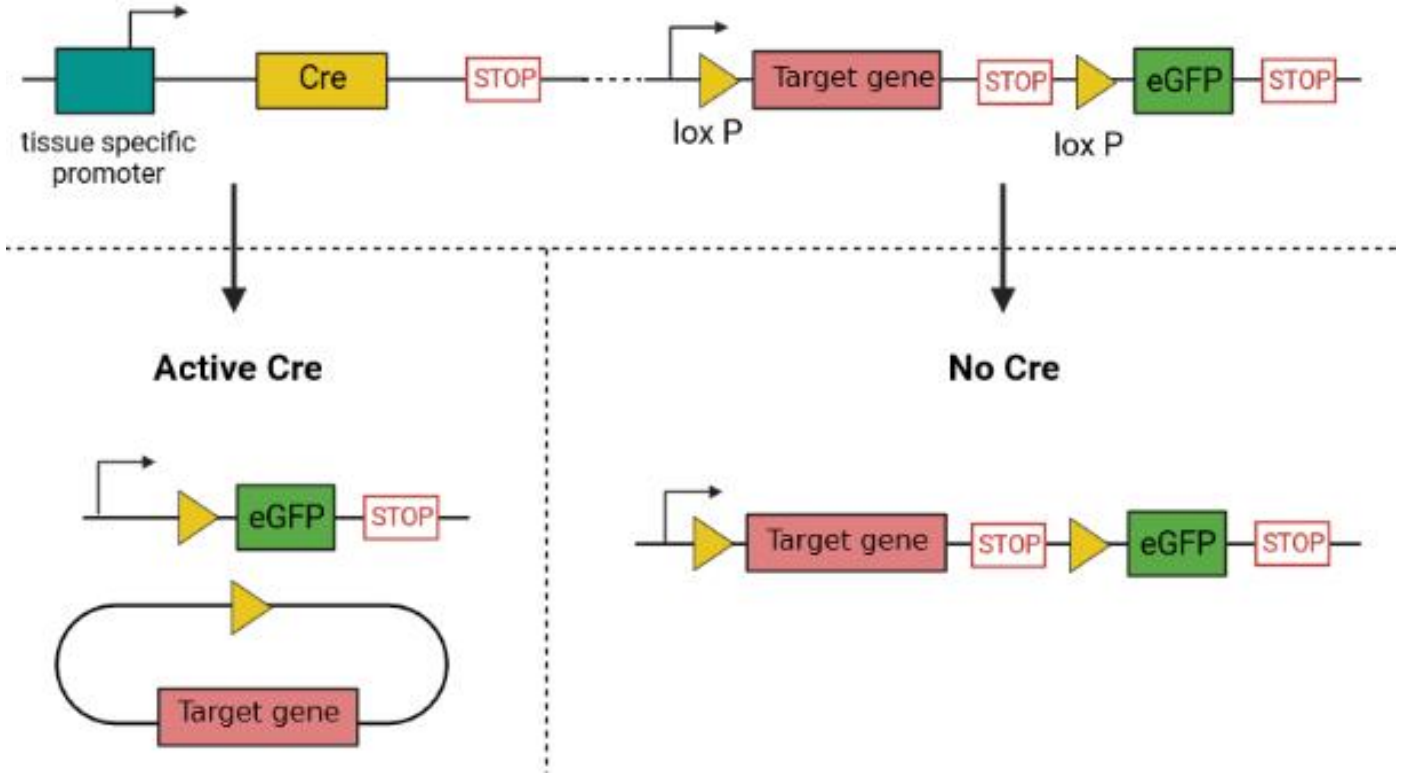
Ethical consideration:

Look at local and governmental regulations and need ethical approval

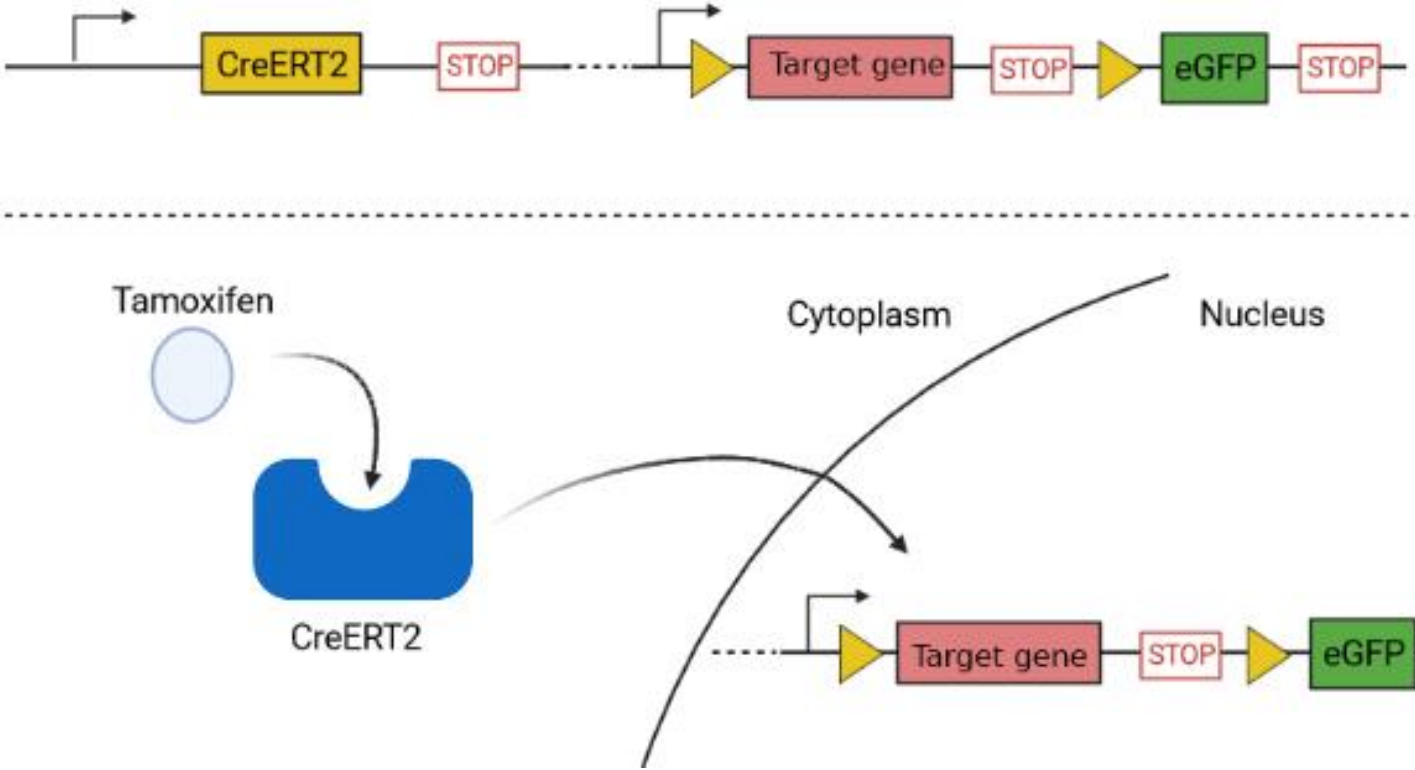
3 types of genetic modification

1. **Stable**: cannot be altered and remains stable over time and generation
    1. Gene knockout allele: deleting a critical exon from a gene in the mouse germline
2. **Conditional**: conditional allele is controlled by a site-specific recombinase
3. **Inducible**: allows temporal control

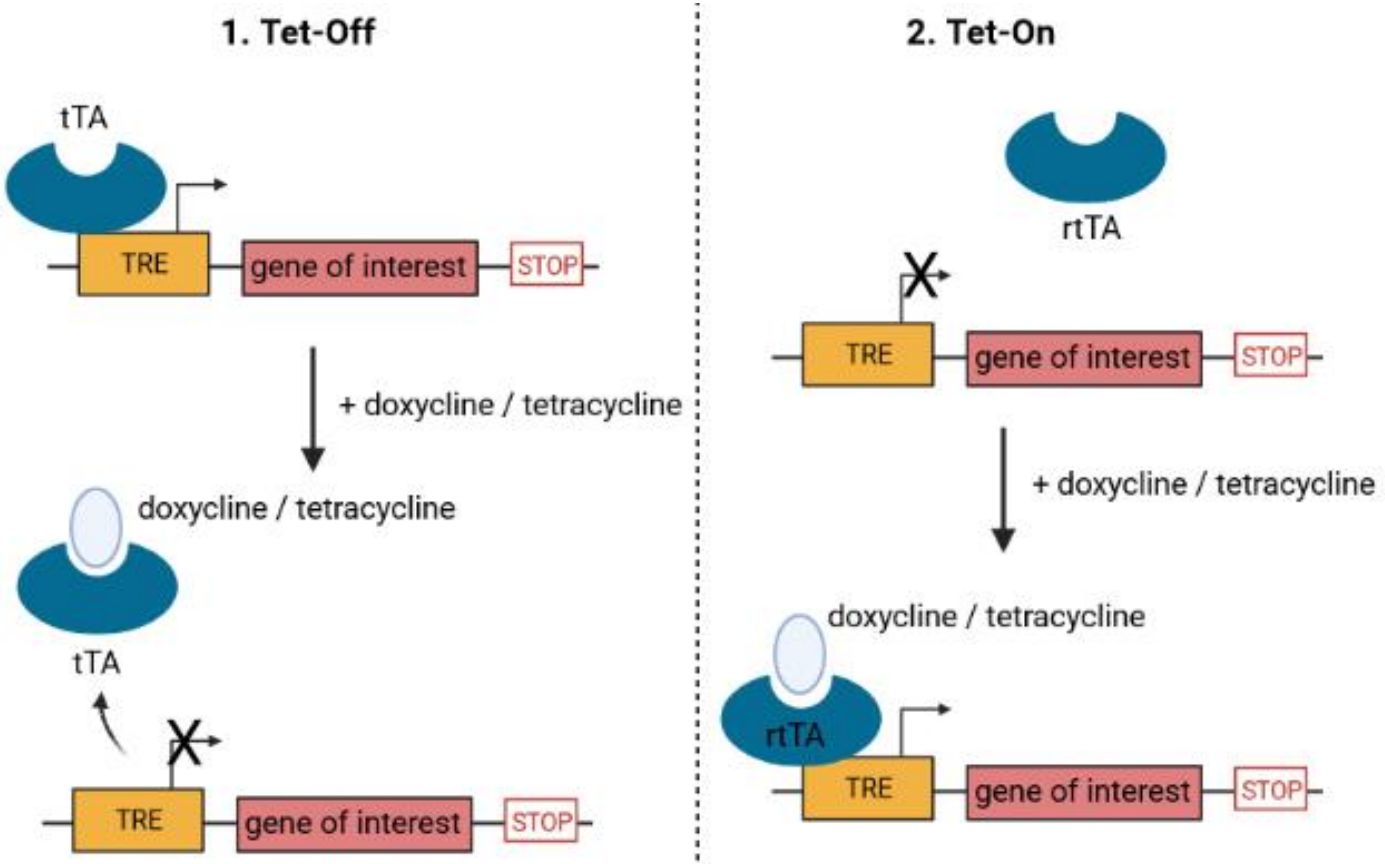
Conditional system: Cre-Lox recombination



Inducible systems: Ligand-dependent inducible systems



Inducible systems: Tet-off and Tet-On

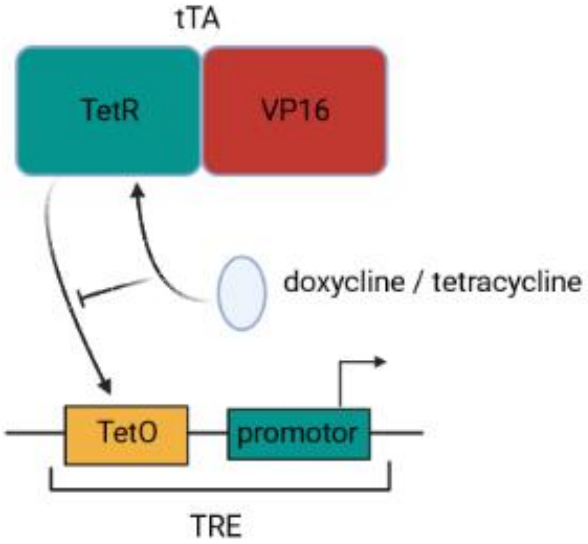


Inducible systems: Tet-off and Tet-On

1. Tet-Off

rtTA = TetR + VP16

tTA: tetracycline transactivator
TetR: tetracycline repressor
VP16: transcriptional activation domain of VP16

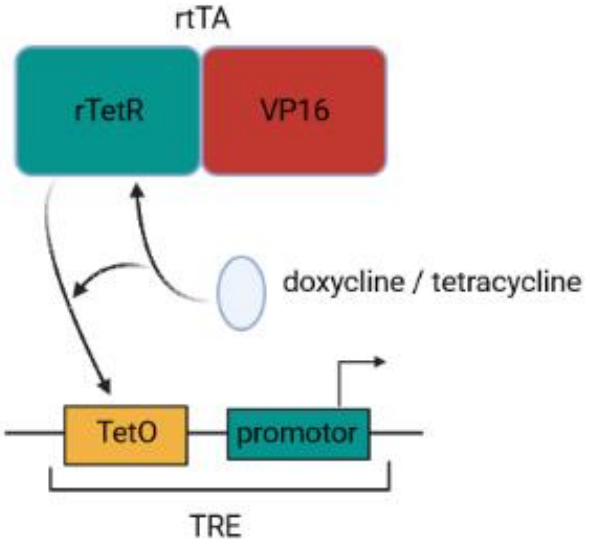


TetO: Tet operator

2. Tet-On

rtTA = TetR + VP16

tTA: tetracycline transactivator
TetR: tetracycline repressor
VP16: transcriptional activation domain of VP16



TetO: Tet operator

Which genetic modification to do?

1. gene inactivation
2. gene modification
3. gene activation

Gene Inactivation

1. Insertion/Deletion (indel) mutation

2. Deletion of a critical exon or an entire gene

3. Gene trap

4. Knock-in

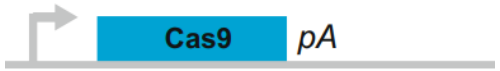
Gene Inactivation

1. Conditional gene inactivation

2. Inducible gene inactivation

   1. Tet-On/Tet-Off

   2. Transgenic mice expressing Cas9
   + recombinant virus that expresses a specific gRNA

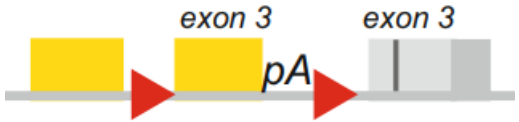


In combination with sgRNA encoding virus

Gene modification

1. Defined point mutations

2. Conditional point mutation



3. Expressed sequence tags
   1. For analysis via immunohistochemistry (e.g. V5-tag)

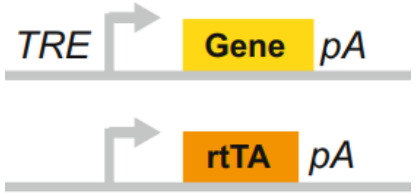
   2. For fluorescent imaging (e.g. with EGFP tag)

Gene activation

1. Overexpression from a transgene

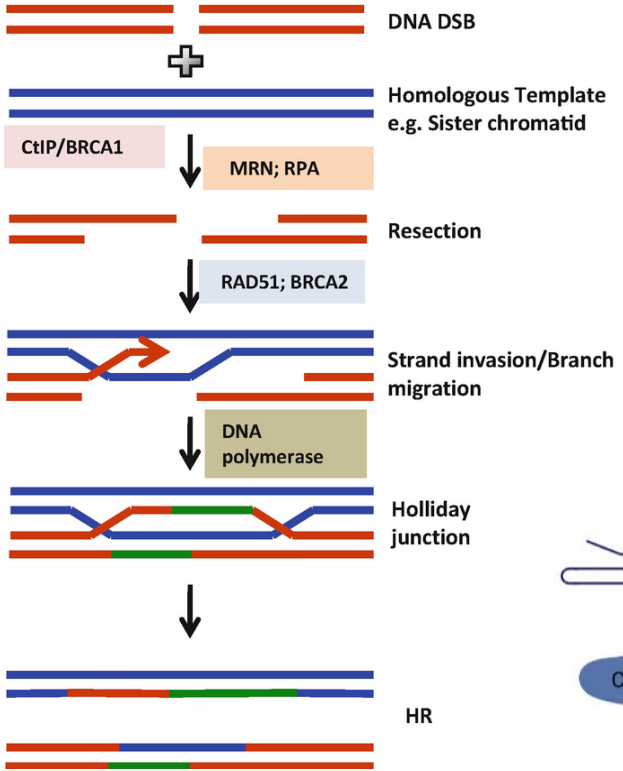
2. Conditional gene activation

3. Inducible gene activation: Tet-On and Tet-Off system



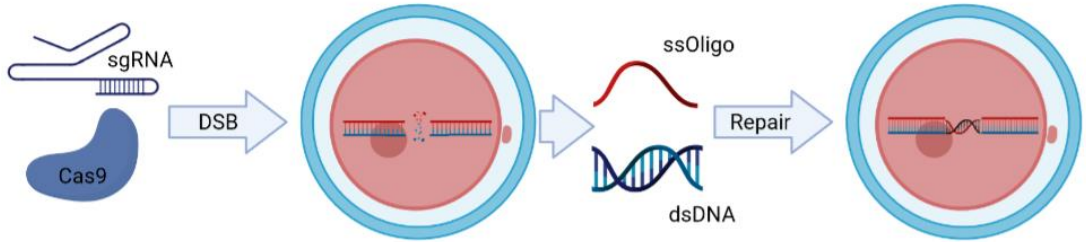
How to introduce such modifications ?

Using built-in cell mechanisms



Homology Directed Repair

- Used for most often for insertion
- Double Strand Break repair mechanism
  - Using a template to insert
  - Using CRISPR to induce a DSB
    - dsDNA or ssOligonucleotide



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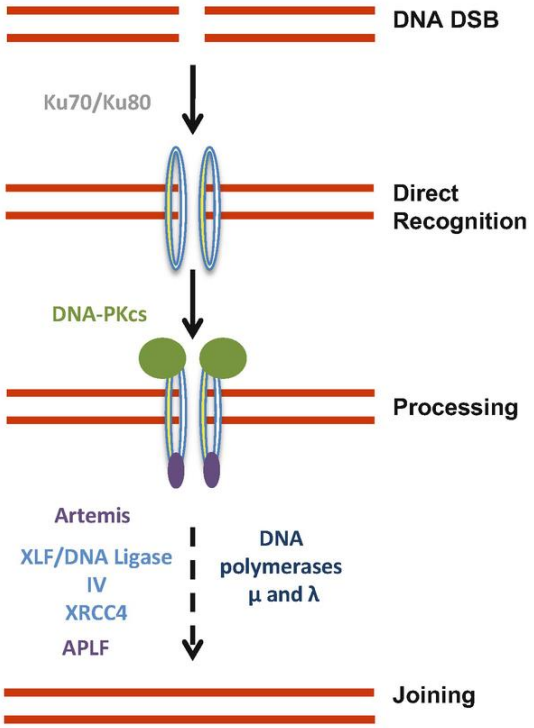
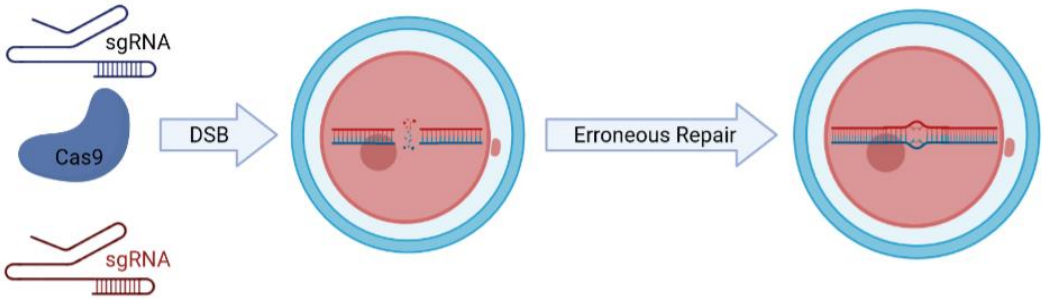


Non-Homologous End Joining

- Often used for deletion
- Less faithful than HDR
- Use errors to our advantage
- Can induce frameshift or null allele
- Can also be used for insertion in spontaneous DSB

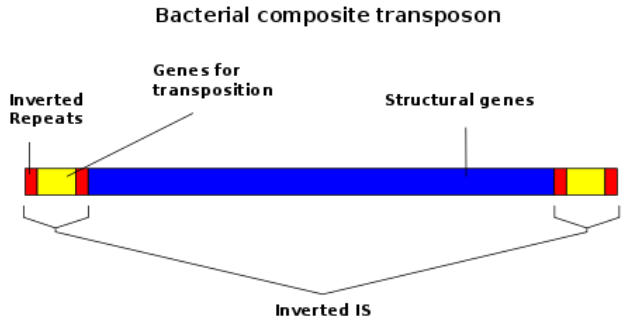


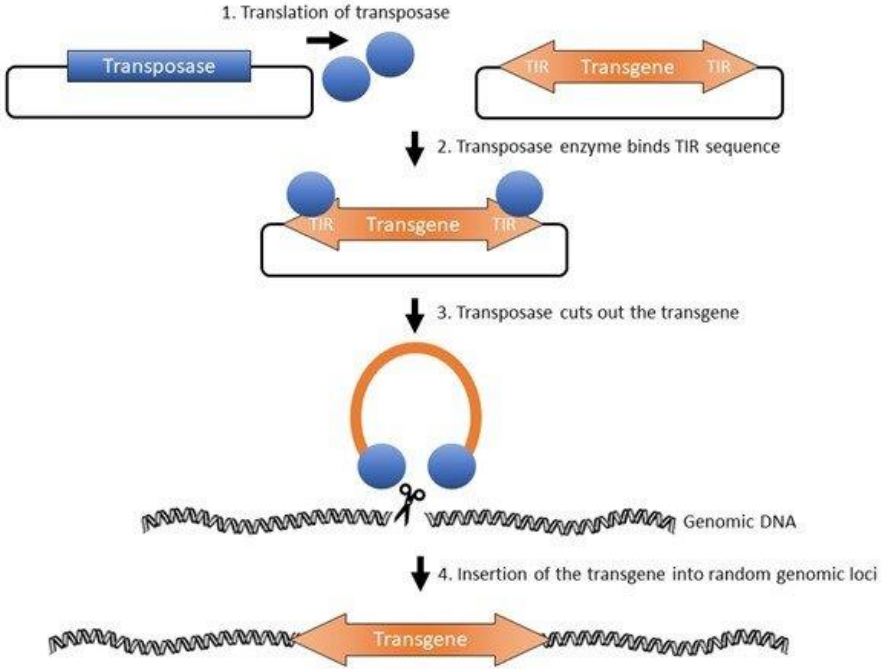
How to introduce such modifications ?

Using external vectors

Transposons

- Clone the DNA between the ITR
  - Used mostly for insertion
    - Several single copies



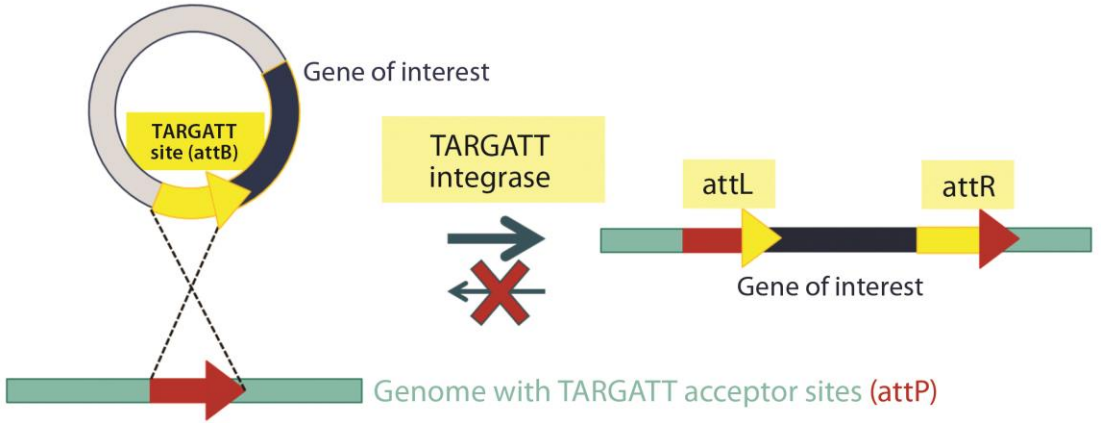


How to introduce such modifications ?

Using external vectors

Recombinases and Integrases

- Efficient, precise and controlled integration, like Rosa26 or Col1a1
- Needs a docking site
- Constituted of a selection cassette and recombination sites
- Needs a target docking site



What are the possible injection routes ?

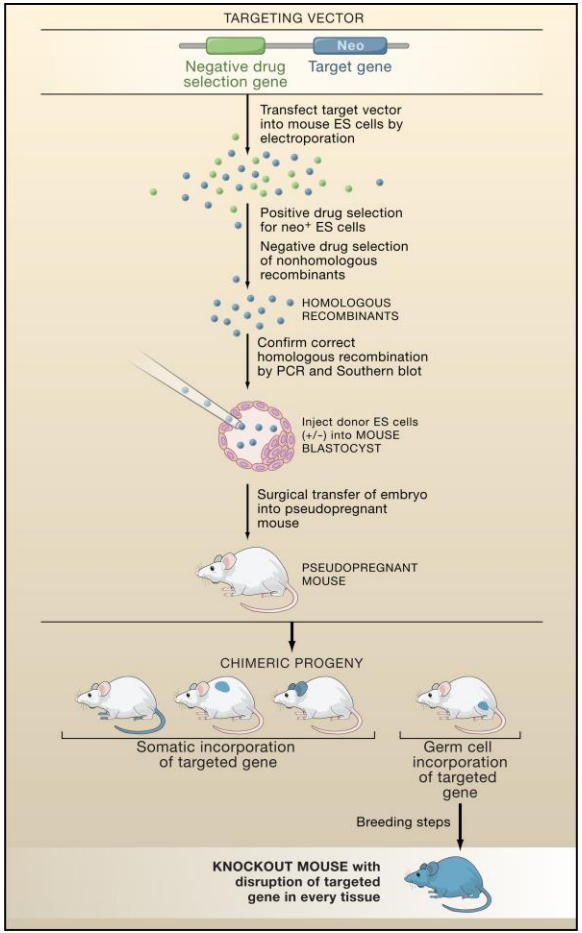
Embryonic Stem Cells (ESCs) route

Classic route:

- Targeting vector introduced in the cells
- Incorporated cells injected into blastocysts
- Establish the line after passing down generations

GEMM route:

- Shortens the time needed for insertion
- Requires less crossbreeding



What are the possible injection routes ?

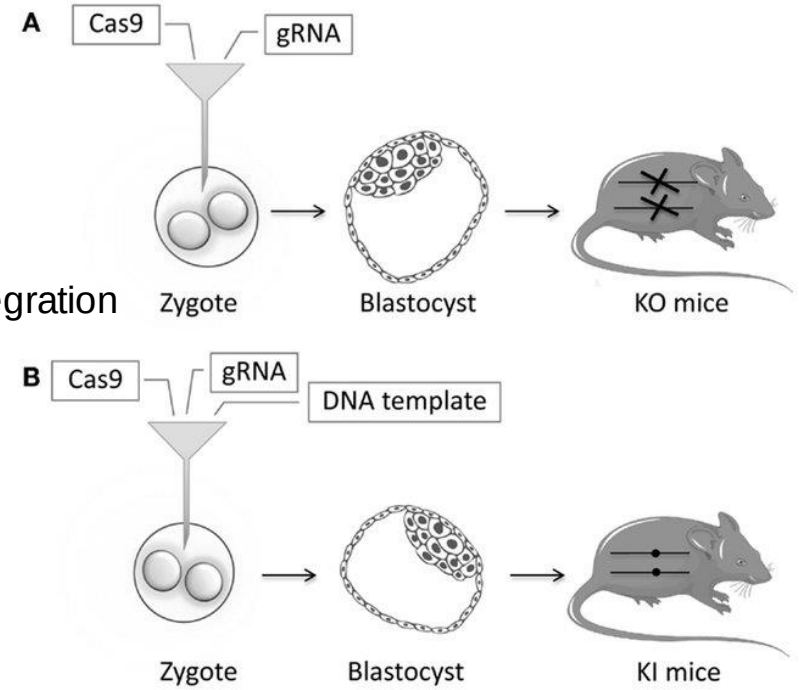
Zygote route

Classic route:

- Inject DNA in the pronucleus of a zygote
- Cleaved embryos injected in oviduct
- Use PCR/Southern Blot to check for proper integration

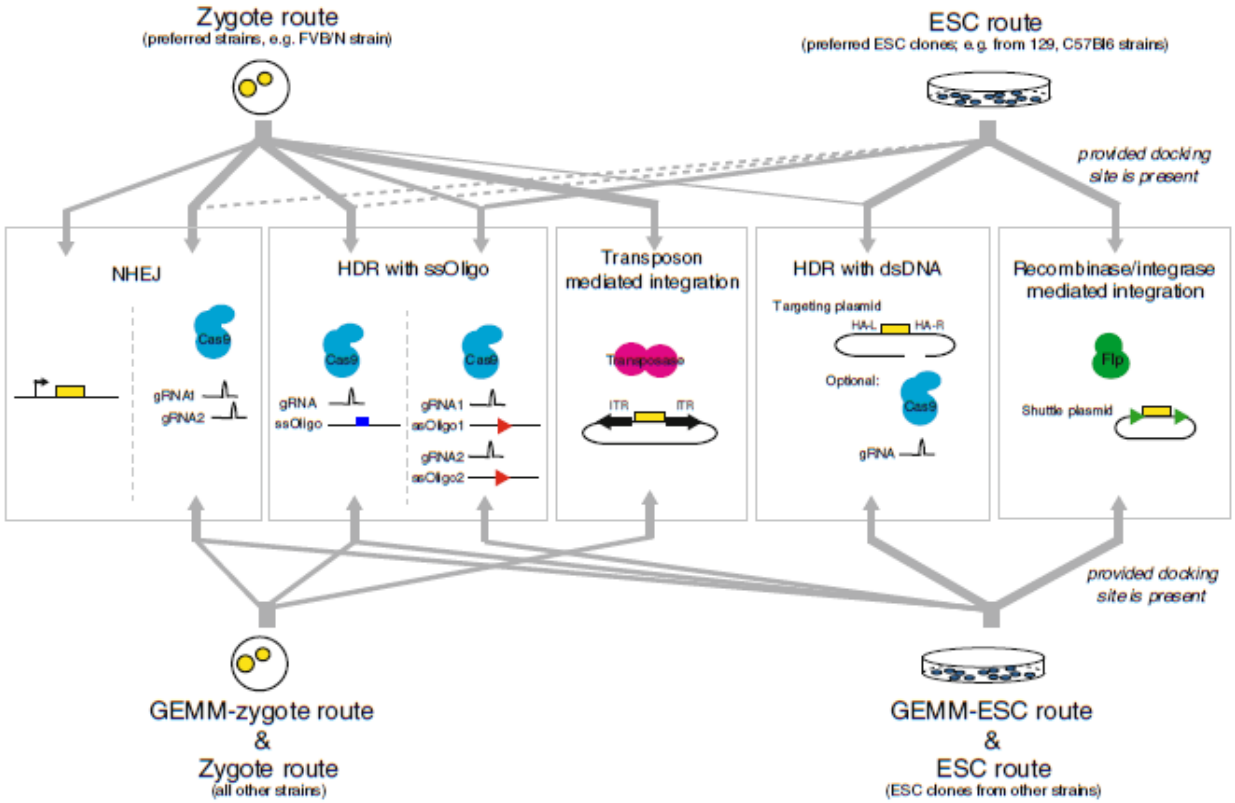
GEMM route:

- Makes modifications easier
- Strain background adds breeding complexity
- Vary in phenotype



What are the possible injection routes ?

Summary

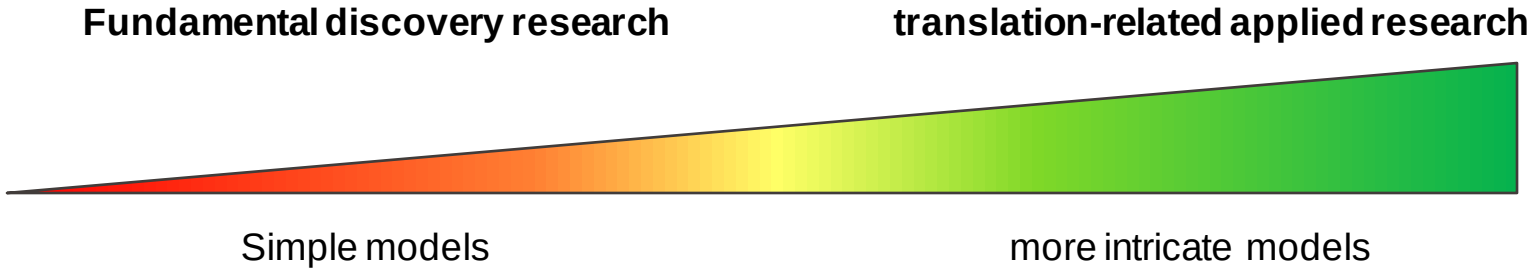


Mouse cancer models

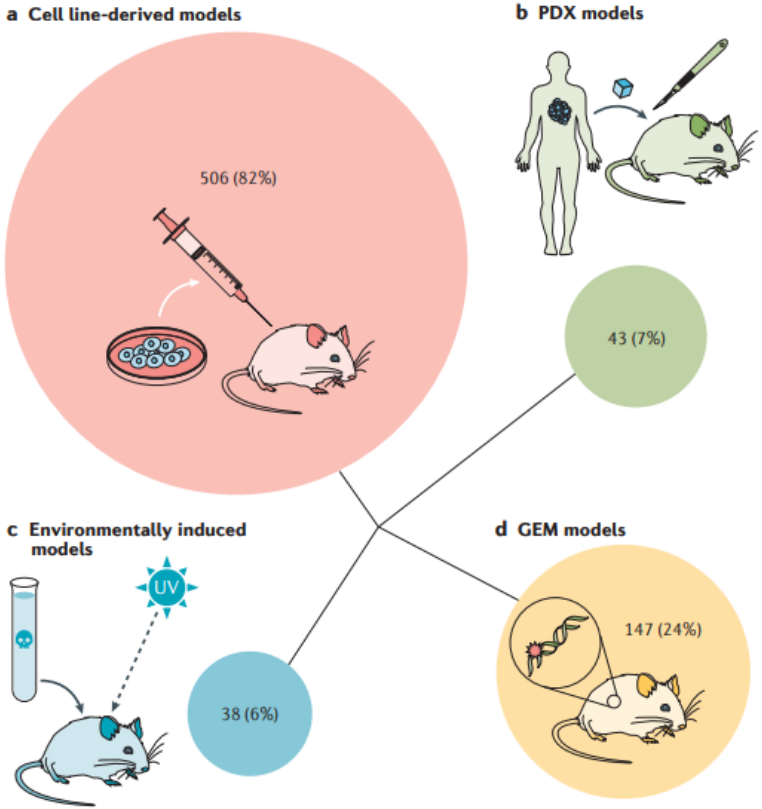
Indispensable intermediate model system bridging reductionist in-vitro research with human studies.

Mimic the complexity of human cancer as a Darwinian process of neoplastically transformed cells in crosstalk with their local, systemic and immune environments.

Different goals for different applications:

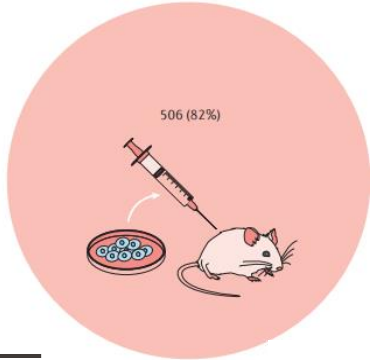


Different mouse cancer models



Preclinical mouse solid tumour models: status quo, challenges and perspectives, Nicolas Gengenbacher, Mahak Singhal and Hellmut G. Augustin, 2016

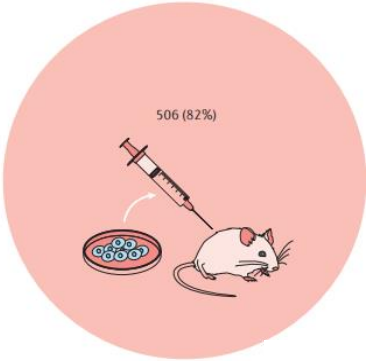
Cell line-derived models



Concept: long-term in-vitro cultured tumor cell lines and their in vivo inoculation in mice.

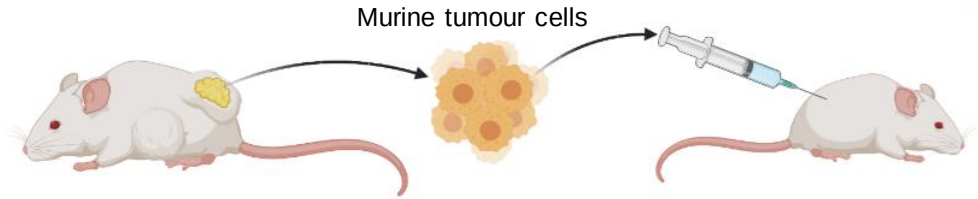
Upsides	Downsides
- low-cost - synchronous tumour growth - easy technical manipulability	- Perturbed tissue architecture and micro-environment (due to the rapid non-autochthonous growth of the tumours) - Loss of genetic heterogeneity and irreversible changes in gene expression are imposed by long-term in vitro propagation

Cell line-derived models

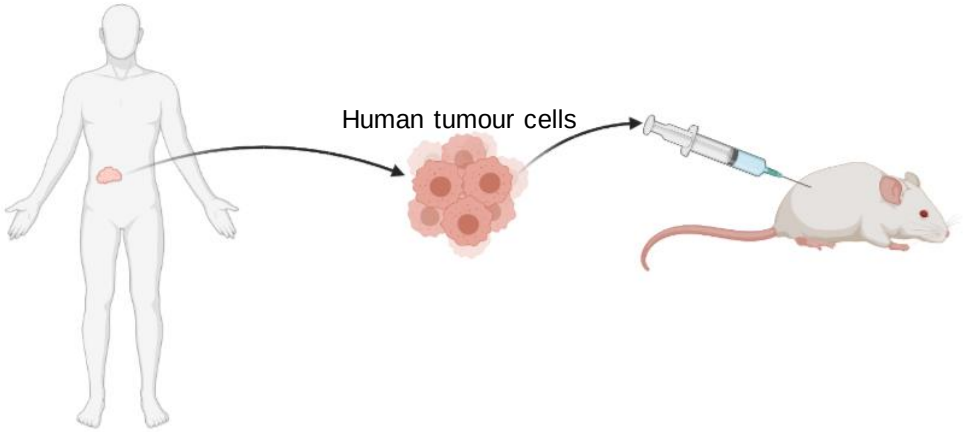


2 types of grafts

Allograft



Xenograft



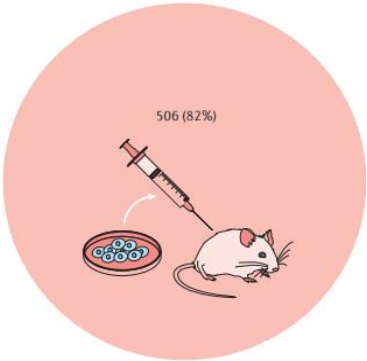
Xenograft: immunodeficient mice strains

« nude » mice	« Scid » mice	« Rag » mice
• Foxn1$^{-/-}$, TF required for both hair follicle and thymic development • Hairless and athymic (T-cell deficient) • **Advantage:** facilitated tumour monitoring	• Prkdc$^{-/-}$, required for DNA repair and seals the double-stranded DNA breaks that occur during somatic recombination of T cell receptors (TCR) and immunoglobulin (Ig) genes. • TCR and Ig genes cannot rearrange, resulting in mice that are both T and B cell deficient.	• Rag1$^{-/-}$ or Rag2$^{-/-}$, required for the somatic recombination of T cell receptors (TCR) and immunoglobulin (Ig) genes. • TCR and Ig genes cannot rearrange, resulting in mice that are both T and B cell deficient.

Xenograft mouse strains: Upsides & Downsides

« nude » mice	« Scid » & « Rag » mice
• **Advantage:** loss of hair and nude skin allows for **facilitated tumour monitoring** • **Disadvantage:** still have B cell response so **not suitable for leucemia** (blood cancers, …)	• **Advantage: T and B-cell deficient** so suitable for blood cancers ; **radiation-sensitive** meaning that DNA repair is impaired • **Disadvantage:** mice need to be shaved to help **tumour visualisation**

Cell line-derived models



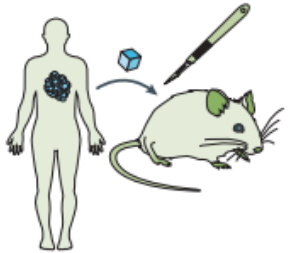
3 methods of cell injection

Orthotopic: engraftment into the anatomically correct organ.

Ectopic: engraftment not in the tissue of origin (usually subcutaneously)

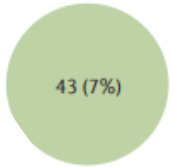
Systemic: injection in the general circulation (Peritoneal or intravenous injection), mostly for studying metastases.

Patient-derived xenograft models (PDX)

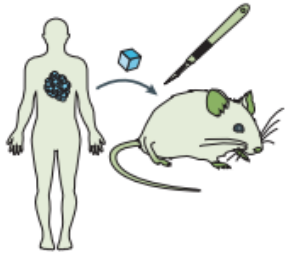


Concept: Subcutaneous implantation of, surgically derived human tumour material into immunodeficient mice.

Upsides	Downsides
- Retain molecular, genetic and histopathological features of the originating tumours.	- High cost
- Incorporate the vast inter-patient and intra-tumour heterogeneity that is inherent to human cancer.	- Low engraftment rates
- Allows direct evaluation of clinically-approved drugs	- Engraftment rates strongly vary between different tumour types and grades.
	- Human stroma gradually replaced by mouse stroma.
	- Necessary use of immunodeficient mouse models

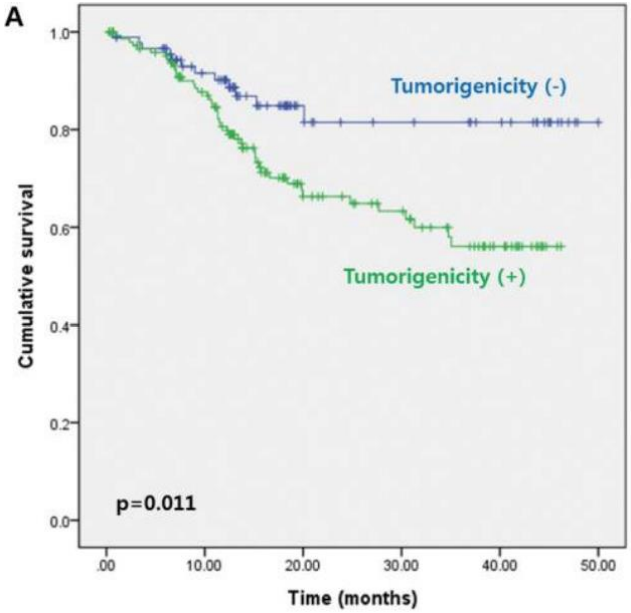


Patient-derived xenograft models (PDX)

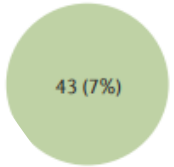


Negative correlation between PDX engraftment and clinical survival of patients.

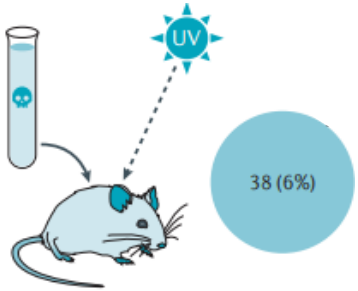
Three-year disease-free survival according to tumorigenicity of the primary colorectal tumor for colorectal cancer patients (stage I–IV)



Correlation between tumor engraftment in patient-derived xenograft models and clinical outcomes in colorectal cancer patients, Oh BY et al., 2015



Environmentally-induced models

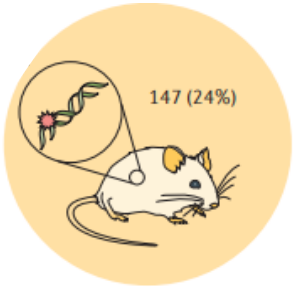


Concept: Tumour induction by environmental carcinogens (chemicals, radiation and pathogens)

Upsides	Downsides
- Closely recapitulate the genetic heterogeneity of their human counterparts. - Represent all stages of multistep carcinogenesis.	- Long latency and high variability of penetrance - Render study design difficult (choosing adequate animal numbers and identifying relevant time points)

Particularly beneficial in defining genetic risk factors and assessing prevention strategies

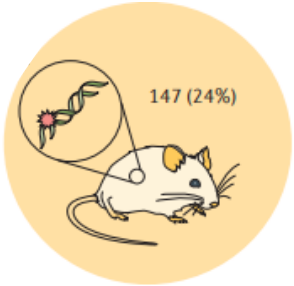
Genetically-engineered mice (GEM)



Concept: The mouse's own genome is altered from birth.

Upsides	Downsides
- Spatially- and temporally-controlled introduction of genotype - Faithfully recapitulate molecular and histological features of human disease	- Expensive & time-consuming - Reduced clonal heterogeneity compared with human tumours - Evaluation of metastasis is challenging, as most GEM models must be sacrificed before developing metastatic disease

Genetically-engineered mice (GEM)

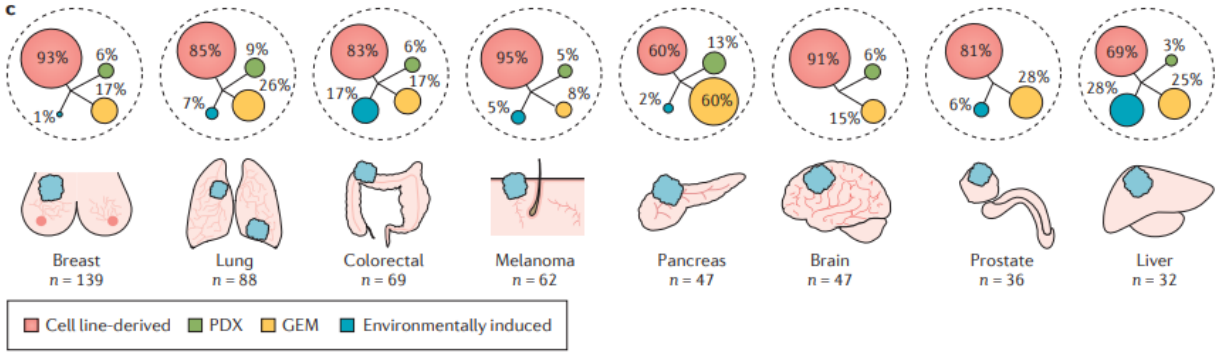


Different types: knock-in, knock-out, inducible (temporal control), tissue-specific (spatial control) or both.

Mouse cohorts can be used for patient stratification by identifying genetic biomarkers of drug resistance and responsiveness as well as for evaluation of combination therapies.

This is by far the best model for testing therapies targeting the tumour microenvironment.

Different cancer types might prefer different models



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Two obvious outliers are found:

- Liver/colorectal cancer studies rely much more on environmentally induced models than their counterparts (28% and 17% respectively, mean 6%). Both cancers are indeed often caused by exposure to environmental factors.

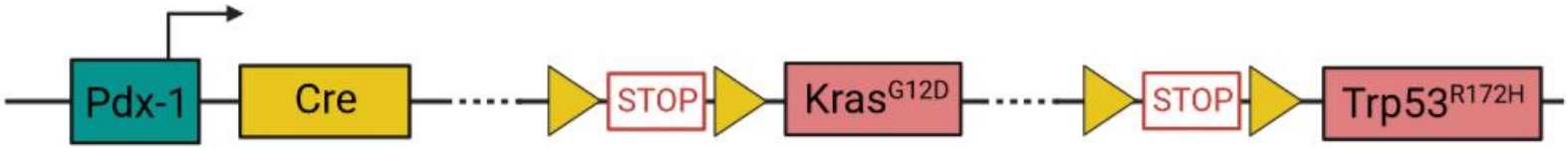
- Pancreatic cancer relies much more on GEM models than its counterparts (60%, mean 24%) ⟹ early discovery of a pancreatic tissue-specific promoter Pdx-1 in 1996*

*Offield, M. F. et al. *PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum.* Development 122, 983–995

KPC mice

Pancreatic ductal adenocarcinoma GEM model, established in 2005 (relatively early for GEM), «gold standard» ever since.

KPC = LSL-**K**ras$^{G12D/+}$; LSL-Trp53$^{R172H/+}$; **P**dx-1-**C**re



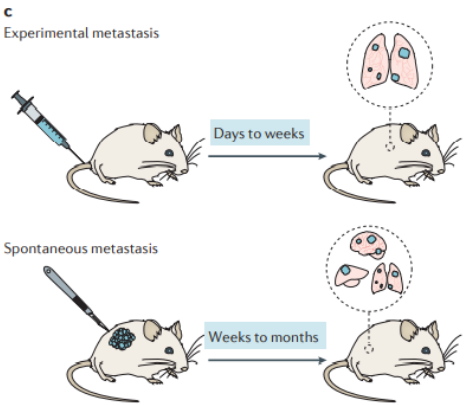
Tissue-specific (pancreas) expression of mutant Kras oncogene and mutant p53 TSG ➡ spontaneous formation of pancreatic adenocarcinoma

Modelling tumour progression & metastasis

Metastasis: 90% of cancer-related mortality, only 25% of studies focused on it.

Experimental: 2/3 of studies; recapitulate only metastatic colonization and circumvent the primary disease, metastasize to a single organ strongly influenced by the site of injection (tail vein -> lung, heart-> brain, spleen->liver)

Spontaneous: 1/3 of studies; recapitulate the entire metastatic cascade, tumour resection is often required to make metastases rate-limiting



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Discussion: mouse cancer models

GEM, PDX and environmentally induced models > cell-line models. However, compared to the latter, they are more time consuming and cost intensive, which explains their relative sparsity.

Metastatic studies not optimized and too sparse.

Many published therapeutic studies directly initiate therapy upon inoculation of tumour cells -> meaningless for human cancer patients.

General conclusion

- Genetic manipulation of mice is indeed an effective tool for analyzing phenotypical traits.
- Rigorous ethical scrutiny around genetics and their engineering
- While not perfect, mouse models remain the gold standard for cancer studies. Additional funding from private and public institutions could push towards more intricate and therapy-oriented mouse models.

Thank you for your attention

Questions?