Rare Bootcamp™

Animal Models as Preclinical Tools to Help Enable Drug Development

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Ultragenyx

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Why are model systems necessary?

Does this drug impact the right pathways?

How frequently do we need to dose?

Is the drug going to the right place?

How do we administer?

Does the disease state impact drug delivery?

How does potency of the drug change over time?

Patient

Candidate Drug

Biodistribution
Dosing
Efficacy
Durability
Safety

Does the drug improve tissue pathology?

What's the right starting dose?

How much of the drug is getting there?

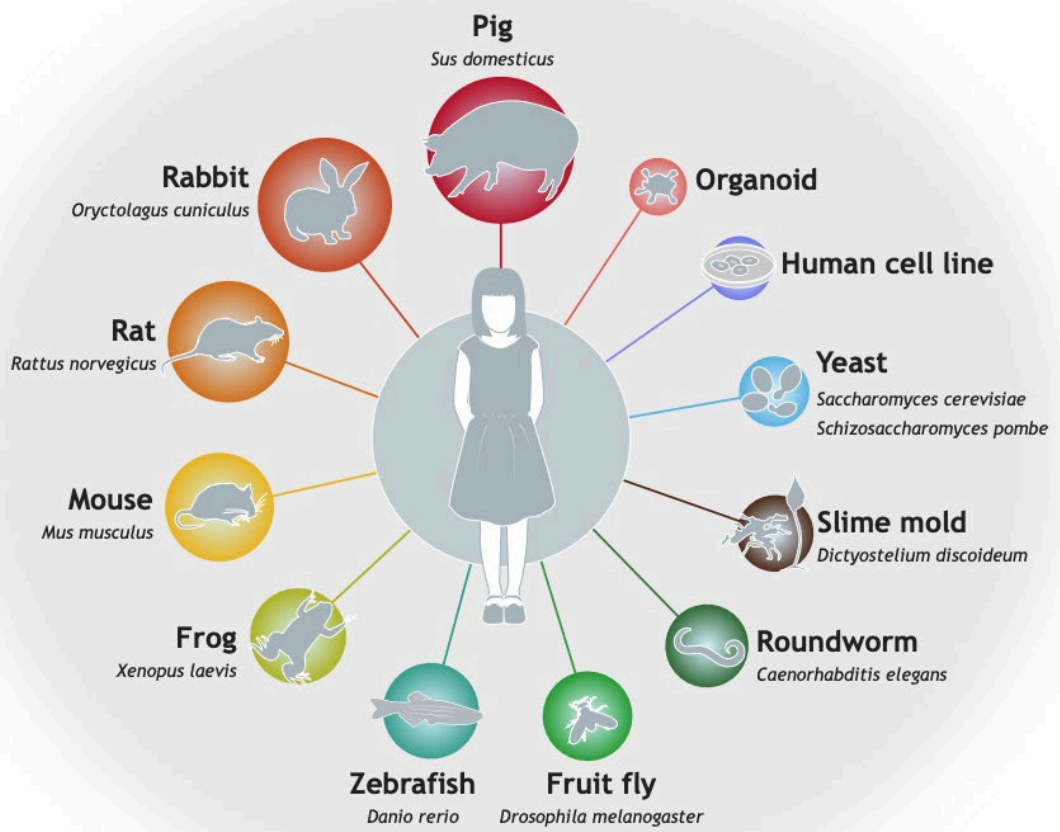
How does the drug improve any behavioral phenotypes?

Are there any off target toxicities?

Is the effect of the drug durable over time?

Are there any on target toxicities?

Multiple model systems exist in the preclinical toolbox



Hmeljak et al., 2019

There is no single, perfect model system for any disease.

Multiple distinct models can, and should, be used to answer specific preclinical questions.

Being intentional about **model selection** and **study design** is vital to successful drug development.

Each model system has advantages and caveats

Large mammals

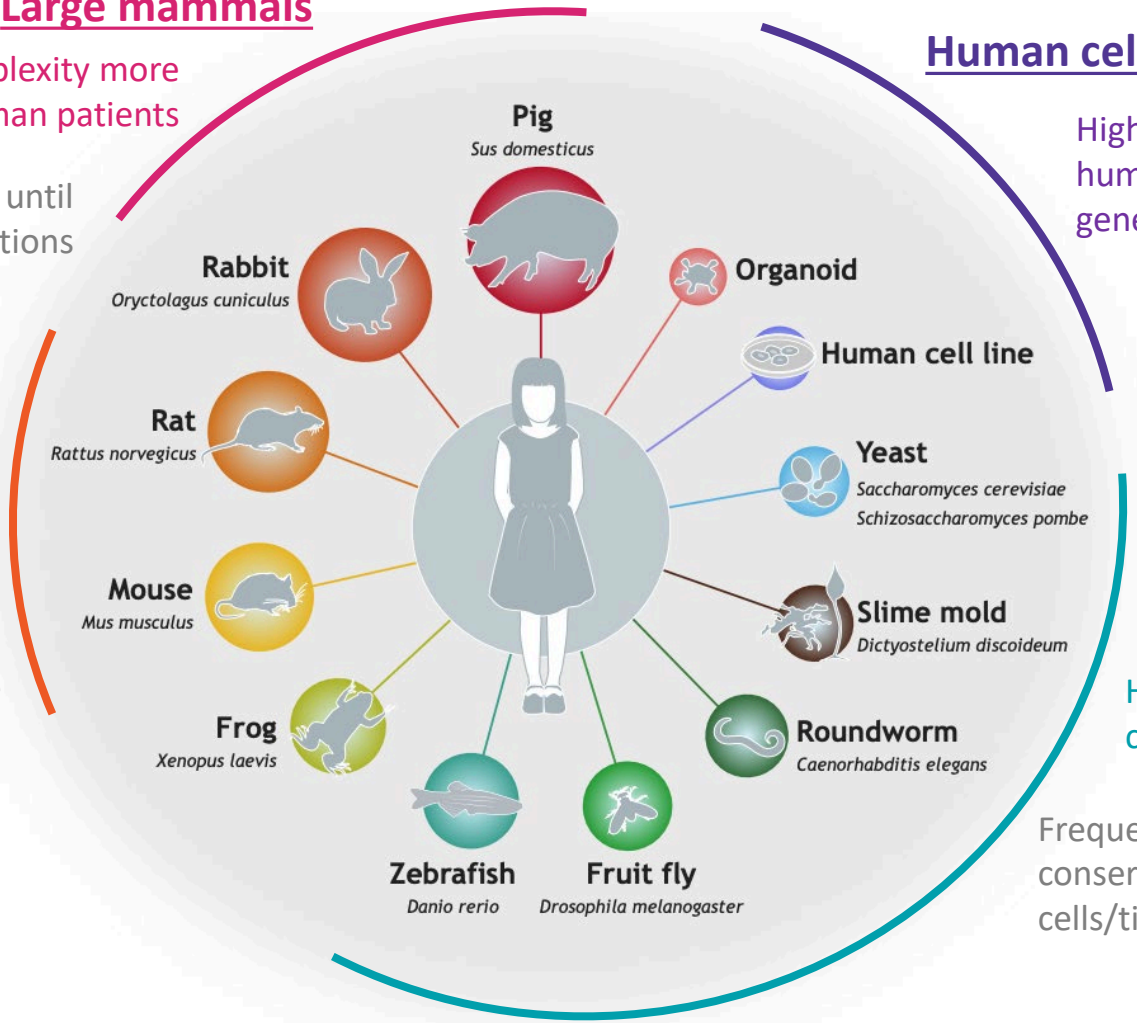
Body size and tissue complexity more similar to human patients

Expensive, low throughput, and until recently few genetic engineering options

Rodents

Frequently used due to genetic conservation, similar mammalian characteristics, and available genetic engineering tools

Reduced body size and tissue complexity relative to human patients, lack of full pathway conservation



Human cells and organoids

High-throughput in vitro model with human molecular pathways and genetics

In vitro limitations on delivery and physiological relevance to a whole body system

Non-mammalian organisms

High-throughput models with well-curated, easily-manipulated genomes

Frequent lack of genetic and pathway conservation relative to human cells/tissues

Modified from Hmeljak et al., 2019

Types of Model Validity to consider in selection

How well does the model predict the behavior of the human disease?

Face Validity

The model has similarities in the anatomical, physiological, and behavioral phenotype of the disease.

Predictive Validity

A model has a response to a known treatment in line with what happens in human patients with the disease.

Construct Validity

The model has similarities in the mechanism of human disease, has nucleic acid and amino acid sequence conservation, and gene expression is in the same cell and tissue types.

Target Validity

The model has downstream molecular mechanisms/targets and upstream regulatory pathways that are intact and conserved with the human disease.

Mice continue to be a valuable model for preclinical work

Table 1 Species-dependent differences

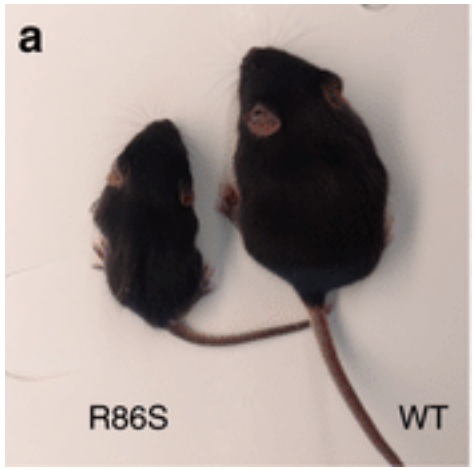
Species	Sexual maturity	Gestation period	Average life span (year)	Average weight (kg)
Human	15–18 years	266 days	75	50
Rhesus monkey	3–5 years	165 days	25	6
Pig	9–11 months	114 days	7	80
Mouse	6–8 weeks	19–21 days	2	0.03

Yang et al., 2021

- Mice have ~97.5% of their DNA in common w/humans
- Multiple sophisticated genetic techniques readily available to generate transgenic models
- A wide toolkit for characterization of molecular, physiological, and behavioral phenotypes
  - Correlation of blood biomarkers, improvement to target organ cell health, and whole animal health or behavior

- Short gestation age, early weaning age and sexual maturity meaning that studies can run quickly
- Relatively cheap

Mouse Nomenclature 101



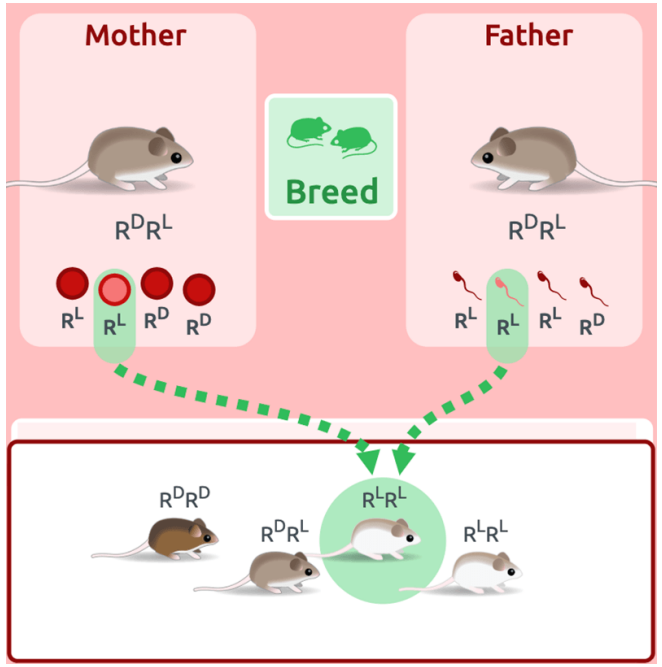
Martin et al., 2020

Transgenic = disease model
R86S is the amino acid mutation in this model

WT = wild-type
The ‘normal’ allele of a particular gene; non-diseased form

Jackson Labs

Strain
Genetic background of the mouse, usually inbred within a biological species. Inbred strains improve subject-to-subject variability.



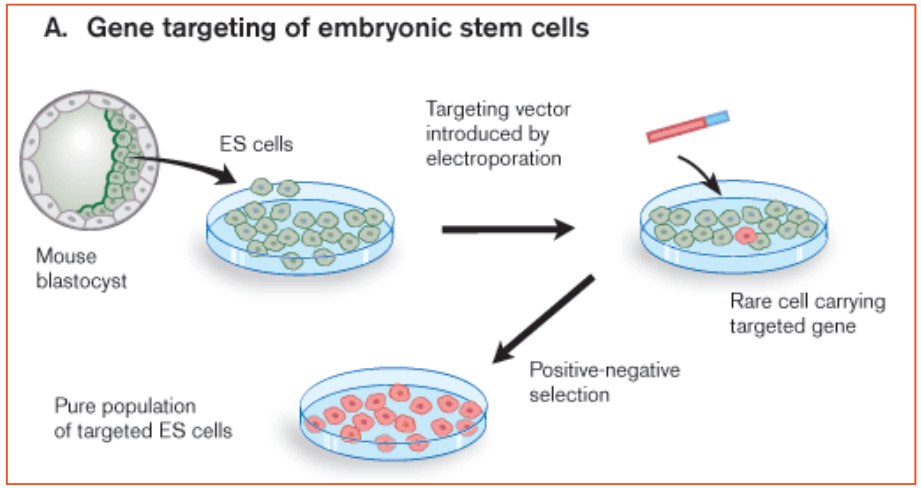
Genotype
Gene$^{+/-}$

Phenotype
Observable characteristic

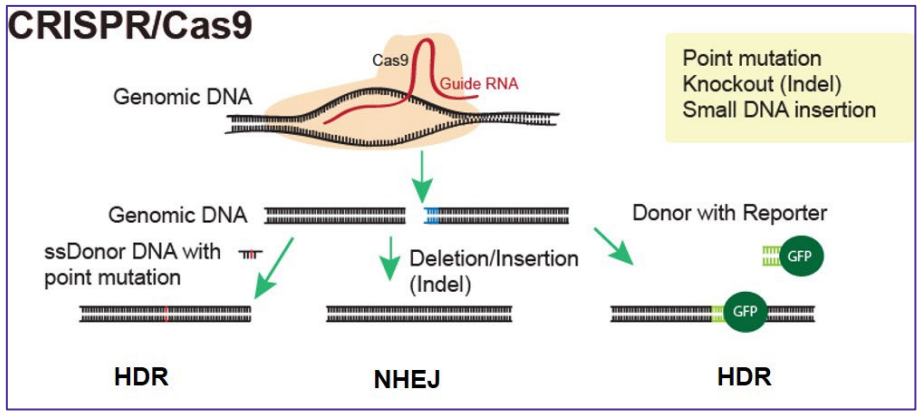
Transgenic- Animal models that have had their genomes altered

A number of technologies have been developed over the years to enable generation of transgenic mice.

- **Embryonic Stem Cell manipulation** using homologous recombination was used for decades to knock-out (KO) or knock-in a gene of interest
  - Conventional KO – the gene is knocked out in all cells at all time
  - Conditional KO – you can control where and when your target gene is knocked out (CreER-LoxP)



- **CRISPR/Cas9 Genome engineering** is a newer and more versatile tool for engineering a wide variety of genetic changes
  - Cas9 (scissors) is guided to a specific sequence with Guide RNA where it cuts the DNA
  - Point mutations, deletions, or insertions of donor DNA can then be made

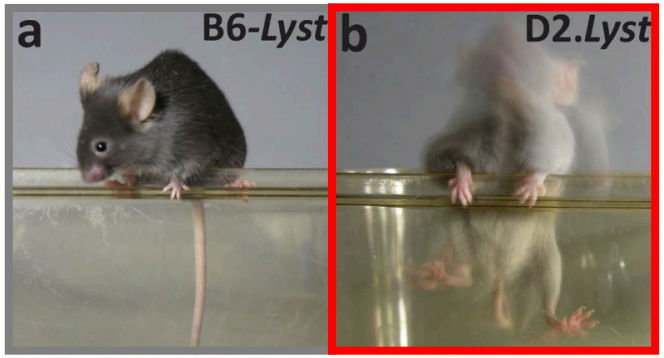


Mouse strain can have an influence on phenotype

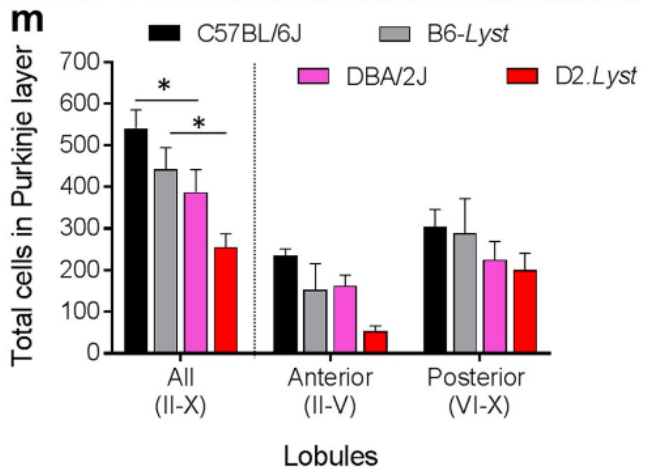
Different mouse strains with the same genotype can yield a different disease phenotype

Chediak Higashi syndrome – *LYST*

B6 | No ataxia
D2 | Ataxia



B6 | Minor Purkinje cell loss
D2 | Major Purkinje cell loss



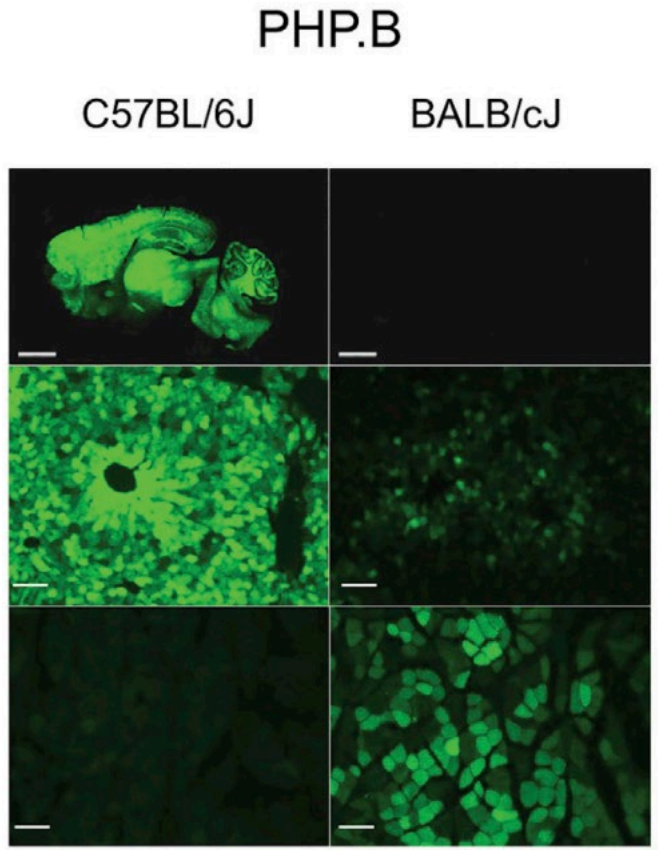
Hedberg-Buenz et al., 2019

Different mouse strains dosed with the same drug can have differential drug delivery or transduction

PHP.B-eGFP

C57BL/6J
High brain delivery

BALB/cJ
Very little brain delivery



Hordeaux et al., 2018

Pigs are emerging as a popular alternative model

Table 1 Species-dependent differences

Species	Sexual maturity	Gestation period	Average life span (year)	Average weight (kg)
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Yang et al., 2021

- More similar anatomy, physiology and metabolism to humans than mice, especially regarding CNS development
- Relative to NHPs, they produce larger litters, have shorter maturation timelines, and lower costs

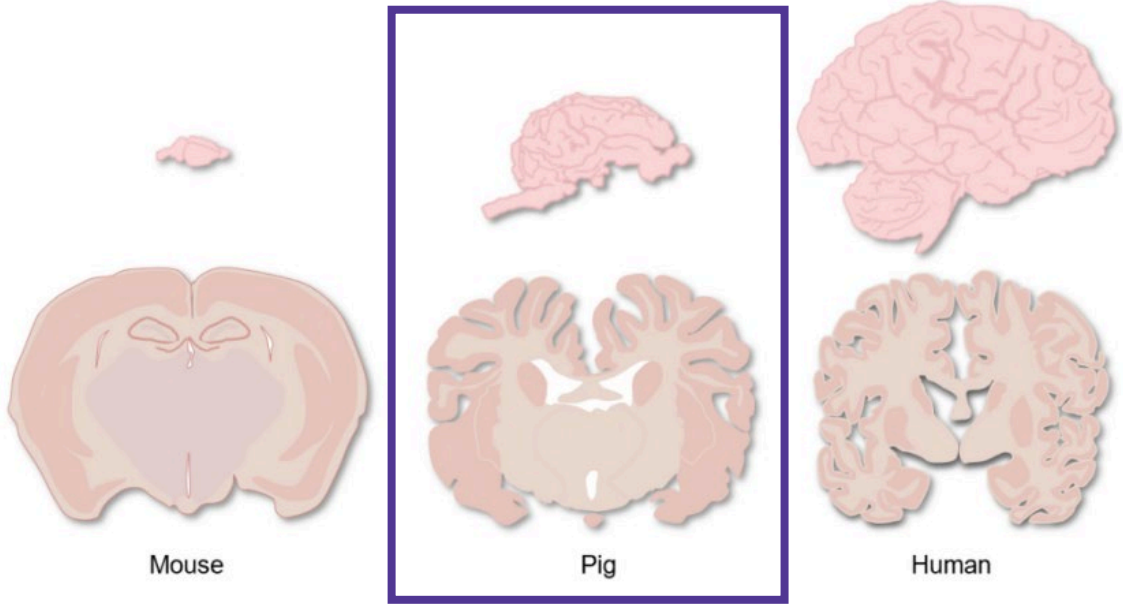

Figure 1. Comparison of brain structures of mouse, pig, and human.

Li et al., 2022

Model choice & study design is dictated by your primary study objective

A lot of different questions need to be answered when developing a drug.

Multiple individual and unique studies should be carefully designed and executed to interrogate those specific questions.

Variables in study design to carefully consider

- **Controls**
  - Positive – a group that has your target phenotype; can be WT mice
  - Negative – model without intervention, or with standard of care
- **Number** of subjects per treatment group
- **Sex** of subjects within groups
- **Age at dosing**, pre vs. post symptom onset
- **Route of delivery** and its reproducibility
- **Length of time** subjects on drug
- **Endpoints**

Thought exercise before running your study

- Draw out different scenarios of your endpoints and form your interpretations.
- Proactively decide "What does success look like?"
- Consider if there are complementary approaches to answering a question that could provide greater confidence

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Thank You

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Resources

Multiple resources for currently existing transgenic models

NIH funded animal resource centers

Rat Resource and Research Center
MU Mutant Mouse Regional Resource Center
National Swine Resource and Research Center

Commercial sources

The Jackson Laboratory
Mouse Genome Informatics
Charles River
Taconic

International resource

International Mouse Phenotyping Consortium