Minimize the use of mice in preclinical research by choosing the right mouse model

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Preclinical Animal Models

How necessary are animal models for modern drug discovery?

- The success rate of new drug development from conception to new drug registration is less than 10% →lack of translation from promising preclinical findings to success in human trial - valley of death

- The FDA Modernization Act 2.0 Allows Animal Trials Alternatives

FDA no longer has to require animal testing for new drugs

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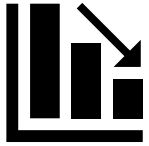
The principle of the 3Rs



Replacement: use of alternative methods to avoid or replace the use of animals in research

- This could include in vitro techniques, computer modeling, or using less sentient organisms.

Reduction focuses on strategies to minimize the number of animals used in experiments

- **Share data – also the negative!**
- Using appropriate statistical analyses to reduce the number of animals needed to find meaningful results

Refinement aims to enhance animal welfare by refining procedures to minimize pain, suffering, and distress

- improving housing conditions
- using less invasive techniques for example imaging

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The process of choosing the correct model

Research question/goal

- Clear and precise
- One per study
- Formulate a hypothesis

Outcome measurement

- Which outcome will answer your question?
- Primary and secondary outcome
- Which methods can assess the parameters/outcome?

Model

- Should be based on research question/hypothesis and outcome measurement

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What makes an animal model a good animal model?

Defining Criteria for Animal Model Validation

- **Face Validity** is defined as how well a model replicates the disease phenotype in humans (similarity of disease/symptoms/signs between humans and the animal model)
- **Construct Validity** is how well the mechanism used to induce the disease phenotype in animals reflects the currently understood disease etiology in humans
- **Predictive Validity** is defined as the measure of how well a model can be used to predict outcomes of the disease in humans

There is no single best model

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Experimental design

- Controls:
  - Positive
  - Comparative
  - Negative
  - Vehicle
  - Sham
- Randomization
  - Physical randomization
  - Computer software
- Blinding: goal should be to be as blinded as possible
- Power analysis

No blinding or randomization increases risk of false positive…

→ contribute to the reproducibility issues

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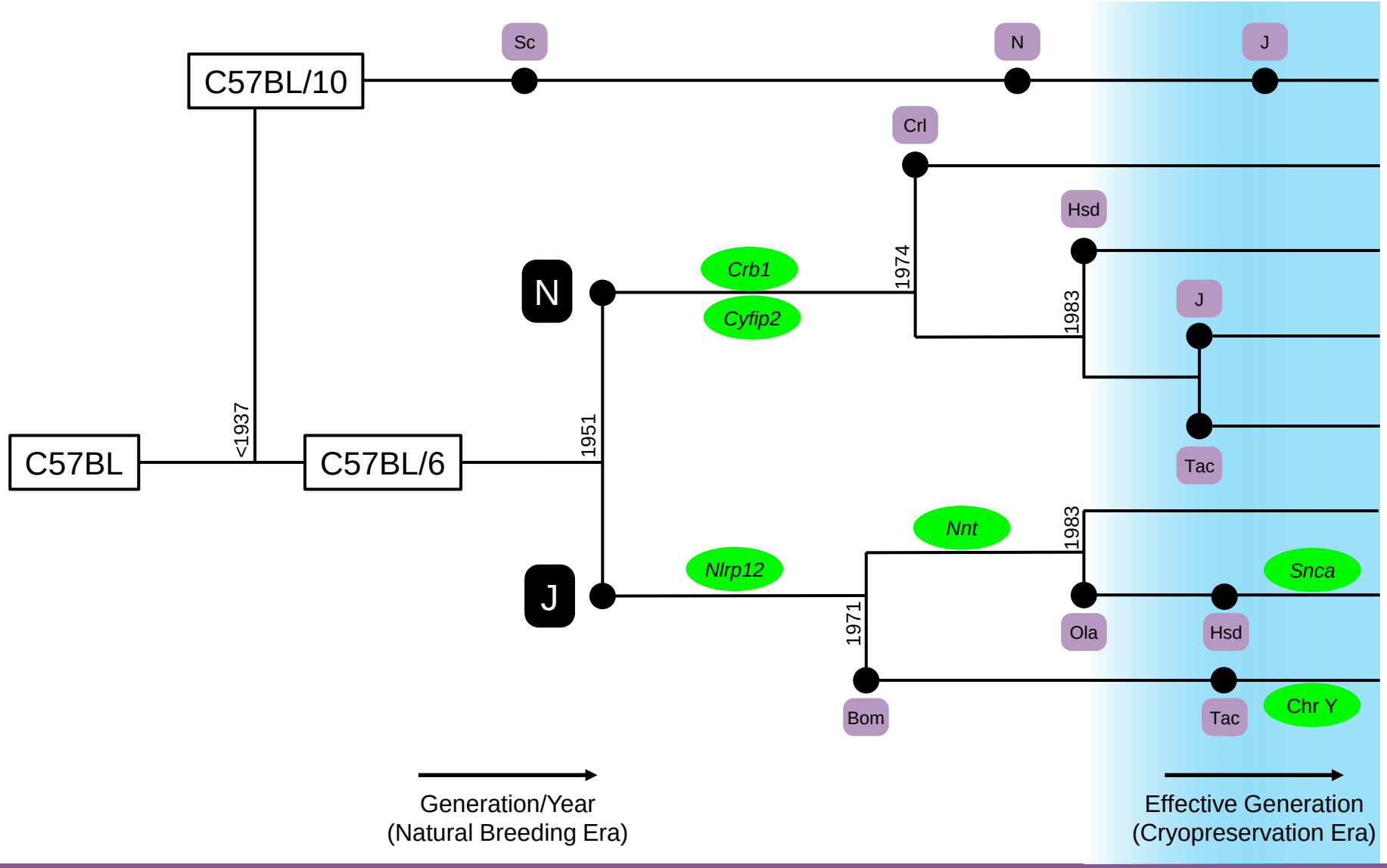
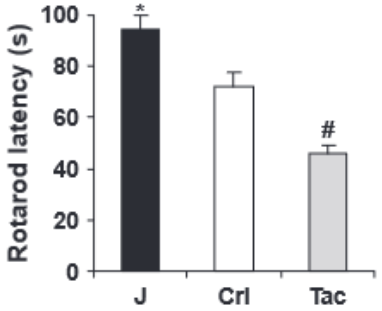
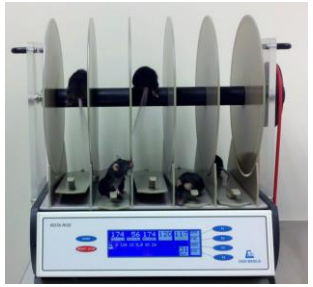
The C57BL/6 Family Tree



Behavioral differences between C57BL/6 substrains





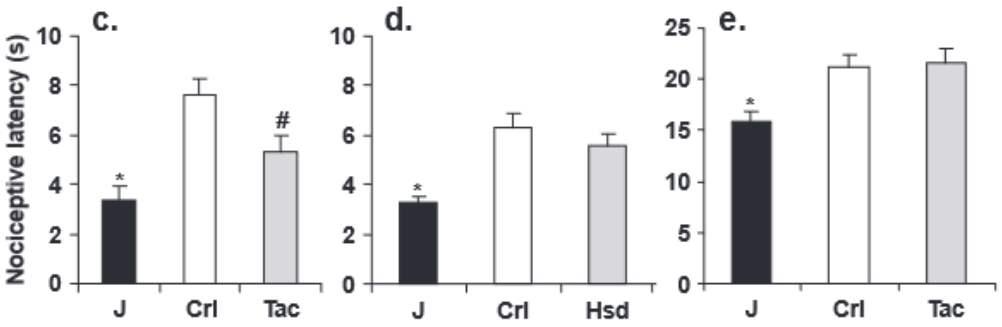
Rotarod picture:
https://en.wikipedia.org/wiki/Rotarod_performance_test#/media/File:Mouse_RotaRod.png

- Altered alcohol preference and consumption
- Altered response to amphetamine
- Altered fear response

Tail-withdrawal latencie (site 1)

Tail-withdrawal latencie (site 2)

Hot-plate latencie



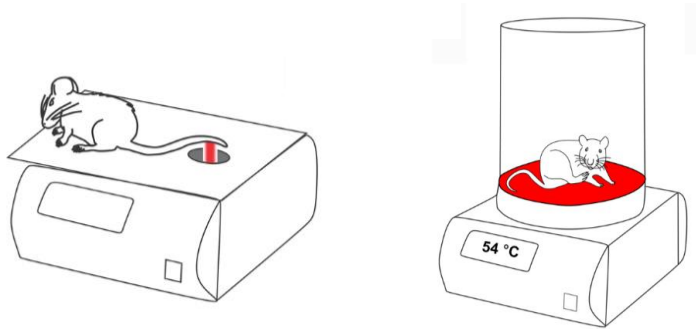


Figure adapted from Deuis JR, et al. (2017) Methods Used to Evaluate Pain Behaviors in Rodents. Front. Mol. Neurosci. doi: 10.3389/fnmol.2017.00284. Licensed under CC BY 4.0 (www.creativecommons.org/licenses/by/4.0/).

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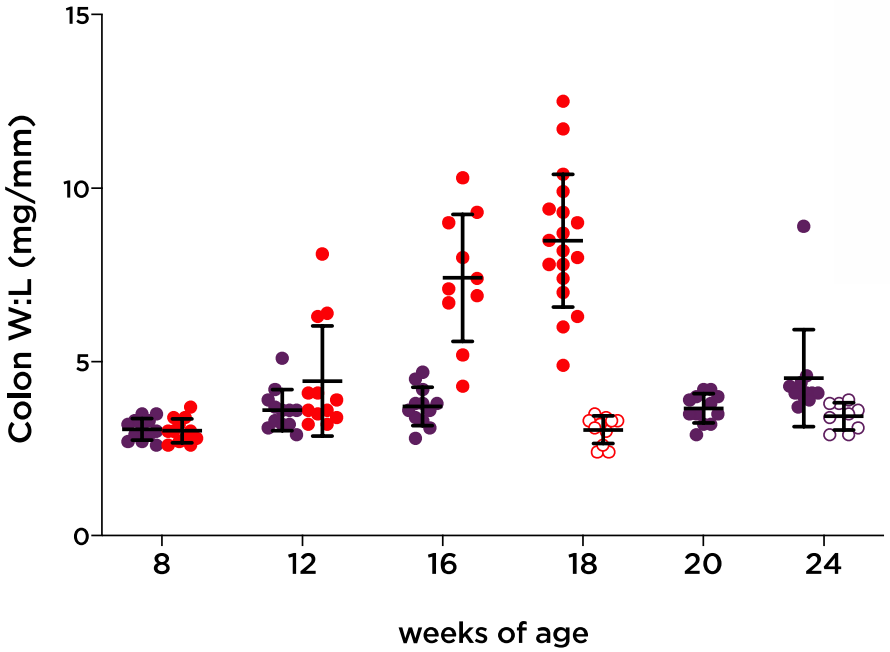
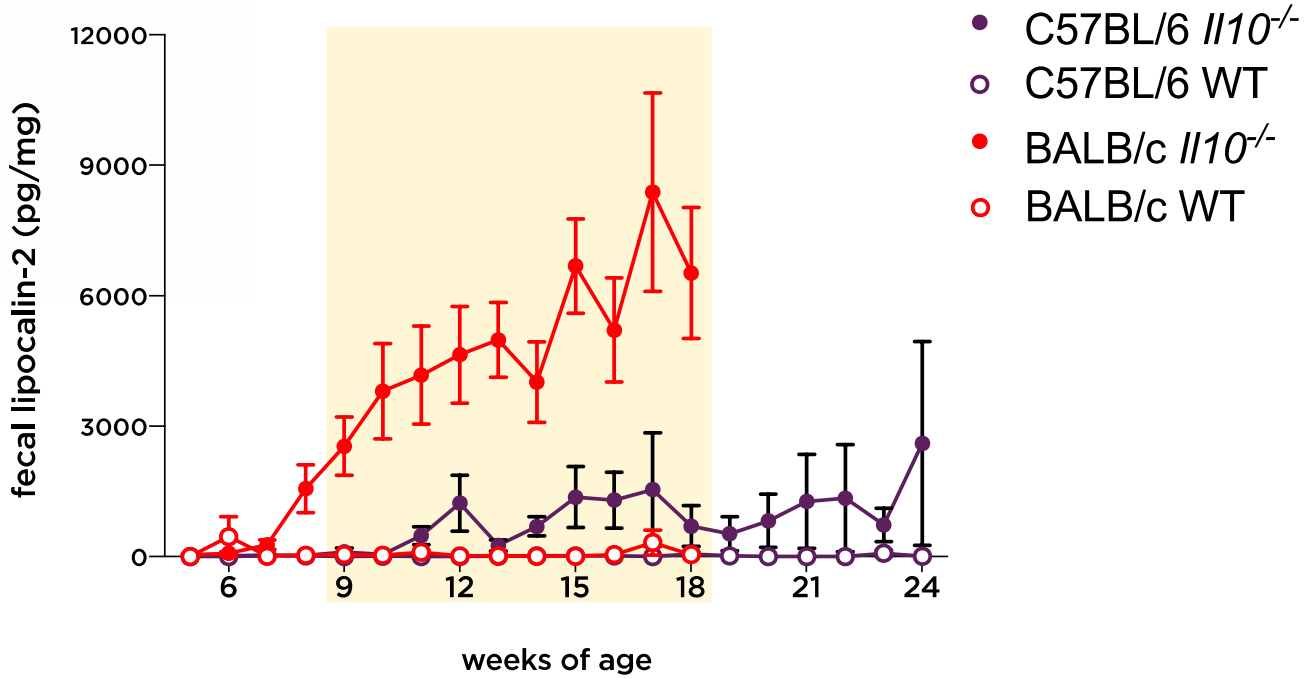
Bryant C. D et al, Behavioral differences among C57BL/6 substrains: implications for transgenic and knockout studies. J Neurogenetics 22 doi: 10.1080/01677060802357388

Importance of strain - Inflammatory Bowel Disease (IBD)

BALB/c vs. C57BL/6 Il10$^{-/-}$



C57BL/6NTac-*Il10*^{em8Tac}



BALB/cAnNTac-*Il10*^{em7Tac}

Data produced in collaboration with Boehringer Ingelheim Pharmaceuticals

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A new player in the game – the microbiome

- The gut microbiome can impact
  - disease development and progression
  - drug efficacy
  - Metabolism/toxicity profile of a drug

The microbiome impacts preclinical animal studies in several ways:

- Variability in results
- Reproducibility issues
- Disease models
- Translatability to humans

Figure created with Microsoft copilot

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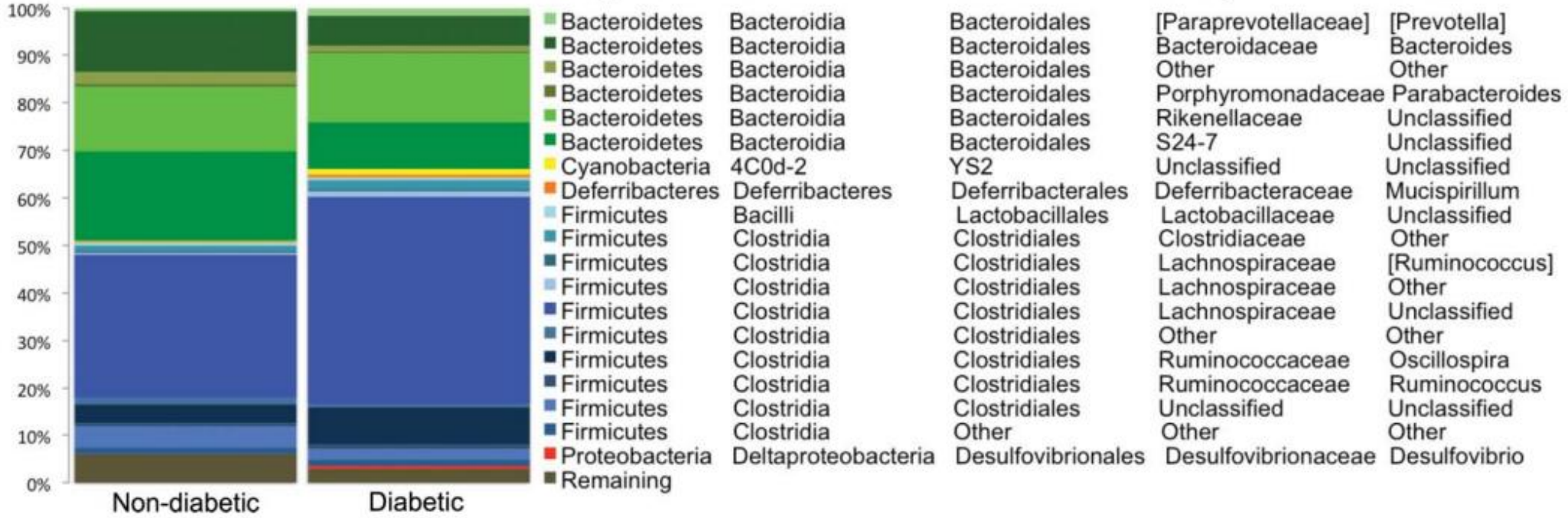
Diabetic Gut Microbiota in NOD Mice

- Spontaneously develop diabetes over time
- Autoimmune pancreatic insulitis begins at 4 weeks of age
- Diabetes incidence and progression varies



Phylum	Class	Order	Family	Genus
Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides
Bacteroidetes	Bacteroidia	Bacteroidales	Other	Other
Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides
Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Unclassified
Bacteroidetes	Bacteroidia	Bacteroidales	S24-7	Unclassified
Cyanobacteria	4C0d-2	YS2	Unclassified	Unclassified
Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Mucispirillum
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Unclassified
Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Other
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Other
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
Firmicutes	Clostridia	Clostridiales	Other	Other
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus
Firmicutes	Clostridia	Clostridiales	Unclassified	Unclassified
Firmicutes	Clostridia	Other	Other	Other
Proteobacteria	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	Desulfovibrio
Remaining				

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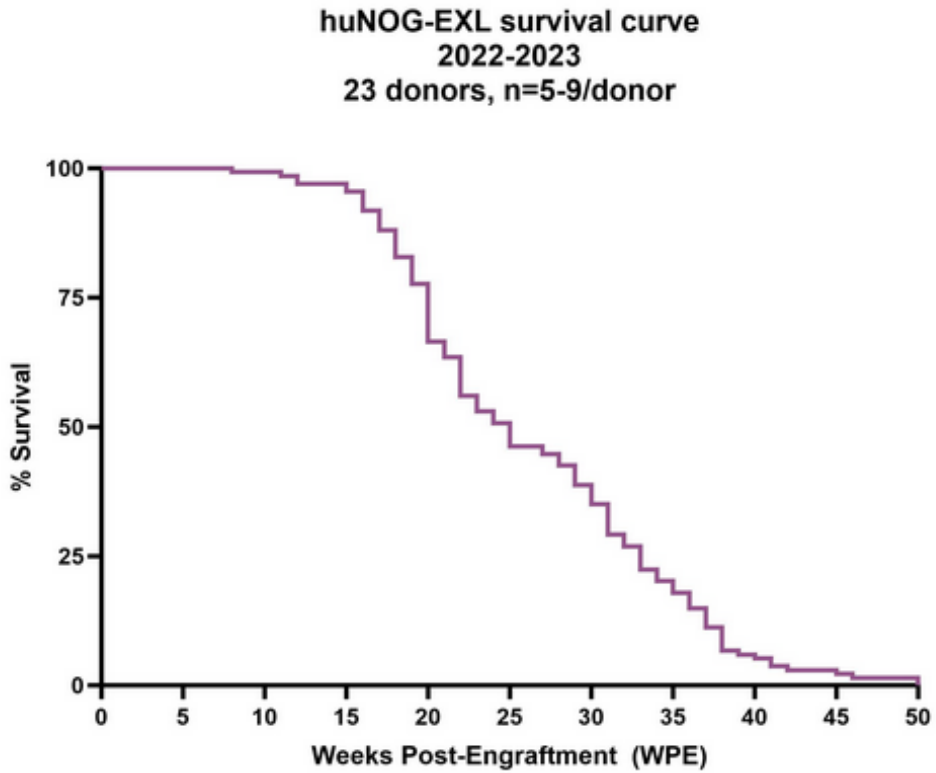
Krych et al., (2015) Gut microbial markers are associated with diabetes onset, regulatory imbalance, and IFN-γ level in NOD Mice. *Gut Microbes* 6(2). doi: 10.1080/19490976.2015.1011876

Importance of knowing the limitations of your model

Importance of knowing the limitations of the model

- Phenotypes to be aware of includes
  - Vision impairment
  - Hearing loss
  - Aggression – perhaps need to single house the mouse
  - Survival

- Example: Humanized immune system (HIS) mice
  - Mice engrafted with human PBMC or HSC
  - Many different models on the market
  - One of the differences between models are the survival



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Using the right model for toxicity testing

The testing of TGN1412, a CD28-specific antibody, resulted in a severe cytokine release syndrome (CRS) during its first human trial in 2006:

Preclinical Testing Limitations: conducted on animals (rodents and non-human primates), which did not predict the human response accurately due to differences in immune cells (CD28 expression on CD4 effector cells are more prominent in humans that in the animal models used)

Lack of Predictive Models: The in vitro tests using human peripheral blood mononuclear cells (PBMCs) also failed to predict the cytokine release because the conditions did not mimic the *in vivo* environment accurately.

Consequence: In humans, TGN1412 caused a massive activation of T-cells, leading to the rapid release of pro-inflammatory cytokines → "**cytokine storm**" which is a severe and life-threatening symptoms for the trial participants.

Humanized immune system mice predicts the outcome

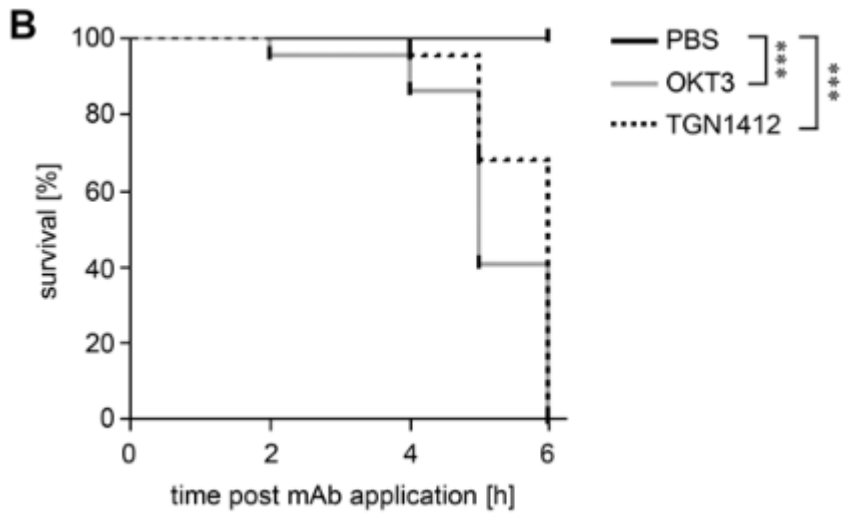

Figure from: Weißmüller S, et al. (2016) TGN1412 Induces Lymphopenia and Human Cytokine Release in a Humanized Mouse Model. PLoS ONE doi:10.1371/journal.pone.0149093. Licensed under CC BY 4.0 (www.creativecommons.org/licenses/by/4.0/).

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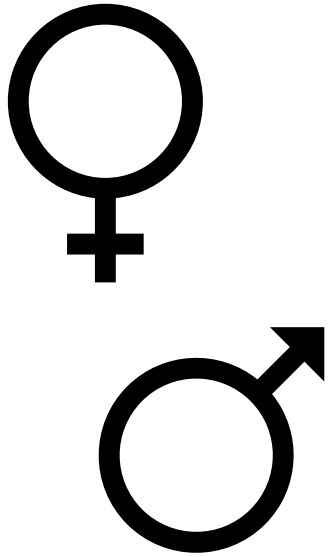
Reduction is not always good

Problem: choosing only one sex in your study

Zolpidem (Ambien)

- A common sleeping aid drug
- Preclinical and clinical trials predominantly involved male subjects
- Consequence: especially women reported doing things like eating, walking, making phone calls and even driving the next day without having memory of it.
- Problem: women metabolize the drug at half the rate of men → should have a lower dose



Up to 80% of mice that is used in research are males

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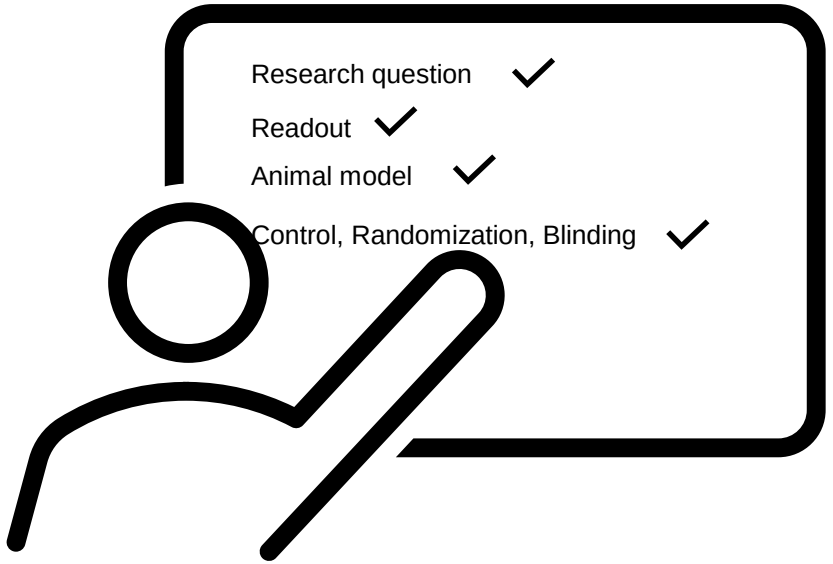
Conclusion

- Choose animal model **AFTER** you have stated a clear research question and determined what the readouts should be
- Mice strains are different
- Good experimental design is crucial

There is no single best model



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Thank You

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