Conflict of Interest

Use of animal models for the development and approval for medical devices and for education of surgeons

External Advisor/ Trainer and Animal Welfare Officer

@Medizinisches Kompetenzzentrum

c/o HCx Consulting GmbH

Medizin im Grünen

Contact:

n.drude@medizin-im-gruenen.de

Responsible PrecliniX: Enhancing Evidence Generation through the 6Rs Framework

Refinement session: Ethical and practical considerations for responsible research using animals

Natascha Drude, PhD

7th November 2023

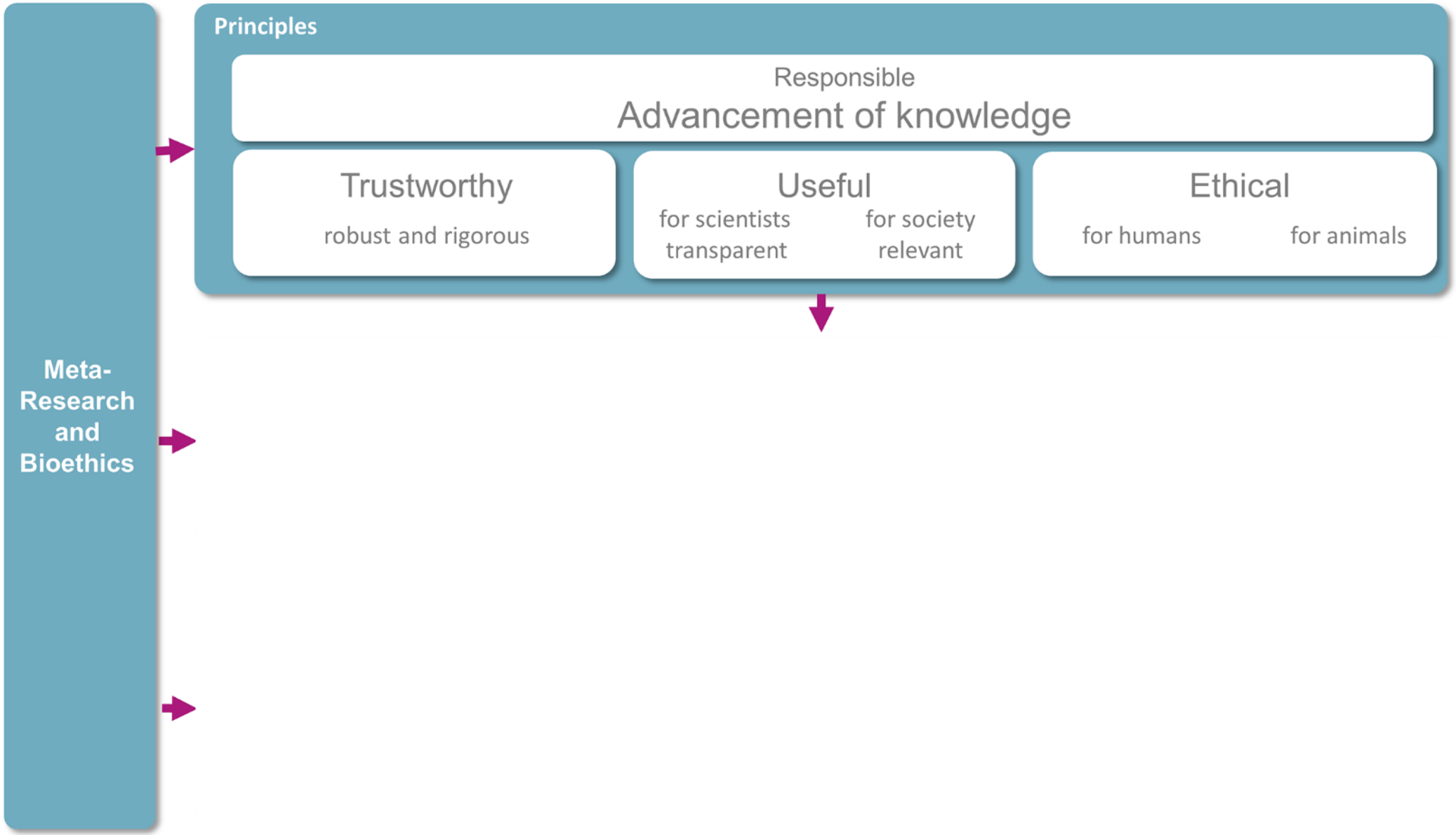
BIH QUEST
Center for Responsible Research

FIN3R Annual Symposium
Improving the quality and translatability of biomedical research through the 3Rs principle

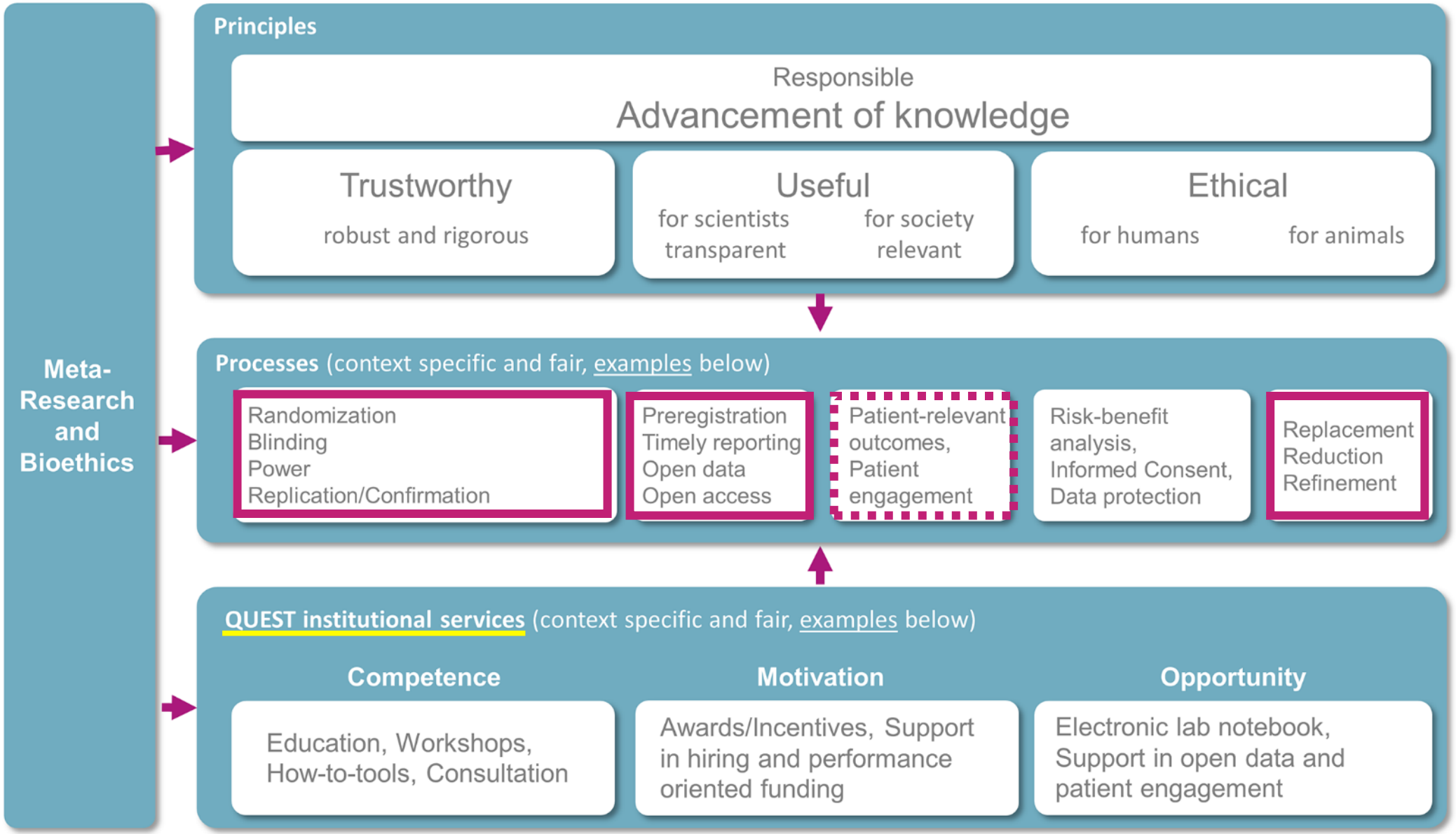
BIH Berlin Institute of Health @Charité

Aus Forschung wird Gesundheit

QUEST Center for Responsible Research



 Strech D, Weissgerber T, Dirnagl U; QUEST Group. PLoS Biol. 2020 Feb 11;18(2):e3000576. doi: 10.1371/journal.pbio.3000576.



 Strech D, Weissgerber T, Dirnagl U; QUEST Group. PLoS Biol. 2020 Feb 11;18(2):e3000576. doi: 10.1371/journal.pbio.3000576.

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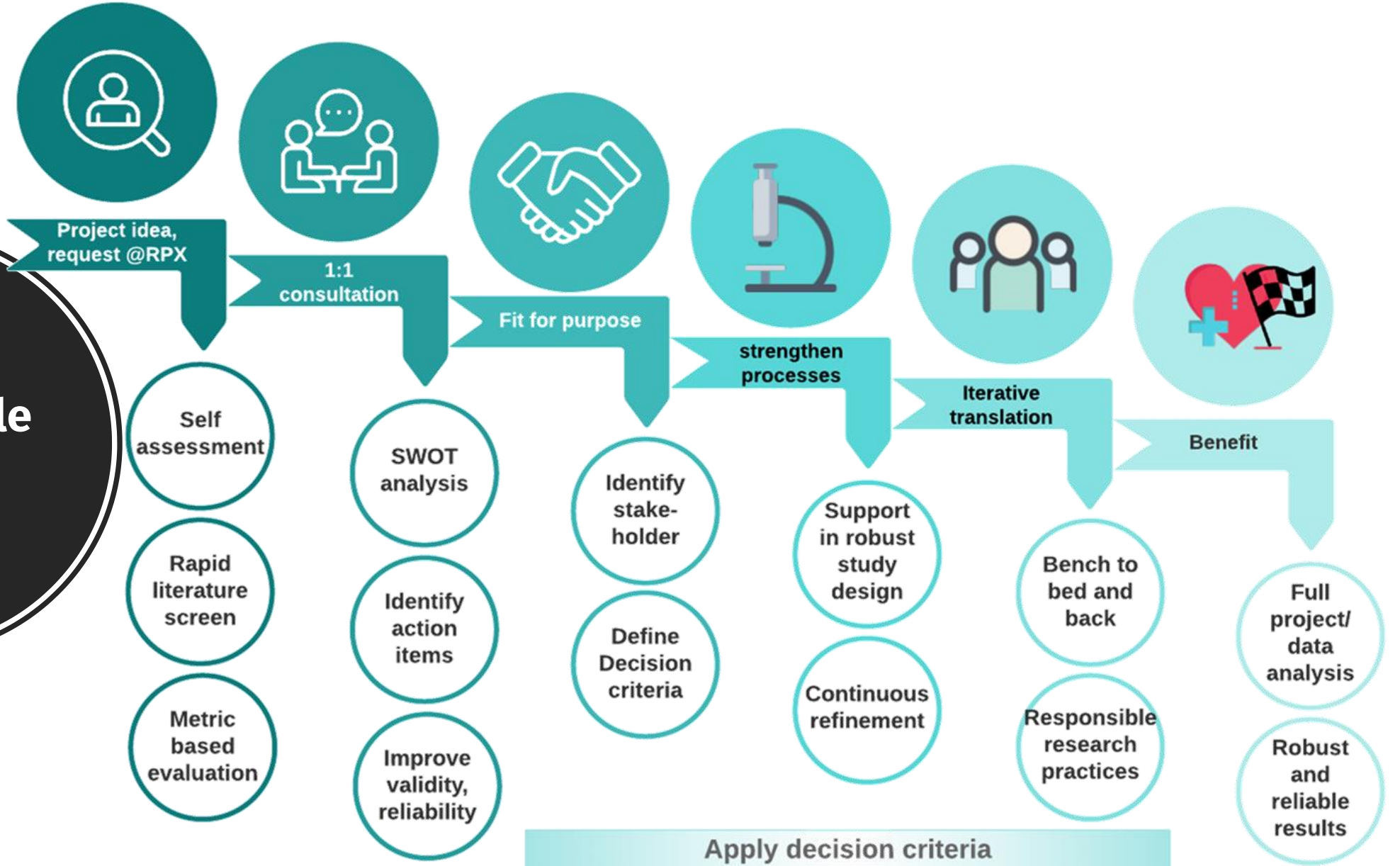
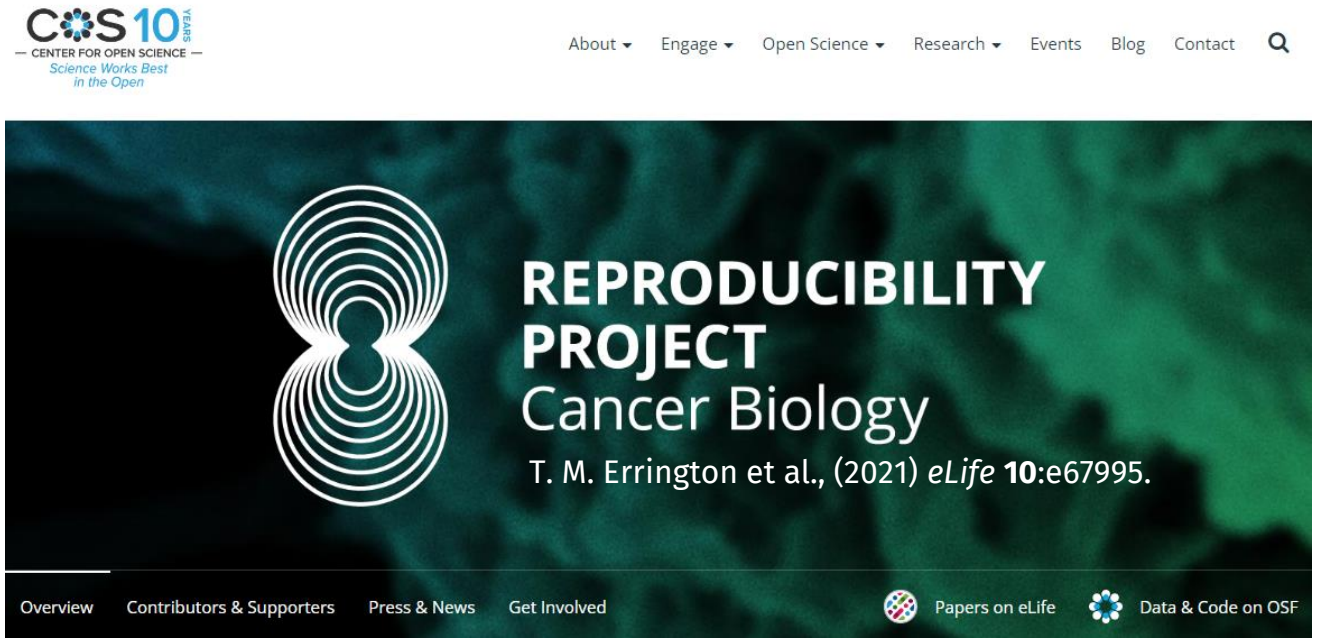
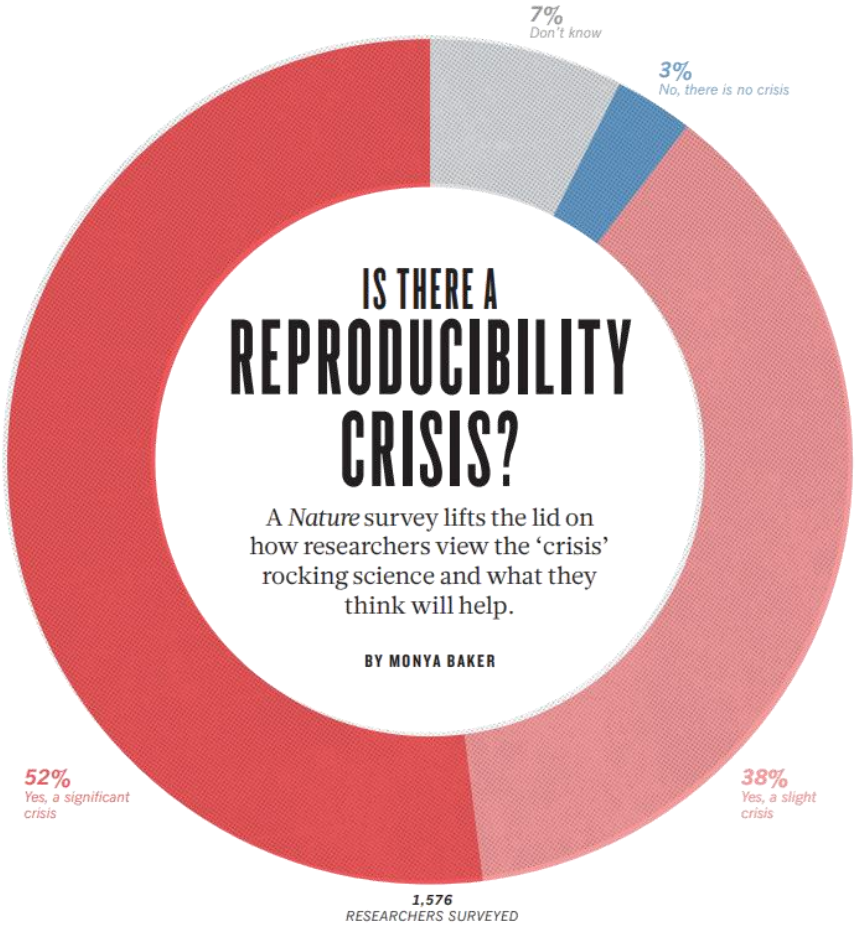
Responsible PrecliniX



Reproducibility crisis and the lack of translation



Kane PB, Kimmelman J. Is preclinical research in cancer biology reproducible enough? Elife. 2021 Dec 7;10:e67527. doi: 10.7554/eLife.67527.



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Failure to connect two worlds, or rather multiple reasons for translational attrition?

- Complexity
- Someone else was there already: Low hanging fruits have been picked
- Lack of robustness and transparency of preclinical research results
- Lack of robustness and transparency of clinical study results
- Lack of resources (including time!)
- …

Seyhan *Translational Medicine Communications* (2019) 4:18
https://doi.org/10.1186/s41231-019-0050-7

Translational Medicine Communications

REVIEW Open Access

Lost in translation: the valley of death across preclinical and clinical divide – identification of problems and overcoming obstacles

Attila A. Seyhan[1,2]

Credit: B. MELLOR

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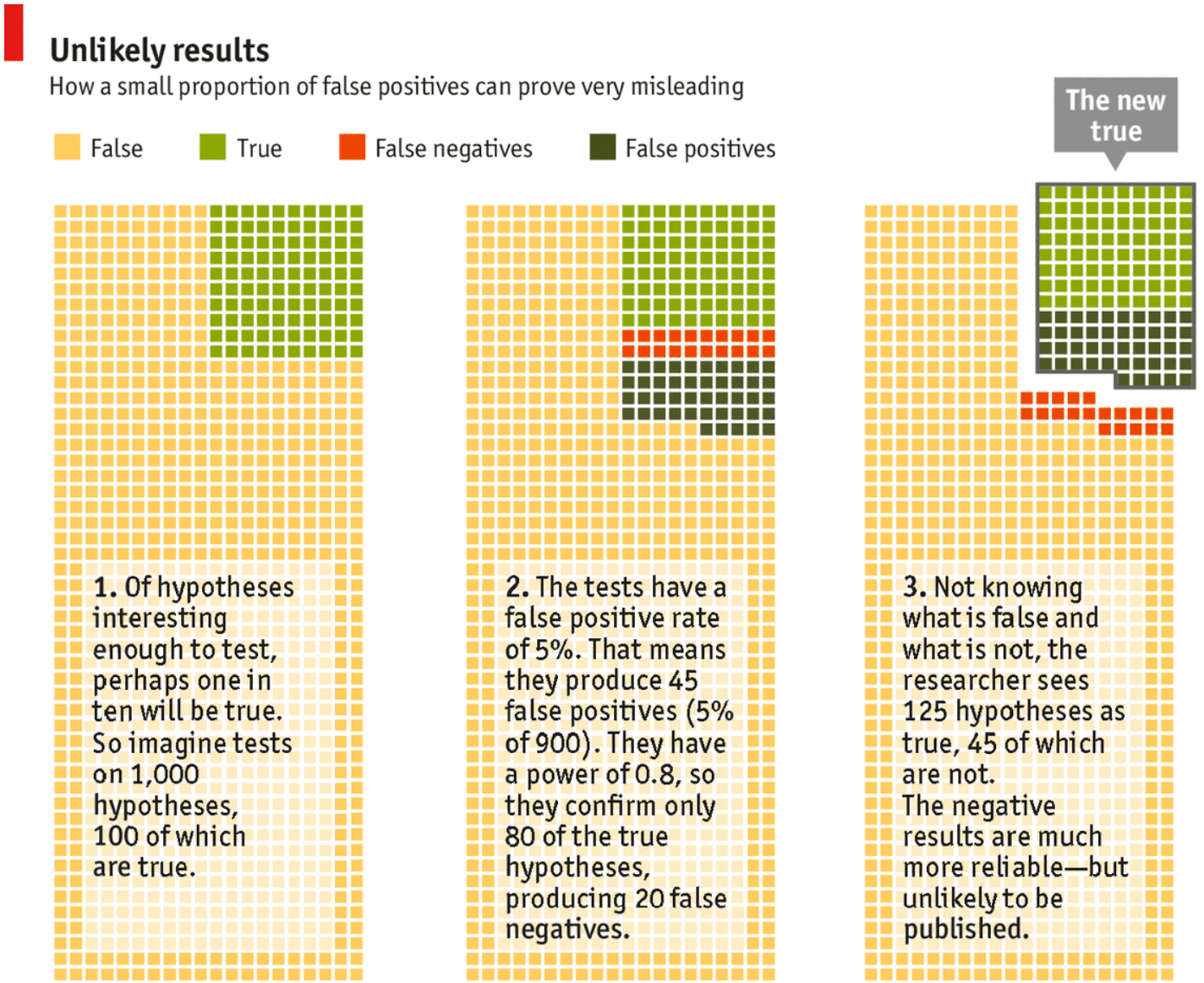
Non-reproducibility as an indicator for cutting edge research?

Typical Experiment

Are you studying something that is rather unlikely (cutting edge?)

False positives:
5% (α = 0.05)

False negatives:
20% (β = 0.80 →power)



Source: *The Economist*

https://www.economist.com/briefing/2013/10/18/trouble-at-the-lab

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Replication versus Non-replication

Commentary

THE EMBO JOURNAL

Rethinking research reproducibility

Ulrich Dirnagl[1,2]

What does it mean if you do not replicate?

Original results false positive?

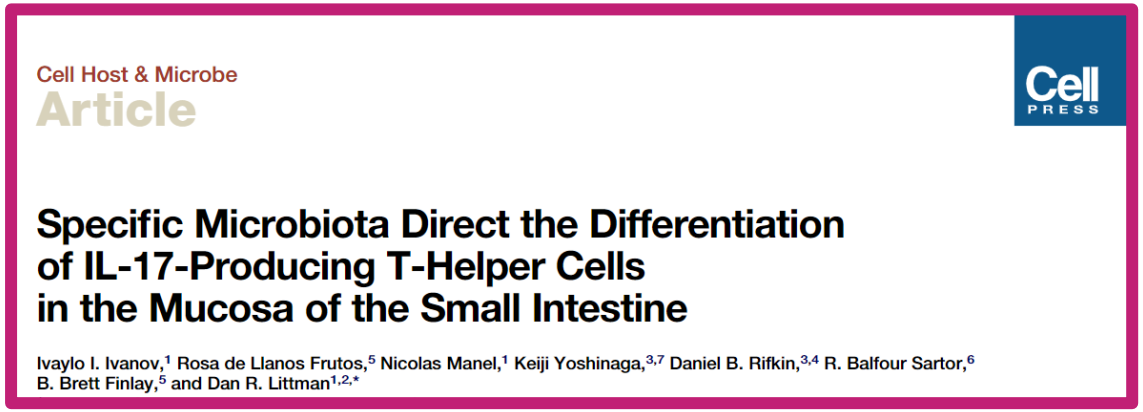
Replication false negative?

Does successful replication mean that the original results were correct?

Could both results be false positives?

Was the study technically competent?

Hidden moderators


Cell Host & Microbe
Article

Cell PRESS

Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine

Ivaylo I. Ivanov,[1] Rosa de Llanos Frutos,[5] Nicolas Manel,[1] Keiji Yoshinaga,[3,7] Daniel B. Rifkin,[3,4] R. Balfour Sartor,[6] B. Brett Finlay,[5] and Dan R. Littman[1,2,*]

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Bias

Systematic error, intentional or unintentional, within the research process

The quality of the study is determined not by the prevention of bias itself, but by the degree to which bias is avoided and possible bias is addressed

There is no one-size-fits-all solution to avoid distortion

Cartoon: Practical Psychology https://www.youtube.com/watch?v=mQKhAue2Js0

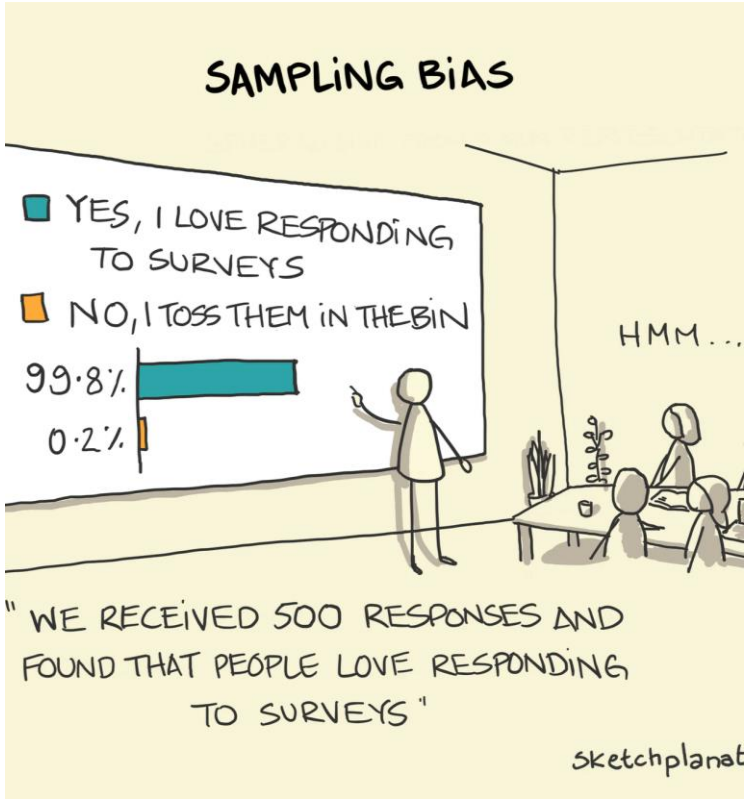
Bias

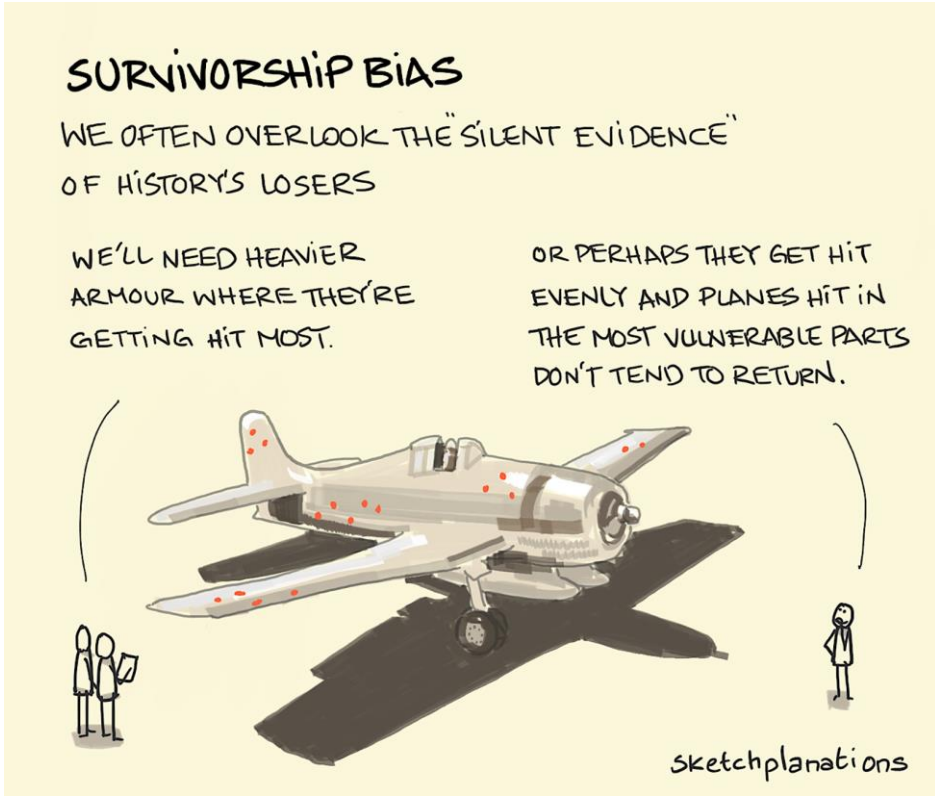
Systematic error, intentional or unintentional, within the research process

The quality of the study is determined not by the prevention of bias itself, but by the degree to which bias is avoided and possible bias is addressed

Cartoon: Practical Psychology https://www.youtube.com/watch?v=mQKhAue2Js0

Hypothesis-driven research -gut feeling and expectations





https://sketchplanations.com/survivorship-bias-silent-evidence

Stephen Sigler, Nature May 1989

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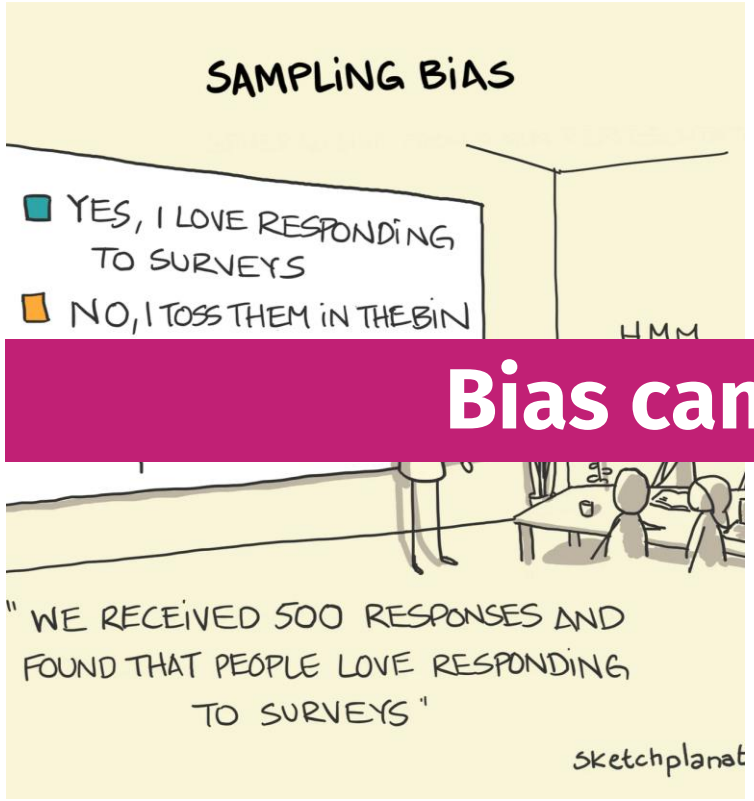
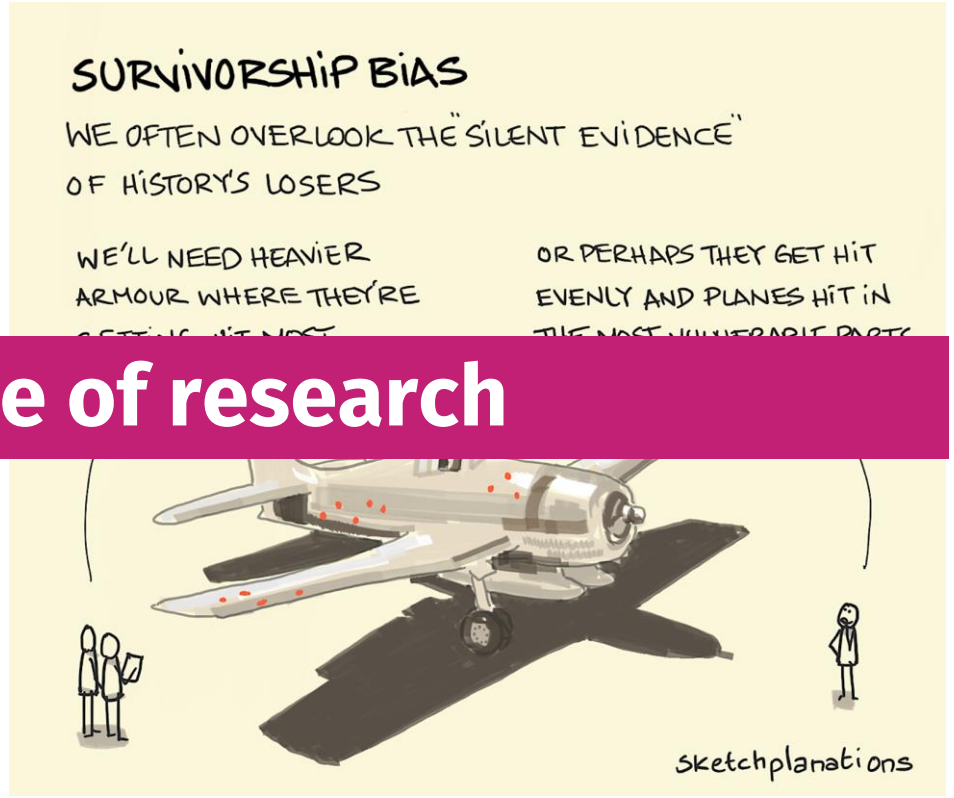
Hypothesis-driven research -gut feeling and expectations



Bias can occur at ANY phase of research



https://sketchplanations.com/survivorship-bias-silent-evidence
Stephen Sigler, Nature May 1989

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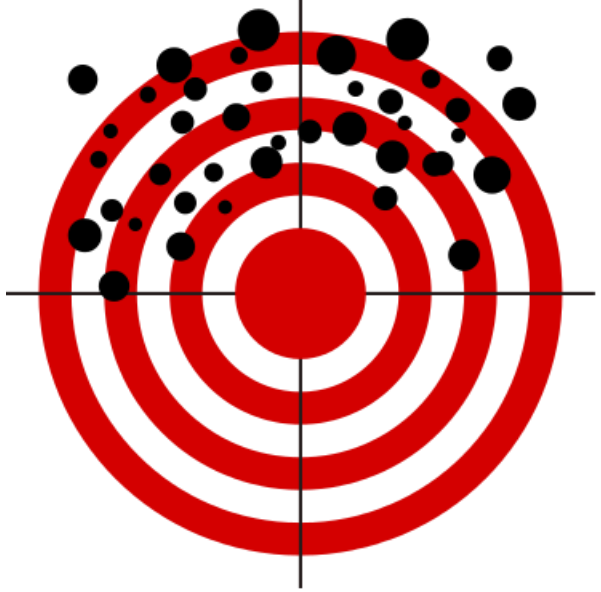
How to minimize risk of bias?
Do we really measure what we want to measure?

Internal validity refers to how far measurements in an experiment reflect causal conclusions or mechanisms

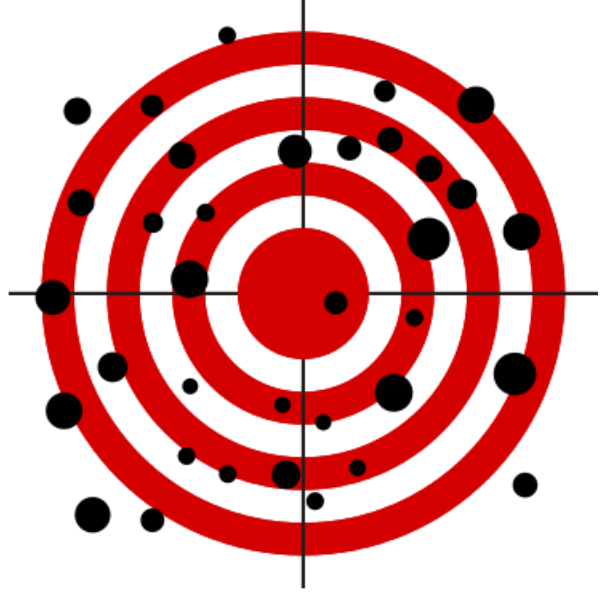
Proper experimental design will aim for high internal validity reducing potential risks of bias

Illustration by Dirk Jan-Hoek (CC-BY), via www.bayesianspectacles.org

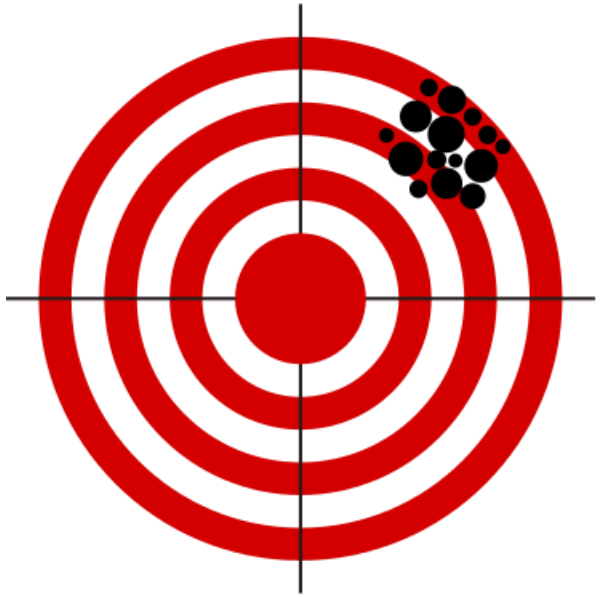
Common figure to understand validity and reliability



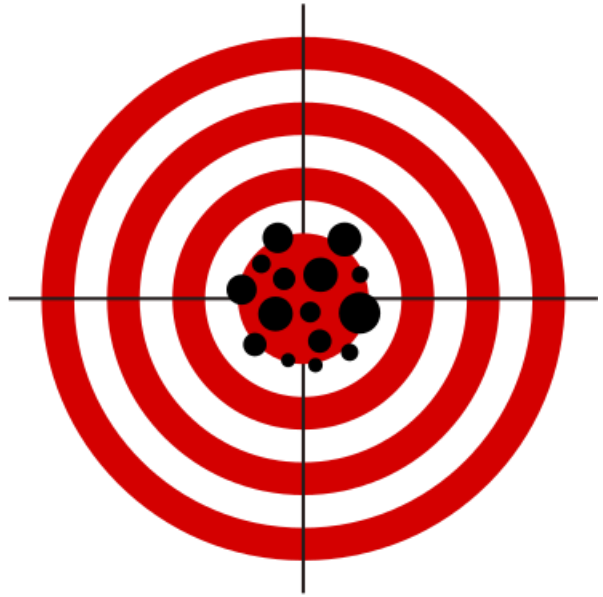
Unreliable & Unvalid



Unreliable, But Valid

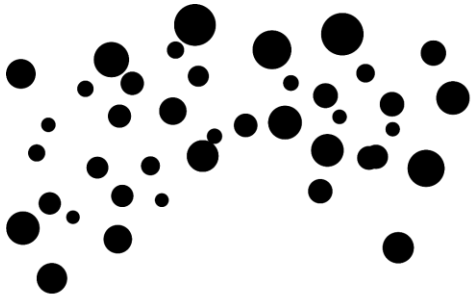


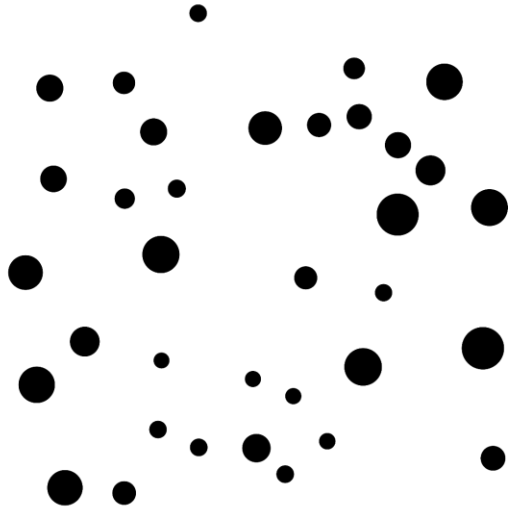
Reliable, Not Valid



Both Reliable & Valid

Which experiment is valid?





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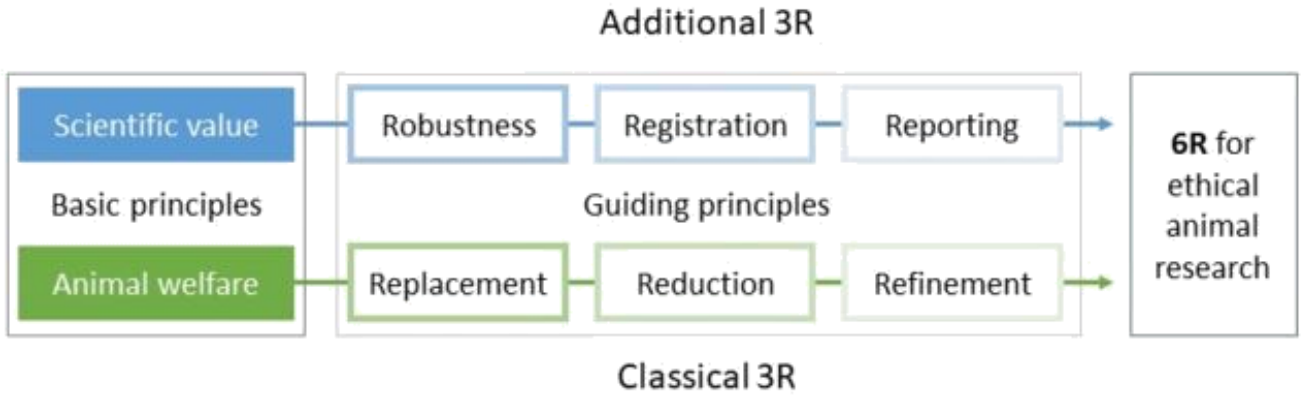
Responsible and robust evidence generation



Trustworthy: Robust and Rigorous

Useful: Registration and Reporting

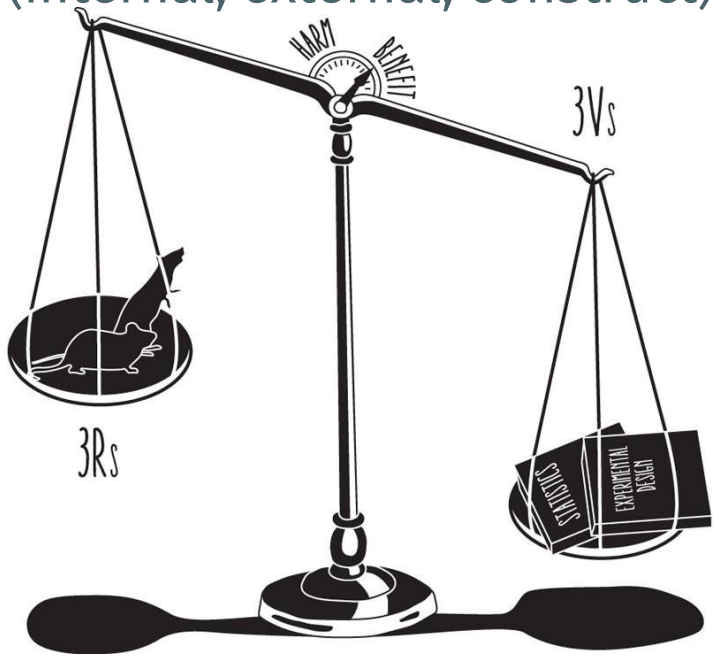
Ethical: 3 (+3) Rs

Strech D, Dirnagl U.; BMJ Open Sci. 2019 Jul 4;3(1):bmjos-2018-000048. doi: 10.1136/bmjos-2018-000048.

Animal Experimentation in Research: The 3Rs Principle and the Validity of Scientific Research

3Vs: scientific validity

(internal, external, construct)



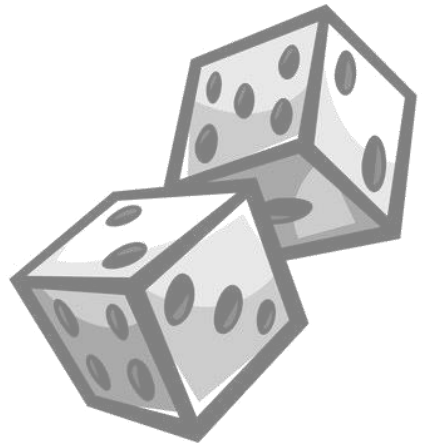
Würbel, H.; *Lab Anim* **46**, 164–166 (2017). https://doi.org/10.1038/laban.1220

Randomization

- Process of **randomly allocating subjects to comparison groups** in a study
- Each subject has the **SAME chance** of receiving each of the possible interventions
- Probability that a subject will receive a particular intervention is **independent** of the probability that any other subject will receive the same intervention

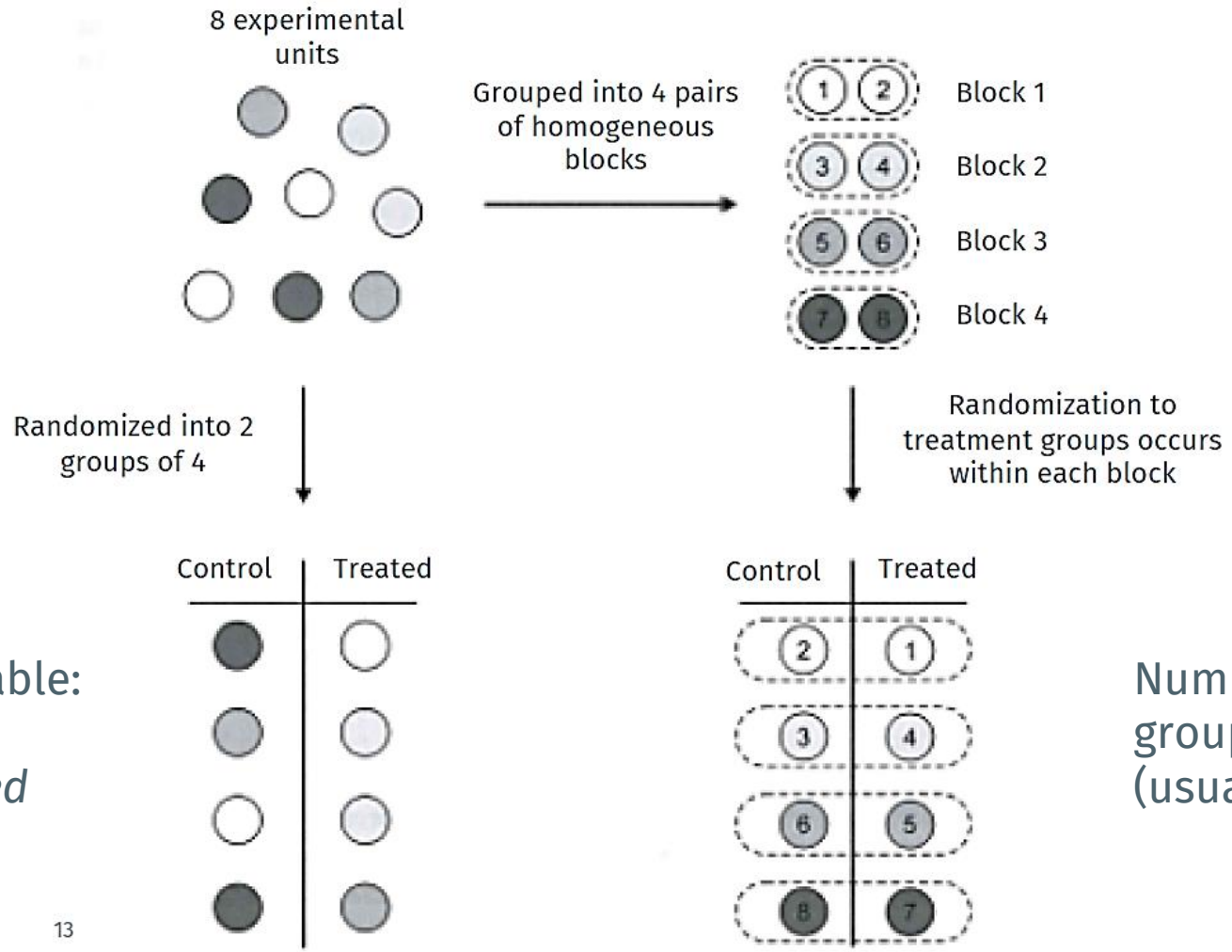
- Random allocation ≠ random sampling

- Equal distribution of the benefits and risks of the interventions



https://arriveguidelines.org/arrive-guidelines/randomisation/4a/explanation

Types of Randomization



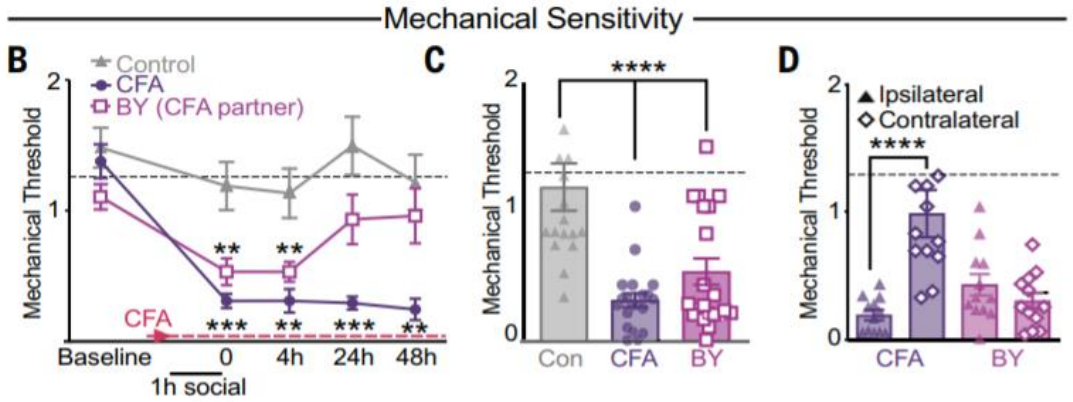
Random number table:
Textbook
Computer generated
Random allocation software

Number of participants in each group within a desired ratio (usually “equal”)

Experimental Design for Laboratory Biologists by Stanley Lazic, 2016 https://doi.org/10.1017/9781139696647

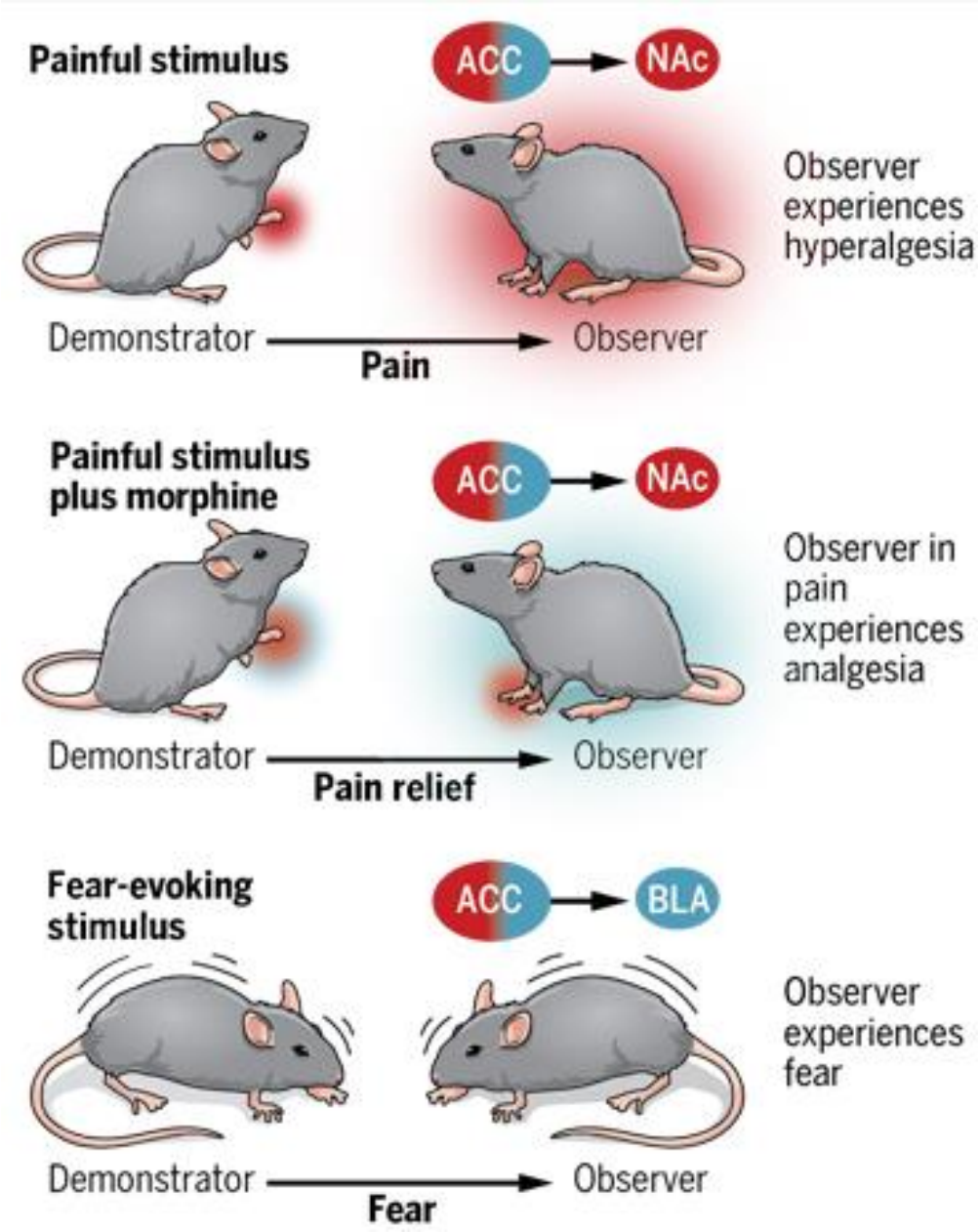
Limitations in preclinical studies?

Social transfer of pain in rodents



Smith et al., Science 371, 153–159 (2021)

Cage effects



Monique L. Smith, Naoyuki Asada, Robert C. Malenka, Science 2021./doi/10.1126/science.abe3040

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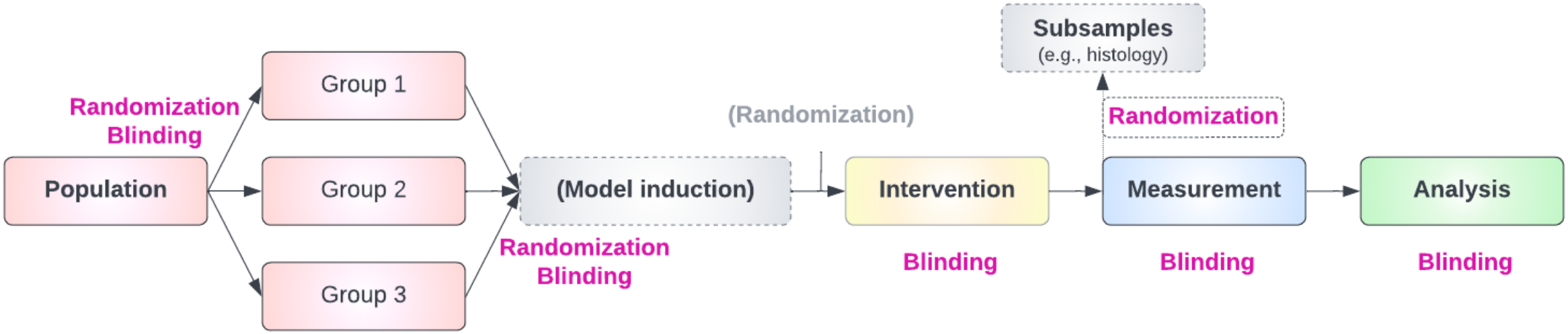
Blinding/ randomization -limitations and feasible strategies



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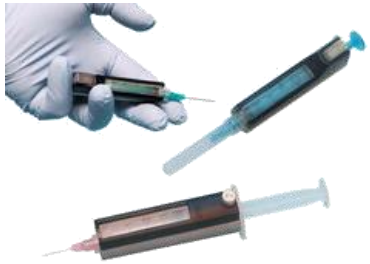
Blinding –limitations?

Preclinical studies often **lack personnel**
–often no monitor who would be aware of e.g., potential adverse events

To avoid bias, the mouse was blinded when self-reporting outcomes. Image credit: Lorris Williams.

Foxn1^{nu} / Foxn1^{nu} mutant *(left)* mouse with wild type control *(right)* littermate

Cai J, Ma L., Genesis. 2011; 49(6):449-59. doi: 10.1002/dvg.20744



Other ways to reduce risk of bias:

Automation
Quality management
Core facilities (outsourcing)
Preregistration

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Preregistration/ publish your protocol

https://preclinicaltrials.eu/

Bf3R German Centre for the Protection of Laboratory Animals

BfR German Federal Institute for Risk Assessment

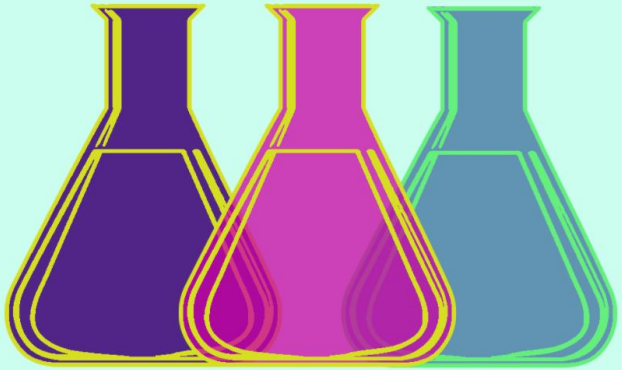
https://www.animalstudyregistry.org

OSF PREREGISTRATION
https://osf.io/registries/osf/new

Registered Reports (Peer-Reviewed)
https://www.nature.com/srep/journal-policies/registered-reports

protocols.io PLOS

https://plos.org/protocols/



Video: Introduction to protocols.io

Published Peer-Reviewed Protocols

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Internal validity and reliability –outlier management versus attrition/drop out

a priori (**preregistration**) based on **e.g. range of accepted values, physiological range, previous experience**

- **Need to be applied in a blinded fashion**

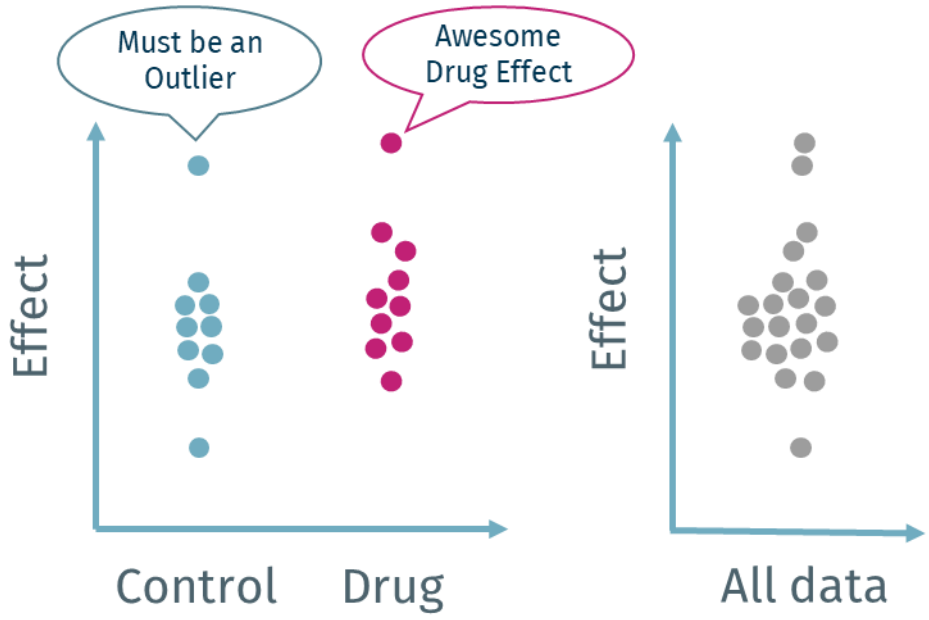
Inclusion/exclusion criteria based on:

- **Animal welfare** (severity assessment and humane endpoint)
- **Scientific outcome** (outlier management)
- **Characteristics of the model** (genotype, phenotype, stage of disease)

Outlier

- technical (failure); extreme values (i.e., >3 SD), animal attrition

Analysis plan



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Attrition/Drop out

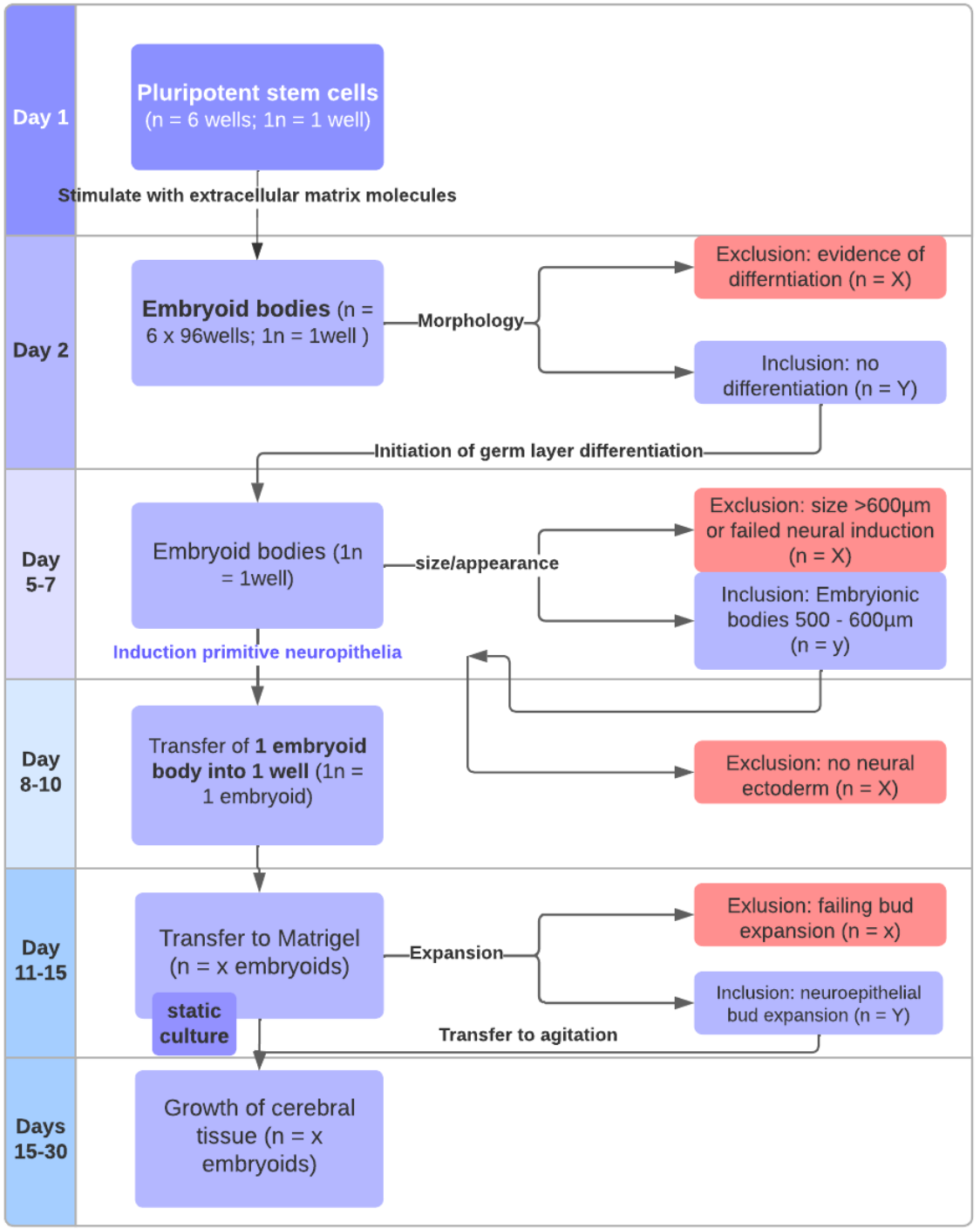
Subject “leaves” during a study

Reason for attrition?

Death?

Loss of follow-up?

Are those who leave different from those who remain?

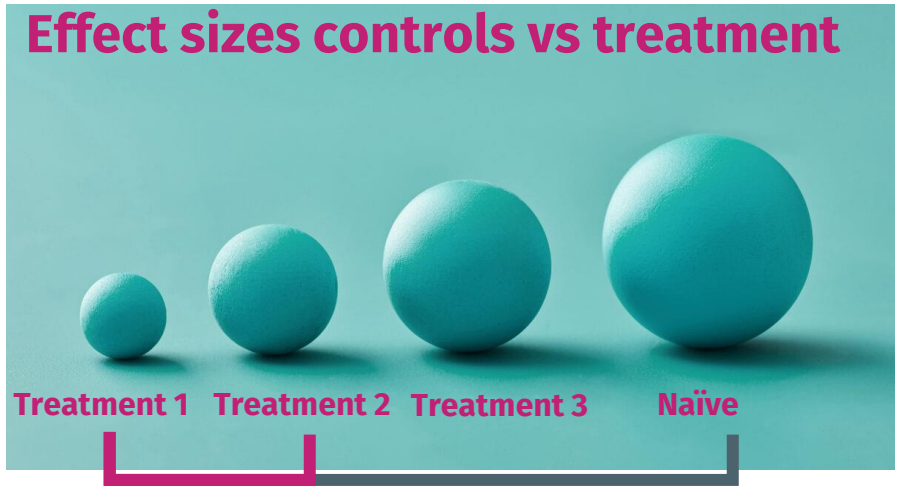


Compared with what?

Control groups:

- Positive and negative control groups?
- **Baseline measures possible?**

<u>Approved</u> comparator drug/intervention in standard clinical care?



What is the **sample size calculation** based on?

Planning and analysis on the level of the **experimental unit**?

- Relevant **confounding variables/ effect modifier**

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How generalisable are your experiments?

Is the targeted mechanism described as causal for the disease in humans?

Correlation or causation?

External validity –generalizability of results

Systematic heterogenization and the “standardization fallacy”

Co-morbidities, different sexes, different strains
Secondary outcomes

EXPERT OPINION ON DRUG DISCOVERY
2023, VOL. 18, NO. 11, 1273–1285
https://doi.org/10.1080/17460441.2023.2251886

Taylor & Francis
Taylor & Francis Group

REVIEW

Check for updates

Mapping strategies towards improved external validity in preclinical translational research

Clarissa F. D. Carneiro, Natascha Drude, Maren Hülsemann, Anja Collazo and Ulf Toelch

Secondary outcomes/ Flanking experiments

Triangulation

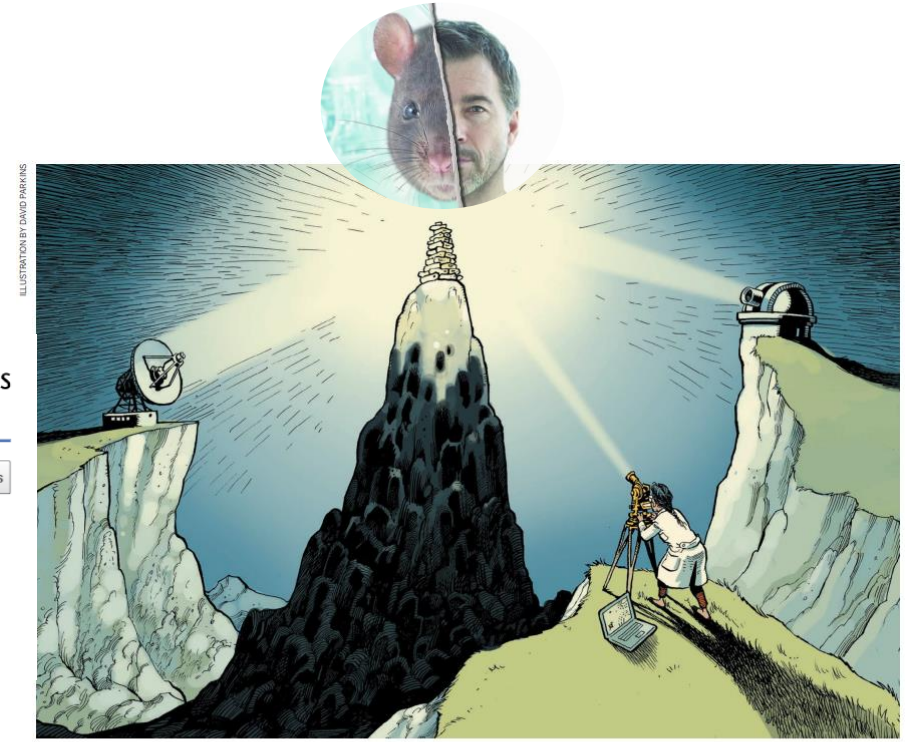

ILLUSTRATION BY DAVID PARKINS

Repeating experiments is not enough

Verifying results requires disparate lines of evidence — a technique called triangulation. **Marcus R. Munafò** and **George Davey Smith** explain.

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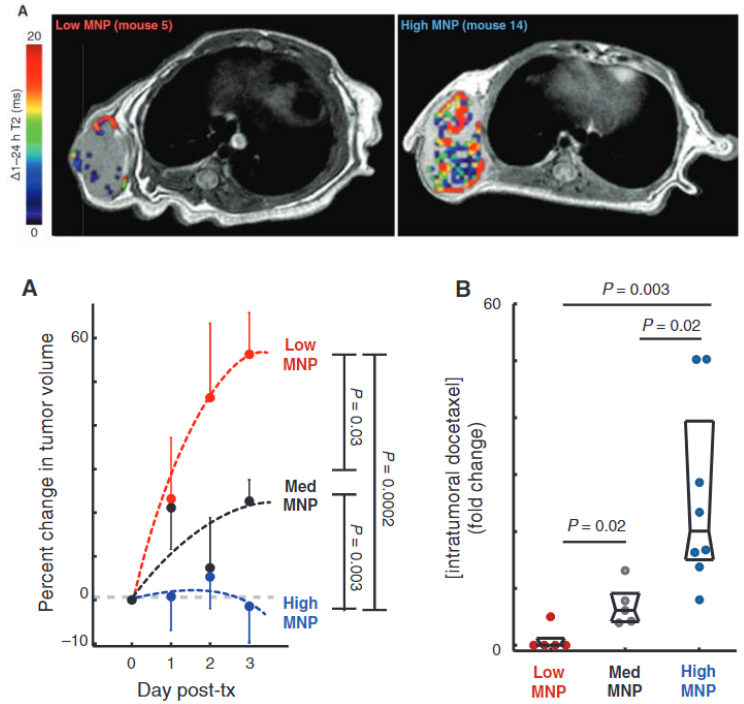
Nature **553**, 399-401 (2018)

Think clinical translation -similarity of the studied model system to human disease conditions

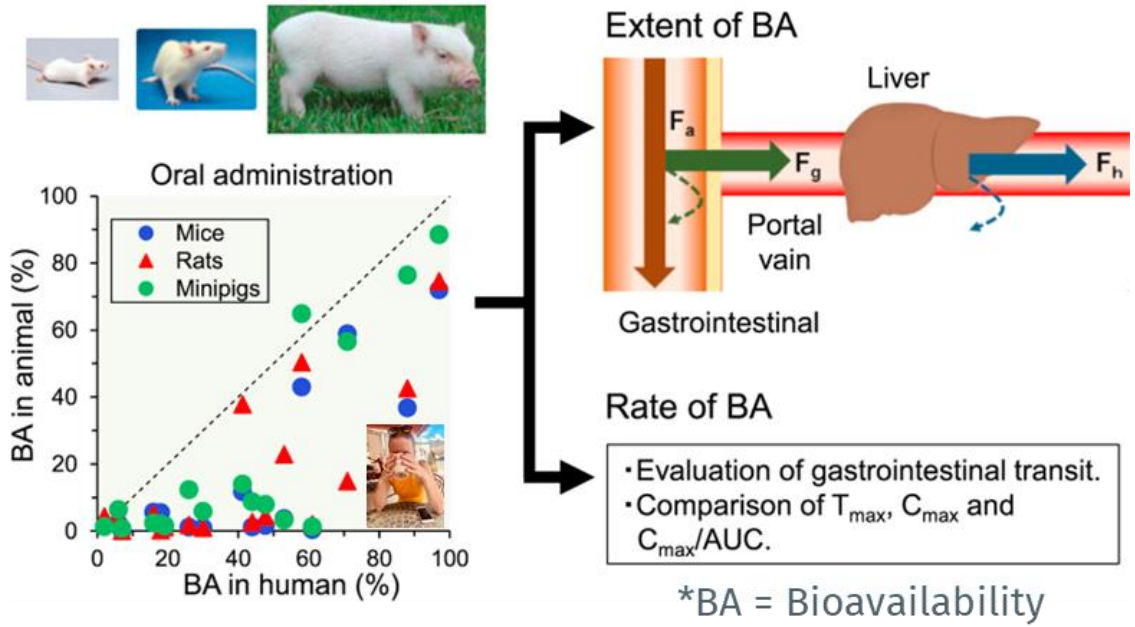
Are clinical biomarkers or companion diagnostics measured that reflect human conditions?



Miles A. Miller *et al.*, *Sci. Transl. Med.***7**,314ra183-314ra183(2015).DOI:10.1126/scitranslmed.aac6522

Clinical relevance of route of administration

- Bioavailability (pharmacokinetics)
- (Drug) dosing



Hiromichi Yoshimatsu, Kunikazu Ishii, Shinji Yamashita, Journal of Drug Delivery Science and Technology, Volume 58, 2020, 101743, https://doi.org/10.1016/j.jddst.2020.101743

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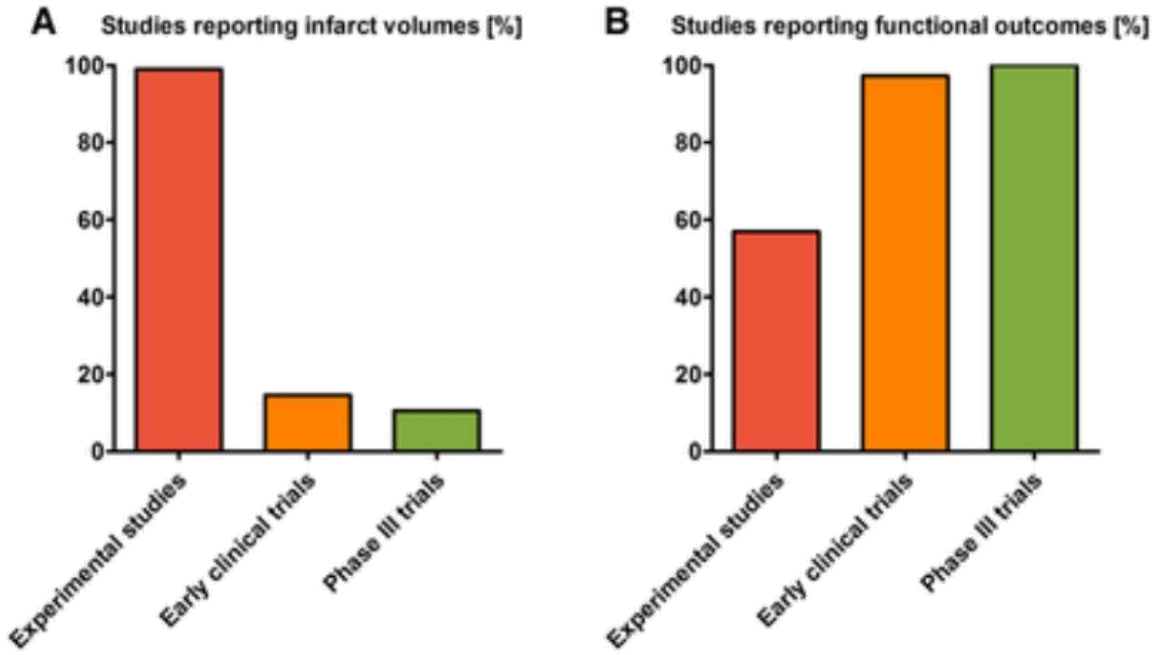
How generalisable are your experiments to humans and patients?

Is the primary outcome adequate for the studied disease?



Schmidt-Pogoda, A et al., (2020), Ann Neurol, 87: 40-51 https://doi.org/10.1002/ana.25643

Do I have convergent and discriminant evidence? (Triangulation)

Converging evidence

Different experimental approaches that support the same claim

Discriminant evidence

Exclude similar alternatives

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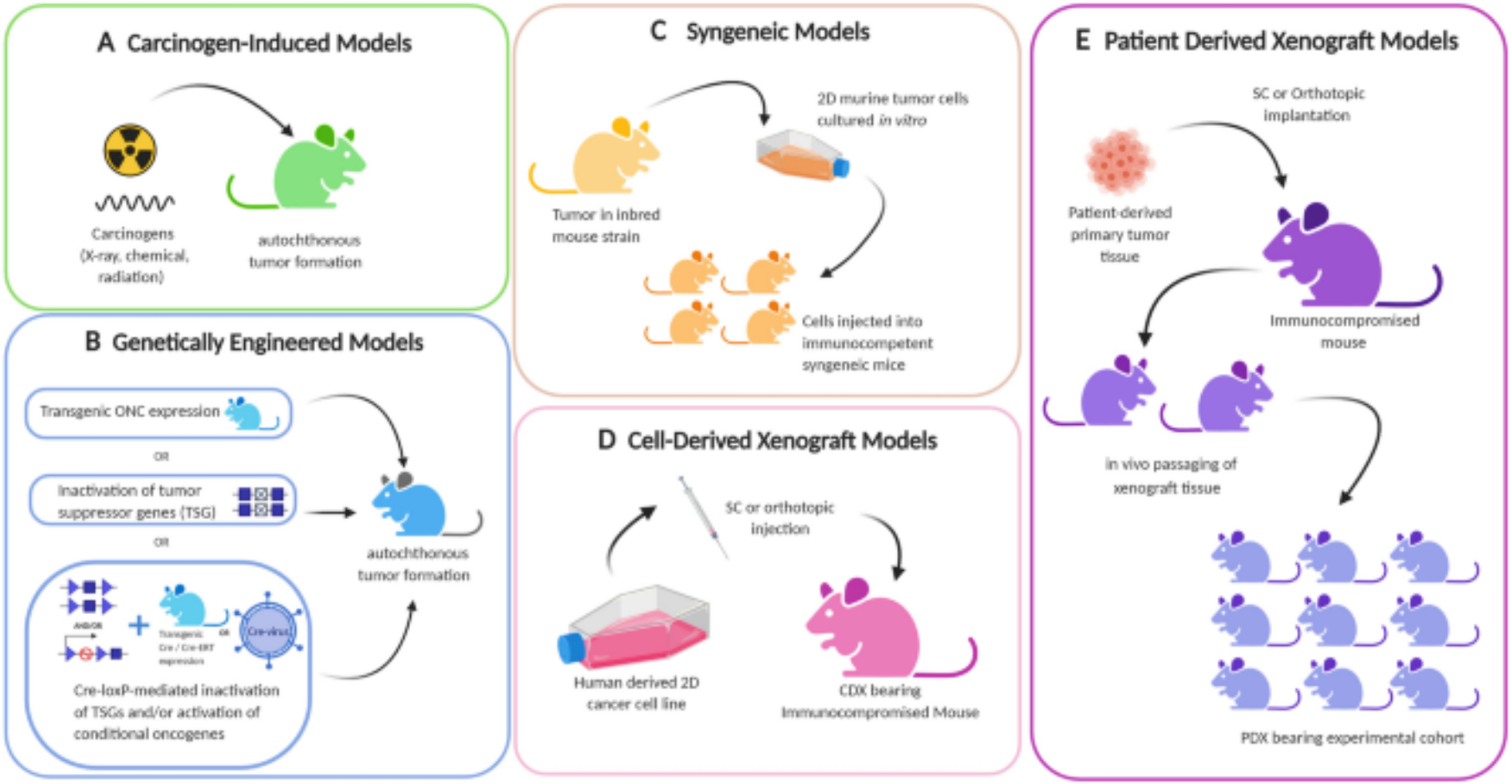
How generalisable are your experiments to humans?

Is the (animal) **model appropriate**? What are we modeling? What are **limitations**?



Bareham, B., Georgakopoulos, N., Matas-Céspedes, A. *et al.*, *Cancer Immunol Immunother* **70,** 2737–2750 (2021). https://doi.org/10.1007/s00262-021-02897-5

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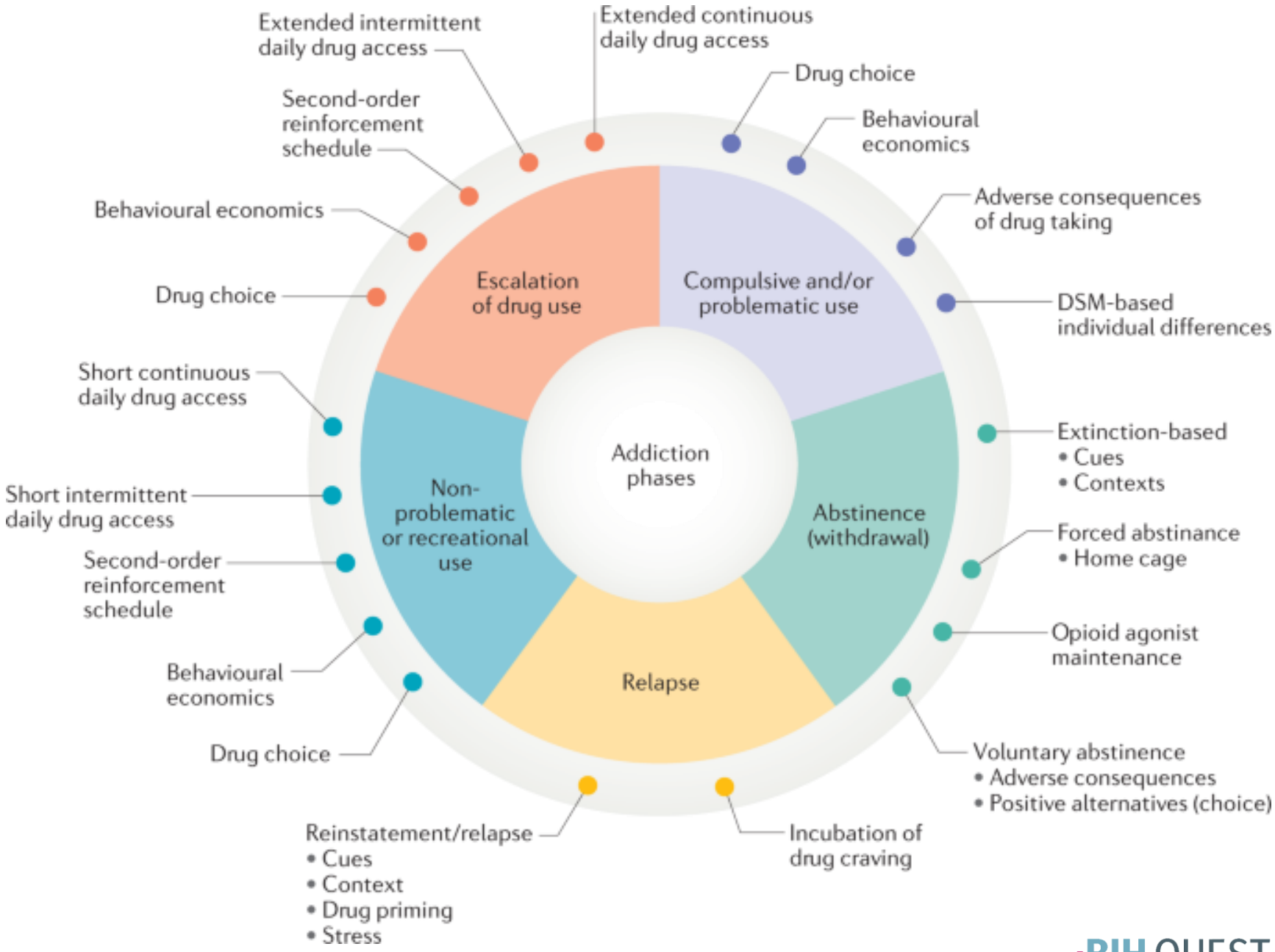
Reverse translation

Understand mechanism and action of successful treatments



Venniro, M., Banks, M.L., Heilig, M. *et al.* Improving translation of animal models of addiction and relapse by reverse translation. *Nat Rev Neurosci* **21**, 625–643 (2020). https://doi.org/10.1038/s41583-020-0378-z

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The immune system -wildlings to increase external validity

RESEARCH

RESEARCH ARTICLE SUMMARY

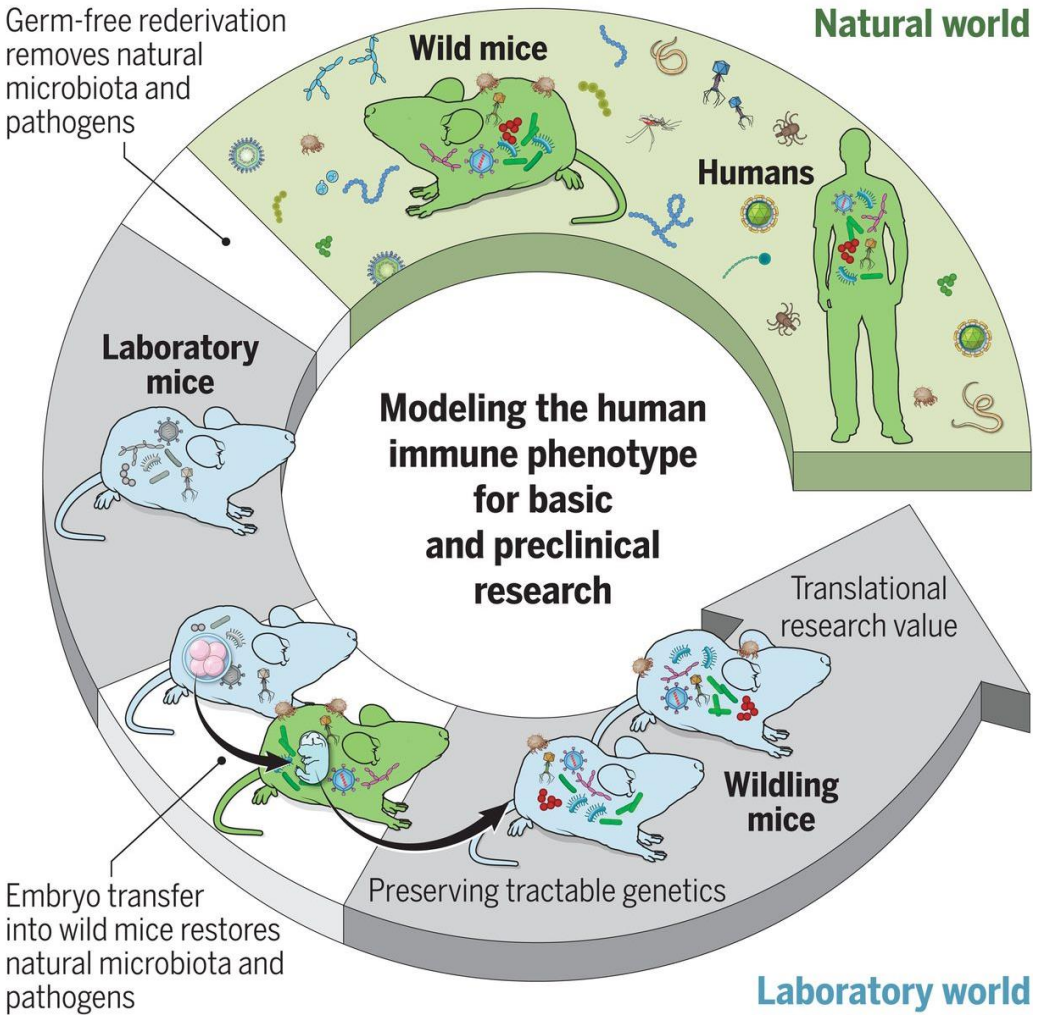
IMMUNOLOGY

Laboratory mice born to wild mice have natural microbiota and model human immune responses

Stephan P. Rosshart*, Jasmin Herz, Brian G. Vassallo, Ashli Hunter, Morgan K. Wall, Jonathan H. Badger, John A. McCulloch, Dimitrios G. Anastasakis, Aishe A. Sarshad, Irina Leonardi, Nicholas Collins, Joshua A. Blatter, Seong-Ji Han, Samira Tamoutounour, Svetlana Potapova, Mark B. Foster St. Claire, Wuxing Yuan, Shurjo K. Sen, Matthew S. Dreier, Benedikt Hild, Markus Hafner, David Wang, Iliyan D. Iliev, Yasmine Belkaid, Giorgio Trinchieri, Barbara Rehermann*

CD28-superagonist (CD28SA) trial: life-threatening activation of inflammatory T cells and cytokine storms

Anti–tumor necrosis factor–alpha (TNF-a) treatment during septic shock ⇨early termination of the study because of harm



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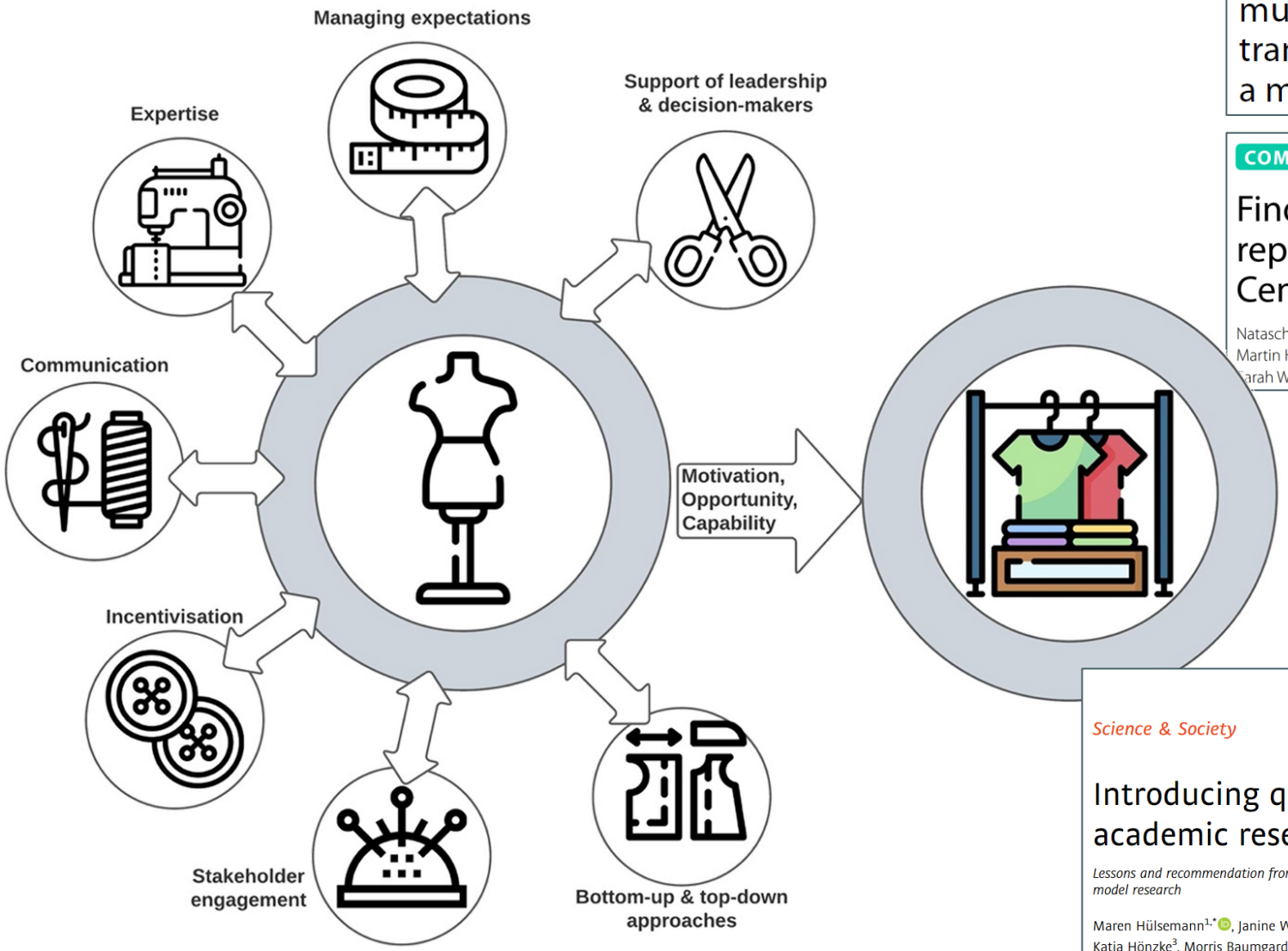
No one-size-fits-all



Drude et al.
Translational Medicine Communications (2022) 7:24
https://doi.org/10.1186/s41231-022-00130-8

Translational Medicine Communications

REVIEW Open Access

Planning preclinical confirmatory multicenter trials to strengthen translation from basic to clinical research – a multi-stakeholder workshop report

COMMENTARY Open Access

Finding the best fit for improving reproducibility: reflections from the QUEST Center for Responsible Research

Natascha Drude[1], Lorena Martinez-Gamboa[1], Tamarinde Haven[1*], Constance Holman[1], Martin Holst[1,2], Silke Kniffert[1], Sarah McCann[1], Torsten Rackoll[1], Robert Schulz[1] and Sarah Weschke[1]

RESEARCH ARTICLE

Measurement challenges and causes of incomplete results reporting of biomedical animal studies: Results from an interview study

Till Bruckner[1,2], Susanne Wieschowski[1,2], Miriam Heider[3], Susanne Deutsch[4], Natascha Drude[1], Ulf Tölch[1], André Bleich[3], René Tolba[4], Daniel Strech[1,2]*

Science & Society

TRANSPARENT PROCESS OPEN ACCESS

EMBO reports

Introducing quality measures in an academic research consortium

Lessons and recommendation from implementing an ad hoc quality management system for organ model research

Maren Hülsemann[1,*], Janine Wiebach[1], Natascha Ingrid Drude[1], Silke Kniffert[1], Laura Behm[2], Katja Hönzke[3], Morris Baumgardt[3], Stefan Hippenstiel[3], Andreas C Hocke[3], Ulrich Dirnagl[1] & Ulf Tölch[1]

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Take home...

Reporting: be transparent about limitations

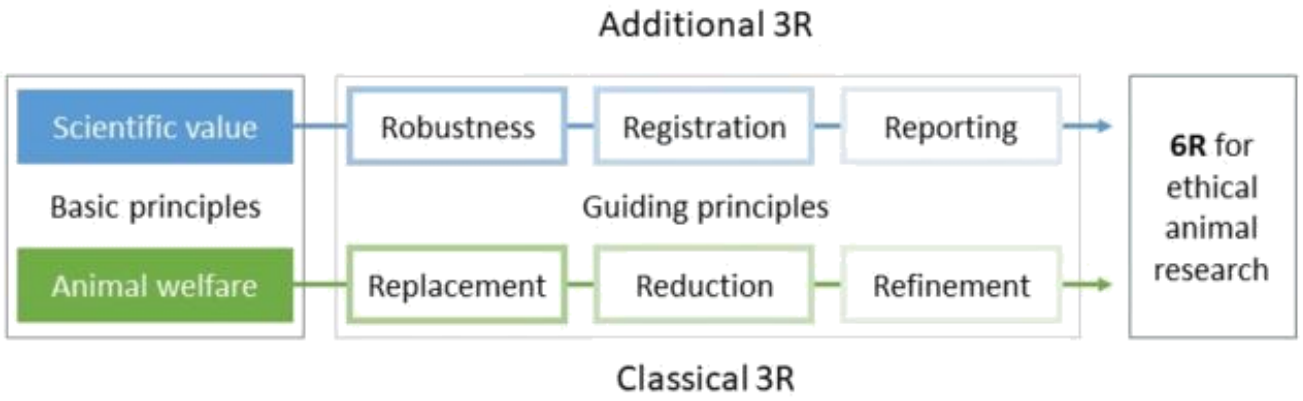
Robustness ≠ box ticking exercise

Registration: explore your options

GUIDELINE Open Access

The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research

Nathalie Percie du Sert[1*], Viki Hurst[1], Amrita Ahluwalia[2,3], Sabina Alam[4], Marc T. Avey[5], Monya Baker[6], William J. Browne[7], Alejandra Clark[8], Innes C. Cuthill[9], Ulrich Dirnagl[10], Michael Emerson[11], Paul Garner[12], Stephen T. Holgate[13], David W. Howells[14], Natasha A. Karp[15], Stanley E. Lazic[16], Katie Lidster[17], Catriona J. MacCallum[18], Malcolm Macleod[19], Esther J. Pearl[1], Ole H. Petersen[20], Frances Rawle[21], Penny Reynolds[22], Kieron Rooney[23], Emily S. Sena[19], Shai D. Silberberg[24], Thomas Steckler[25] and Hanno Würbel[26]



Think clinical translation early on

What is your next step, if you succeed?

What are Go and No/Go Decision criteria?

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Thank you!

Please do not hesitate to contact us!

QUEST Center for Responsible Research

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Courses and Workshops

BIH Charité (Junior) Clinician Scientist Programm

GEFÖRDERT VOM

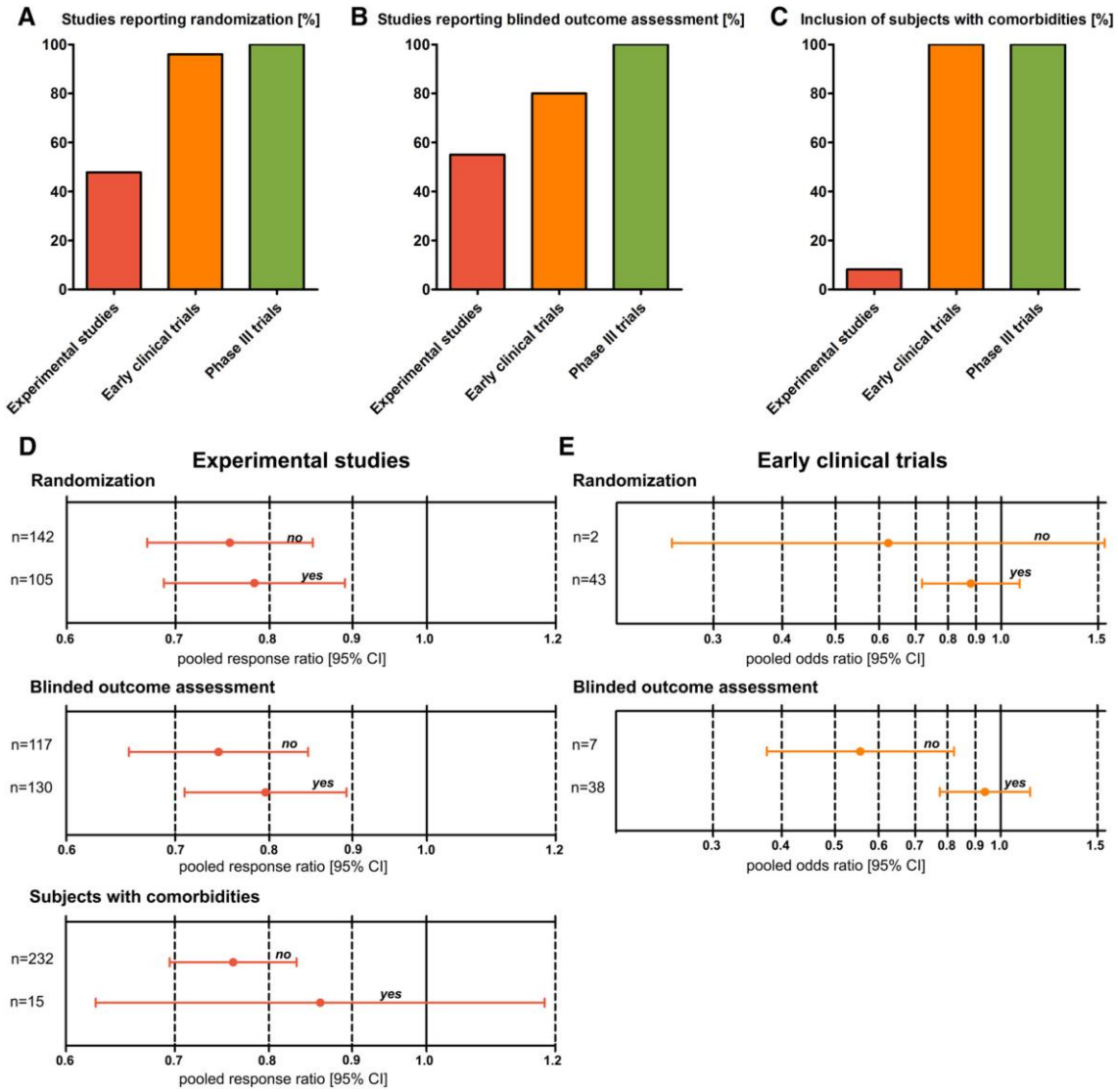
Bundesministerium für Bildung und Forschung

Critical questions to ask to ensure internal validity

1. Are measures to avoid risk of bias implemented?
   - Randomization
   - Blinding
   - Inclusion/Exclusion criteria

2. Are critical control conditions included?
3. Is the primary outcome defined?
4. Are Quality control measures in place?
   - Are protocols established, standardized and available for review?

Blinding and Randomization



Research Article | Open Access

Why Most Acute Stroke Studies Are Positive in Animals but Not in Patients: A Systematic Comparison of Preclinical, Early Phase, and Phase 3 Clinical Trials of Neuroprotective Agents

Antje Schmidt-Pogoda MD, Nadine Bonberg MSc, Mailin Hannah Marie Koecke, Jan-Kolja Strecker PhD, Jürgen Wellmann PhD, Nils-Martin Bruckmann MD, Carolin Beuker MD, Wolf-Rüdiger Schäbitz MD, Sven G. Meuth MD, PhD, Heinz Wiendl MD, Heike Minnerup MD, MSc, Jens Minnerup MD ... See fewer authors

First published: 12 November 2019 | https://doi.org/10.1002/ana.25643 | Citations: 28

Neglecting quality criteria contributes to an **overestimation of treatment efficacy** in experimental studies and early clinical trials

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External validity…

…refers to how well the outcome of a study can be expected to apply to other settings, such as other study conditions, animal strains/species.

Translational validity…

…refers to the extent to which a scientific finding can be translated from preclinical to clinical contexts.

Primary (and secondary) Outcome

Should be defined at the time of study design (a priori)

What is your most important measure? (The measure that you use to assess the effect of an intervention)

What did you base your sample size calculation on?

HARVARD CATALYST | THE HARVARD CLINICAL AND TRANSLATIONAL SCIENCE CENTER

Examples:

Results Field	Incorrect Examples:	Corrected Examples:
Outcome Measure Title:	"Efficacy"	"Percentage of subjects that experienced a decrease in [measure: PSA] levels greater or equal to 75% at day 30 post-treatment with [drug]"
	"To assess survival"	"median overal survival"; "number of participants alive at 2 years"

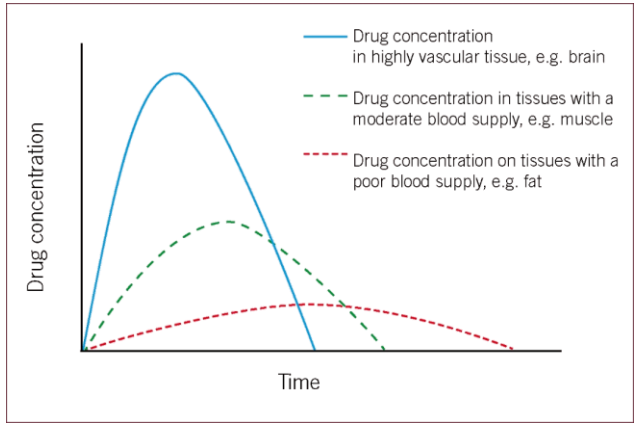
https://catalyst.harvard.edu/wp-content/uploads/regulatory/CTR3_OutcomeMeasures.pdf

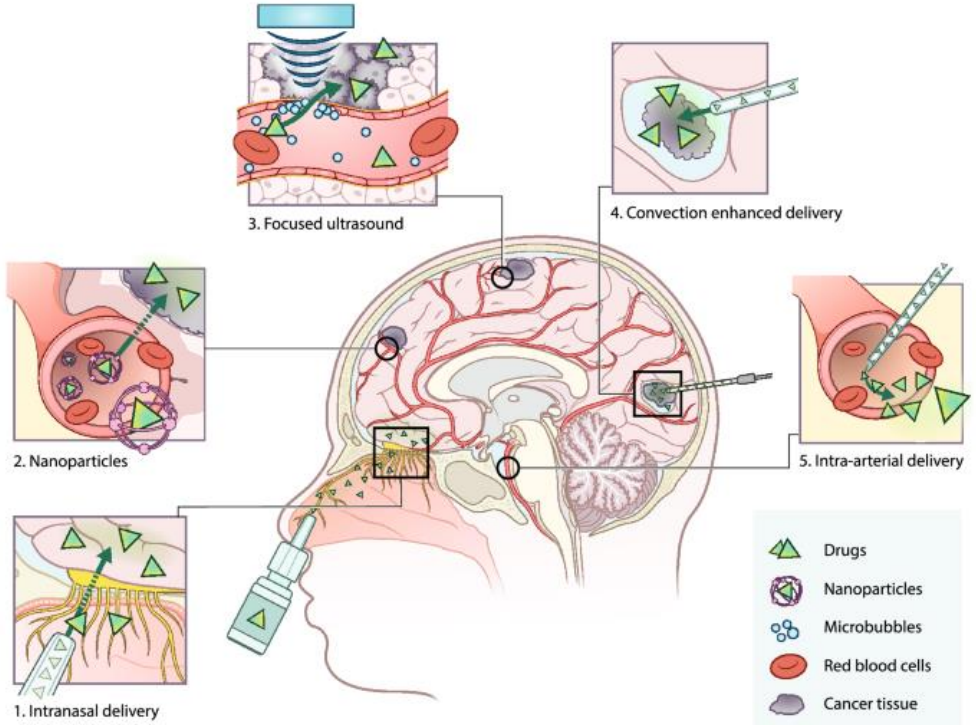
Have pharmacodynamics and pharmacokinetics been investigated?

Route of Administration? What is clinically relevant?

Bioavailability

Pharmacokinetics





Haumann, R., Videira, J.C., Kaspers, G.J.L. *et al. CNS Drugs* 2020.
https://doi.org/10.1007/s40263-020-00766-w

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