# Sex inclusive preclinical research: current landscape and misconceptions

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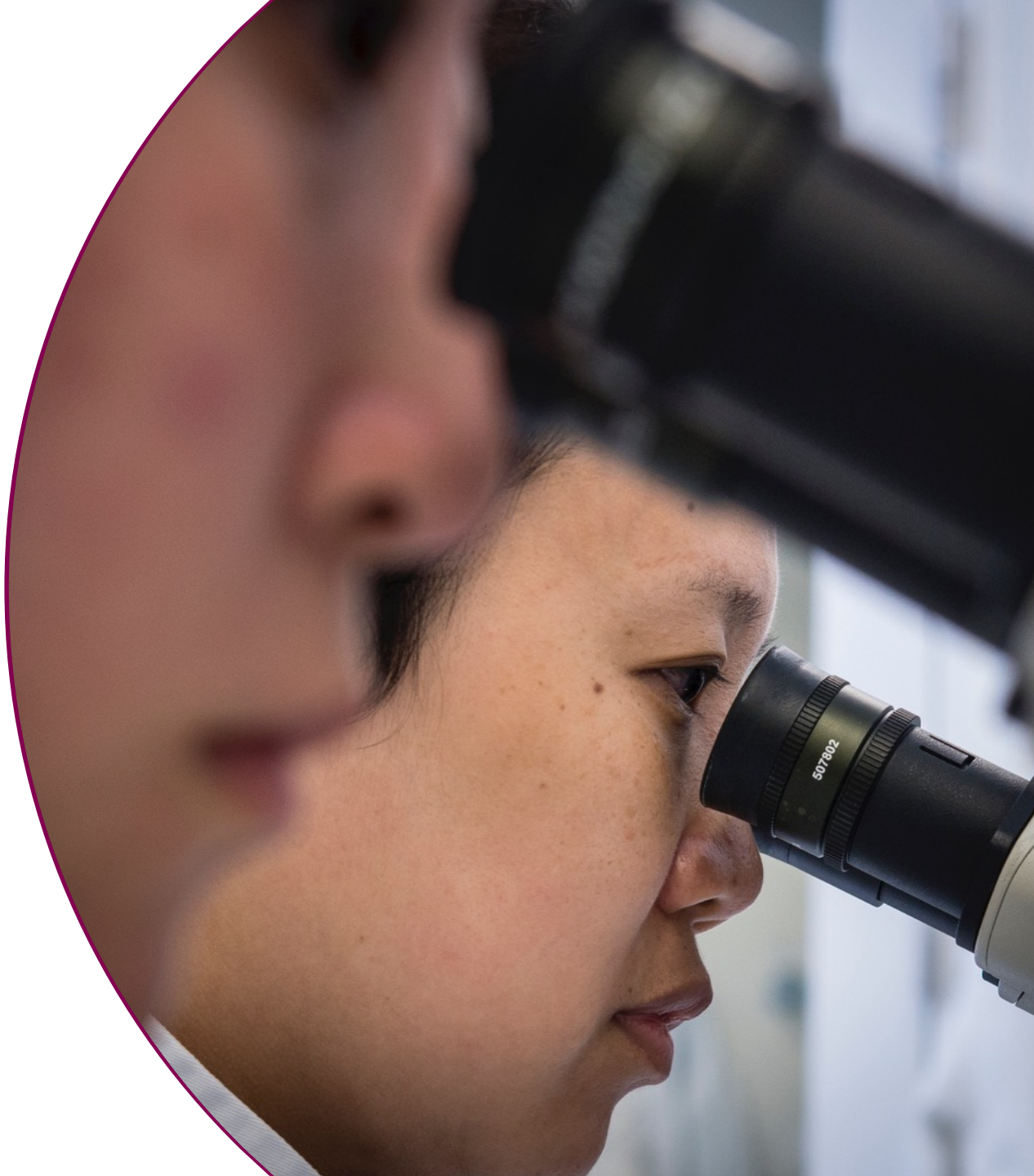

# Outline

1. The preclinical sex inclusion landscape
3. What is sex inclusive research?
3. What are the barriers?
4. A key barrier: statistical power concerns
5. Moving towards inclusion as the default: The Sex Inclusive Research Framework
6. Conclusions

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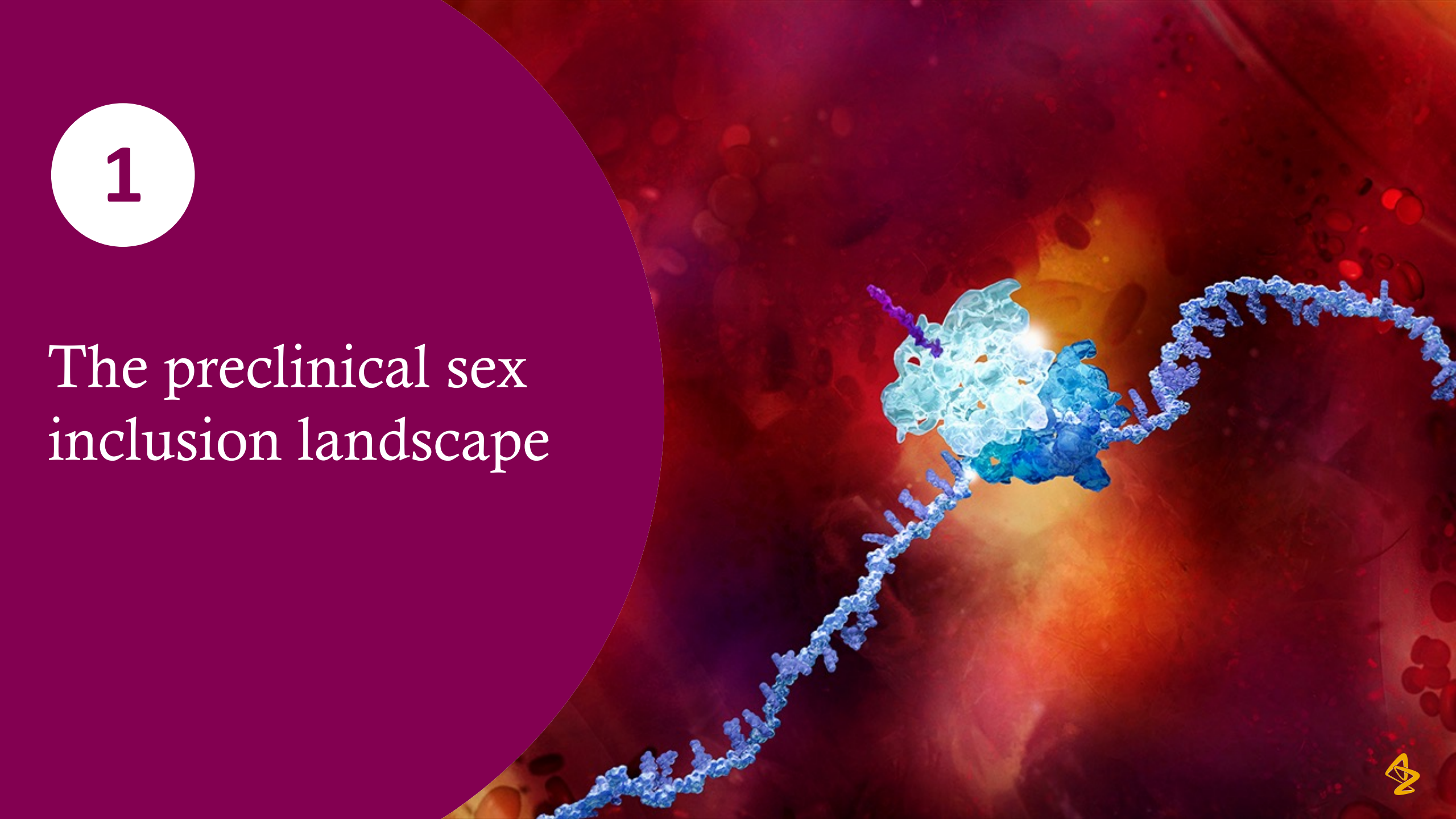
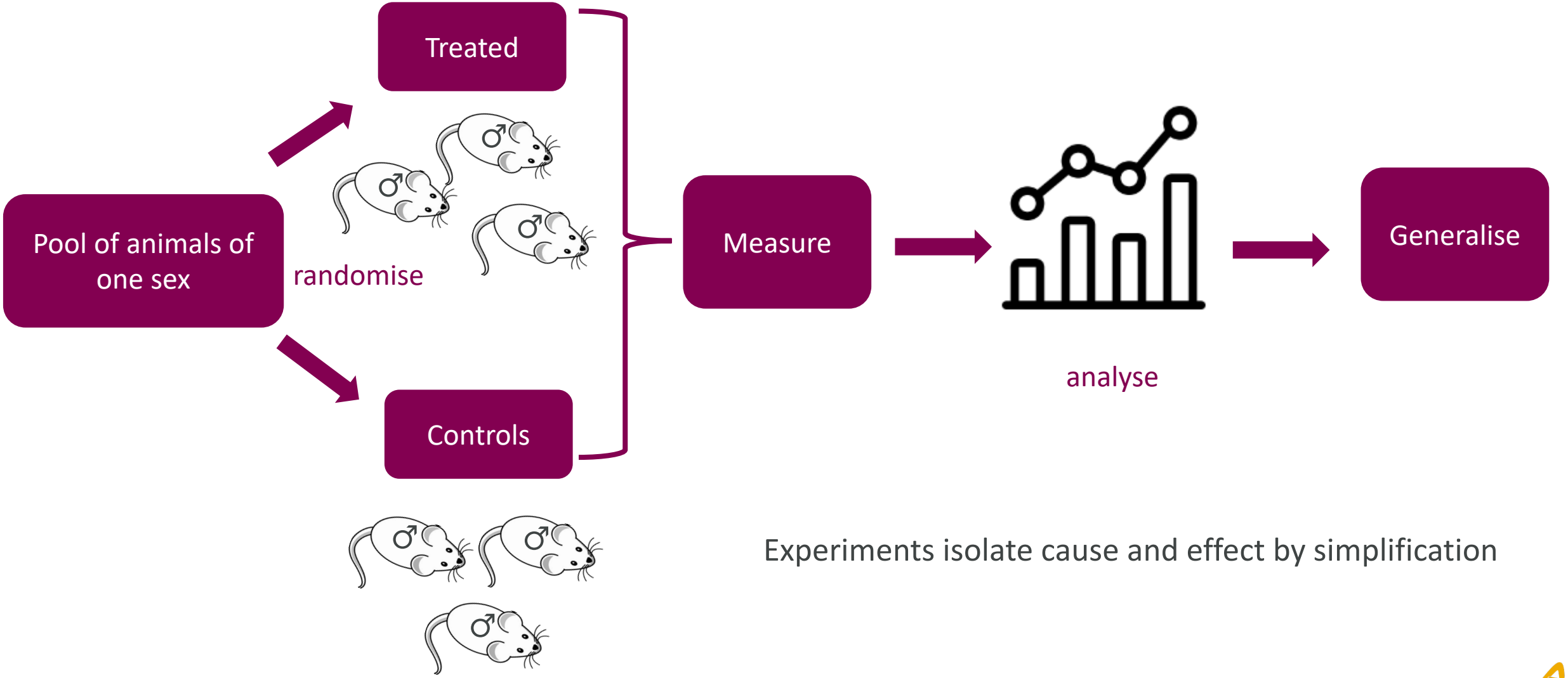
# The preclinical sex inclusion landscape



# Classic design - driven by minimising N




Experiments isolate cause and effect by simplification

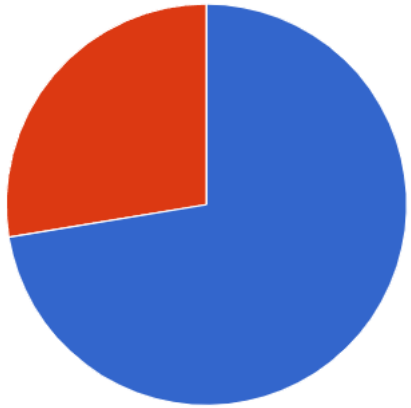
# Embedded neglect of sex within preclinical research

- Reporting:
  - *In vivo*: Sex not specified – 22% did not specify Yoon et al 2014
  - *In vitro*: 75% did not report the sex Shah 2014
- Experimental design:
  - *In vivo*: comparison across 9 fields of biology, 2009 to 2019 Beery 2020
    - 6/9 significant improvement, 1(Pharmacology) reduction to 29% , average 26% to 48%
  - *In vitro*: 69 -80% male only Taylor 2011, Shah 2014
- Analysis (*In vivo*):
  - When both sexes (N=356), only 42% sex-based analysis Beery 2020
  - Those reporting sex differences: 1/3 did not test statistically

Garcia-Sifuentes & Maney 2021

# Emerging evidence that our knowledge base is biased

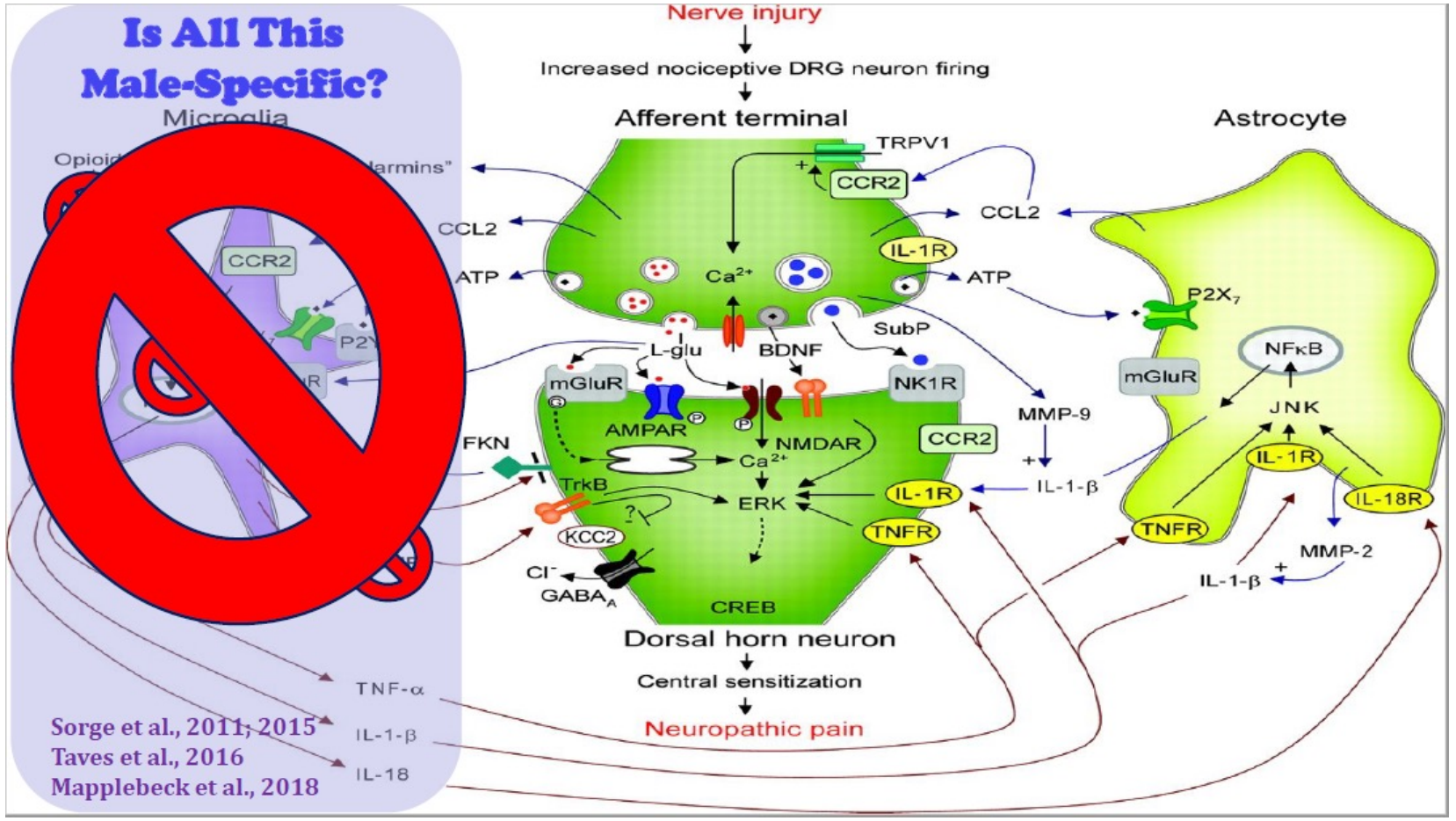
**27.6% female only**



**72.4% male only**

Pain processing

N=127




Mogil (2020) Nature Reviews Neuroscience

# MRC survey

- Majority of MRC researchers (95% of in vivo researchers and 88% of cell users) saw benefit of considering diversity
  - Translatability
  - Reproducibility
  - Detecting sex specific effects

  - Barriers/Concerns
    - Cost of experiments and complexity of research design
    - Compliance with the principles of the 3Rs (animal usage for *in vivo* researchers)
    - Commercial availability of samples (cell researchers)

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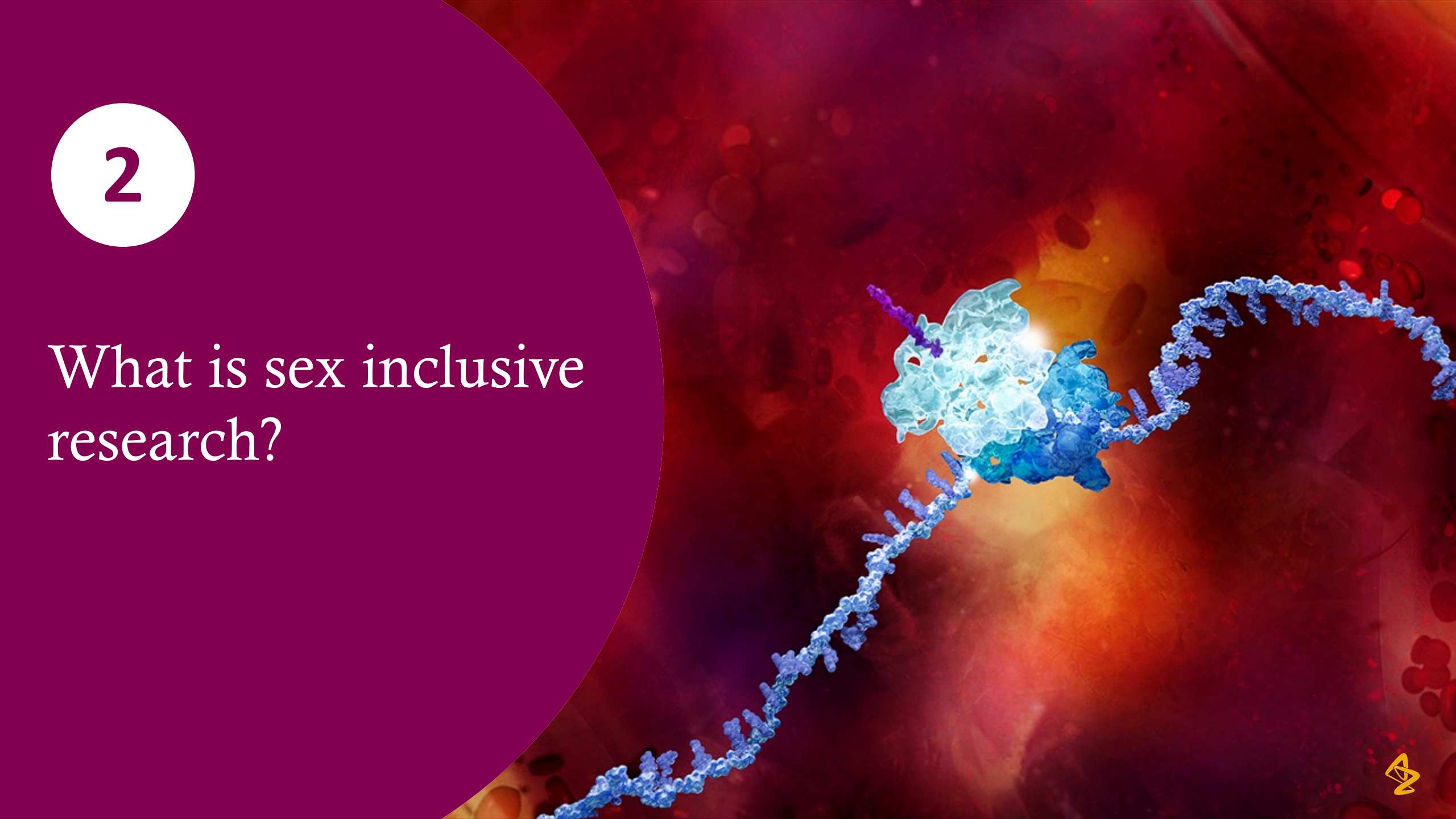
# What is sex inclusive research?



# Sex and Gender Equity in Research (SAGER) guidelines

- Principles
  - Use the term sex and gender carefully
  - Where the subjects of research comprise organisms capable of differentiation by sex, the research should be designed and conducted in a way that can reveal sex-related differences in the results, even if these were not initially expected.
  - Where subjects can also be differentiated by gender (shaped by social and cultural circumstances), the research should be conducted similarly at this additional level of distinction

Heidari 2016 Research Integrity and Peer Review

# Funding bodies are driving change

- Movement from recommendation to requirement and active questions in funding process

| Body | Year |
|---|---|
| NIH | 2016 – required incorporation both *in vivo* and *in vitro* |
| Canadian Institute of Health Research | 2010 – questions in grant application |
| Irish Research Council | 2013 – questions in grant application |
| European Commission | 2020 – required incorporation both *in vivo* and *in vitro* |
| MRC | 2022 – inclusion of both sexes the default for *in vivo* and *in vitro* |
| CRUK | *2023 – inclusion of both sexes the default for animal, tissues or cells* |

- WT funding - MESSAGE (Medical Science Sex and Gender Equity) project
  - Co-develop a sex and gender policy framework for funders and regulators in the UK

# Common themes

## Requirement

- Where subject of research comprises organisms that can be differentiated by sex:
- Inclusion of both sexes as default for studies involving animals and human and animal tissues and cells
- Analysis should account for sex
- Raw data reported with sex detailed
- Non inclusive research by exceptions

## Vision

- Not to study sex differences but rather estimate a generalisable effect
- Experiments are powered to detect the effect of interest across the two sexes
- If the effect is very different between the sexes then this will become apparent

Take home: Goal of sex inclusive research is to estimate an effect that represents both sexes.

# Exception?

- Where the sex of the sample cannot be determined
- Pure molecular studies  [when conducted outside of a cellular system]
  - e.g.  Association and dissociation interactions between proteins
- Sex-specific conditions or phenomena
  - e.g. ovarian cancer
- Acutely scare resources
  - e.g.  rare disease

- If you can provide strong justification.

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BJP British Journal of Pharmacology

REVIEW ARTICLE | Free Access

## Sex bias in preclinical research and an exploration of how to change the status quo

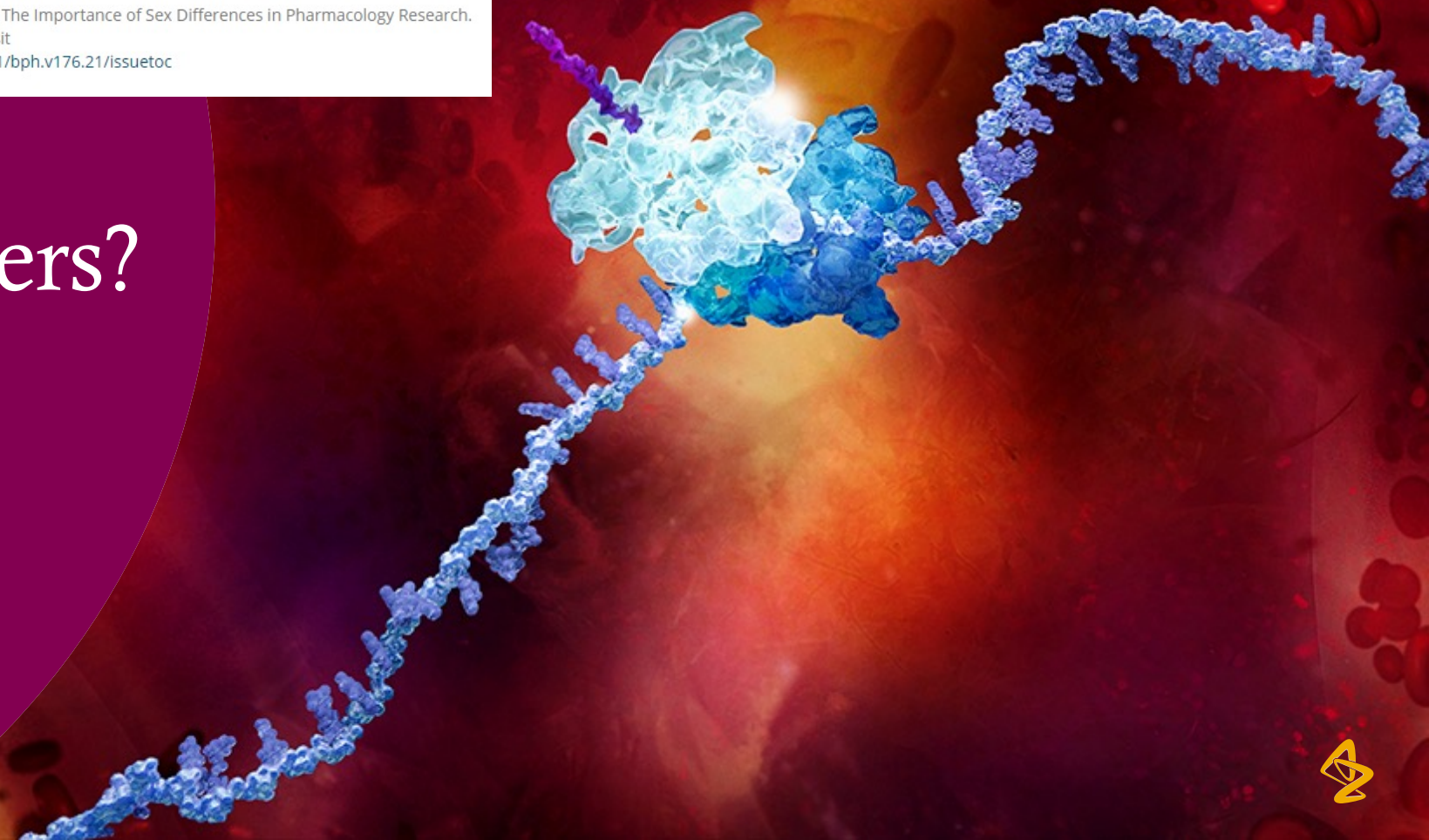
Natasha A Karp, Neil Reavey

First published: 12 November 2018 | https://doi.org/10.1111/bph.14539 | Citations: 20

This article is part of a themed section on The Importance of Sex Differences in Pharmacology Research. To view the other articles in this setion visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v176.21/issuetoc

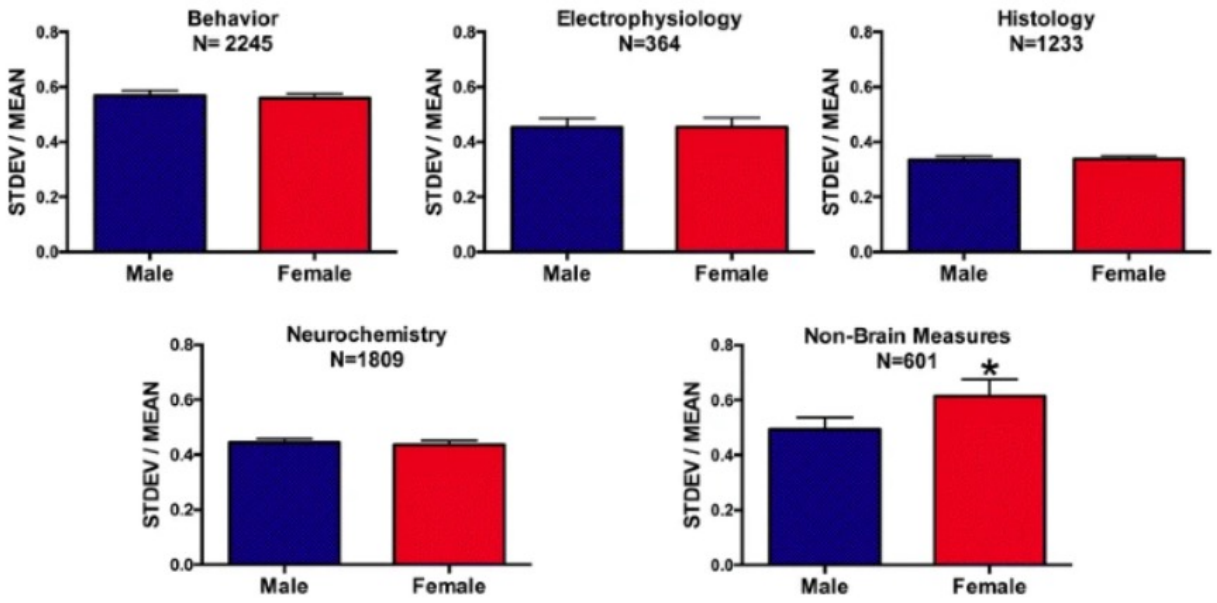
# What are the barriers?

- **Misconceptions**
- **skill gap**
- **practical concerns**
- **3R interpretation of Reduction**



# **Misconception**: hormonal cycles: females more variable

**Rats** Becker 2016 BSD




"Female rats were not more variable at any stage of the estrous cycle than male rats."

**Mice** Prendergast 2014 NNBR

- meta-analysis 293 published articles
- behavioral, physiological, morphological, and molecular traits
- CV distribution = no differences
- At trait level – for three types of traits males were more variable than females

"Randomly cycling female mice were no more variable than males on any trait."

# Inclusion isn't at odds with the 3R mindset

1. Breeding – produces both males and female animals

2. Reduction in N across experiments – more efficient to include both sexes

| | **Standard** | **Contemporary** |
|---|---|---|
| **Reduction** | Methods which minimise the number of animals used per experiment | Appropriately designed and analysed animal experiments that are robust and reproducible, and truly add to the knowledge base |

https://www.nc3rs.org.uk/the-3rs

# Fear of change

"To date, sex hasn't explained variation in my model"

- Lack of data regarding sex differences does not indicate there are none
- The goal isn't to identify sex differences but to estimate generalisable effects and be able to detect very large differences when they do occur
- Meta analysis has found that data analysis is often poorly conducted and hence historic conclusions can be misleading [https://elifesciences.org/articles/70817]

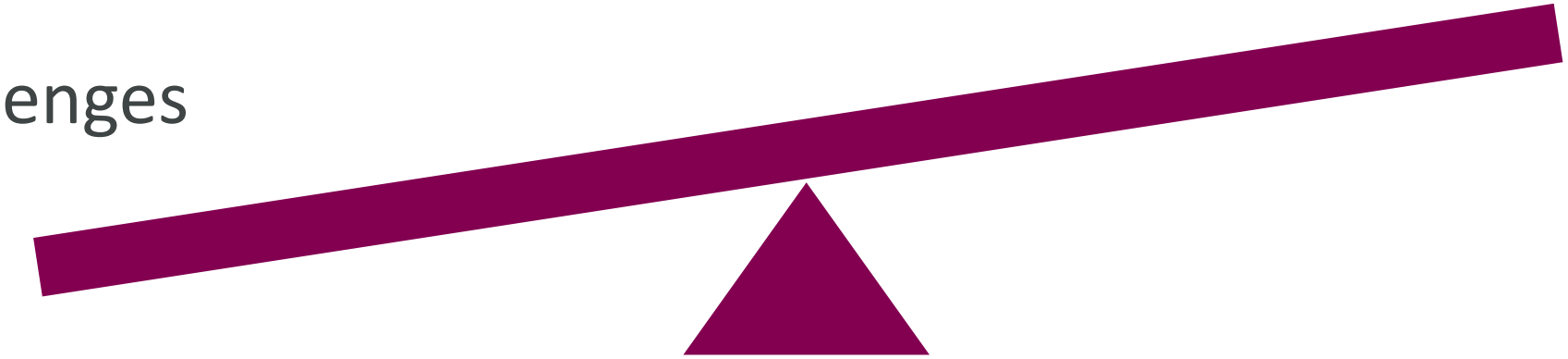
"My prior work has only considered in one sex"

- Unfortunately it carries lots of risk.
- " To change is difficult. Not to change is fatal" William Pollard

# Single sex justification

Viable experiment

Challenges



The justification could be appropriate following exploration for that study of logistical, ethical, or cost implications relative to the benefit of using both sexes of animals in a research proposal.

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# A key barrier: statistical power concerns

# **Misconception**: It will double/greatly increase my animal usage

**"I am essentially testing my treatment effect twice (once in each sex) so I will need to double the sample size I would have used"**

**"Including both sexes will introduce additional variability into my experiment, therefore I will need to increase the sample size to retain the same level of statistical power"**

**"Keep doing what you are already doing but change half the animals in your study to female"**

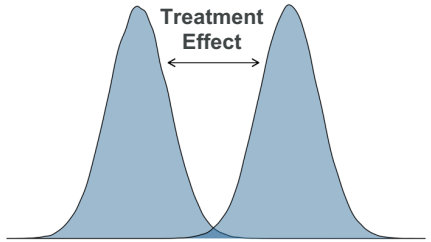
*McCarthy **2015** Schizophrenia Bulletin*

# A simulation-based approach for exploring factorial power

1) **Generate simulated data**

2) **Apply statistical test and extract p values**

3) **Repeat process 1000 times for each effect size of interest and calculate the % of significant p values as the statistical power for that scenario.**



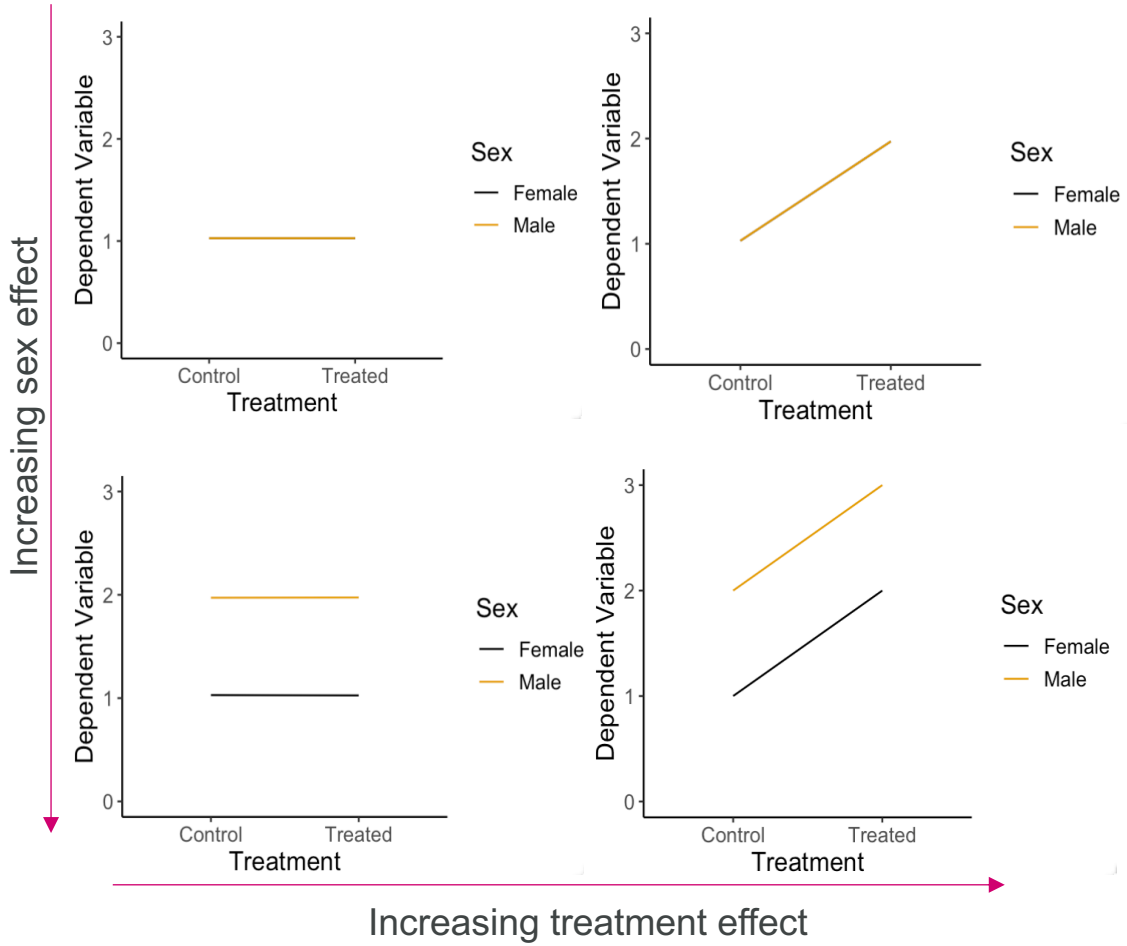

**x 1000**

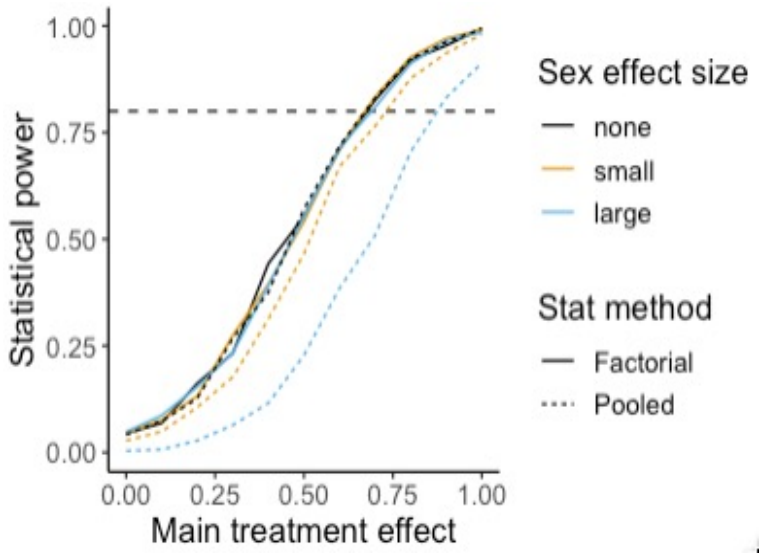
**Scenarios**

1. Baseline effect of sex
2. A differential effect of sex on treatment in the same direction
3. An effect in one sex only
4. Opposite effects of sex by treatment

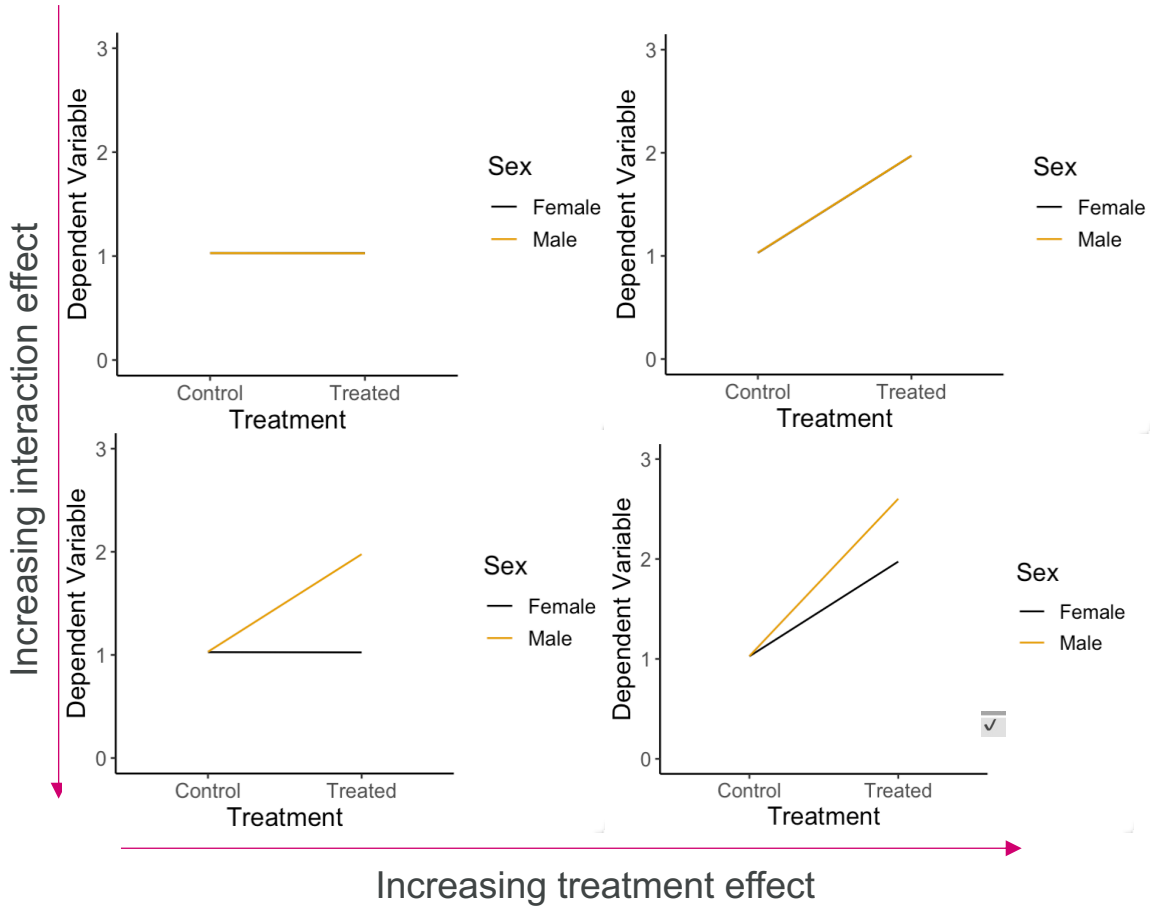
*Phillips, Haschler, & Karp., PLoS Biology 2023*

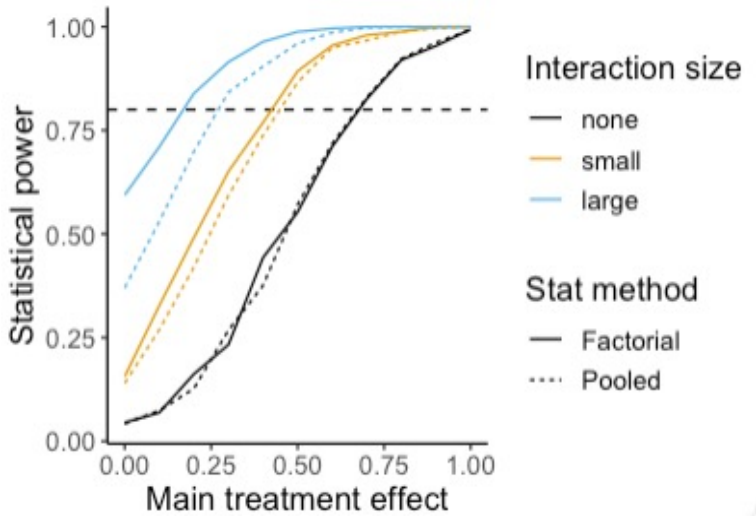
# 1: When there is a baseline effect of sex?



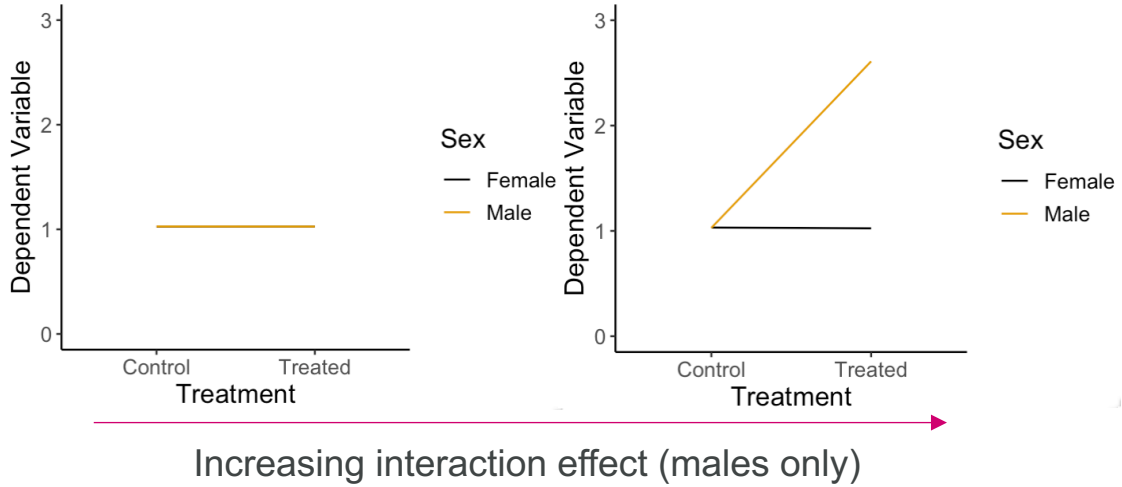



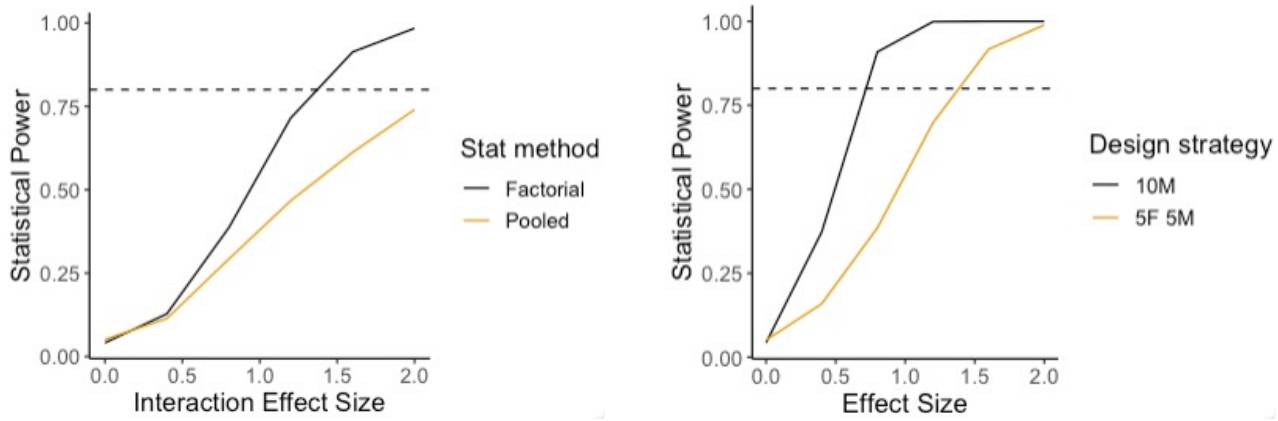
# 2: When there is a differential effect in the same direction?



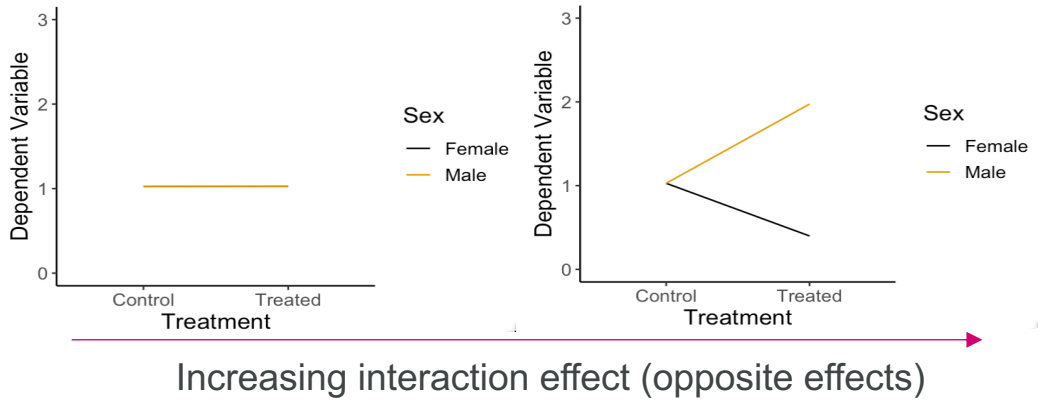



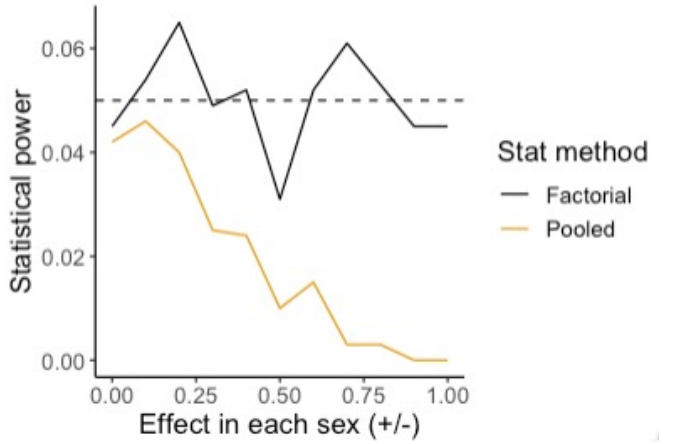
# Pooling compromises treatment power when there is an effect in one sex

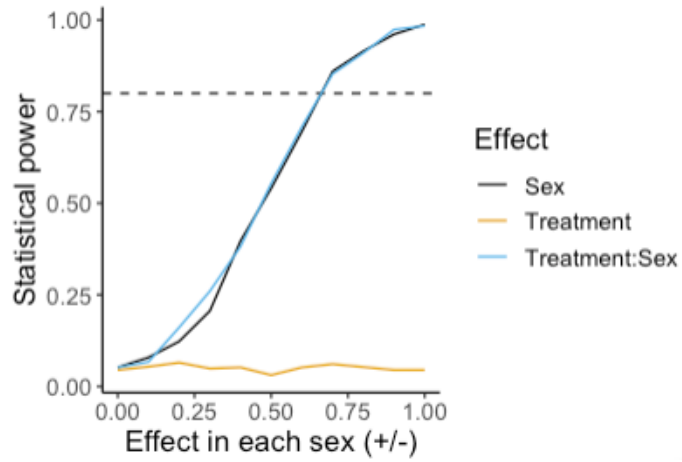


# Power is passed to the interaction term when the effects are in opposite directions



# Simulation conclusions:

A factorial approach provides power benefits, compared to a pooling t-test approach.

Disaggregation of data by sex loses statistical power and doesn't test for the interaction.

Typically, there is no loss of power to detect a treatment effect when including both sexes.

When power is lost, the knowledge gained is vital as the power is transferred to the interaction term.

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# Moving towards inclusion as the default: The Sex Inclusive Research Framework

# SIRF: Sex Inclusive Research Framework

## Why?

- Regulatory bodies need a resource to determine whether a research proposal is appropriate
- Frequently, barriers are misconceptions
- Need transparency in the decision-making process
- We need educational resource to help move into considered justification to assess whether sex inclusion is a possibility.

## What?

- Decision tree of 12 questions and associated supporting information
- Delivers 1 or more classifications
- Options:
- **Green**: Proposal is appropriate
- **Amber**: Caution is required (I.e., the proposed design/analysis carries some risk)
- **Red**: Justification for single sex study in the proposal is not sufficient

Manuscript: https://osf.io/twjg6/

# Driving cultural change: from exclusion to inclusion

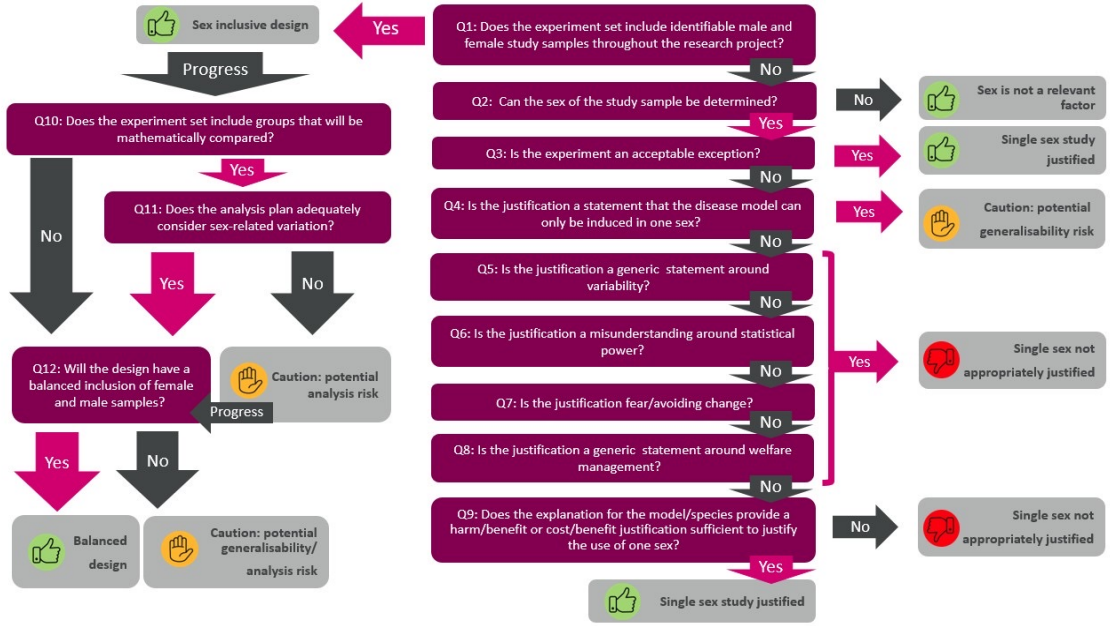
SIRF Working Group

**Lead & Chair – Dr Natasha Karp**

DRAFT

Refine & development of supporting resources

- Published dataset
- Ethical review bodies
- Funding body review round





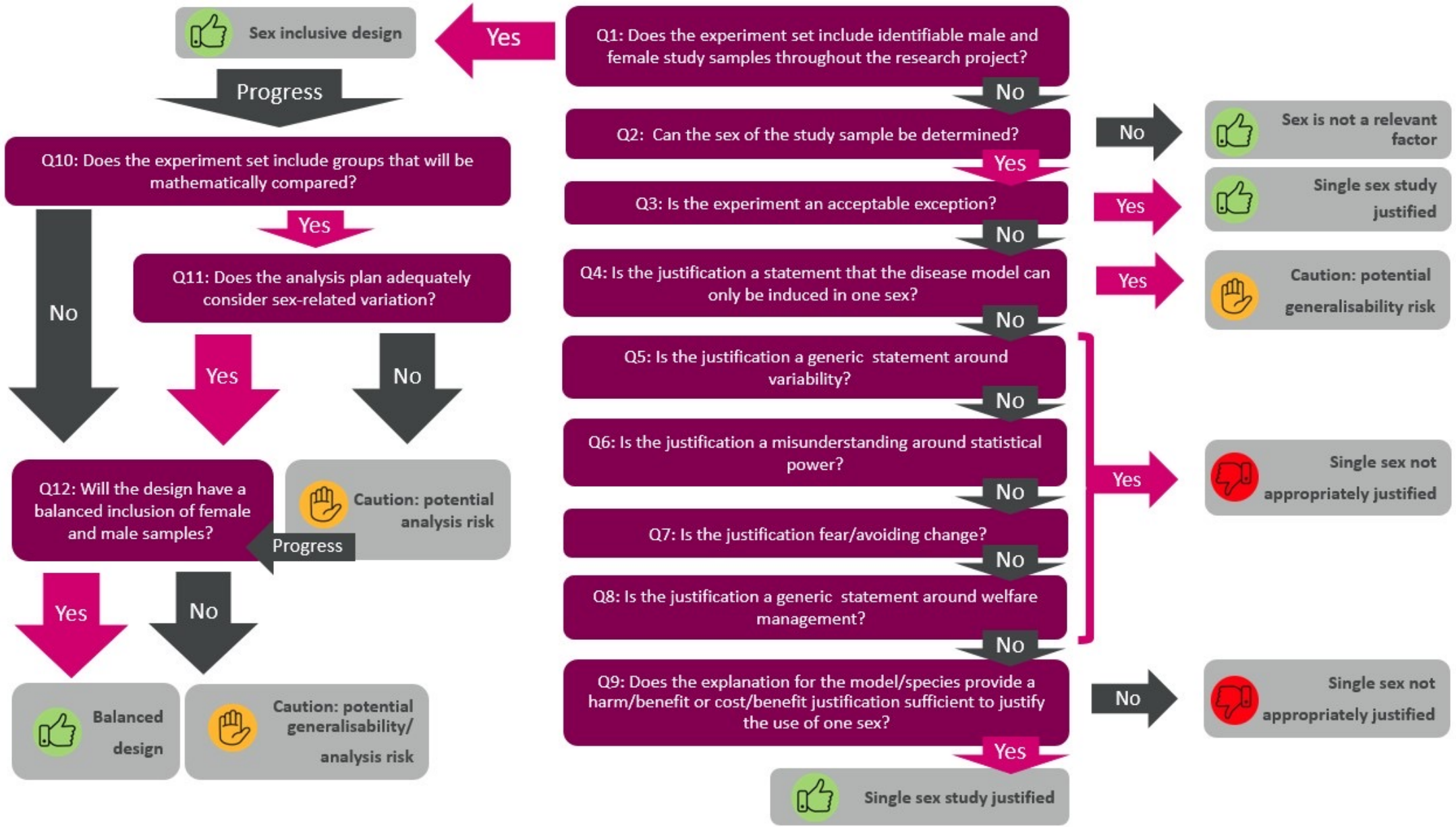
Nature Communications Manuscript (in revision)

Website

Interactive tool: generates a report

FAQs

Recorded Seminar


Q1: Does the experiment set include identifiable male and female study samples throughout the research project?
Yes
Sex inclusive design
No
Q2: Can the sex of the study sample be determined?
No
Sex is not a relevant factor
Yes
Q3: Is the experiment an acceptable exception?
Yes
Single sex study justified
No
Q4: Is the justification a statement that the disease model can only be induced in one sex?
Yes
Caution: potential generalisability risk
No
Q5: Is the justification a generic statement around variability?
No
Q6: Is the justification a misunderstanding around statistical power?
No
Q7: Is the justification fear/avoiding change?
No
Q8: Is the justification a generic statement around welfare management?
No
Yes
Single sex not appropriately justified
Q9: Does the explanation for the model/species provide a harm/benefit or cost/benefit justification sufficient to justify the use of one sex?
No
Single sex not appropriately justified
Yes
Single sex study justified
Progress
Q10: Does the experiment set include groups that will be mathematically compared?
Yes
No
Q11: Does the analysis plan adequately consider sex-related variation?
Yes
No
Q12: Will the design have a balanced inclusion of female and male samples?
Caution: potential analysis risk
Progress
Yes
No
Balanced design
Caution: potential generalisability/ analysis risk

# 160 days post website launch – impact?

## Endorsed

- **Funders**: MRC, CRUK, NC3Rs, MRC, NIH – ORWH (Office of Research on Women's Health) and NIH – OLAW (Office of Laboratory Animal Welfare)
- **Regulators**: EU Member State National Contact Point (Directive 2010/63/EU) presentation and newsletter
- **Supporting bodies**: MESSAGE (WT funded Medical Science Sex and Gender Equity project), LASA, FC3R, Norecopa, LASS, Finnish 3RC

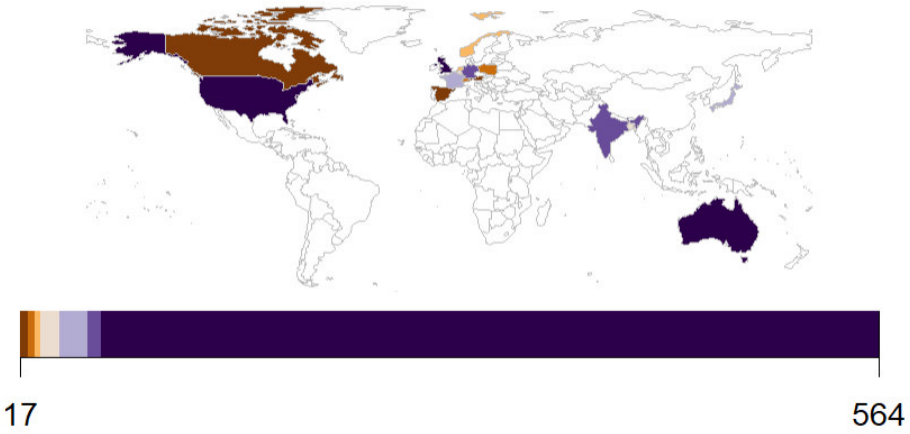
## Website analytics

Pageviews: **1771**

Av time: **3:36 mins**

Countries: **52**

New visitors: **1047 - 7%**



# 6

# Conclusions

Research suggests that sex is a significant source of variation for both *in vivo* and *in vitro*.

Improving translation requires us to embrace variation. Sex is a first step to improve generalisability.

However, sex bias is culturally embedded in our research pipelines, impacting the reporting, design, and analysis.

Many of the barriers are misconceptions.

Including both sexes, is not at odds with a reduction mindset.

The expectations are changing. We need to work out how to consider sex mindfully and effectively.

# Acknowledgements



**The SIRF working group**

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