

# Statistical Considerations for Sex Inclusion in Basic Science Research

Denise M. Scholtens, Ph.D.

Associate Professor, Department of Preventive Medicine

Associate Director, Division of Biostatistics

[dscholtens@northwestern.edu](mailto:dscholtens@northwestern.edu)

# BCC: Biostatistics Collaboration Center

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- Not Pictured:
1. David A. Aaby, MS  
Senior Stat. Analyst
  2. Tameka L. Brannon  
Financial | Research  
Administrator

# BCC: Biostatistics Collaboration Center

## What We Do

Our mission is to support FSM investigators in the conduct of high-quality, innovative health-related research by providing expertise in biostatistics, statistical programming, and data management.



www.jpeds.com • THE JOURNAL OF PEDIATRICS

ORIGINAL ARTICLES

Risk Factors for Recurrent *Clostridium difficile* Infection in Children: A Nested Case-Control Study

In Drug- and Alcohol-Use in a Longitudinal Study

RESEARCH ARTICLE  
Clinical and epidemiological profile of...  
Downloaded from <http://ard.bmj.com/>

Object Study of m...  
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'Gastr...  
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Phiv...  
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PCR...  
NCD...  
Ck...  
Pa...  
984

I WAS a fanatical tennis player for decades until my increasingly painful arthritis forced me to stop playing. The knees were replaced two years later, but by then, I had filled in my life with other activities, including a daily three-mile walk and aerobic work, and cycling for exercise and errands. Now I also walk a puppy four times a day. I have made many new friends and, much to my surprise, I don't miss tennis at all. Osteoarthritis is something nearly all of us can expect to face if we live long enough. A quarter of the population has it, and the percentage is expected to rise significantly in the years ahead. Two-thirds of people with arthritis are younger than 65, the Centers for Disease Control and Prevention reports. "Arthritis affects more than 52 million adults in the United States and is the most common cause of disability," the CDC states. Yet it often takes a back seat to other chronic ailments in treatment and public awareness. "When the pain of arthritis results in a decline in physical activity, as it commonly does, the risk of developing a chronic health problem like heart disease and Type 2 diabetes rises and performing the tasks of daily living becomes increasingly difficult. The big question now is how the growing number of adults with arthritis will cope with a painful, disabling and as yet incurable disease. Although several commonly affected joints — hips, shoulders, ankles, wrists and elbows as well as knees — can be replaced by artificial ones, not everyone is affected to a candidate for surgery, and the operation itself leaves some people with activity limitations. According to Patricia A. Parise, a professor of psychology and the director of the Behavioral Research Institute on Aging at the University of Michigan, "As we get older, if we don't get up and move around as much as we can, then we soon won't be able to move at all." In a 10-year study of more than 2,000 men and women with arthritic knees, Jungwha Lee and her colleagues found that fewer than 10 percent met the national guidelines of doing 150 minutes a week of moderate physical activity. But if they improved their physical activity, "they functioned better and had less disability," said Dr. Lee, a biostatistician at Northwestern University Feinberg School of Medicine in Chicago. In a second study of 188 individuals ages 49 to 83 who had X-ray evidence of knee arthritis, participants monitored their daily activity with accelerometers that measured duration and intensity of activity. The researchers found that, on average, participants spent two-thirds of their waking hours being sedentary. The less active they were in the course of the day (apart from any moderate or vigorous activity they did), the more heavily they walked and the less able they were to get up from a chair. In a study that Pamela A. Semanik, Dr. Lee and colleagues published last month in



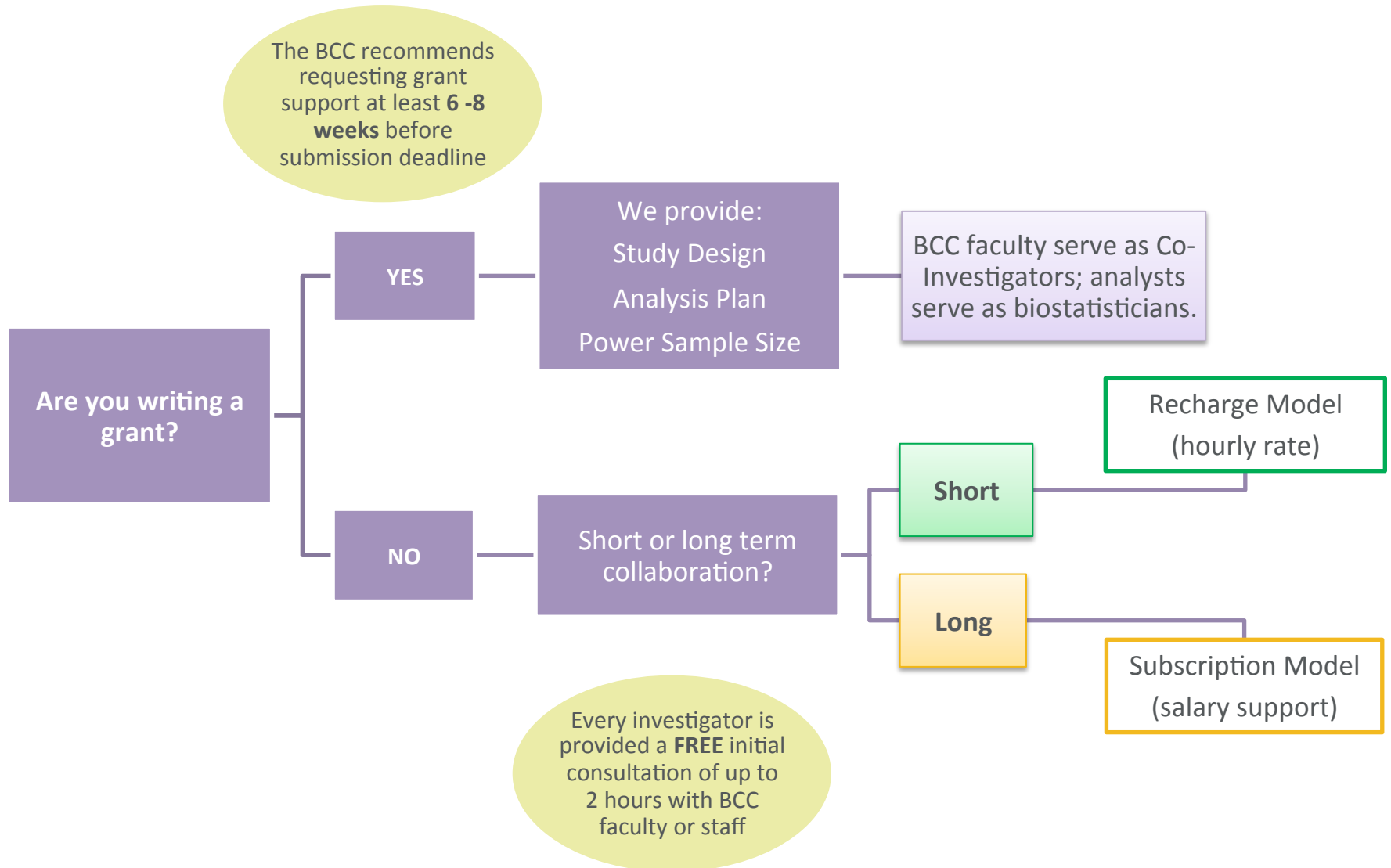
"People control their pain by doing less physical activity," Dr. Lee said. "But being more active can delay the functional decline that accompanies aging. Any activity is better than being sedentary." She added: "You don't need a gym membership to promote good health. Build movement into your daily routine. Don't park right next to the store. If your job involves prolonged sitting, set an alarm and stand up every 20 minutes. Use a remote printer. Take a lap around the floor after using the restroom. Go for a walk during lunch." Injury to joints is a major risk factor for developing arthritis, Patricia G. Conaghan of the University of Leeds in England wrote in an essay. "It's impossible to stop all these. We can, however, control our weight, and that's a major battle for all Western societies. Overweight and obesity place far greater stress on major joints like the hips and knees than they are designed to withstand for three score and 10 and then some. Even a 10 percent reduction in body weight can be beneficial. So can muscle strengthening. Dr. Conaghan wrote: "If you are having difficulty getting out of chairs or walking up stairs, chap you likely have weak muscles in your thigh. The sensation of the leg 'giving way' is mostly due to thigh muscle weakness. So strengthening leg muscles alone reduces knee pain from osteoarthritis." For a "smarter exercise," he recommended "water laps in a swimming pool. Then, with stronger muscles, he said, activity can be increased, say, by using an exercise bike or cross-trainer, to help maintain muscle strength and also burn extra calories if weight loss is needed." When people fail to delay the progress of arthritis, a multidisciplinary study that Dr. Conaghan directed showed, it is likely to progress more quickly, causing difficulty with stairs to pain while walking, then standing, lying or sitting, and finally walk in bed. Elizabeth M. A. Hootner of the Leeds Institute of Rheumatic and Musculoskeletal Medicine was the lead author of the study, published in January in Arthritis Care & Research.

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ctors (comorbidity, body...  
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sedentary group was...  
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# BCC: Biostatistics Collaboration Center

## How We Do It



# BCC: Biostatistics Collaboration Center

## Contact Us

- Request an Appointment
  - <http://www.feinberg.northwestern.edu/sites/bcc/contact-us/request-form.html>
- General Inquiries
  - [bcc@northwestern.edu](mailto:bcc@northwestern.edu)
  - 312.503.2288
- Visit Our Website
  - <http://www.feinberg.northwestern.edu/sites/bcc/index.html>

Biostatistics Collaboration Center | 680 N. Lake Shore Drive, Suite 1400 | Chicago, IL 60611



Topic for today:

# Sex as a variable in basic science research

# Sex as a Biological Variable

## Recent History

Collins & Tabak  
Nature  
Comment  
*Policy: NIH plans to enhance reproducibility*

January 2014

‘Crucial experimental design elements that are all too frequently ignored include blinding, randomization, replication, sample-size calculation and the effect of sex differences.’

**nature** International weekly journal of science

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Archive > Volume 505 > Issue 7485 > Comment > Article

NATURE | COMMENT

### Policy: NIH plans to enhance reproducibility

Francis S. Collins & Lawrence A. Tabak

27 January 2014

Francis S. Collins and Lawrence A. Tabak discuss initiatives that the US National Institutes of Health is exploring to restore the self-correcting nature of preclinical research.

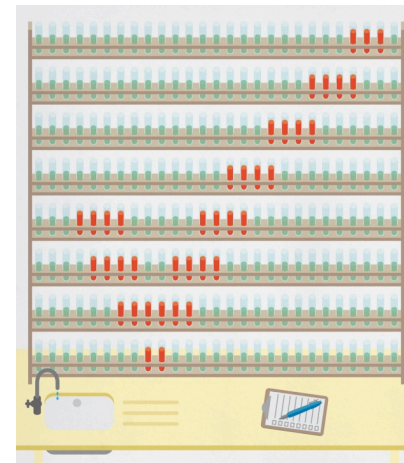
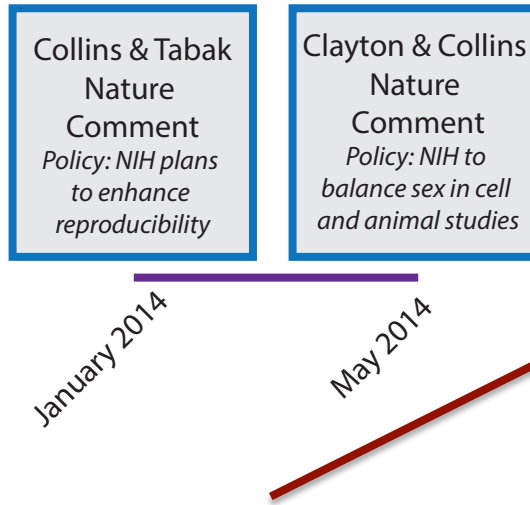


Figure from Collins & Tabak, Nature (2014)

# Sex as a Biological Variable

## Recent History



‘Furthermore, inadequate inclusion of female cells and animals in experiments and inadequate analysis of data by sex may well contribute to the troubling rise of irreproducibility in preclinical biomedical research, which the NIH is now actively working to address.’



NATURE | COMMENT

### Policy: NIH to balance sex in cell and animal studies

Janine A. Clayton & Francis S. Collins

14 May 2014

Janine A. Clayton and Francis S. Collins unveil policies to ensure that preclinical research funded by the US National Institutes of Health considers females and males.

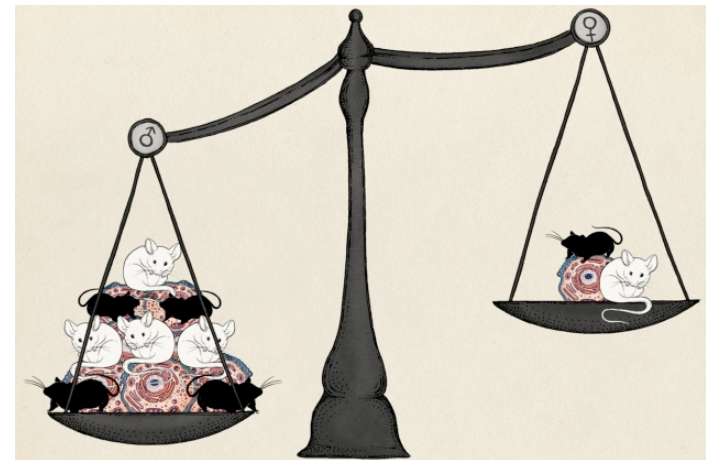
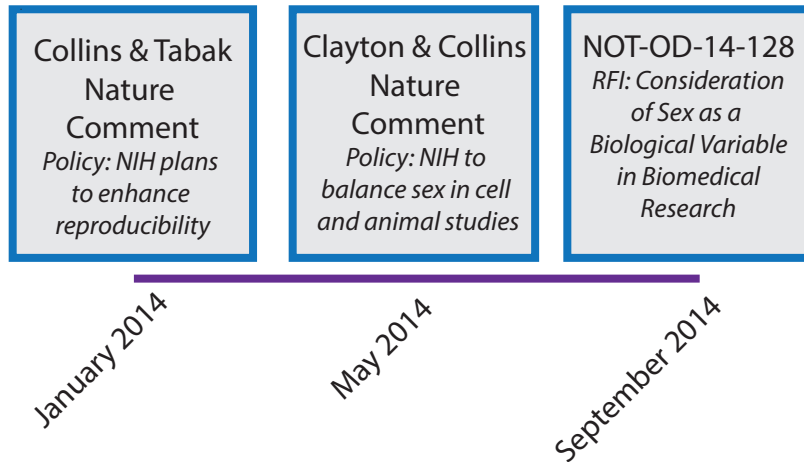


Figure from Clayton & Collins, Nature (2014)



# Sex as a Biological Variable

## Recent History



## NIH Request for Information: Consideration of Sex as a Biological Variable in Biomedical Research

*Analysis of Public Comments*

May 19, 2015

Specific concerns related to...

Sample sizes

From NOT-OD-14-128:

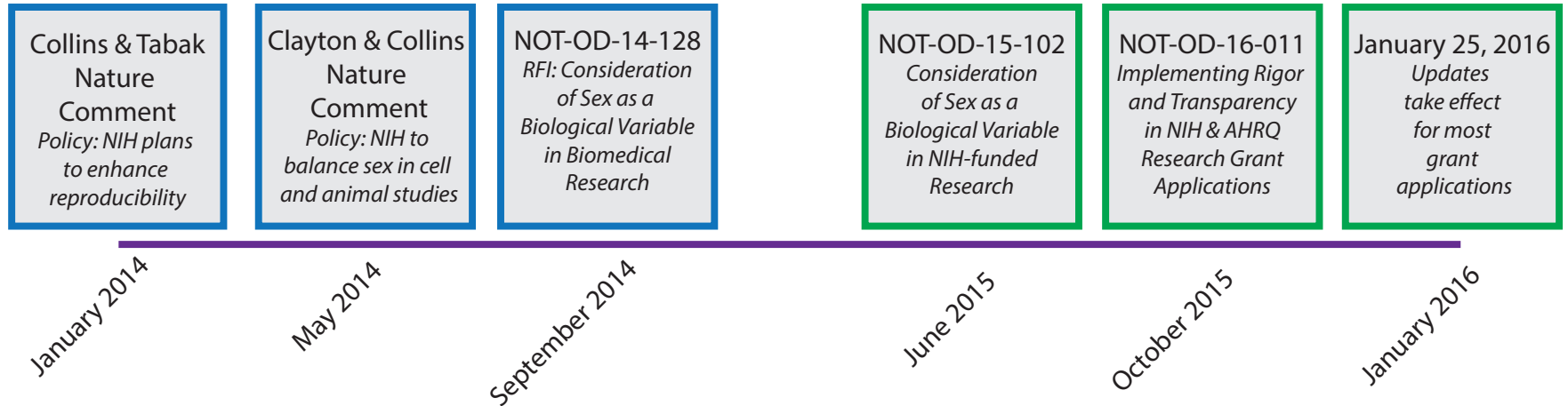
‘Although we have made major progress in achieving balance of sex in human studies — women now account for roughly half of the participants in NIH-funded clinical trials — we have not seen a similar pattern in biomedical research. Animal studies have typically focused on males, and investigators studying cell models have often not reported the sex of the individual from which the cells were obtained. Even if both sexes are included in a study design, resulting data may not be analyzed or disaggregated by sex. ....

By developing a policy to ensure that sex is considered in NIH-funded studies, NIH will ensure that sex and sex differences are examined in all aspects of biomedical research.

This will lead to a stronger foundation upon which to build clinical research and clinical trials.’

# Sex as a Biological Variable

## Recent History



<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html>

<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html>

# Research Toolbox

Northwestern University's Women's Health Research Institute



[https://www.womenshealth.northwestern.edu/sites/womenshealth/files/u926/Statistical Considerations in Basic Science Sex Inclusive Research.pdf](https://www.womenshealth.northwestern.edu/sites/womenshealth/files/u926/Statistical%20Considerations%20in%20Basic%20Science%20Sex%20Inclusive%20Research.pdf)

## 5 Scenarios

pertaining to sex inclusion in basic science research

Sample size / experimental design

Statistical analysis plans

Grant applications / manuscripts

Gentle guide only

(i.e. contact the BCC)

## Very simple experimental setting

- *Suppose an investigator wants to evaluate the effect of a treatment compared to control in a basic science setting involving cell lines.*
- *The investigator plans to measure a continuous outcome variable Z in the cells.*
- *'Female' and 'male' refer to chromosomal female (XX) and chromosomal male (XY), respectively.*

## 3 Questions resulting in 5 Scenarios

- *Is there strong biological justification to study cells of only one sex?*
- *In the control condition, is the mean of  $Z$  the same for males and females?*
- *Does treatment affect  $Z$  in the same way for females and males?*

# Scenario 1

| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 1        | Yes  | -  | -  | No   |

## Sample size / experimental design:

Sample size calculations can be performed without consideration of the sex variable

## Statistical analyses:

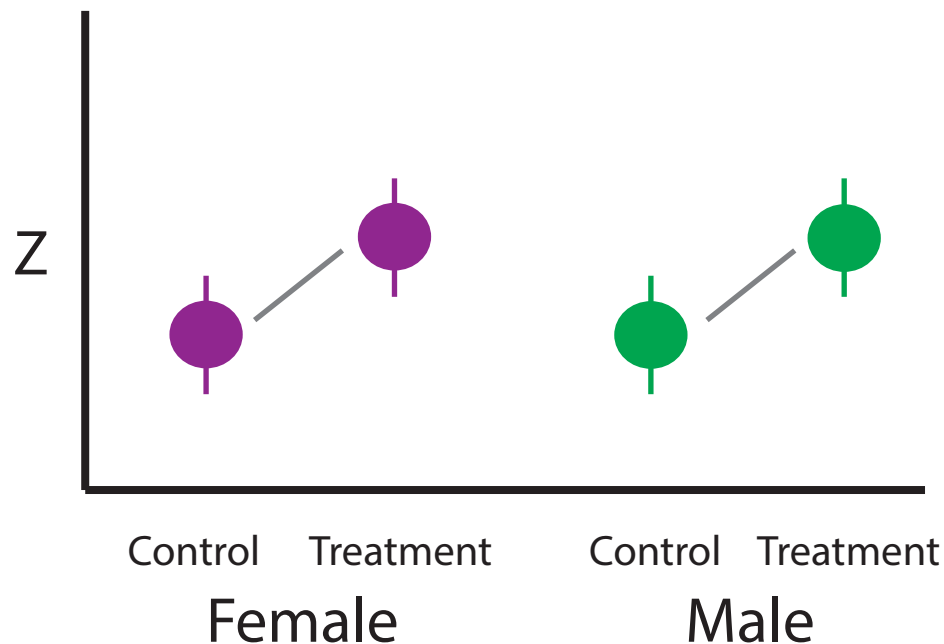
Statistical analyses do not need to consider sex as a variable

## Grant applications / manuscripts:

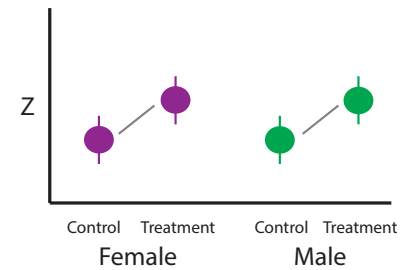
Include strong biological justification for studying only one sex

## Scenario 2

| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 2        | No   | Yes  | Yes  | Possibly   |



# Scenario 2



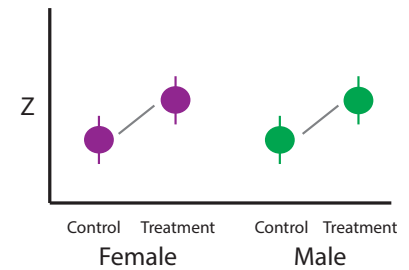
| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 2        | No   | Yes  | Yes  | Possibly   |

## Sample size / experimental design:

**IF** it can be reasonably assumed (either based on preliminary data or published literature or both) that mean levels of Z in the control condition are similar for both sexes **AND** that treatment has the same effect in both sexes, **THEN** sample size calculations will be no different than calculations made in Setting 1.



# Scenario 2



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 2        | No   | Yes  | Yes  | Possibly   |

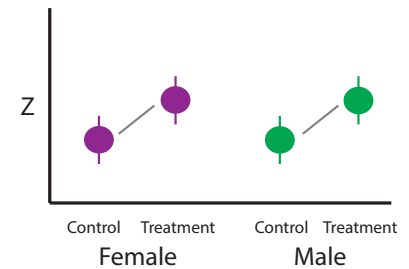
## Statistical analyses:

When analyzing collected data, the assumption that Z has the same mean in cells of both sexes in the control condition should be examined using descriptive statistics (i.e. means) and/or plots.

The similarity of the treatment's effect on Z for cells of both sexes should also be examined using descriptive statistics and/or plots.

**IF** assumptions **ARE** met, it **MAY** be appropriate to analyze data from the female and male cells together, without statistical control for sex.

# Scenario 2



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 2        | No   | Yes  | Yes  | Possibly   |

### Statistical analyses con't:

**IF** assumptions **ARE NOT** met, move to Scenarios 3, 4 or 5.

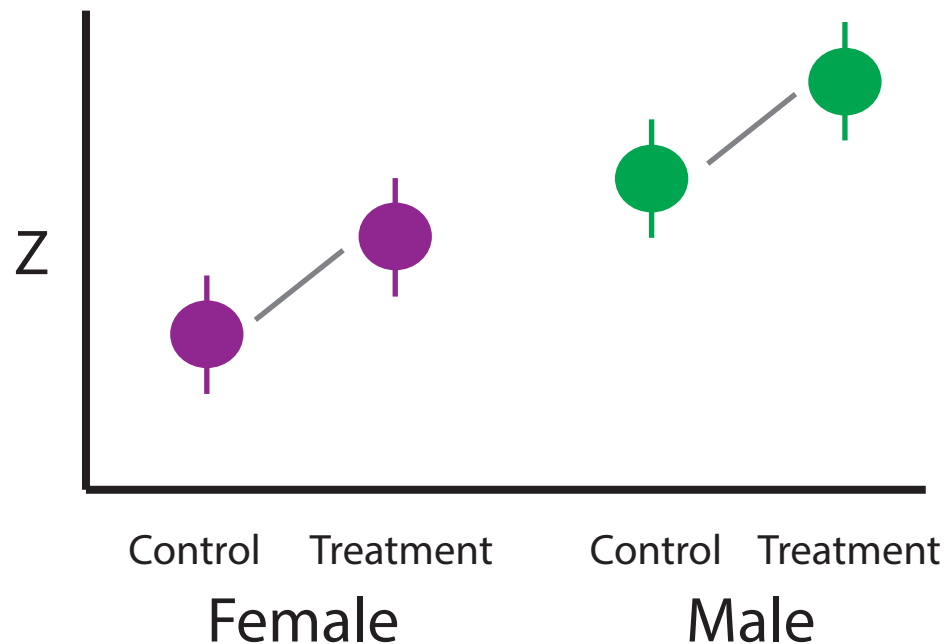
### Grant applications / manuscripts:

For grants, include strong justification for assuming equal means and treatment effects. Incorrect assumptions may result in experiments with too few samples.

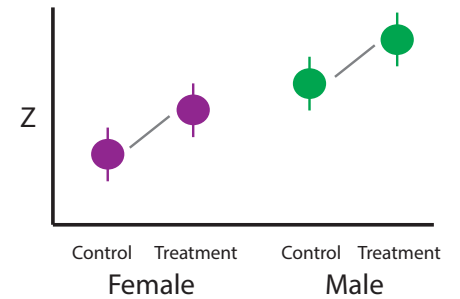
For manuscripts, descriptive statistics or plots confirming assumptions of equal means and treatment effects (or lack thereof) should be reported.

# Scenario 3

| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 3        | No   | No   | Yes  | Yes  |



# Scenario 3



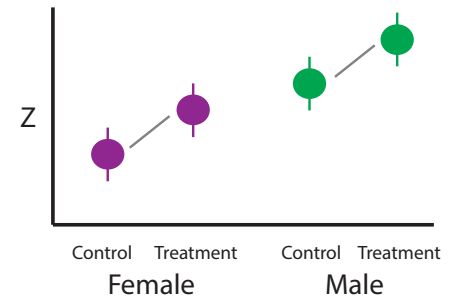
| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 3        | No   | No   | Yes  | Yes  |

## Sample size / experimental design:

When planning the experiment, sample size requirements are likely to be higher than in studies of one sex only. Actual requirements will depend on the anticipated extent to which the mean of Z in the control condition differs for female and male cells.

Consult your statistician.

# Scenario 3



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 3        | No   | No   | Yes  | Yes  |

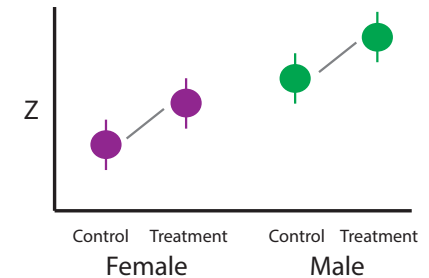
## Statistical analyses:

When analyzing collected data, sex should be included as a ‘covariate’ in all analyses, including regression.

The similarity of the treatment’s effect on Z for cells of both sexes should be examined using descriptive statistics and/or plots.

**IF** treatment effects **ARE** equal, it is appropriate to analyze data from the female and male cells together, including statistical adjustment for sex with a common treatment effect.

# Scenario 3



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 3        | No   | No   | Yes  | Yes  |

## Statistical analyses:

**IF** treatment effects **ARE NOT** equal, move to Scenarios 4 or 5.

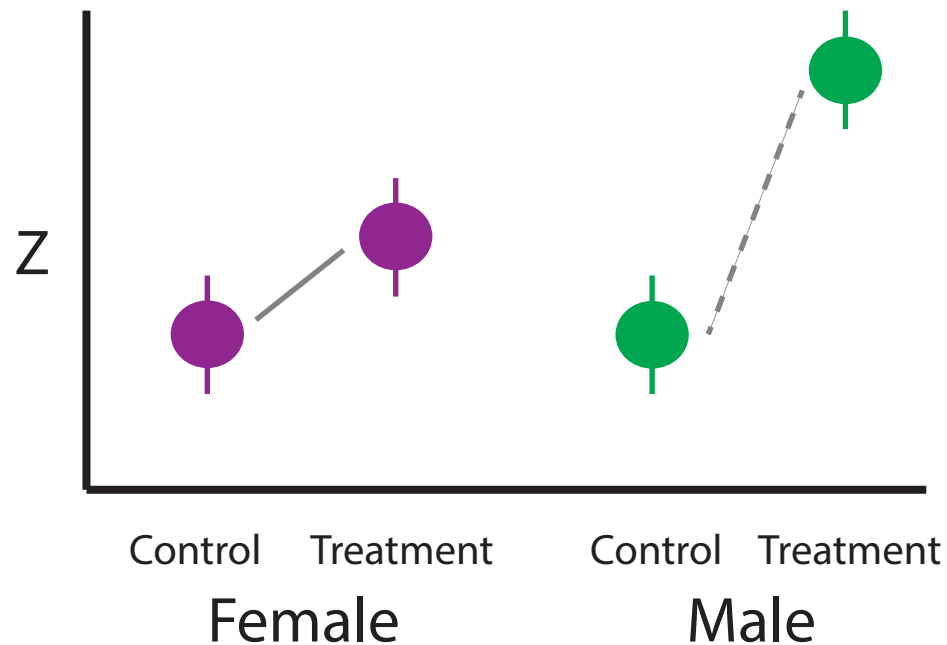
## Grant applications / manuscripts:

For grants, include strong justification for the assumption that treatment effects do not differ by sex.

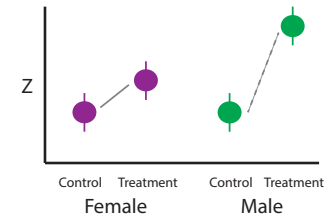
For manuscripts, it may or may not be necessary to report results separately for both sexes. It will depend on the setting. However, descriptive statistics or plots confirming equal treatment effects should be reported.

# Scenario 4

| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 4        | No   | Possibly   | No, not of primary interest                                    | Yes  |



# Scenario 4



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 4        | No   | Possibly   | No, not of primary interest                                    | Yes  |

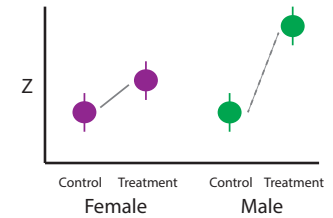
## Sample size / experimental design:

When planning the experiment, sample size requirements will be higher than in studies of one sex only. Actual requirements will depend on the extent to which the mean of Z in the control condition and/or the treatment’s effect on Z are anticipated to differ for female and male cells.

Consult your statistician.



# Scenario 4



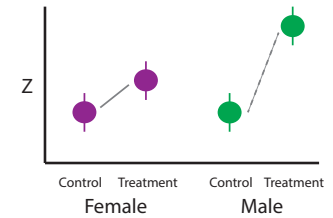
| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 4        | No   | Possibly   | No, not of primary interest                                    | Yes  |

## Statistical analyses:

When analyzing collected data, sex should be included as a ‘covariate’ in all analyses, including regression. An ‘interaction term’ between treatment and sex should also be included to account for the difference in treatment effect according to sex.

Descriptive statistics and/or plots will be helpful to demonstrate possible differences in means and treatment effects.

# Scenario 4



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 4        | No   | Possibly   | No, not of primary interest                                    | Yes  |

## Grant applications / manuscripts:

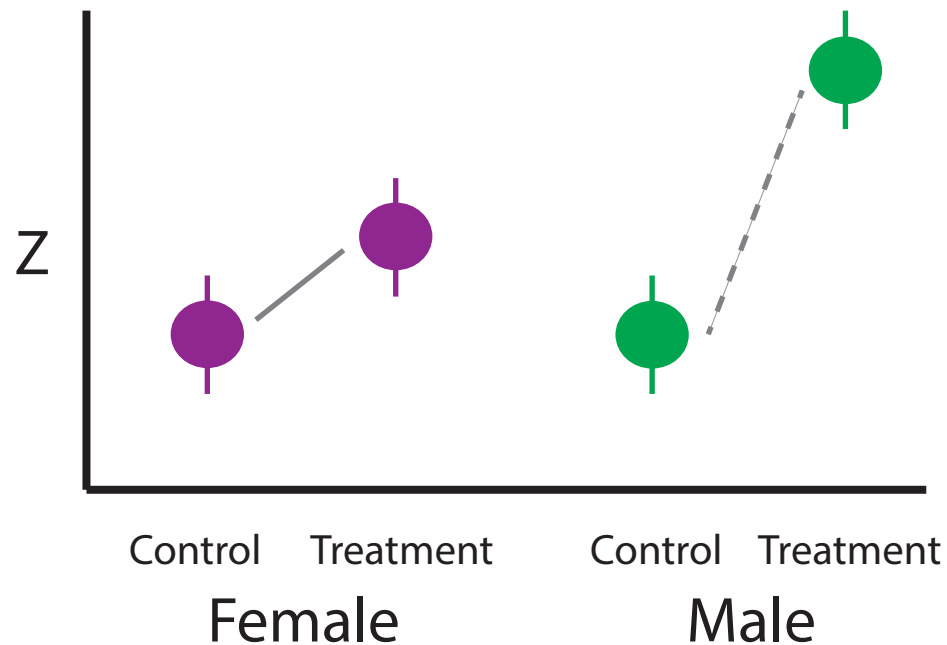
For grants, there should be justification for why it is not of primary interest to formally demonstrate differences in treatment effects for males and females.

For manuscripts, it is important to report means of Z in the control condition and after treatment separately for female and male cells.

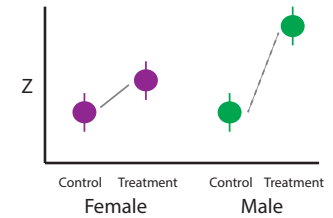
In Scenario 4, formally showing that the treatment effect is statistically significantly different for males and females **WILL NOT** be a main point in the manuscript.

# Scenario 5

| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 5        | No   | Possibly   | No, and of primary interest                                    | Yes  |



# Scenario 5



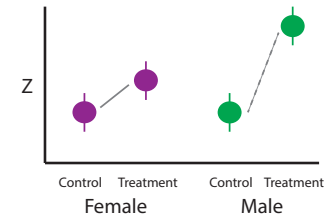
| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 5        | No   | Possibly   | No, and of primary interest                                    | Yes  |

## Sample size / experimental design:

When planning the experiment, sample size requirements will be higher than in studies of one sex only. Specifically, the study should have adequate sample size to test whether the interaction term between treatment and sex is statistically significantly different from zero. Actual requirements will depend on the extent to which the mean of Z in the control condition and/or the treatment's effect on Z are anticipated to differ for female and male cells.

Consult your statistician.

# Scenario 5



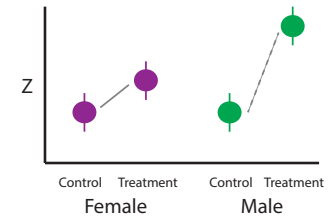
| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 5        | No   | Possibly   | No, and of primary interest                                    | Yes  |

## Statistical analyses:

When analyzing collected data, sex should be included as a ‘covariate’ in all analyses, including regression. An ‘interaction term’ between treatment and sex should also be included to account for the difference in treatment effect according to sex.

Analyses should include formal statistical hypothesis testing on the interaction term.

# Scenario 5



| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 5        | No   | Possibly   | No, and of primary interest                                    | Yes  |

## Grant applications / manuscripts:

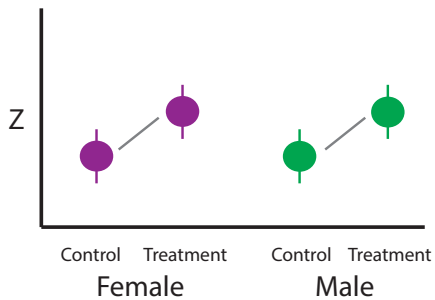
For grants, it is important to justify why it is necessary to formally conclude that there are treatment differences for males and females. This will be crucial to justifying the additional expense for higher sample size.

For manuscripts, it is important to report means of Z in the control condition and after treatment separately for female and male cells.

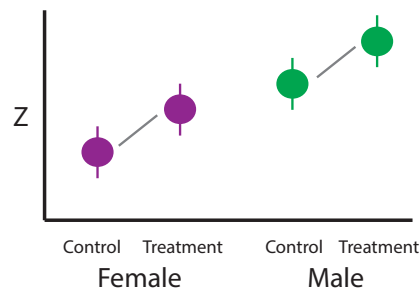
In Scenario 5, formally showing that the treatment effect is statistically significantly different for males and females **WILL** be a main point in the manuscript.

| Scenario | Is there strong biological justification to study cells of only one sex? | In the control condition, is the mean of Z the same for males and females? | Does treatment affect Z in the same way for females and males? | Is sex a variable that should be included in experiment planning and statistical analyses? |
|----------|--|--|--|--|
| 1        | Yes  | -  | -  | No   |
| 2        | No   | Yes  | Yes  | Possibly   |
| 3        | No   | No   | Yes  | Yes  |
| 4        | No   | Possibly   | No, not of primary interest                                    | Yes  |
| 5        | No   | Possibly   | No, and of primary interest                                    | Yes  |

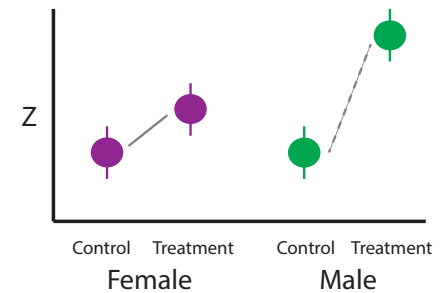
Scenario 2



Scenario 3



Scenario 4/5



# What if I'm not studying cell lines with a continuous outcome and not just treatment v. control?

Same story, different details

- Details that might differ:
  - Mice instead of cell lines
  - Categorical outcome
  - Multiple treatments



# What if I'm not studying cell lines with a continuous outcome and not just treatment v. control?

Same story, different details

- If outcome levels and treatment effects are the same or highly similar for males and females (and this has been checked, not just assumed) then analyses can for the most part be carried out without worrying about sex as a variable.
- Sex is not adding variability in this case.
- Experiments can also be planned without worrying about needing higher sample size than a study of one sex only.
- Experiments should be planned so that both males and females are studied so that appropriate conclusions can be made.

# What if I'm not studying cell lines with a continuous outcome and not just treatment v. control?

Same story, different details

- If outcome levels and/or treatment effects differ for males and females, then statistical analyses do need to account for sex as a variable.
- Sex is adding variability in this case.
- Experiments will in general require higher sample size.
- The highest sample size will typically be required for formally showing that treatment effects differ for males and females.
- Experiments should be planned so that both males and females are studied so that appropriate conclusions can be made.

# What if I planned my experiment incorrectly?

Help!!

- What if I assumed Scenario 2 would apply, but descriptive statistics and/or plots suggest Scenarios 3, 4 or 5 are more applicable?
- Statistical modeling can still be performed, but sample size may limit statistical power for making correct conclusions.
- More samples may be required.
- Consult a statistician.

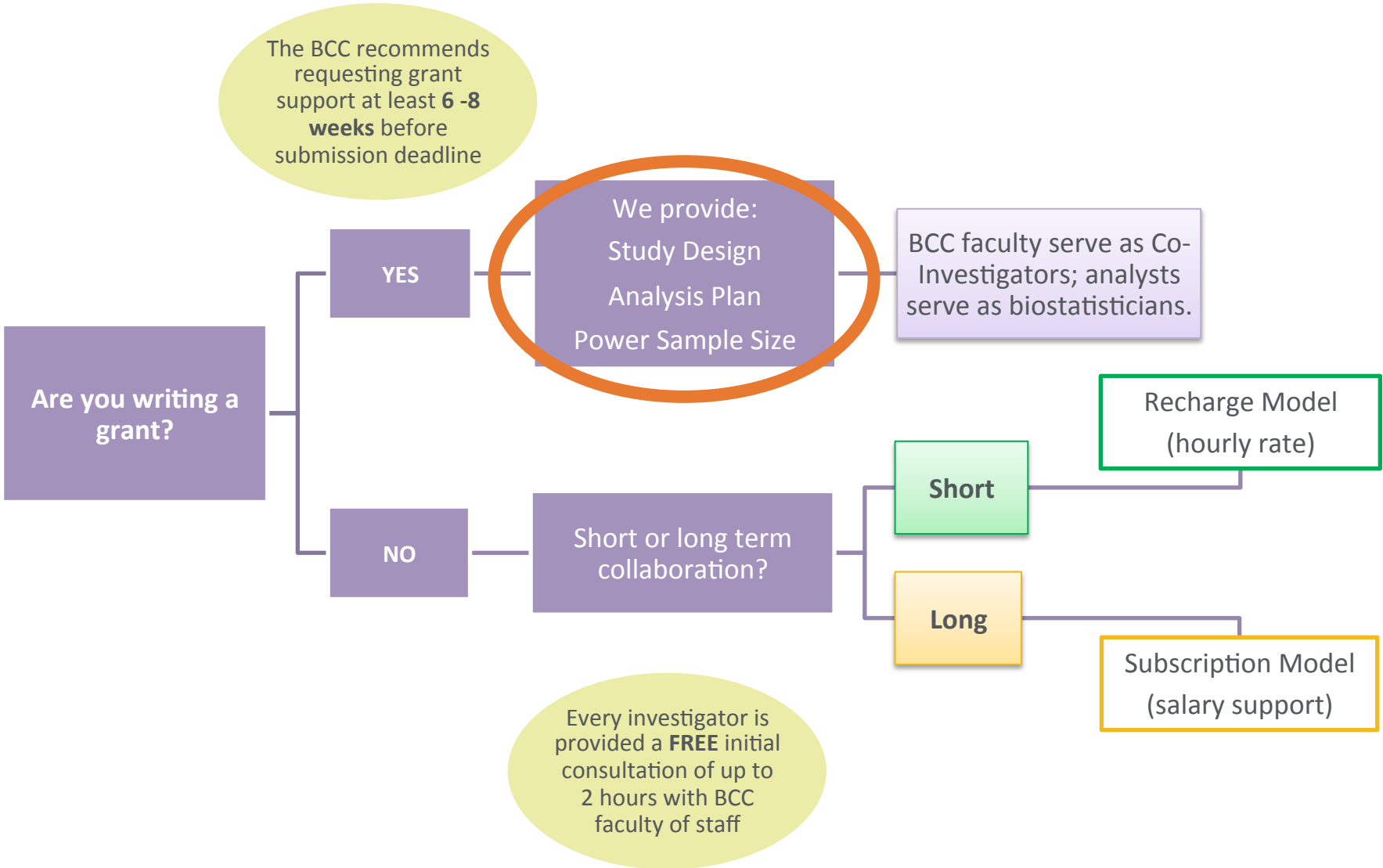
# What if I planned my experiment incorrectly?

Help!!

- What if I assumed Scenario 4 or 5 would apply, but descriptive statistics and/or plots suggest Scenarios 2 or 3 are more applicable?
- You're in good shape.
- If you designed your experiment with adequate sample size for Scenarios 4 or 5, you will have plenty of statistical power for Scenarios 2 or 3.

# BCC: Biostatistics Collaboration Center

## How We Do It



# Statistically Speaking ...

## What's next?

Tuesday, October 11 **Statistical Considerations for Sex Inclusion in Basic Science Research**  
Denise M. Scholtens, PhD, Associate Professor, Division of Biostatistics Associate Director, Department of Preventive Medicine

Friday, October 14 **The Impact of Other Factors: Confounding, Mediation, and Effect Modification** Amy Yang, MS, Sr. Statistical Analyst, Division of Biostatistics, Department of Preventive Medicine

Tuesday, October 18 **Statistical Power and Sample Size: What You Need and How Much**  
Mary Kwasny, ScD, Associate Professor, Division of Biostatistics, Department of Preventive Medicine

Friday, October 21 **Clinical Trials: Highlights from Design to Conduct** Masha Kocherginsky, PhD, Associate Professor, Division of Biostatistics, Department of Preventive Medicine

Tuesday, October 25 **Finding Signals in Big Data** Kwang-Youn A. Kim, PhD, Assistant Professor, Division of Biostatistics, Department of Preventive Medicine

Friday, October 28 **Enhancing Rigor and Transparency in Research: Adopting Tools that Support Reproducible Research** Leah J. Welty, PhD, BCC Director, Associate Professor, Division of Biostatistics, Department of Preventive Medicine

All lectures will be held from noon to 1 pm in Hughes Auditorium, Robert H. Lurie Medical Research Center, 303 E. Superior St.