

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

BCC: Biostatistics Collaboration Center

Who We Are



Leah J. Welty, PhD
Assoc. Professor
BCC Director



Joan S. Chmiel,
PhD
Professor



Jody D. Ciolino,
PhD
Asst. Professor



Kwang-Youn A. Kim,
PhD
Asst. Professor



Masha Kocherginsky,
PhD
Assoc. Professor



Mary J. Kwasny, ScD
Assoc. Professor



Julia Lee, PhD, MPH
Assoc. Professor



Alfred Rademaker, PhD
Professor



Hannah L. Palac, MS
Senior Stat. Analyst



Gerald Rouleau, MS
Stat. Analyst



Amy Yang, MS
Senior Stat. Analyst

Not Pictured:

1. David A. Aaby, MS
Senior Stat. Analyst

2. Tameka L. Brannon
Financial | Research
Administrator

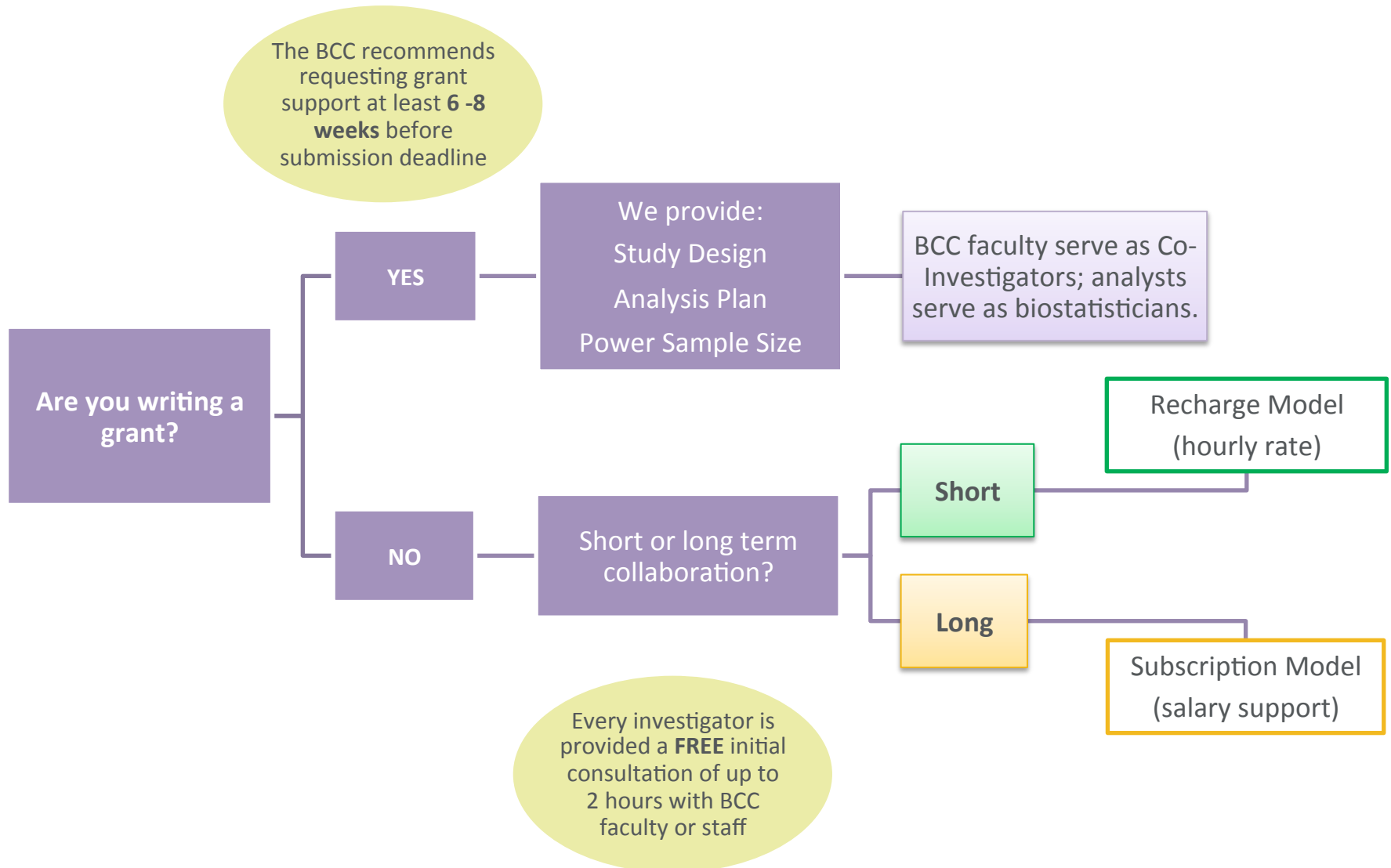
BCC: Biostatistics Collaboration Center

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.

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
 - 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.’

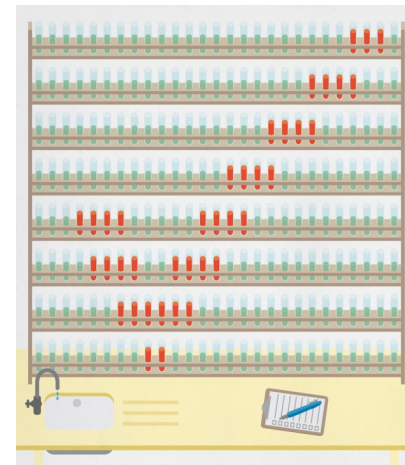
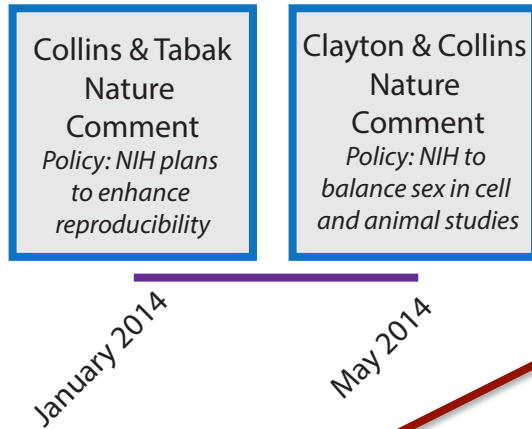


Figure from Collins & Tabak, Nature (2014)

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.’

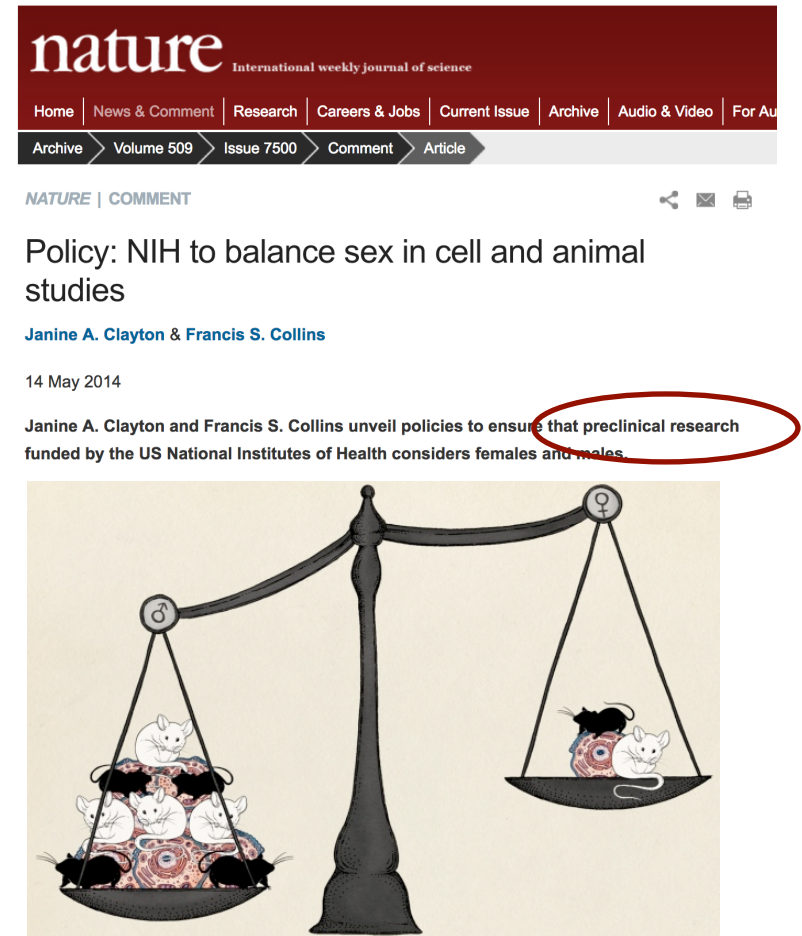
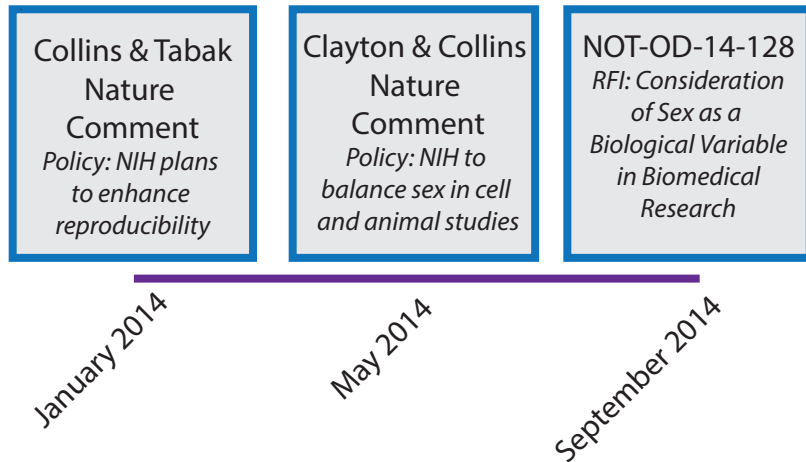


Figure from Clayton & Collins, Nature (2014)

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...

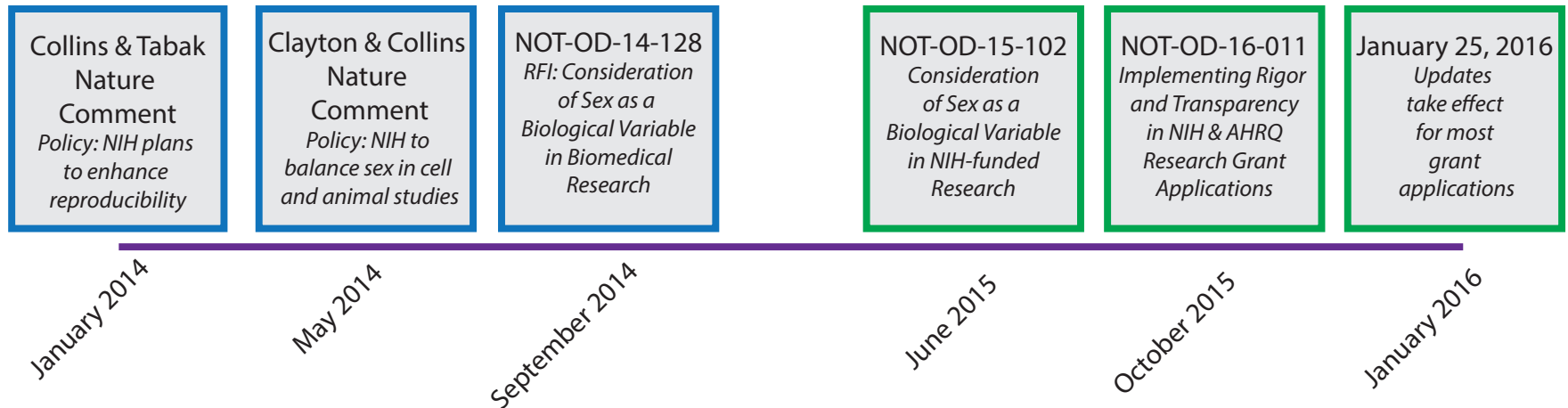
Sample sizes

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:

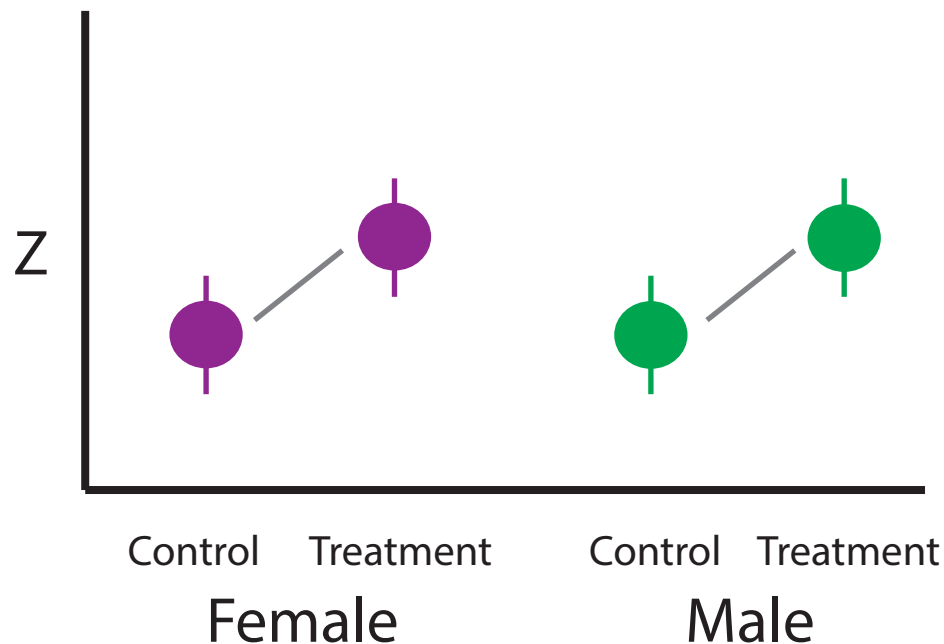
Statistical analyses do not need to consider sex as a variable

Grant applications / manuscripts:

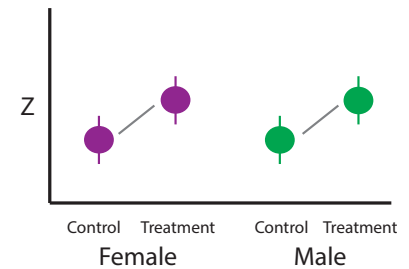
Include strong biological justification for studying only one sex

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

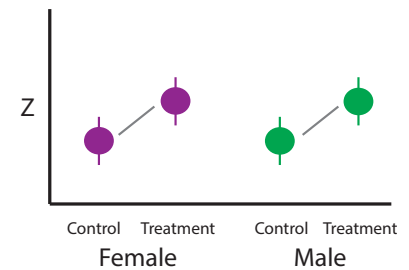


Scenario	Is there strong biological justification to study cells of only one sex?	In the control condition, is the mean of Z the same for males and females?	Does treatment affect Z in the same way for females and males?	Is sex a variable that should be included in experiment planning and statistical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

Sample size / experimental design:

IF 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

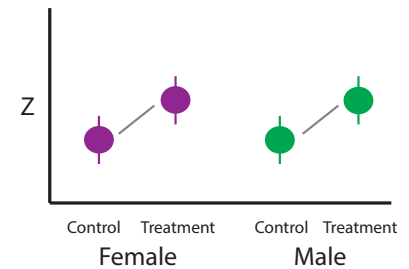
Statistical analyses:

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.

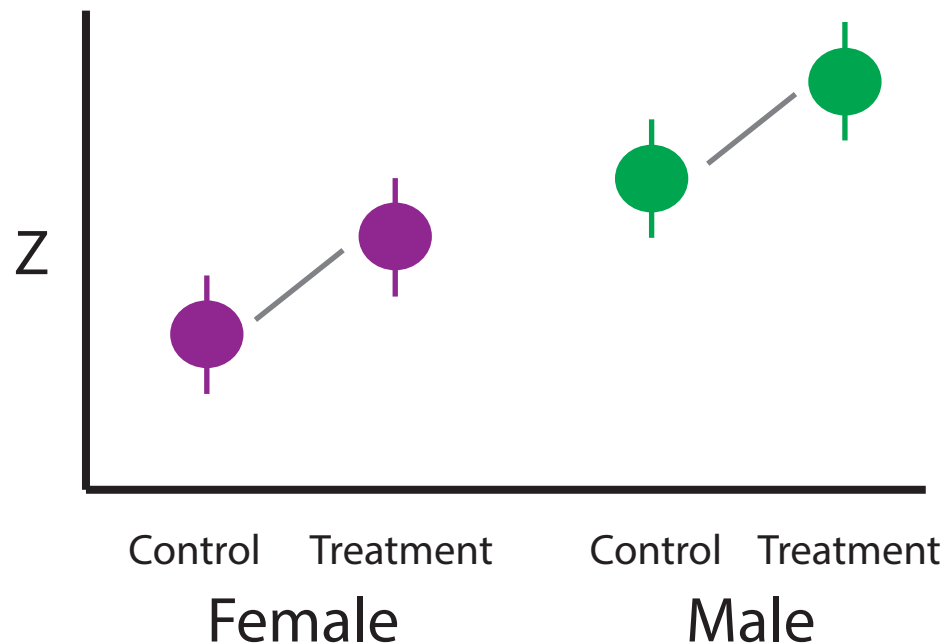
Grant applications / manuscripts:

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

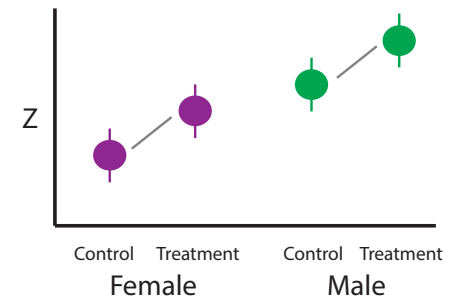
For manuscripts, descriptive statistics or plots confirming assumptions of equal means and treatment effects (or lack thereof) should be reported.

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



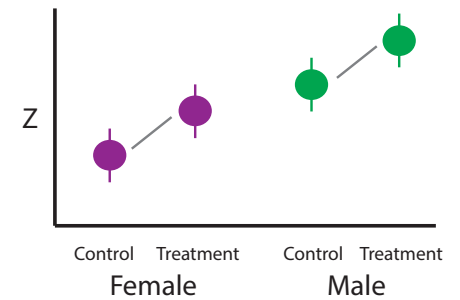
Scenario	Is there strong biological justification to study cells of only one sex?	In the control condition, is the mean of Z the same for males and females?	Does treatment affect Z in the same way for females and males?	Is sex a variable that should be included in experiment planning and statistical 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

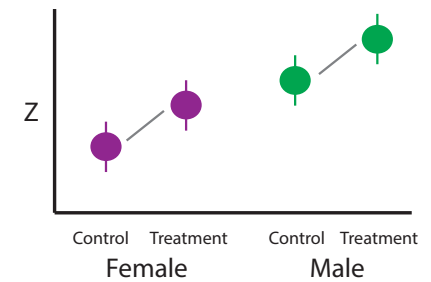
Statistical analyses:

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.

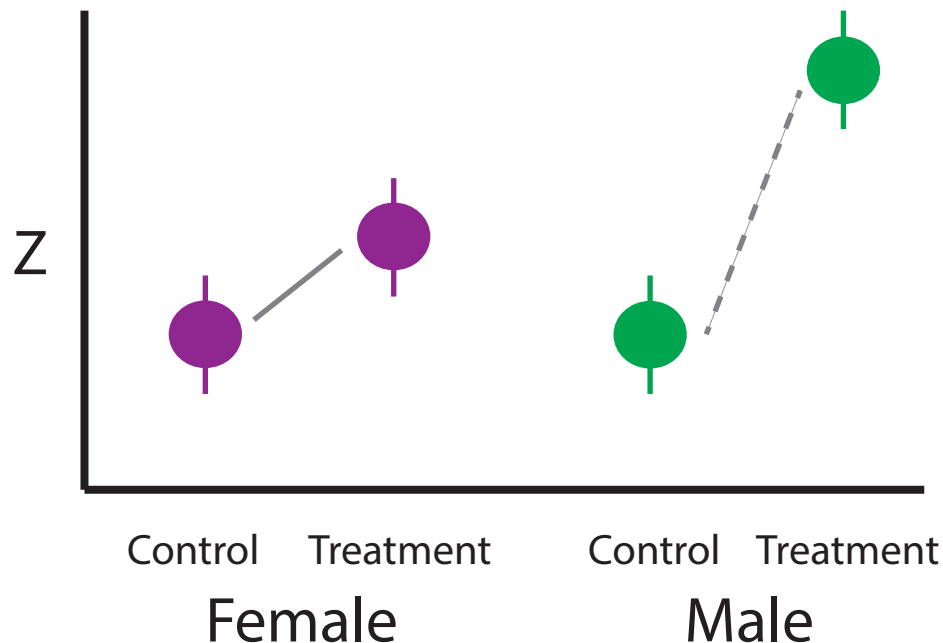
Grant applications / manuscripts:

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

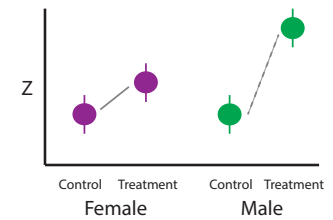
For manuscripts, it may or may not be necessary to report results separately for both sexes. It will depend on the setting. However, descriptive statistics or plots confirming equal treatment effects should be reported.

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



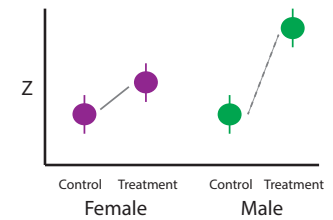
Scenario	Is there strong biological justification to study cells of only one sex?	In the control condition, is the mean of Z the same for males and females?	Does treatment affect Z in the same way for females and males?	Is sex a variable that should be included in experiment planning and statistical 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



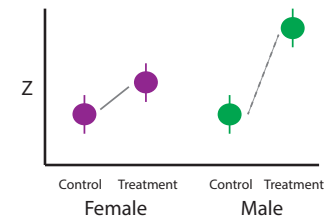
Scenario	Is there strong biological justification to study cells of only one sex?	In the control condition, is the mean of Z the same for males and females?	Does treatment affect Z in the same way for females and males?	Is sex a variable that should be included in experiment planning and statistical 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:

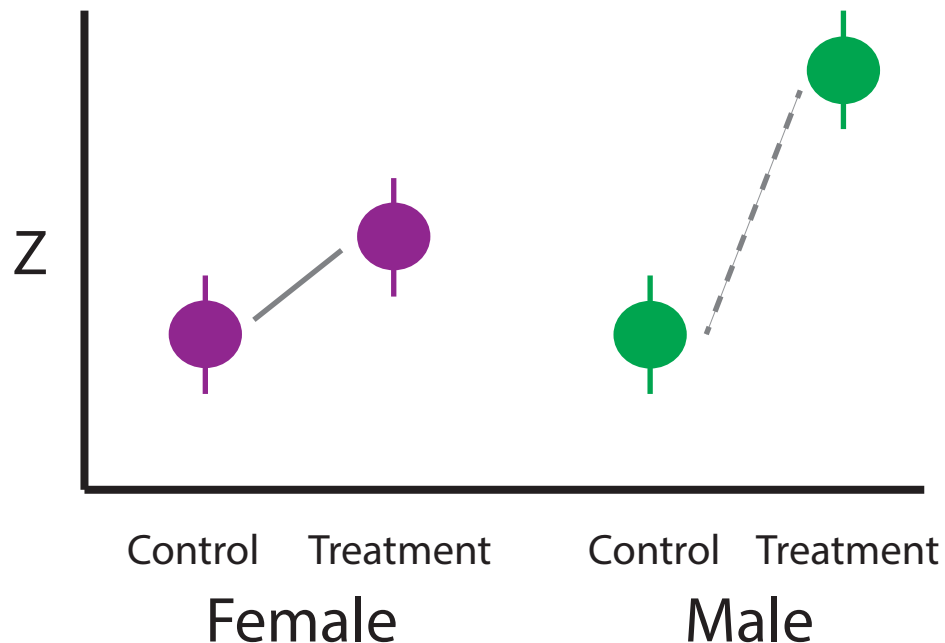
For grants, there should be justification for why it is not of primary interest to formally demonstrate differences in treatment effects for males and females.

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

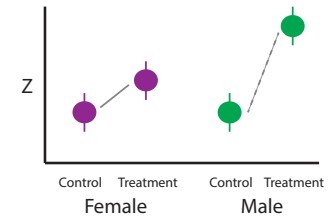
In Scenario 4, formally showing that the treatment effect is statistically significantly different for males and females **WILL NOT** be a main point in the manuscript.

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



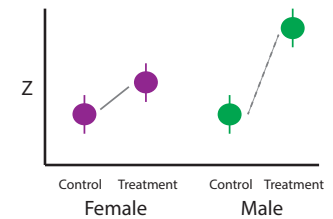
Scenario	Is there strong biological justification to study cells of only one sex?	In the control condition, is the mean of Z the same for males and females?	Does treatment affect Z in the same way for females and males?	Is sex a variable that should be included in experiment planning and statistical 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



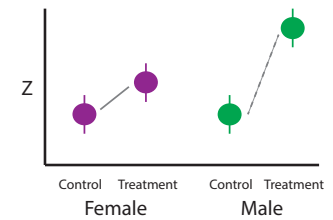
Scenario	Is there strong biological justification to study cells of only one sex?	In the control condition, is the mean of Z the same for males and females?	Does treatment affect Z in the same way for females and males?	Is sex a variable that should be included in experiment planning and statistical 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:

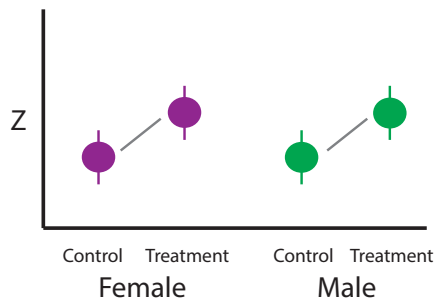
For grants, it is important to justify why it is necessary to formally conclude that there are treatment differences for males and females. This will be crucial to justifying the additional expense for higher sample size.

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

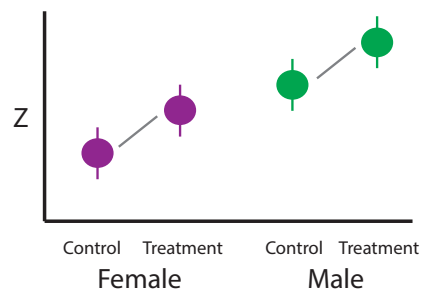
In Scenario 5, formally showing that the treatment effect is statistically significantly different for males and females **WILL** be a main point in the manuscript.

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

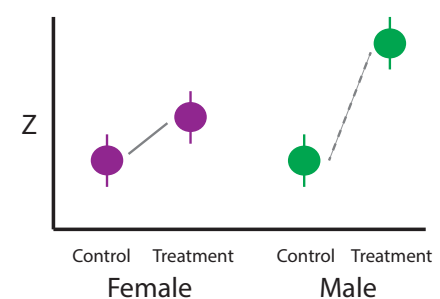
Scenario 2



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.

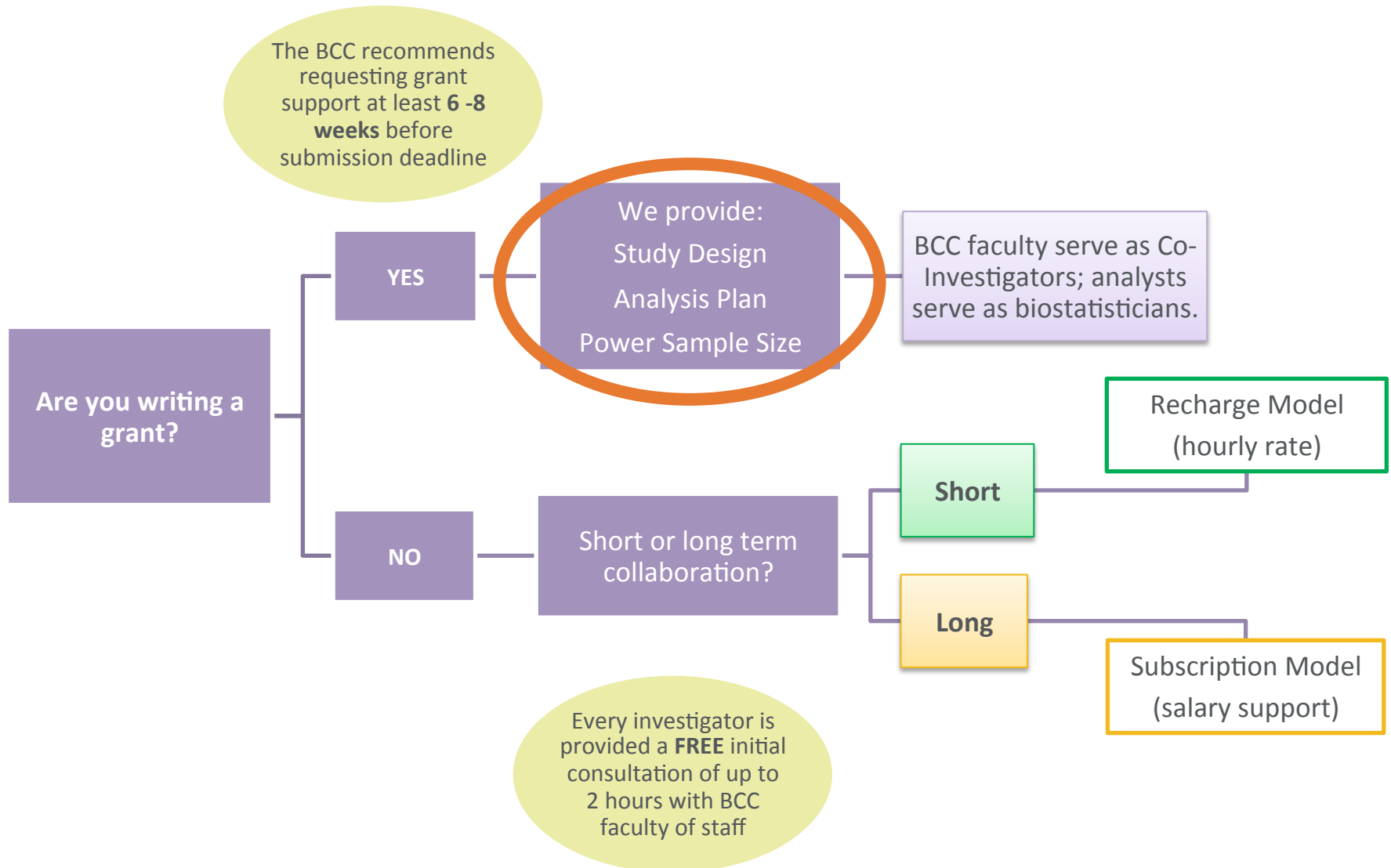
What if I planned my experiment incorrectly?

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.