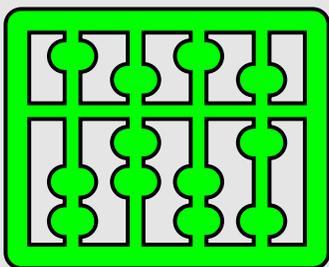


Sample Sizes for Cell and Animal Studies

BERDC Special Topics Talk 8



DaCCoTA

DAKOTA CANCER COLLABORATIVE
ON TRANSLATIONAL ACTIVITY

Dr. Mark Williamson

Biostatistics, Epidemiology,
and Research Design Core

Opening

Goal: Untangle sample size for cell and animal studies

- Experimental design 
- Experimental unit 
- Cell and animal-specific considerations  
- Additional considerations 
- Methods for sample size calculation 
- Examples 

Before Moving On:

Pre-test: https://und.qualtrics.com/jfe/form/SV_oBby5MZ6USU5WU6

Design with Goal in Mind

What question are you trying to answer?

- What is the population of focus? [1,2]
- Are your samples representative? [2]
- What is the day-in and day-out reality? [3]

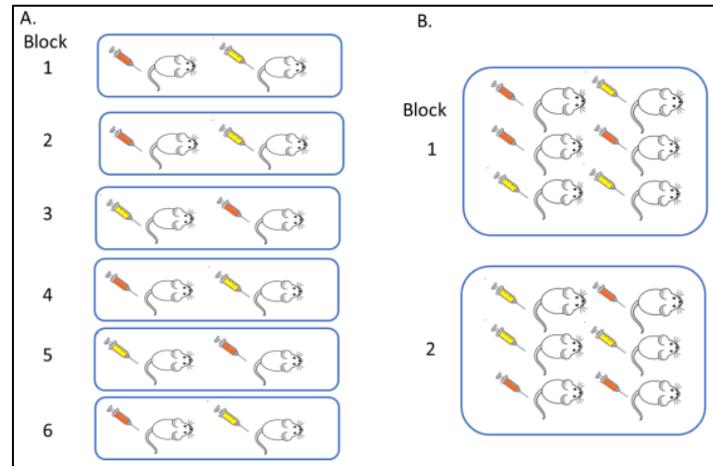
Use design well [4]

- Craft your design
- Determine levels
- \uparrow design \approx \uparrow inference

Too many vs. too few [5]

- *too many* -> *partial waste*
- *too few* -> *total waste*

Define the experimental unit



Defining Replication

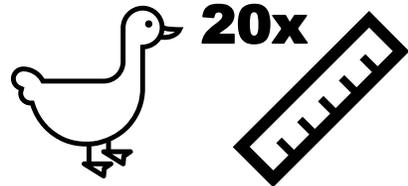
Number of samples \approx replication

- Benefits -> 'better' stats
- Drawbacks -> time, money, effort
- Key point -> tradeoffs

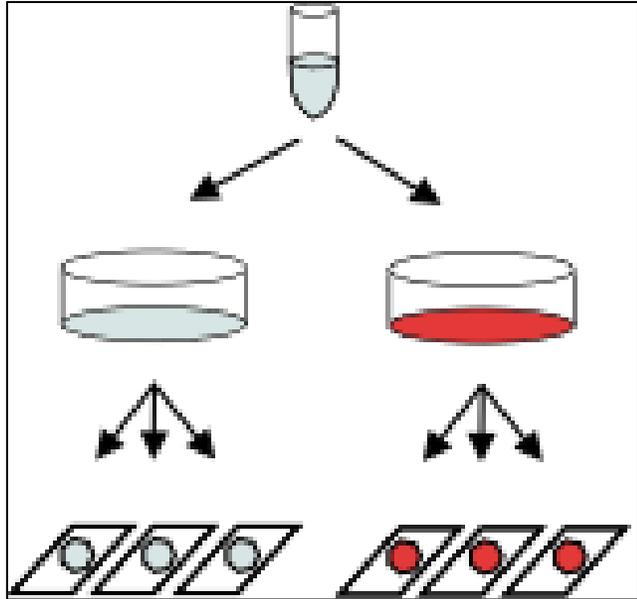
Replication versus Pseudoreplication

- Biological vs. Technical [6,7]

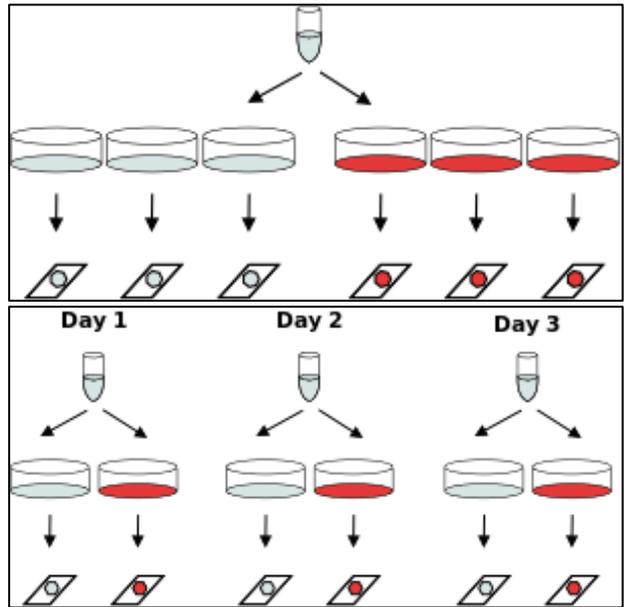
Blatant pseudoreplication



Nuanced pseudoreplication



Better design, but need better terms



Technical replication ~ Pseudoreplication

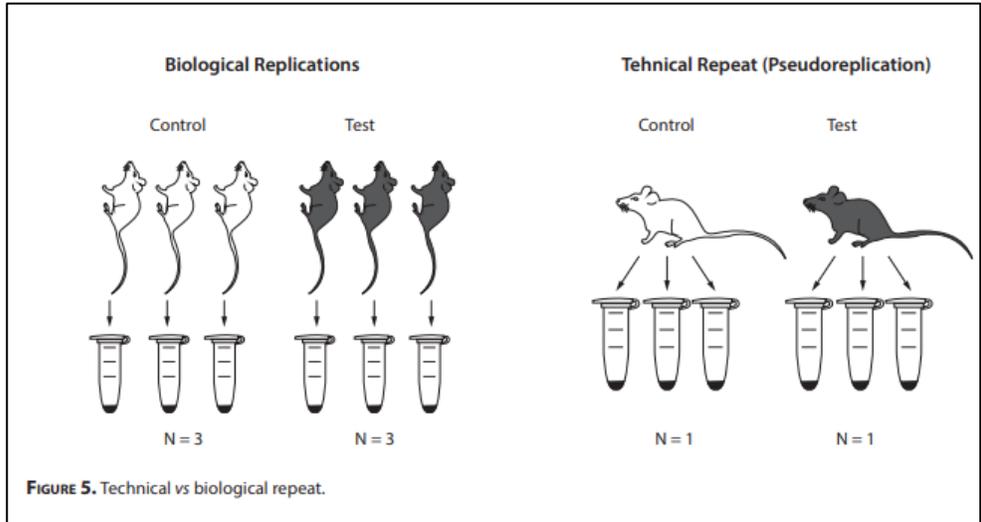


FIGURE 5. Technical vs biological repeat.

Defining Replication

Number of samples \approx replication

- Benefits -> 'better' stats
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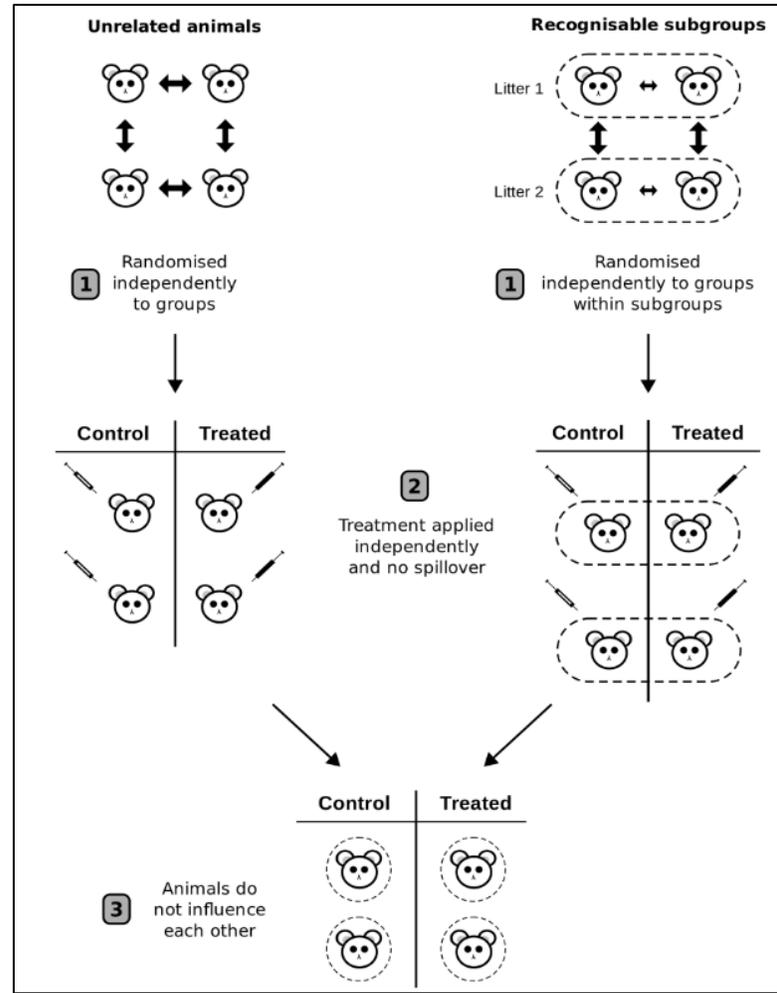
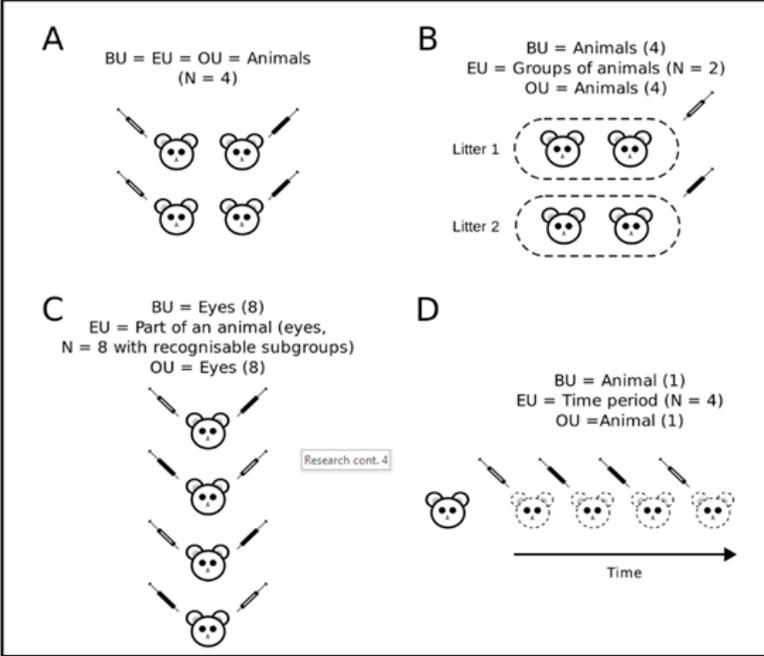
Replication versus Pseudoreplication

- Biological vs. Technical [6,7]
- Biological, **Experimental**, and Observational Units [8]

Criteria for genuine replication [8]

- 1) EUs independently randomized to treatment conditions
- 2) treatment independently applied to each EU
- 3) EUs must not influence each other

The Experimental Unit is the level at which the intervention is made and is the point where replication matters



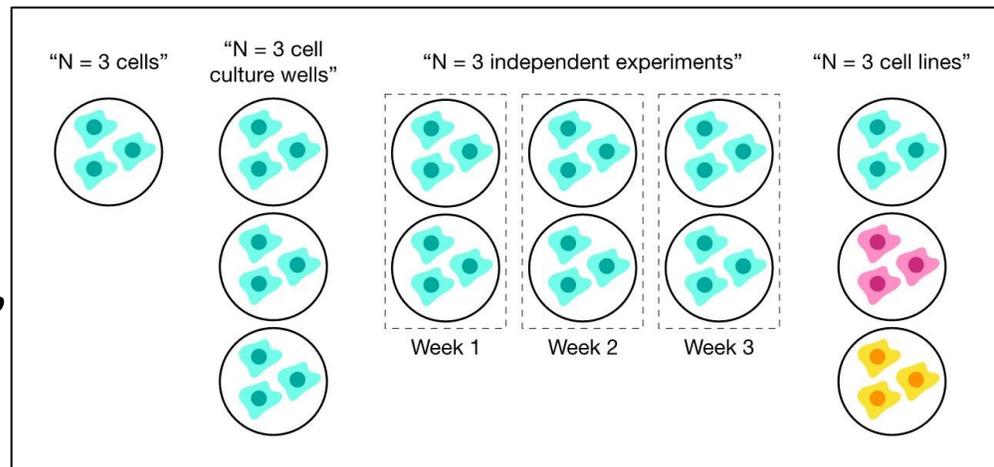
Cell-specific Considerations

What is the experimental unit for cell studies?

- Not canonically defined [9,10]
- Rarely cells themselves
- Some contenders are independent experiments, culture wells, monoclonal cell lines, etc. [2]
- Cell line guidelines
 - Best setup is samples from unique individuals [8]
 - Second best is sample from unique cell lines
 - Acceptable alternative -> different days/conditions

What are actual sample size numbers?

- Statistical ideal is around thirty [3]
- Commonly in single digits or as low as three [1]
- Practical minimum is also three



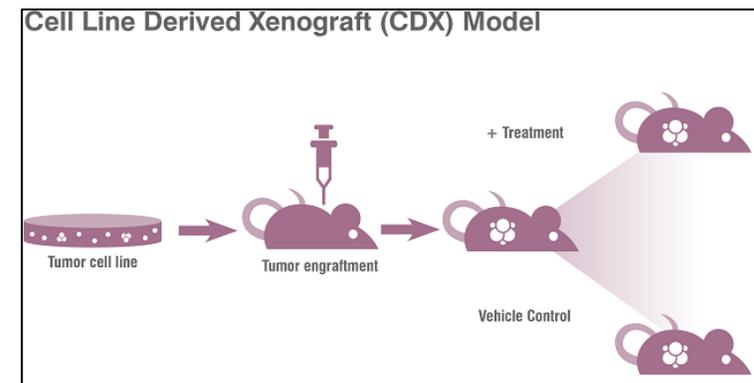
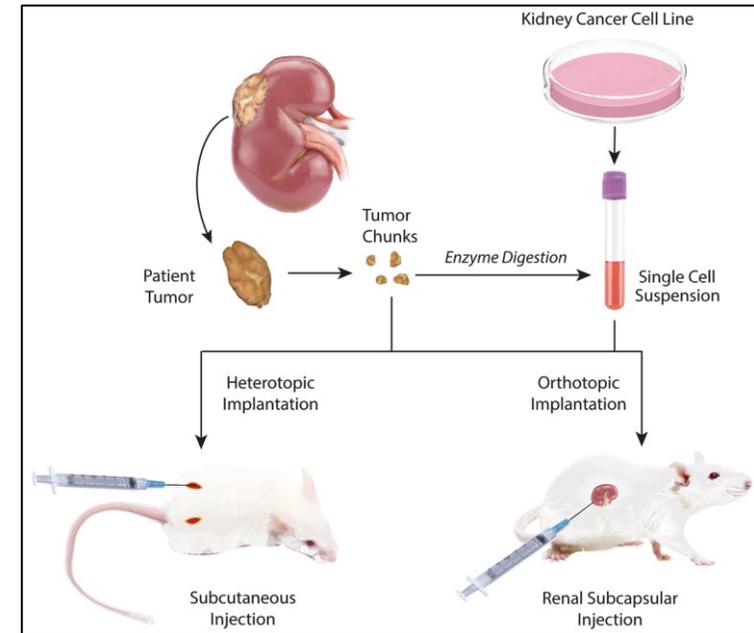
Animal-specific Considerations

What is the experimental unit for animals (mice)?

- Typically the individual mouse
- Depends on experimental exposure -> may be cage or litter instead [8]
- PDX or CDX model guidelines
 - EU may be tumors
 - Multiple flanks possible
 - Pay attention to levels

What are actual sample size numbers?

- Basic -> 12, same as rule of thumb for pilot studies [11]
- More involved -> Resource Equation Model [12]
- Will need to account for premature death [13,14]

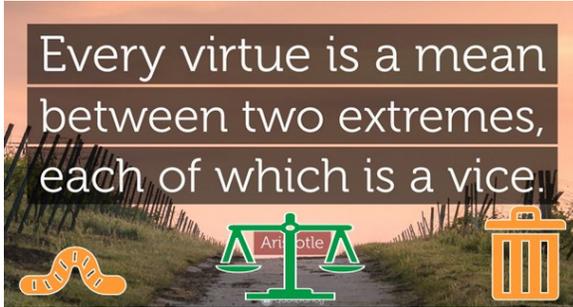
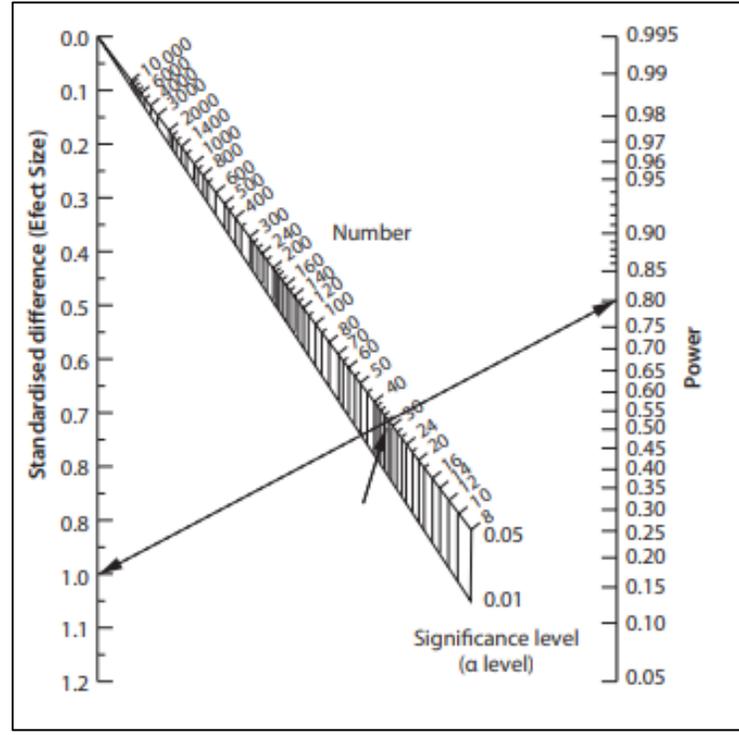


Sample Size Calculation Methods

- 1) Power Analysis
- Software -> G*Power, R, etc.
- 2) Resource Equation Model
- Used for animal studies
- Equation for min/max
- 3) Literature Search
- Methods
- Rationale



$E = \text{Total \# of animals} - \text{Total \# of groups}$
$\text{Total \# of animals} = E + \text{Total \# of groups}$
• $X = \text{\# of animals}$
• $E_{\min} = 10; E_{\max} = 20$
• Get group # and attrition correction
• Calculate min and max



Same/Similar model system
Same/Similar experimental design
Number of samples/group
Number of groups

Perineural invasion in mouse models (literature search example)
• Cancer study 1 (n=10/group, 3 groups; n=7/group, 2 groups; n=8/group, 2 groups)
• Cancer study 2 (n=10/group, 2 groups)
• Cancer study 3 (n=7/group, 2 groups, 6 time points; n=5/group, 2 group, 6 time points)

Additional Considerations

Ways to increase power w/o increasing sample size [15]

Not this kind of power



THE INSULT THAT MADE A MAN OUT OF "MAC"

HEY! QUIT KICKING THAT SAND IN OUR FACES!

LISTEN HERE, MAC. I'D SMASH YOUR FACE -- ONLY YOU'RE SO SKINNY YOU MIGHT DRY UP AND BLOW AWAY.

SEE YOU LATER, GIRLIE...

THE BIG BULLY! I'LL GET EVEN SOME DAY.

OH DON'T LET IT BOTHER YOU LITTLE BOY!

NO, I'M AFRAID NOT, I'M PRETTY BUSY GOOD-DAY!

IT WAS NICE TO MEET YOU GRACE. CAN I EVER COME AROUND SOME EVENING?

DARN IT! I'M SICK AND TIRED OF BEING A SCARECROW! CHARLES ATLAS SAYS HE CAN GIVE ME A REAL BODY. ALL RIGHT! I'LL GAMBLE A STAND AND GIVE HIS FREE BOOK!

BOY! IT DIDN'T TAKE ATLAS LONG TO DO THIS FOR ME! LOOK AT THOSE MUSCLES BULGE OUT NOW!

THERE'S THAT BIG STIFF AGAIN, SHOWING OFF IN FRONT OF GRACE AND THE CROWD. WELL, IT'S MY TURN THIS TIME!

WHAM! -- NOW IT'S YOUR TURN TO DRY UP AND BLOW AWAY!

OH MAC! YOU ARE A REAL MAN AFTER ALL!

The 97-lb. Weakling Who Became "The World's Most Perfectly Developed Man!"

THEY used to think there wasn't much hope for me. I weighed only 97 pounds. I was a sticky scarecrow. Then I discovered *Dynamic-Tension*. It gave me the body that twice won the title, "The World's Most Perfectly Developed Man." If my own risk I'll give you PROOF in just 7 days that my same method can make you over into a NEW MAN of giant power and energy.

I'LL PROVE You Can Have a Body Like Mine!

I'LL PROVE that *Dynamic-Tension* without any pills and bulges that may strain your heart or other vital organs--can make you a healthy, confident, powerful **HE-MAN!** In just a few minutes a day! Mail Coupon NOW for my illustrated book, "Everlasting Health and Strength." Tells all about *Dynamic-Tension*. Shows actual photos. It's a valuable book! And it's FREE. Send for your copy today. Address me personally: Charles Atlas, Dept. 68, 115 E. 23rd St., New York, N. Y.

or unnatural dieting or weights. **FREE BOOK**

CHARLES ATLAS
Holder of the title: "The World's Most Perfectly Developed Man."

THIS STERLING SILVER CUP BEING GIVEN AWAY
This valuable cup stands about 14 inches high on a black mahogany base. I will award it to my pupil who makes the most improvement in his development within 7 days.

CHARLES ATLAS, Dept. 68
115 East 23rd Street, New York, N. Y.

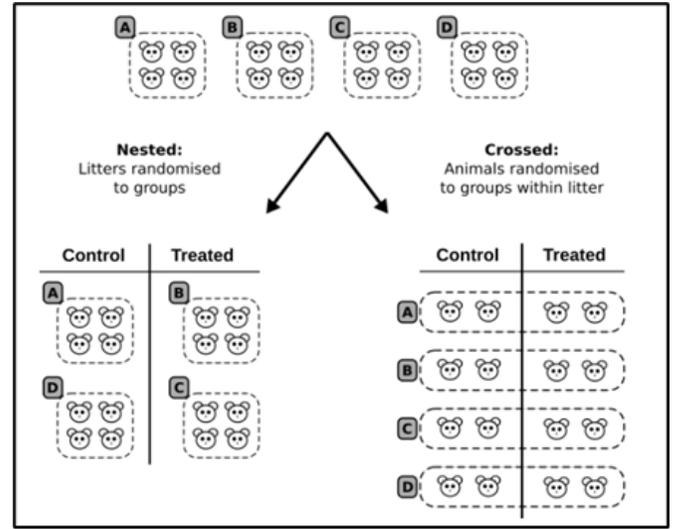
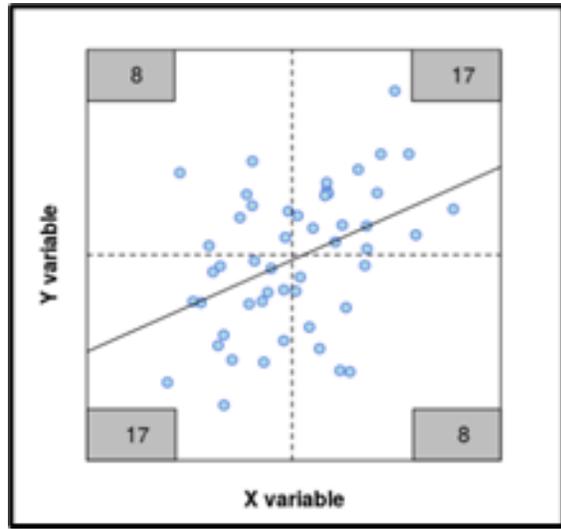
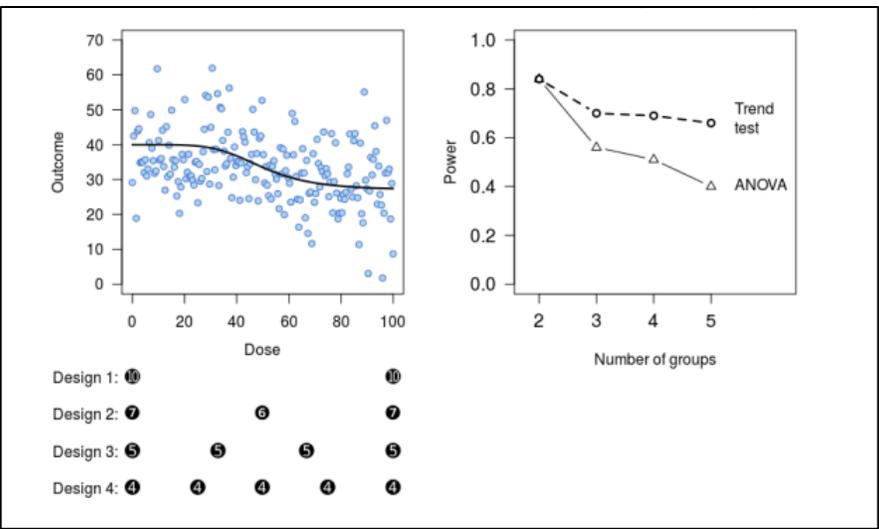
I want the proof that your system of *Dynamic-Tension* will make a New Man of me--give me a healthy, husky body and big muscle development. Send me your free book, "Everlasting Health and Strength."

Name
(Please print or write plainly)
Address
City State

Additional Considerations

Ways to increase power w/o increasing sample size [15]

- Fewer factor levels for continuous predictors
- Focused hypothesis test
- Don't dichotomize or bin continuous variables
- Cross rather than nest factors



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Ways to increase power w/o increasing sample size [15]

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Statistical versus clinical significance [13]

Statistical design and sample size

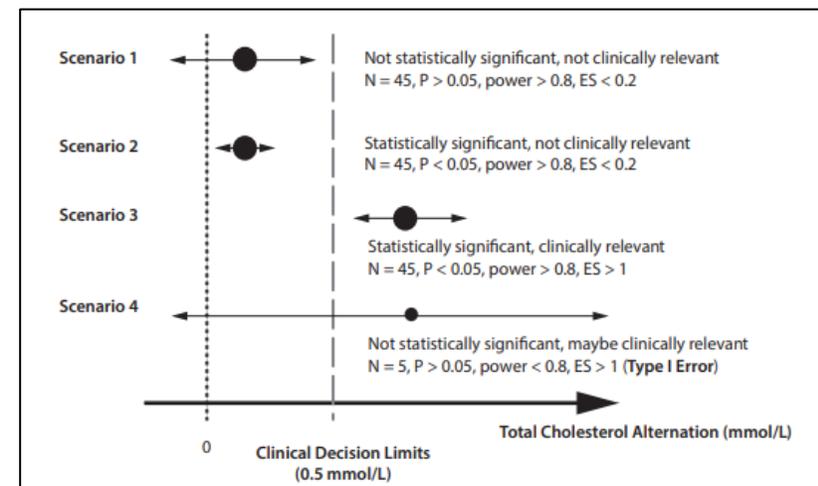
- Examples on binary data, single group analysis, time to an event, etc. [16]
- Other useful examples [17] and tidbits [13]

Sample size is not well reported

- 26% of animal studies (experiment on adults measured on offspring) did not report sample size [8]
- 44% of preclinical trials examined (n=233) had insufficient sample size description [18]

Still a place for technical replicates

- Reproducibility of experiment [3]
- Bayes can help make valid inferences, even with pseudoreplication [19]

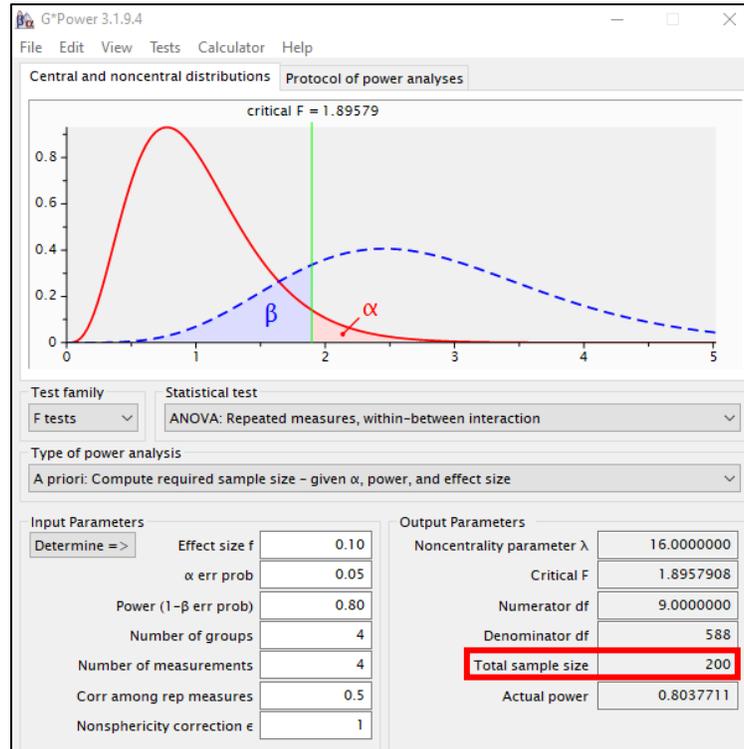
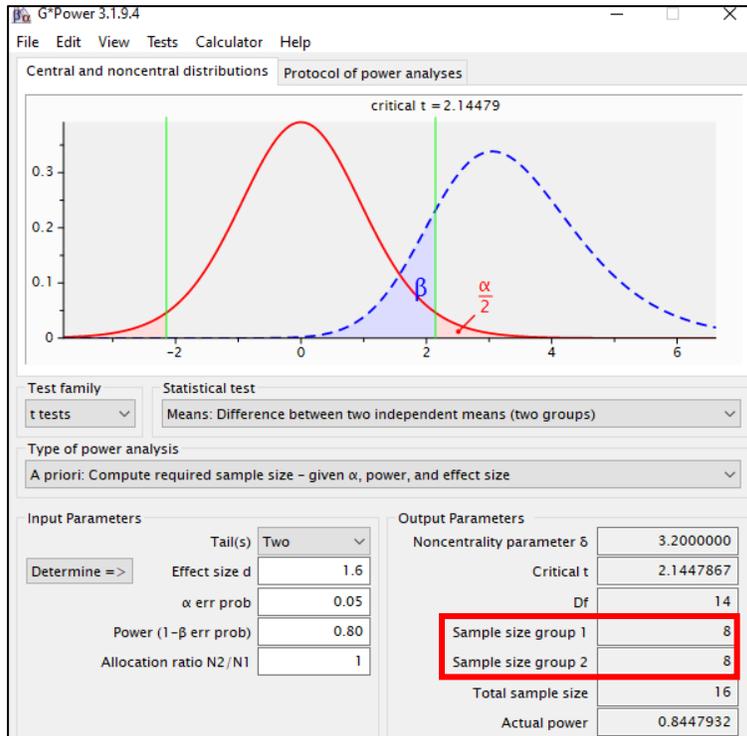


Examples

Cell line study

1) Power Analysis via G*Power

- 2 groups (control, treatment)
- 4 groups, 4 time points



Examples

Cell line study

- 1) Power Analysis via G*Power
 - 2 groups (control, treatment)
 - 4 groups, 4 time points

Patient derived xenograft study

- 2) Resource Equation Model
- 3) Literature Search

E = Total # of animals – Total # of groups
Total # of animals = E + Total # of groups
• X = # of animals
• E_{min} = 10; E_{max} = 20
• Get group # and attrition correction
• Calculate min and max
4 groups; 20% attrition (divide by 0.80) $X(\text{min}) = (10 + 4)/0.80 = 17.5$ $X(\text{max}) = (20 + 4)/0.80 = 30$ $17.5/4 = 4.375 \rightarrow 5 \text{ per group (min)}$ $30/4 = 7.5 \rightarrow 8 \text{ per group (max)}$ 5-8 animals per group

Patient-derived xenografts studies
• Mouse study 1 (n=8/group , 4 groups)
• Mouse study 2 (n=5/group , 4 groups)
• Mouse study 3 (n=6/group, 5 groups)
• Mouse study 4 (n=5/group, 5 groups)

Wrap-up

- Sample sizes depends on the research question and research design
- Sample size should be counted at the level of experimental unit
 - ★ Gold standard for cell studies are individual sample-derived cultures
 - ★ Gold standard for animal studies are individual animals with direct intervention
- Sample size can be determined in several ways
 - 👍 Rule of thumb (3-10 cell lines, 12 animals per group)
 - 💪 Power analysis (w/ estimated or approximate effect size)
 - 🐭 Resource equation model (animal studies)
 - 📖 Literature search (similar systems and designs)

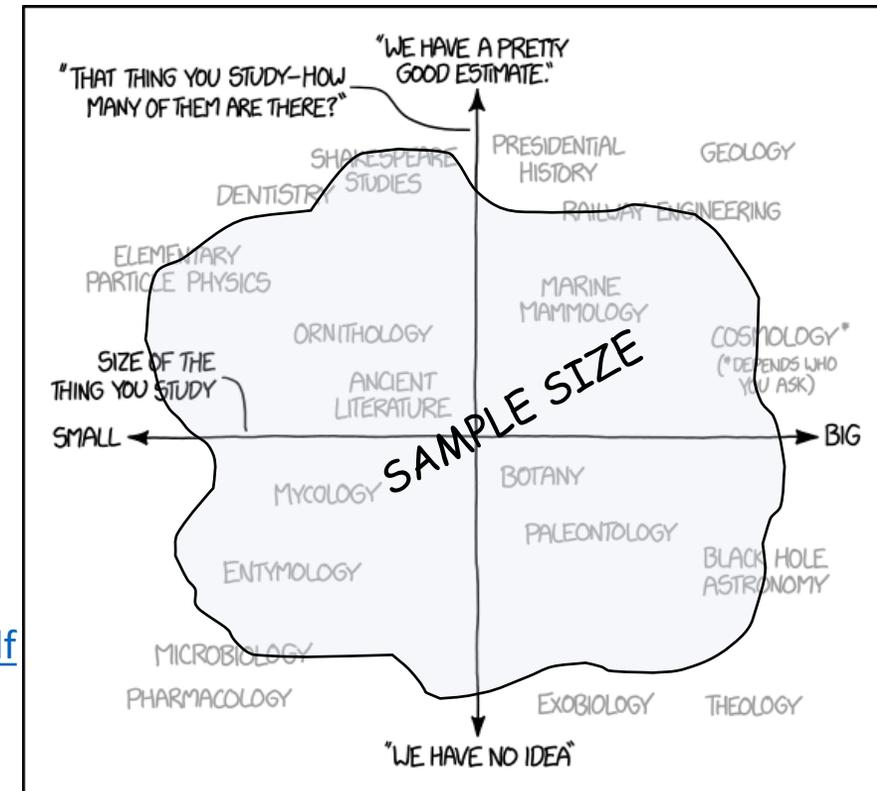
Please take the post-test and survey:

Post-test: https://und.qualtrics.com/jfe/form/SV_7NIfX6kCVqhE8oC

Survey: https://und.qualtrics.com/jfe/form/SV_eJNC2rBthtsTf2S

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<https://xkcd.com/1991/>

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