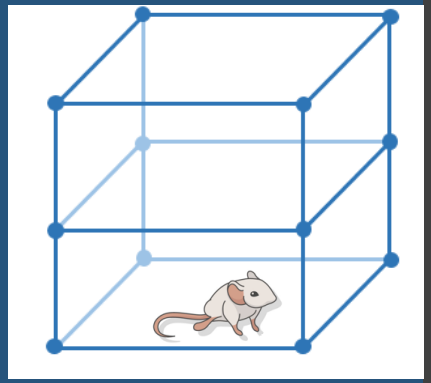
Many factors, few prior information –

some thoughts, approaches and examples to consider for sample size calculation in preclinical animal trials



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Picture from Freepik

Some thoughts and approaches about many factors…

- factors: sex, genotype, time points, treatment, cage,…
  - fixed, random effects
  - crossed, nested

- when setting up a new animal model:
  - common approach: one factor at a time not very efficient
  - large factorial designs more efficient
- when generating hypotheses:
  - small factorial designs
  - block designs
- when testing hypotheses a stricter experimental procedure should be done.
  - block designs
  - planned comparisons or other multiple comparison procedures

Preclinical animal experiment → biometric report

10.1. Versuchstyp

A	☐	Technisch erforderliche (z.B. die Verwendung von Tieren zur Gewinnung von Material)
B	☐	Hypothesen generierender Versuch **mit geringen Tierzahlen**, bei dem noch keine spezifizierten Hypothesen geprüft werden können (Pilot-Versuch, Grundlagenklärung)
C	☒	Hypothesen überprüfender Versuch → **in diesem Fall je ein Formblatt zur biometrischen Planung pro Teilversuch beifügen** (alternativ biometrisches Gutachten)
D	☐	Sonstige nicht-hypothesengenerierende Versuche (z.B. Versuchsvorhaben zu Aus-, Fort- und Weiterbildungszwecken)

in english

10.1. experiment type

A technically necessary (e.g gaining material)

B hypothesis generating experiment with fewer animals

C hypothesis testing experiment with one biometric planning form per sub-sample (alternative biometric report)

D other non-hypothesis generating experiments (e.g. training, educational purposes)

Challenges in planning and analysis of preclinical animal experiments
(sample size calculation perspective)

- small sample sizes

  → parametric vs. non-parametric analysis

- sparse prior data

  → statistical test for the actual animal experiment may not be applicable for sample size calculation

- mistaken statistical test- and result-thinking („other research groups do it the same way“)

  → inaccurate experimental design

→ limited validity of calculated sample size

> “In principle, the true sample size can only be derived from accurate prior information [...] The sample size is therefore a planning parameter whose quality depends on the accuracy of the prior information. One cannot expect more from a planning procedure than the best possible use of prior information.” (Bock, 1998)

Motivation
(full factorial design)

- Goal: investigate treatment X with >1 other fixed factors (sex, dosage,…)
- Problem:
  - importance of the other fixed factors (interaction effects) for statistical analysis often not clear to the applicant of the animal experiment
  - applicable prior data for sample size calculation
- in practice of translational animal experiments: 2 means and 1 SD from transferable treatments (factor A with 2 levels)
  → Missing but needed: information about other fixed factors within the applicable data

→ solution proposal: simulations of possible factorial designs to evaluate a valid sample size

“Standard” sample size calculation

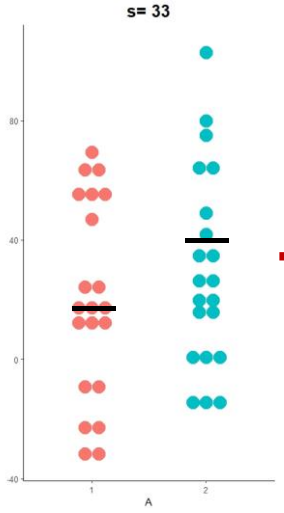
- Example: final design: 2x3 full factorial design (6 groups)
  information: $\mu_1 = 20$, $\mu_1 = 40$, $\sigma = 20$

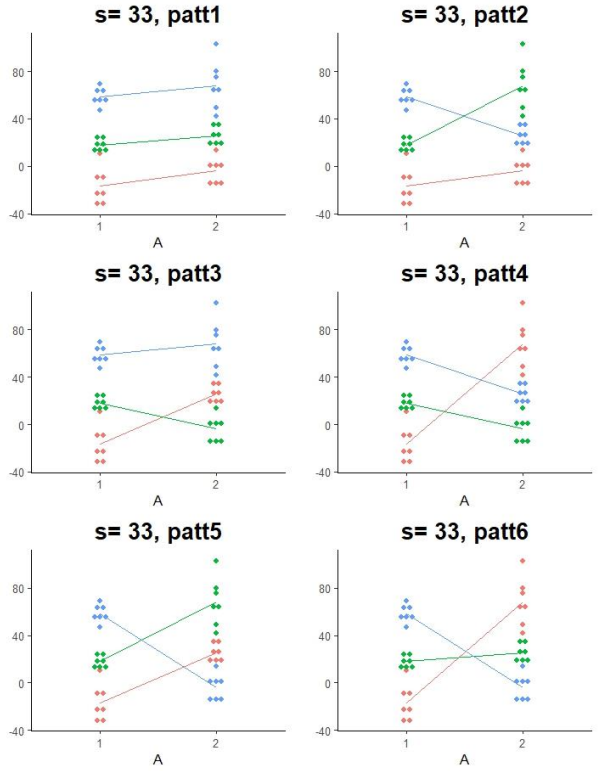
- $\alpha = 0.05$ (two-sided), $1 - \beta = 0.8$
- Resource equation method (Mead, 1990): $df_\epsilon = 10 - 20$ $[= 2 * 3(n-1)]$ → $N = 12$ $(n_{2x3} = 3)$ or $N = 18$ $(n_{2x3} = 4)$
- Rule of thumb: at least 3 animals per combination of the factor levels (Bate and Clark, 2014) → $N = 18$
- Unpaired t-test: $\mathrm{N_t} = 34$ $(n_t = 17^*)$ → $N = 36$ $(n_{2x3} = 6)$
- Wilcoxon-Mann-Whitney: $N_W = 40$ $(n_W = 20^*)$ → $N = 42$ $(n_{2x3} = 7)$

* by nQuery Advanced 9.3.1

Full factorial design – simulations

- different distribution assumptions of the simulated data (normal, lognormal, laplace, exponential)
- different scaling factors s (variation) and mean differences of the simulated data
- different number of other fixed factors (B,*C*) [besides factor A with 2 levels]
- with different number of levels (2,3,4,*5*)
- and different interaction patterns!





naïve arrangement
of additional factor levels

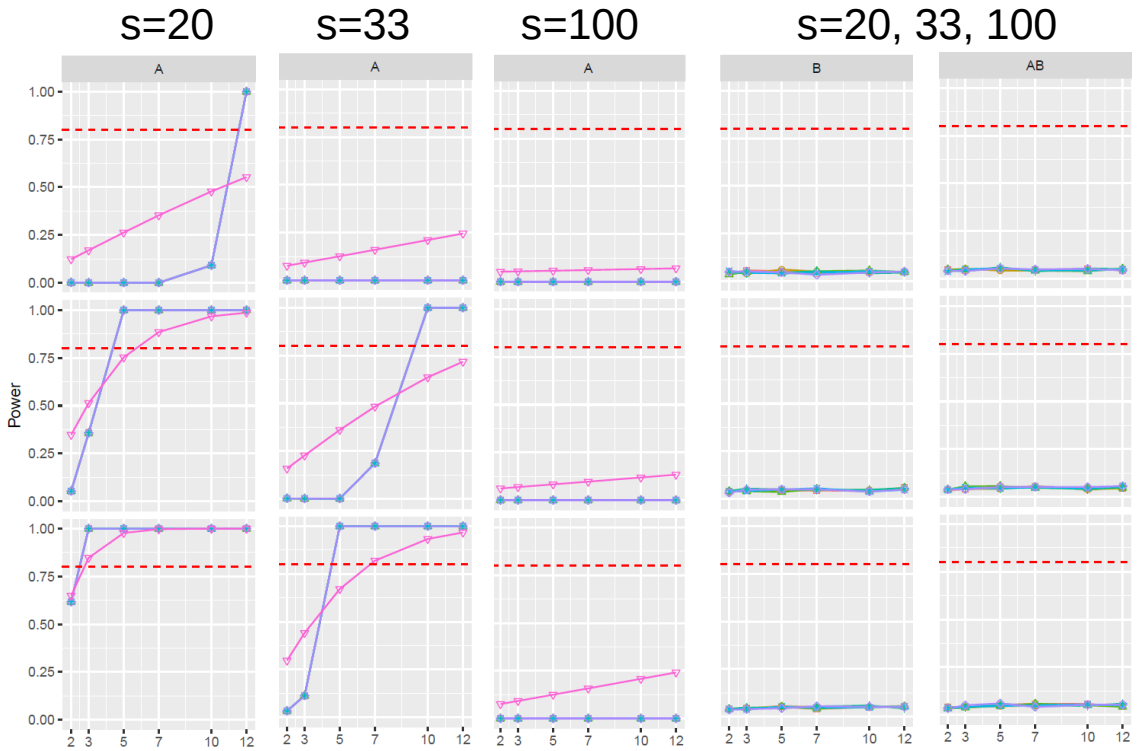
Full factorial design – results (1/4)

random
~normal

ΔMean=10

ΔMean=20

ΔMean=30



Power 80%

simulated observations per cell

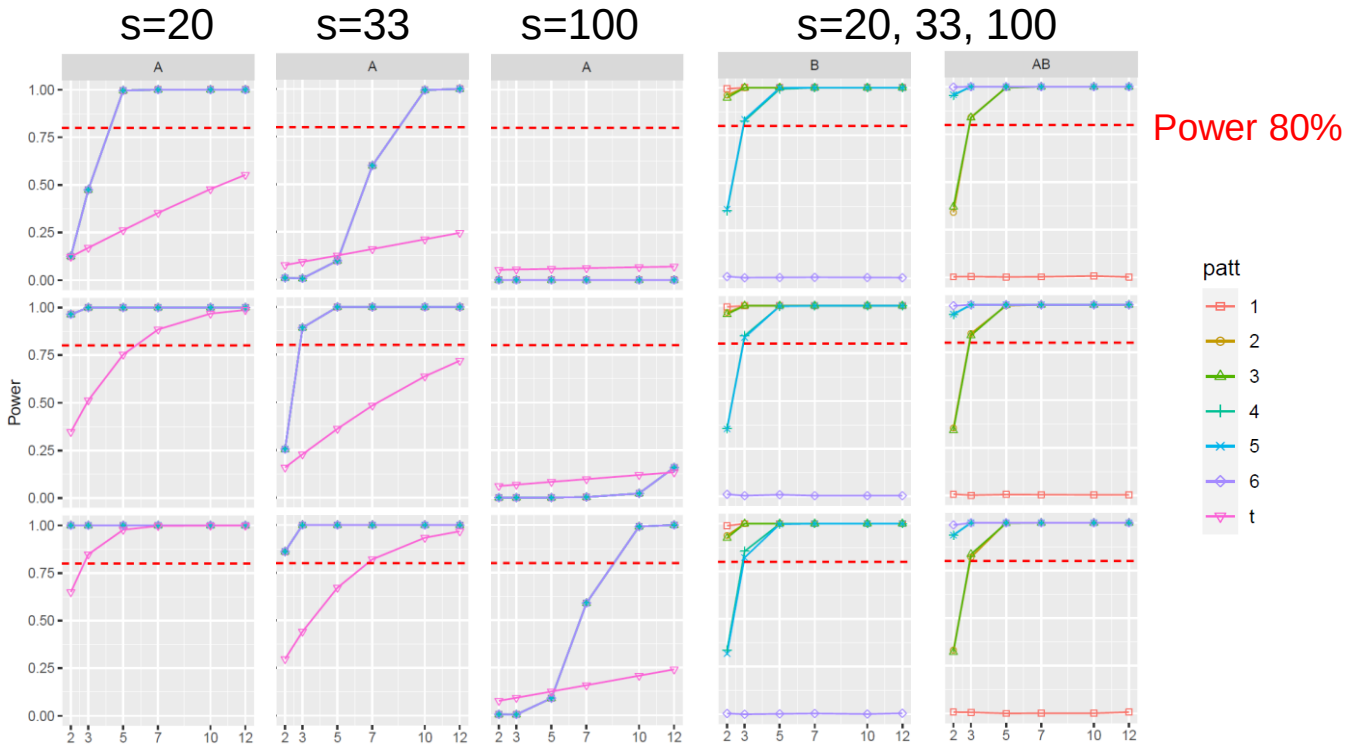
Full factorial design – results (2/4)

naïve
~normal

ΔMean=10

ΔMean=20

ΔMean=30



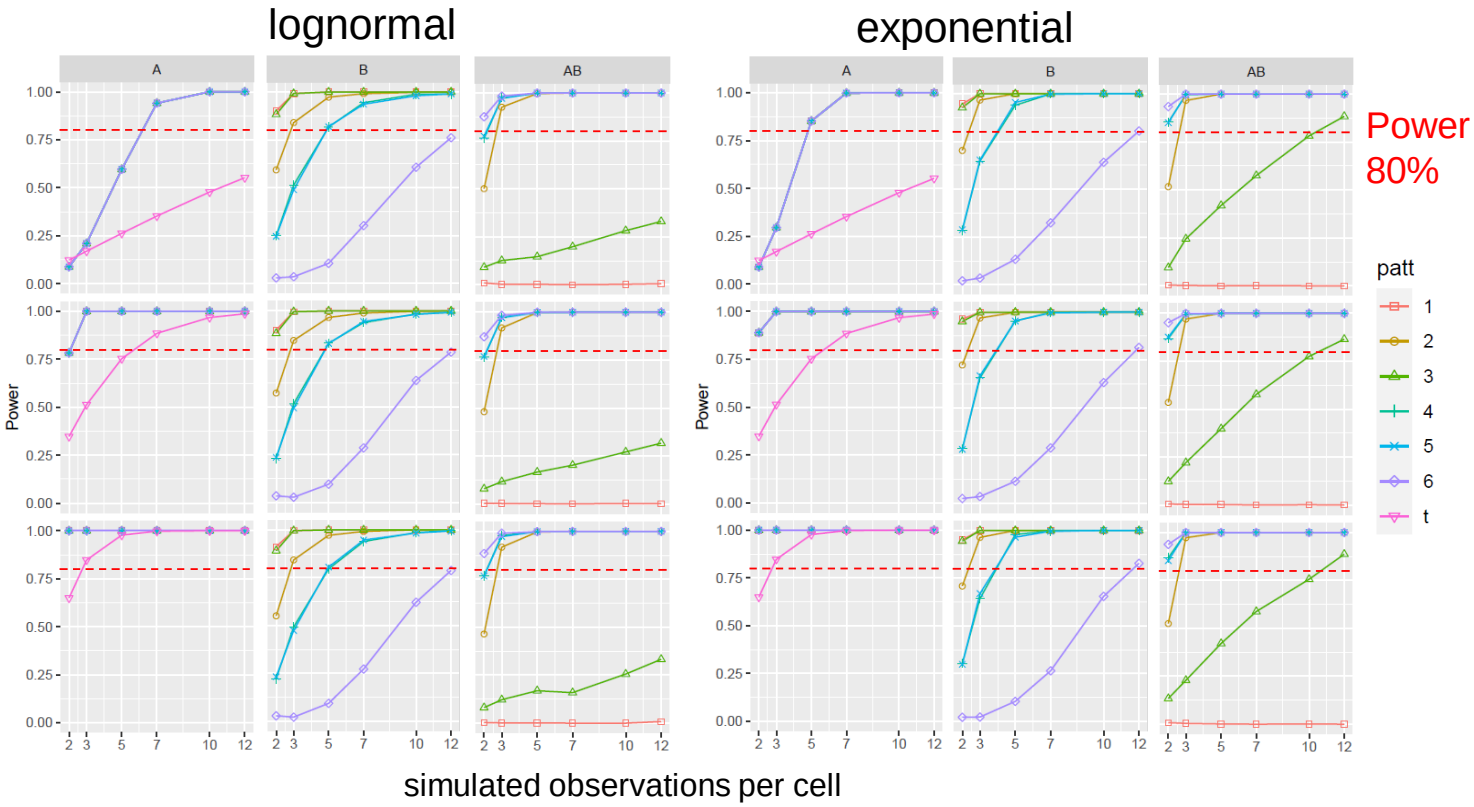
Full factorial design – results (3/4)

naïve, s=20
~non-normal

ΔMean=10

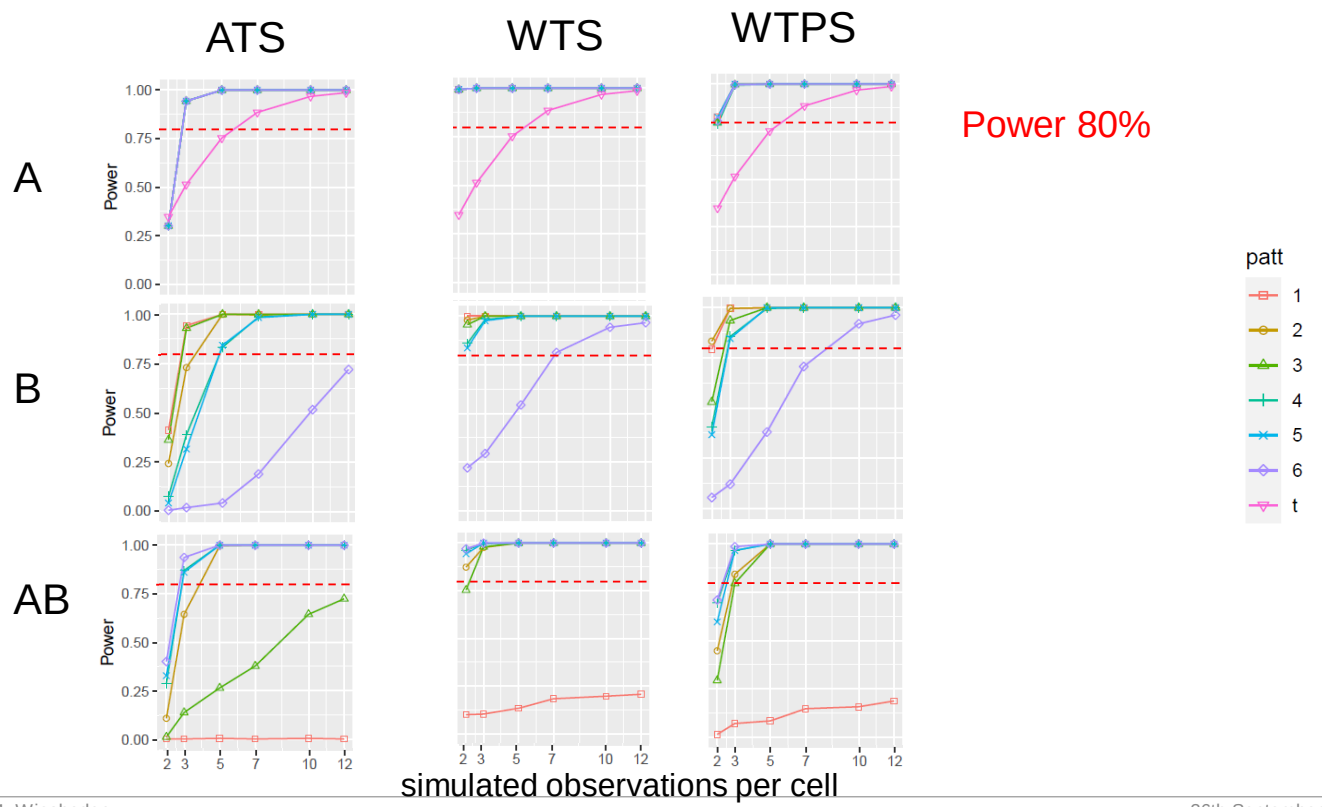
ΔMean=20

ΔMean=30



Full factorial design – results (4/4)

naïve, s=20,
ΔMean=20
~exponential



Power 80%

Summary, discussion, limitations

- simulations of possible interaction patterns within the given/assumed data can help to evaluate a meaningful sample size
  - help for applicant of the animal experiment
  - explorative vs. confirmatory analysis

- check:
  - full factorial design assumptions after naïve arrangement of additional factor levels
  - simulated data in the range of the outcome

- increasing complexity with more factors and levels

References

- Bate, S. T., and Clark, R. A. *The design and statistical analysis of animal experiments* (Cambridge university press, 2014).
- Bock, J. *Bestimmung des Stichprobenumfangs für biologische Experimente und kontrollierte klinische Studien* (Oldenbourg, Munich; Vienna, 1998)
- Cohen, J. *Statistical Power Analysis for the Behavioral Science* (Lawrence Erlbaum Associates, 1988).
- Dean, A., Voss, D. and Draguljic, D. *Design and Analysis of Experiments* 2nd ed. (Springer, New York, 2017).
- Mayer, B. and Muche, R. Die limitierte Aussagekraft formaler Fallzahlplanung im Rahmen von Tierversuchen der medizinischen Grundlagenforschung. In: *Tierärztliche Praxis Ausgabe K: Kleintiere/Heimtiere* 41.06. 367–374 (2013).
- Mead, R. *The design of experiments: statistical principles for practical applications* (Cambridge university press, 1990)
- Shaw, R., Festing, M. F. W., Peers, I. and Furlong, L.. Use of factorial designs to optimize animal experiments and reduce animal use. In: *ILAR journal* 43, 223-232 (2002).

Thanks for your attention

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