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Less is more: Dose-response in preclinical xenograft experiments

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Life forward

Agenda

01 Background xenograft experiments

02 New experimental design based on evaluation of historical data

03 Pilot trial

04 Conclusion & Outlook

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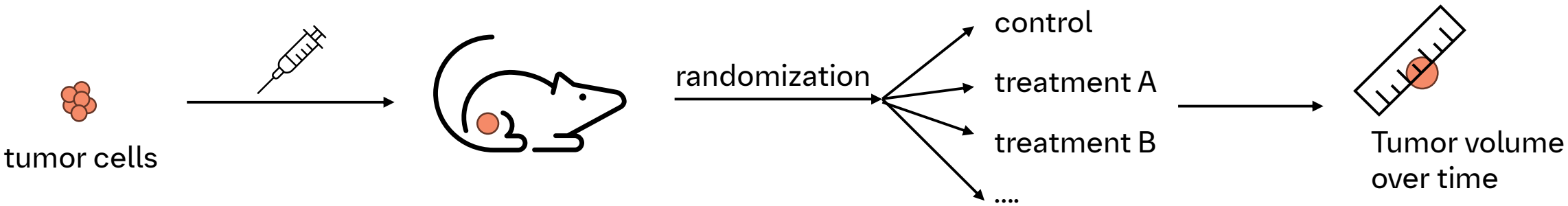
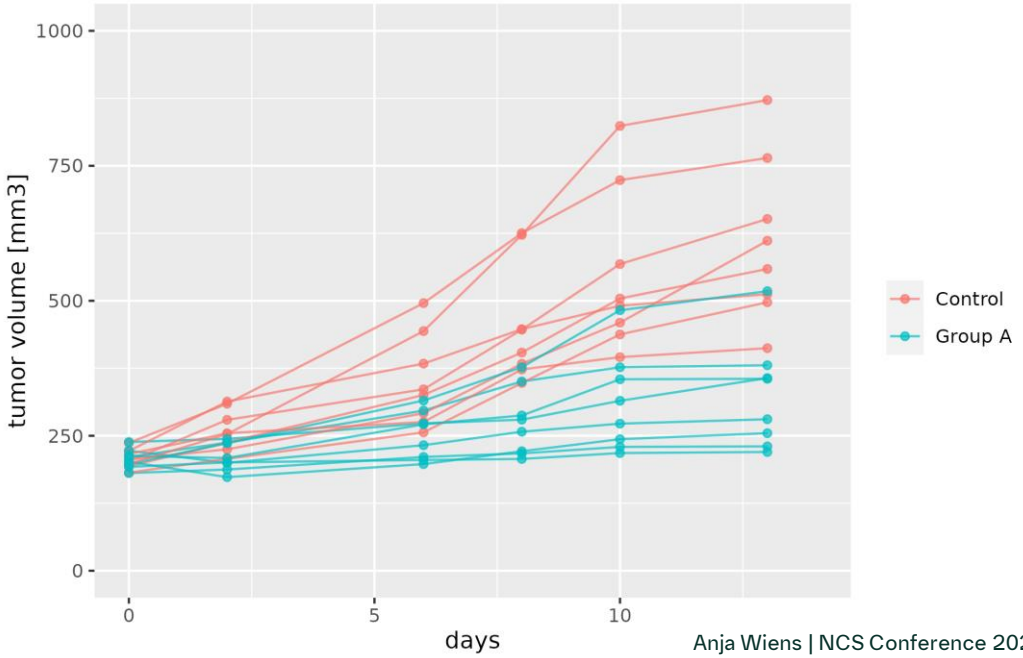
Xenograft experiments



- Tumor-bearing animals are treated and observed over a period of time (longitudinal data, parallel design)
- Primary endpoint: Tumor volume [mm^3]
- Measurements every 2-3 days up to 12 weeks
- Animals are euthanized if tumor is too large (>1500 mm^3) or other well-being issues



Historical set up: focus on proof of concept

- Projects contain many experiments with at most three active doses
- Basis for planning: Statistically significant difference of control vs. any dose level with relevant effect

	Dose [mg/kg]									
Experiment	0	2.5	3	4	5	6	8	10	30	60
A	x		x					x	x	
B	x			x		x	x			
C	x	x			x			x		
D	x							x		
E	x							x		
F	x							x		
G	x				x					
H	x									x

→Long time span until full picture of dose-response model (1-2 years)

→Large number of animals used (repetition)

→Design not optimized for modelling dose-response

→Handle variability between experiments

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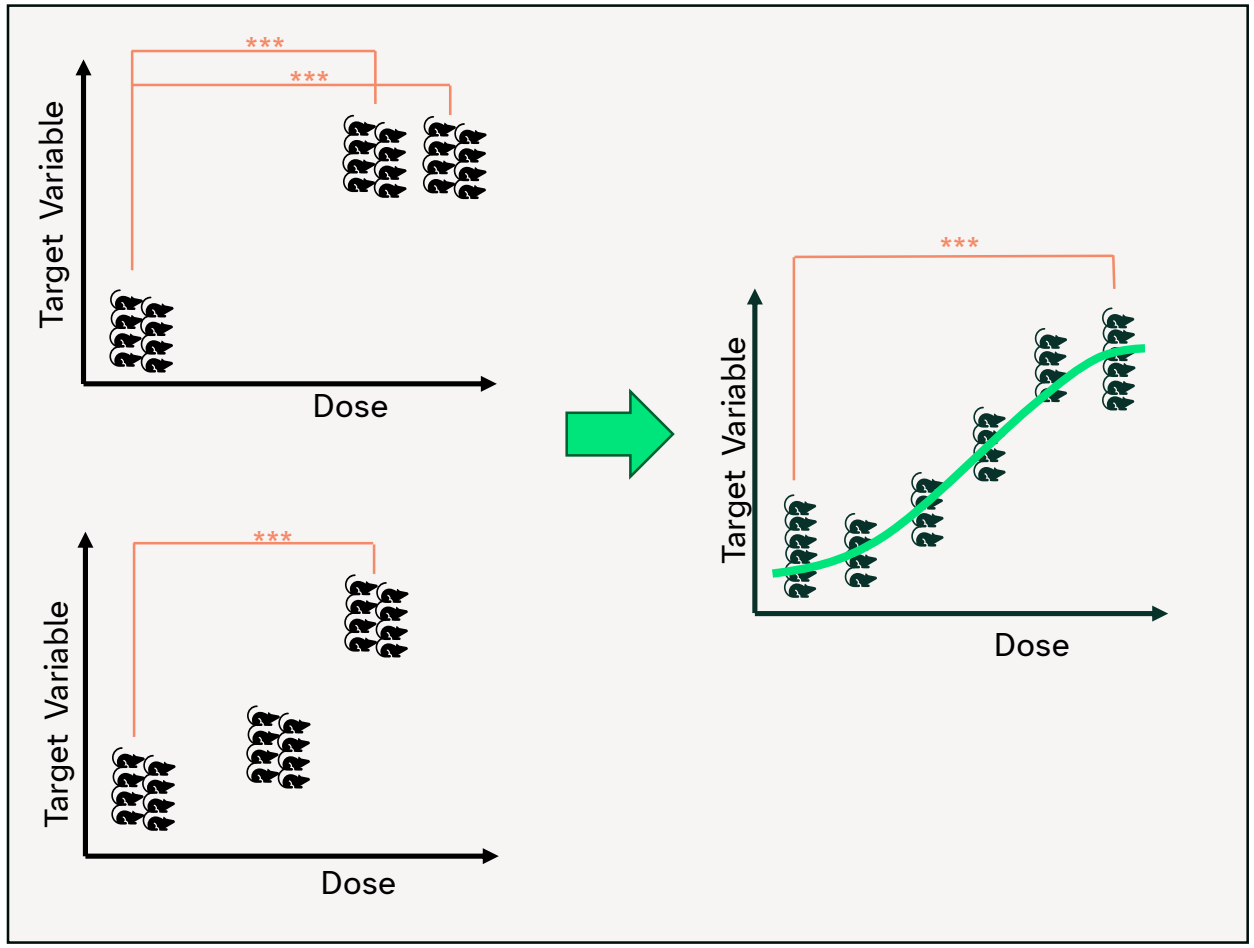
Rethinking of experimental design

- Fewer experiments in a project (combination of efficacy and dose-response)
- Less animals per group but more dose levels with adequate spacing/range

→Speed up timelines, reduce number of animals in the total project, improved modelling, better starting point for combinations

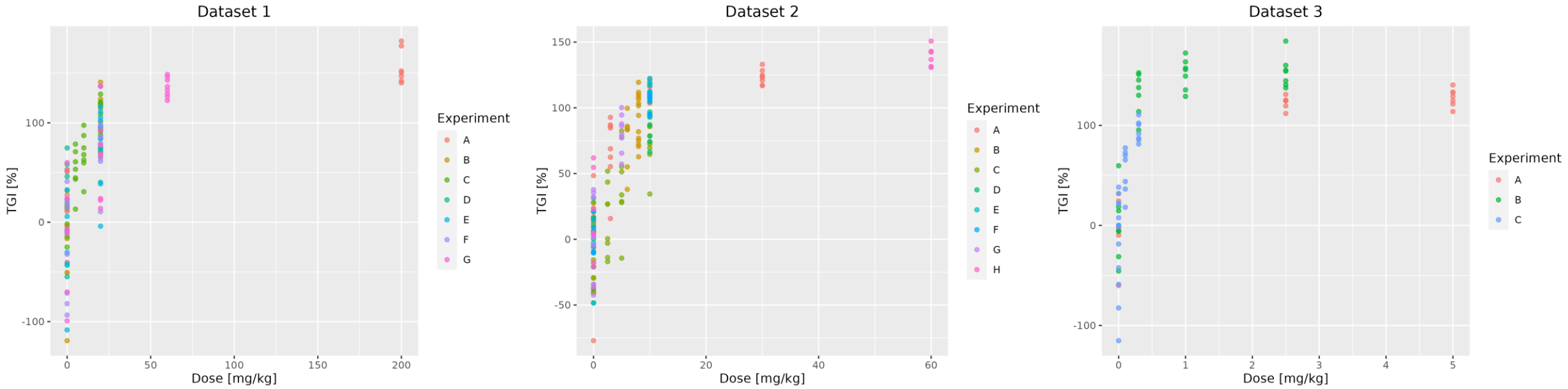
Action points:

- Retrospective power analysis of 3 projects
- Proposal & pilot trial
- Roll-out of new strategy



Pooling of the experiments within projects

- Normalized endpoint tumor growth inhibition (TGI) to pool retrospectively different experiments
  - Individual growth compared to control group
  - $\mathrm{TGI} \sim 0$: No efficacy, $0 < \mathrm{TGI}$: test better than control, $\mathrm{TGI} > 100$: tumor regression



MCP-Mod (Multiple Comparison Procedure and Modelling techniques)[1]

A strategy using one framework contains:

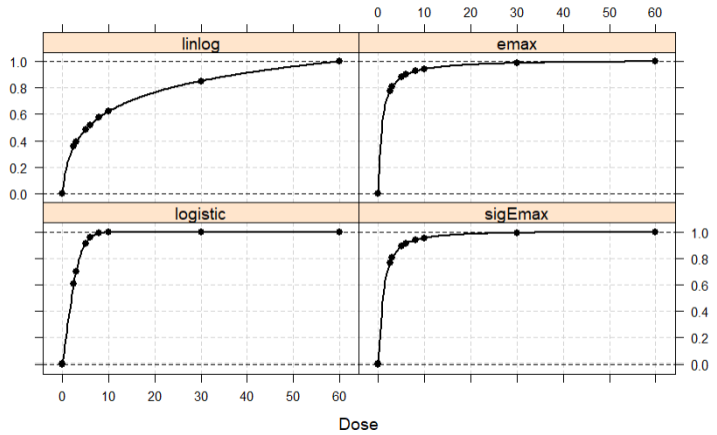
1. Multiple Comparison Procedures (MCP Step)
    - Dose as **qualitative** factor
    - Robust, but inference restricted to dose levels under investigation
2. Model-based approaches (Mod Step)
    - Dose as **quantitative** factor
    - Fitted model used to estimate an adequate dose to achieve desired response
    - Flexible, but validity will highly depend on correct choice of model

[1]Pinheiro J, Bornkamp B, and Bretz F. Design and analysis of dose-finding studies combining multiple comparisons and modeling procedures. J Biopharm Stat. 16, 639–656 (2006).

MCP-Mod (Multiple Comparison Procedure and Modelling techniques)

MCP Step

- Assessment of dose-response test using contrast tests (efficacy)
- Model selection or model averaging out of significant models




```
Multiple Contrast Test:
          t-Stat  adj-p
linlog    25.001 <0.001
logistic  24.580 <0.001
sigEmax   24.141 <0.001
emax      24.085 <0.001

****************************************
Model selection criteria (AIC):
****************************************
  linlog     emax logistic  sigEmax
1580.156 1574.060 1576.344 1573.673

Selected model: sigEmax
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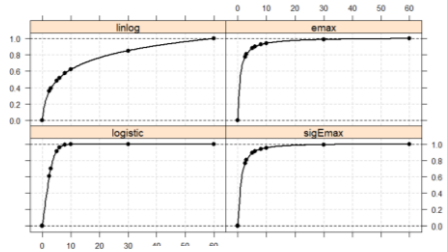


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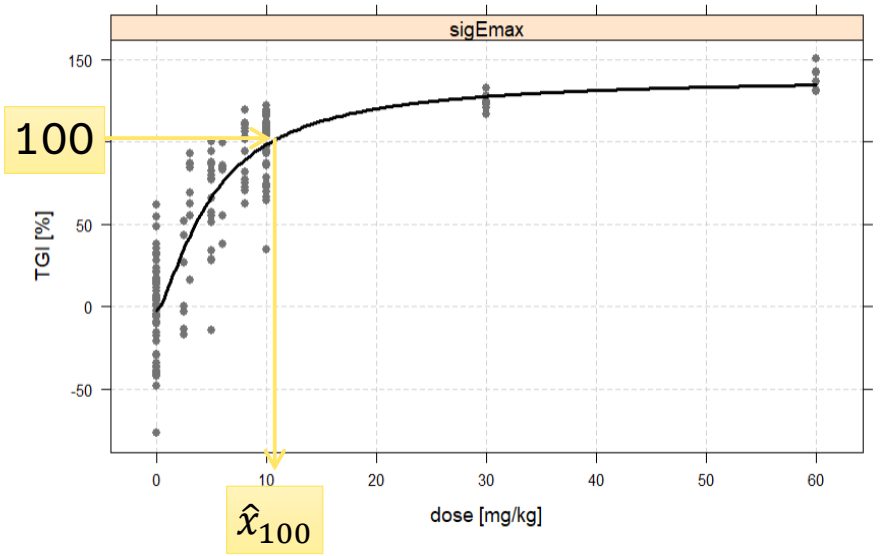
Mod Step

- Target dose estimation based on selected model or averaging



Power?

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Power analysis

Aim: Power with reduced number of animals

Analysis 1: Efficacy

- Control group vs. high-dose group or MCP Step
- Power = Probability to achieve significant treatment effect

Always ~100%, even with n=3

Analysis 2: Precision of estimated dose to achieve TGI=100% ($\hat{x}_{100}$)

- Precision of dose-response curve is represented as $\hat{x}_{100}$
- Power = Probability to estimate dose-response relationship with required accuracy, i.e., estimated $\hat{x}_{100}$ with the reduced sample should fall within a pre-defined interval.

bootstrapping

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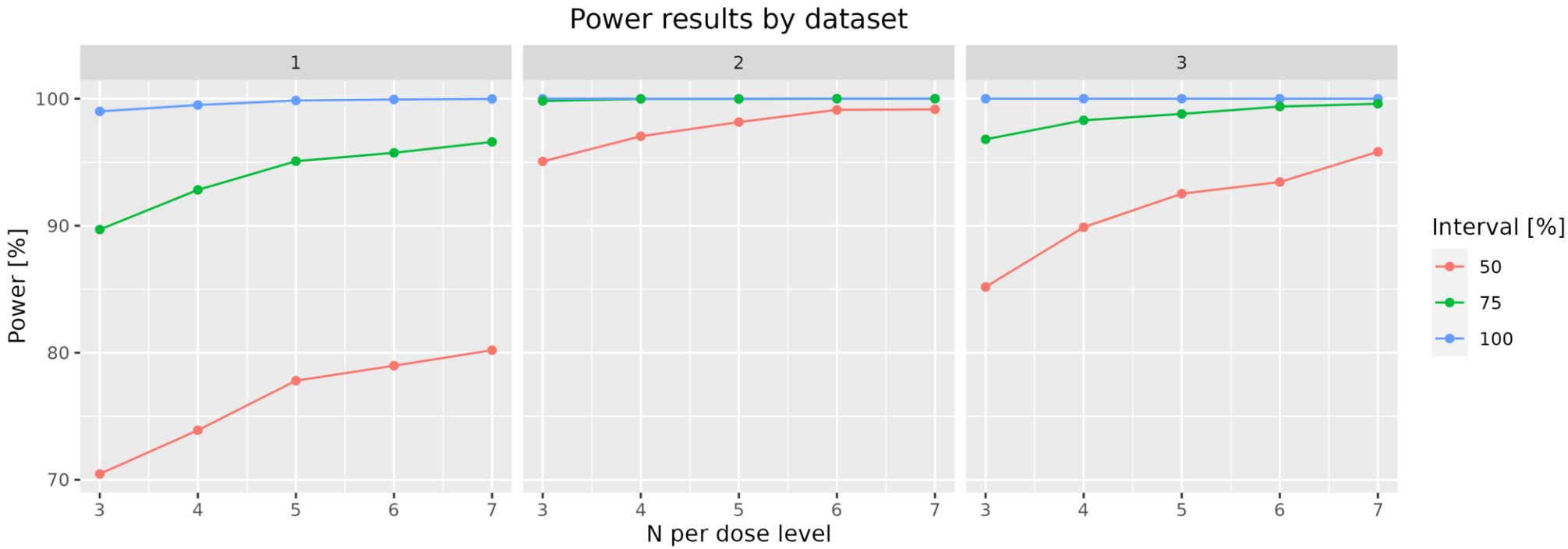
Power analysis 2

Power = Percentage of estimated $\hat{x}_{100,i}$ ($i = 1, \ldots, 5000$ bootstrap samples) within a pre-defined interval

δ% interval: $\left[\hat{x}_{100} - \frac{\delta}{2}\,\hat{x}_{100}; \hat{x}_{100} + \delta\,\hat{x}_{100}\right]$, e.g., 100% interval: $[\hat{x}_{100}/2\,;\,2\,\hat{x}_{100}]$



- Tumor model with high variability
- 6 dose levels

- 9 dose levels

- 6 dose levels

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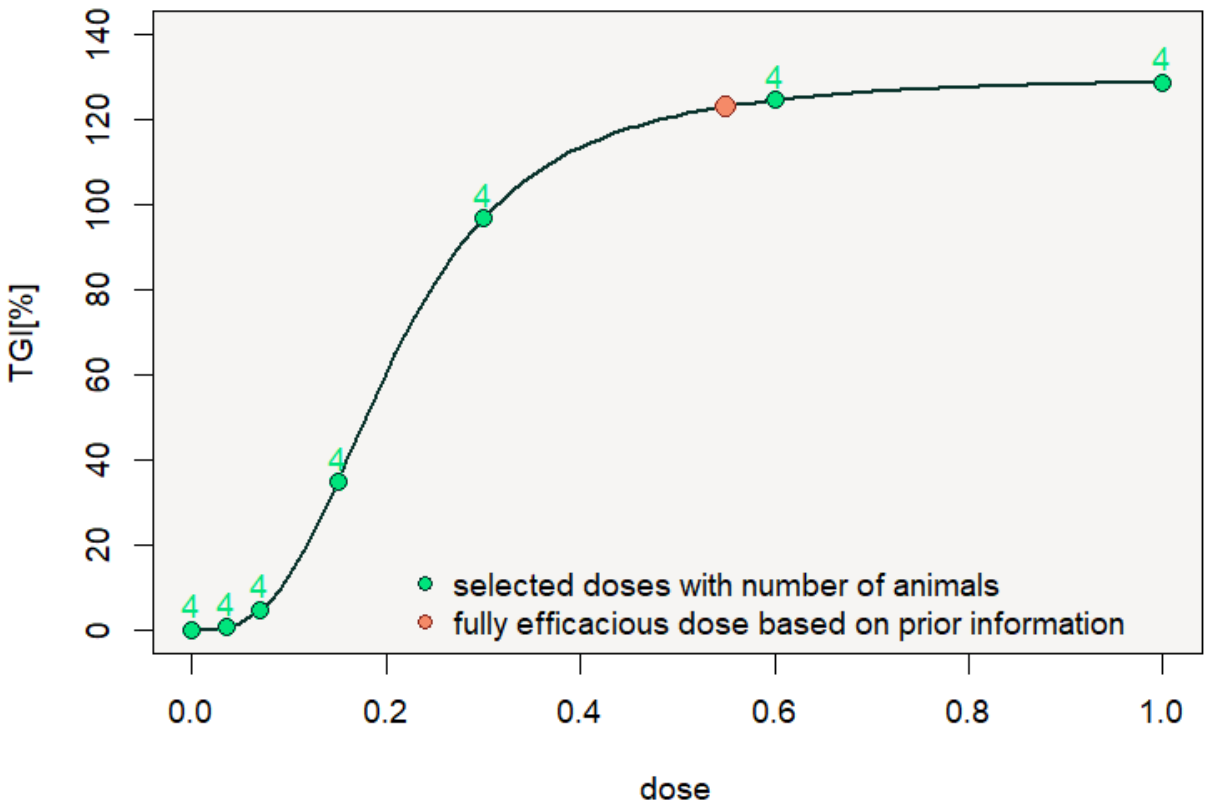
Proposal



- 4-7 active doses[2]
- Two doses on plateau (max effect verified)
- At least one dose with minor effect
- More animals for vehicle group and highest dose can be considered (e.g., 6 or 8 animals)
- At least 10-fold dose range (ratio of highest and lowest dose group ≥ 10)
- Doses approx. equally spaced on logarithmic scale if no optimality criterion can be applied

[2]Bornkamp et al. Innovative approaches for designing and analyzing adaptive dose-ranging trials,. J Biopharm Stat. 17, 965–995 (2007).

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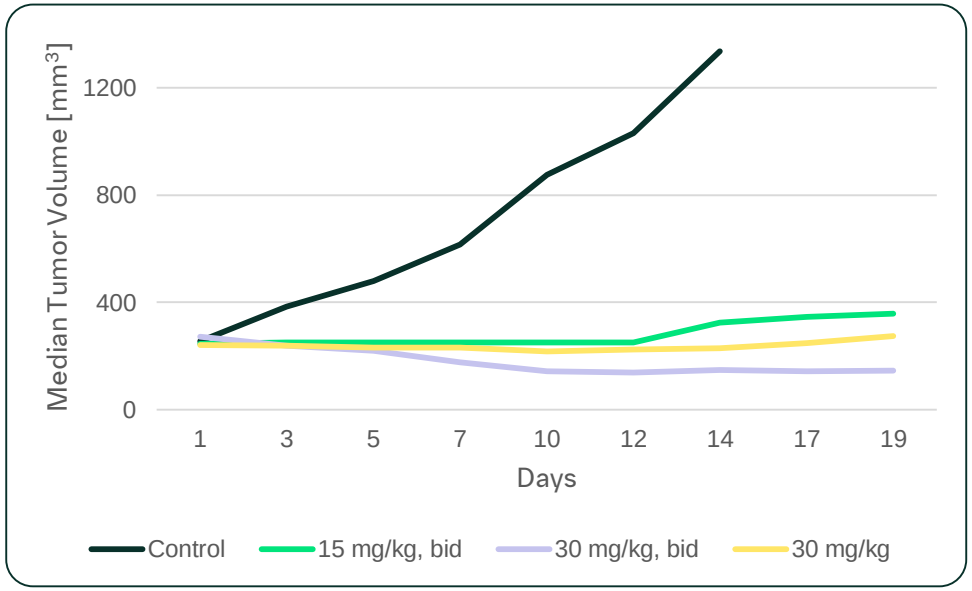
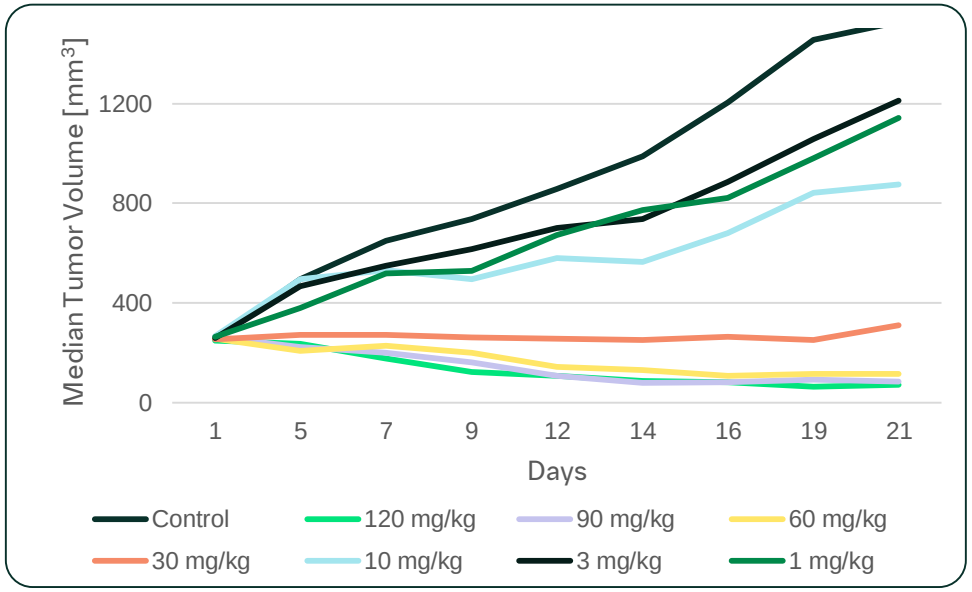
Pilot experiment: Historical set up and new design combined

- 4 groups tested with 8 animals each (32 animals)
- Conclusion: proof of concept ✓
- Open questions: Would lower dosages be efficacious as well? Which dose is appropriate for combination studies? How is human dose estimation supported?



- 8 groups tested with 4 animals each (32 animals)
- Conclusion: proof of concept ✓ AND additional information on dose-response



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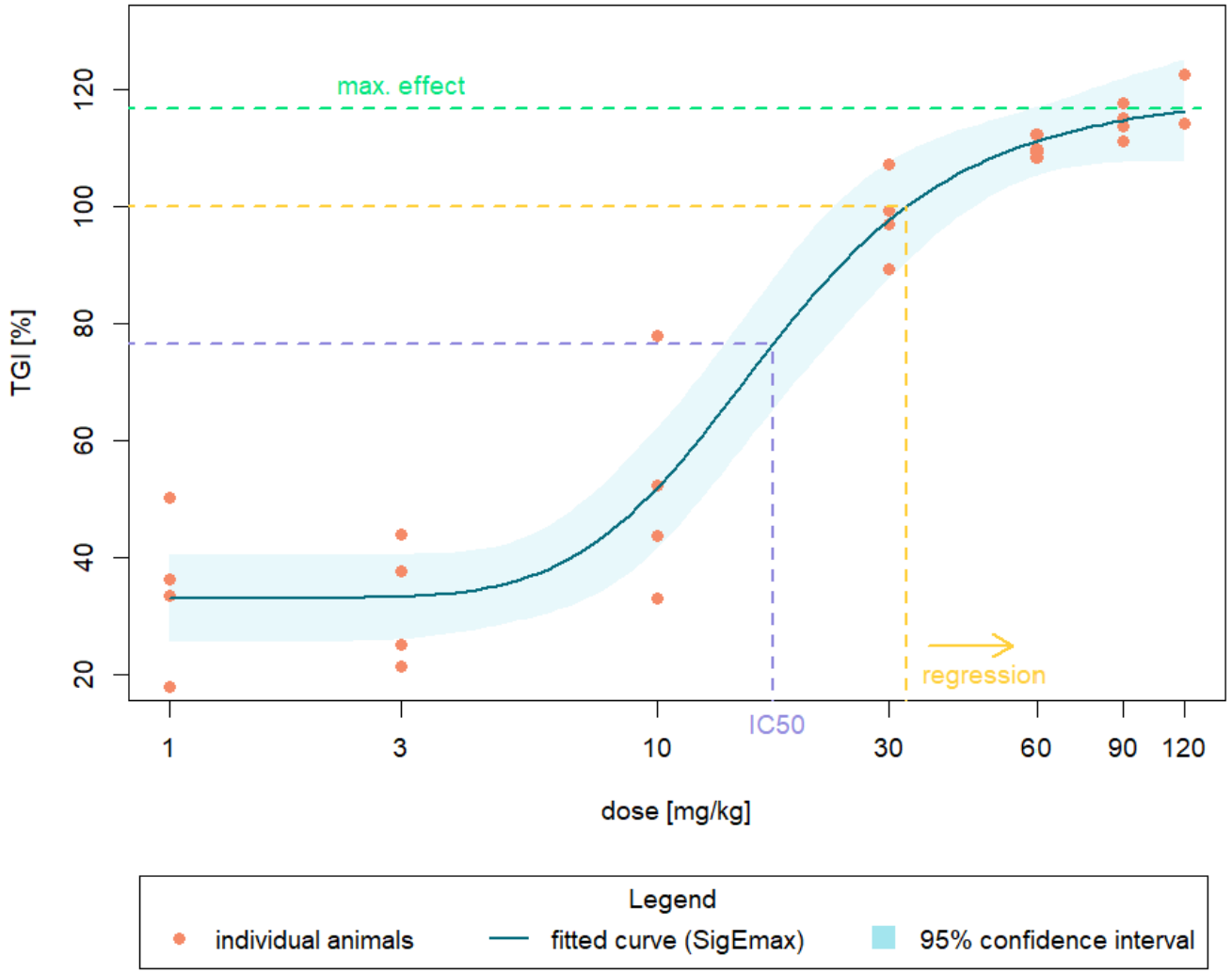
Dose response curve from pilot trial



- Two animals in highest dose dropped out due to side effects
- Key readouts:
  - Significant treatment effect
  - Maximal effect
  - Dose range for tumor regression
  - IC_{50} (concentration that gives half-maximal response)
- Pilot trial fulfilled the expectations

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Conclusion

Advantages

- Time efficient
- Improved dose-response estimation in addition to proof-of-concept
  - Human dose estimation improved
  - Knowledge on curve shape for similar compounds / pathways / tumor model
  - Improved starting point for combination studies
- Number of animals might be reduced
- If possible, additional PK/PD measurements

Extra considerations

- Handling of more groups in the laboratory
- Sample size planning and statistical analysis more complex

“Less is more” design is now the standard approach for monotherapy efficacy experiments!

Outlook

- Re-evaluation of performed experiments
- Standardization of dose-response curve fitting
- Combination therapies with two or more compounds

Thank you!

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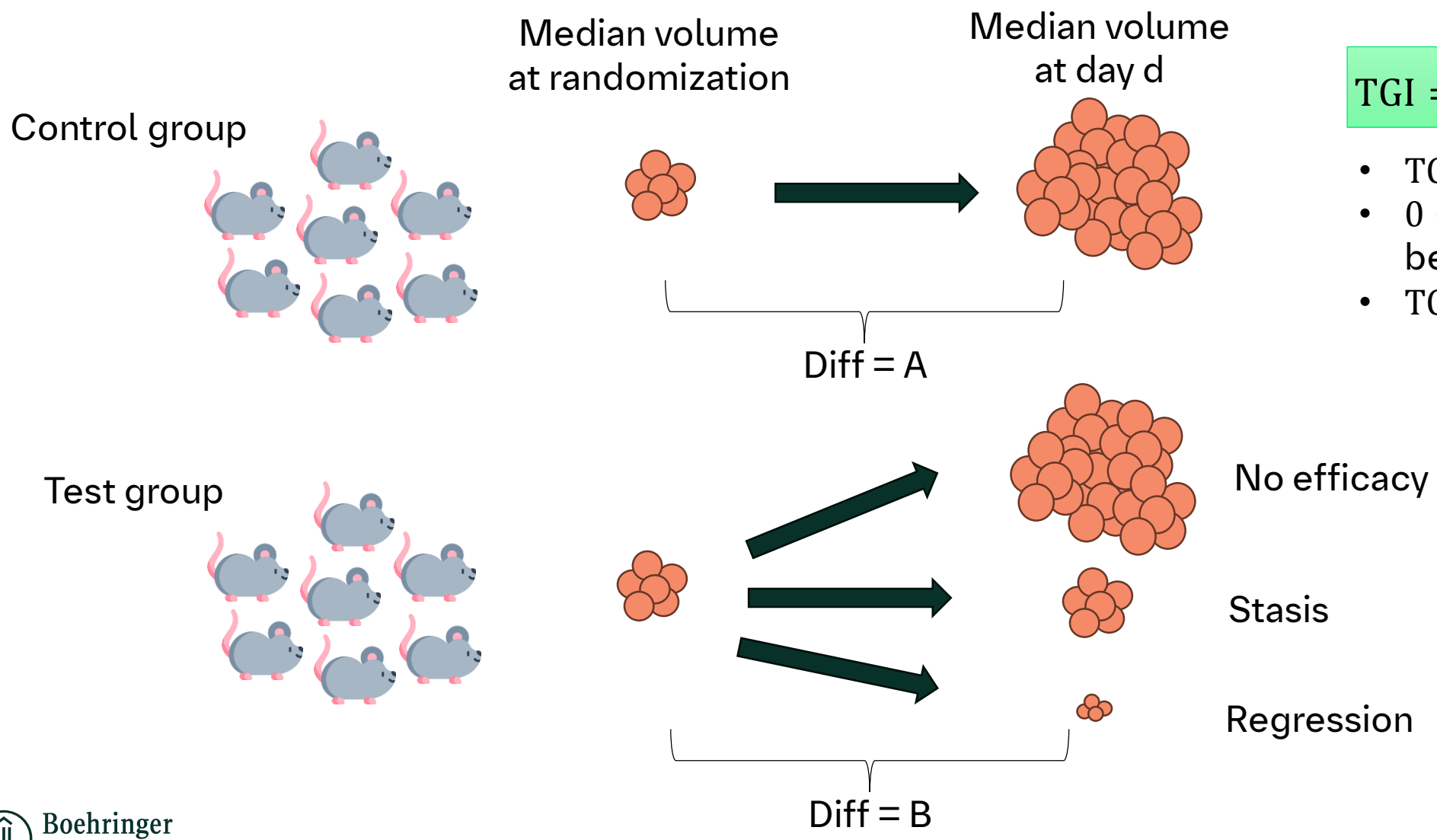
Efficacy parameter tumor growth inhibition (TGI)



$$TGI = \left(1 - \frac{B}{A}\right) \cdot 100\%$$

- $TGI \sim 0$: No efficacy
- $0 < TGI < 100$: test better than control
- $TGI > 100$: regression