Multilevel mixed models for single-animal designs

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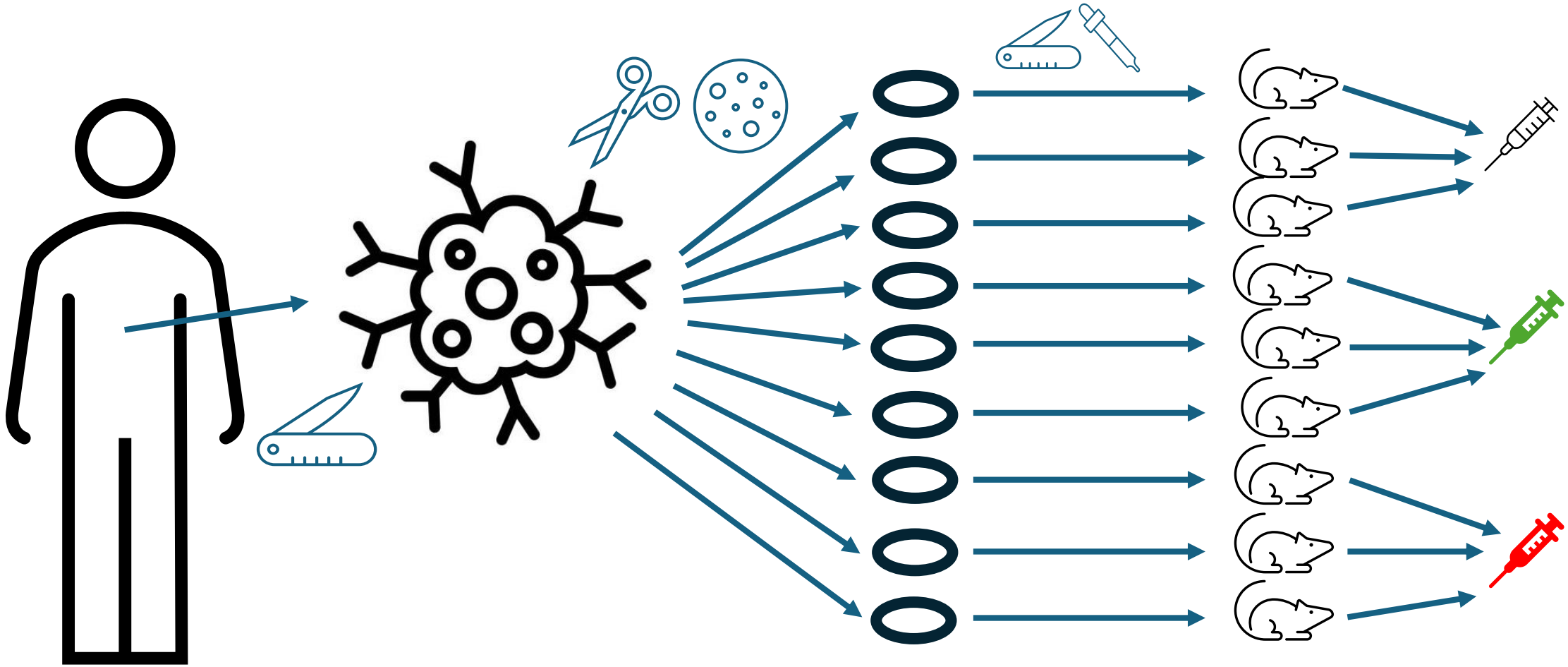
Design

Xenograft experiments in oncology

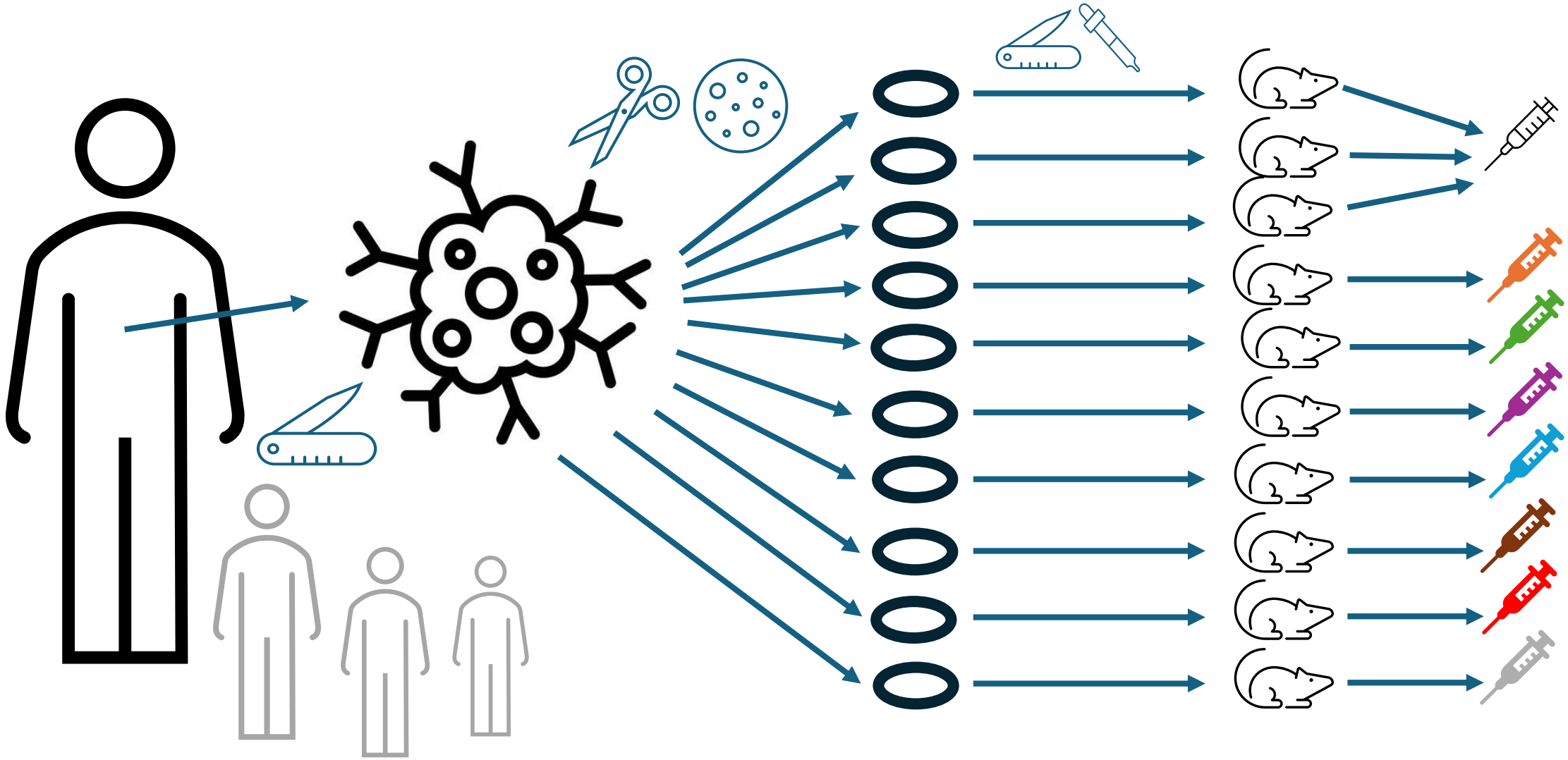


Innovative xenograft experiment's design

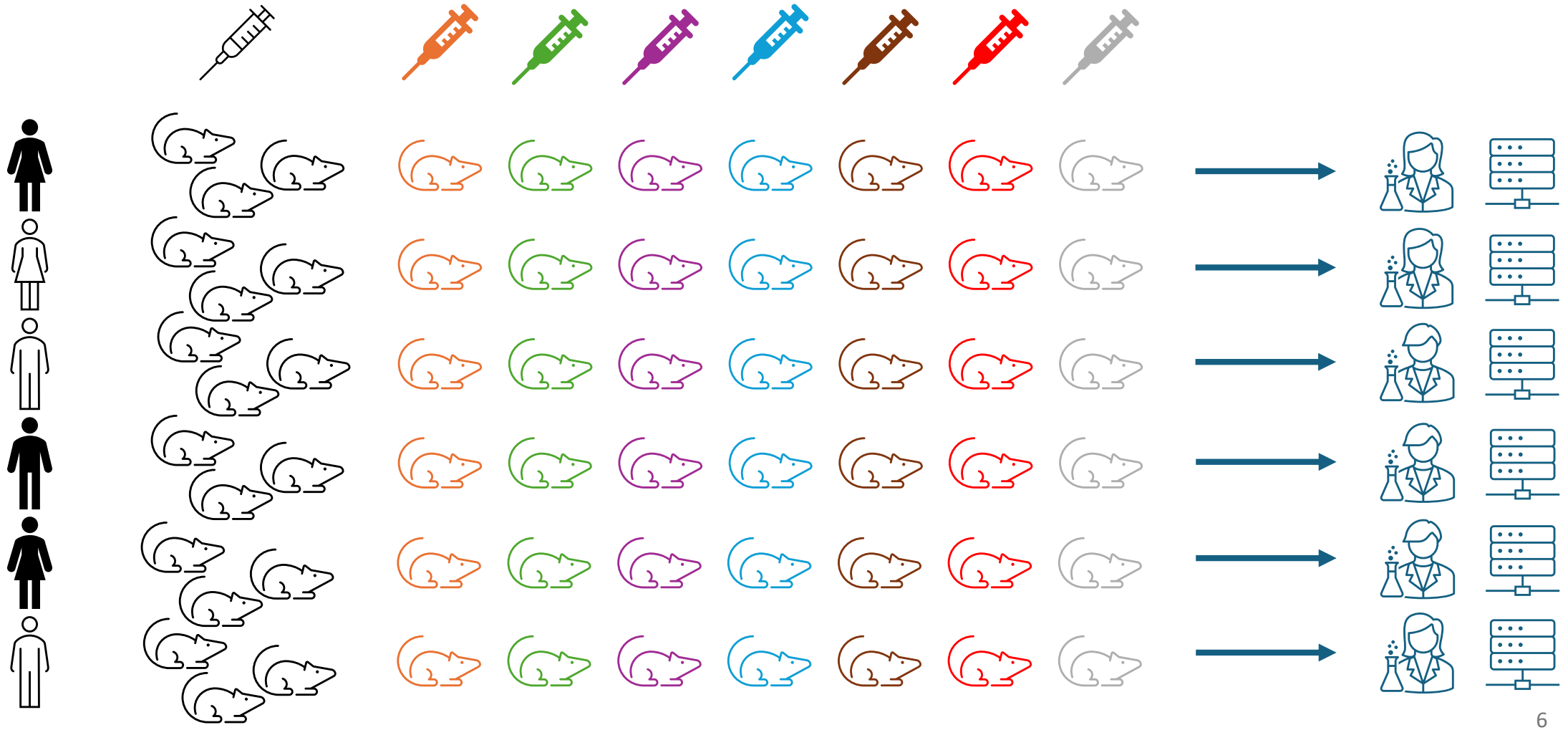
- IMI project on cancer in children (ITCC-P4) 2017-2023
- several different donors
  - biopsies of 'same type' cancers
  - more realistic representation of natural variability
- project start accompanied by animal-sparing design idea:

  N=1 (single-animal) design

Single-animal design



Single-animal design

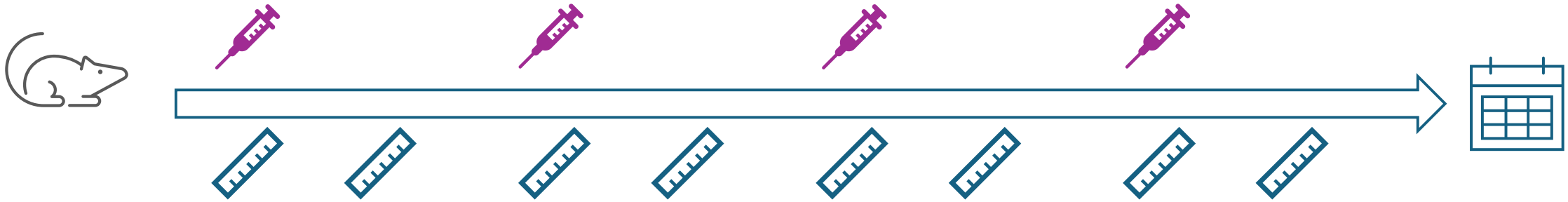


Modelling

ITCC-P4 – developing statistical strategies

- details for design (staggered start, …)
- randomization
- mixed model evaluation
  - estimate tumor growth rates
  - comparison between treatments

Measurement data



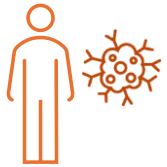
- longitudinal (e.g., twice a week over several weeks)
- subcutaneous tumor length and width
  - tumor volume derived
  - log-transformed tumor volume as primary endpoint

Multiple data levels

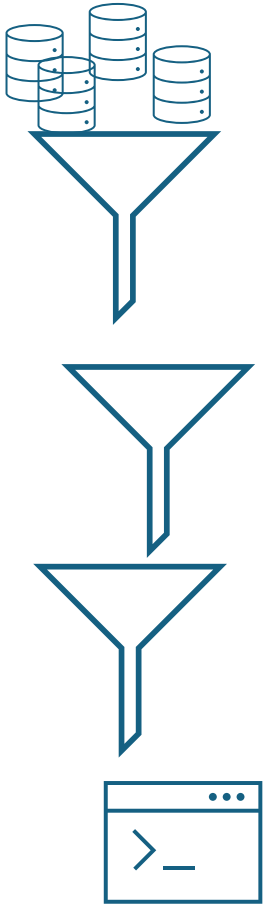
1. Measurement at time $t = 1, \ldots, T$

2. Animal $i = 1, \ldots, m_j$
   - $m_1 = 3$ for vehicle group
   - $m_j = 1$ for treatment group j

3. Treatment group $j = 1, \ldots, n$



4. Donor $k = 1, \ldots, p$



Multilevel mixed model

- assumed normal distribution of log-transformed tumor volume
- level 1: time-dependent linear model for exponential tumor growth:

$$X_{tijk} = \alpha_{ijk}^{(1)} + \beta_{ijk}^{(1)} * t + \varepsilon_{tijk}^{(1)}$$


t


i


j


k

- next levels:

  parameters from level before as “dependent variables”, e.g.:

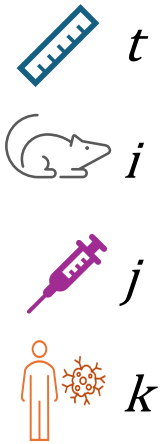
$$\beta_{ijk}^{(1)} = \beta_{jk}^{(2)} + \varepsilon_{ijk}^{(2\beta)} \quad \text{(add random slope per animal)}$$

$$\beta_{jk}^{(2)} = \beta_{k}^{(3)} + \gamma_{j}^{(3)} \quad \text{(add fixed treatment effect on slope)}$$

Resulting model specification

- decisions which fixed and random effects are reasonable
  - all grafted tumors start roughly at same volume: global intercept α
  - individual characteristics of donors and animals can affect growth:

    random slopes $\varepsilon_k^{(4\beta)} \sim N(0, \sigma^2_{(4\beta)})$ and $\varepsilon_{ijk}^{(2\beta)} \sim N(0, \sigma^2_{(2\beta)})$
  - fixed treatment effects $\gamma_j^{(3)}$ on growth (of primary interest)



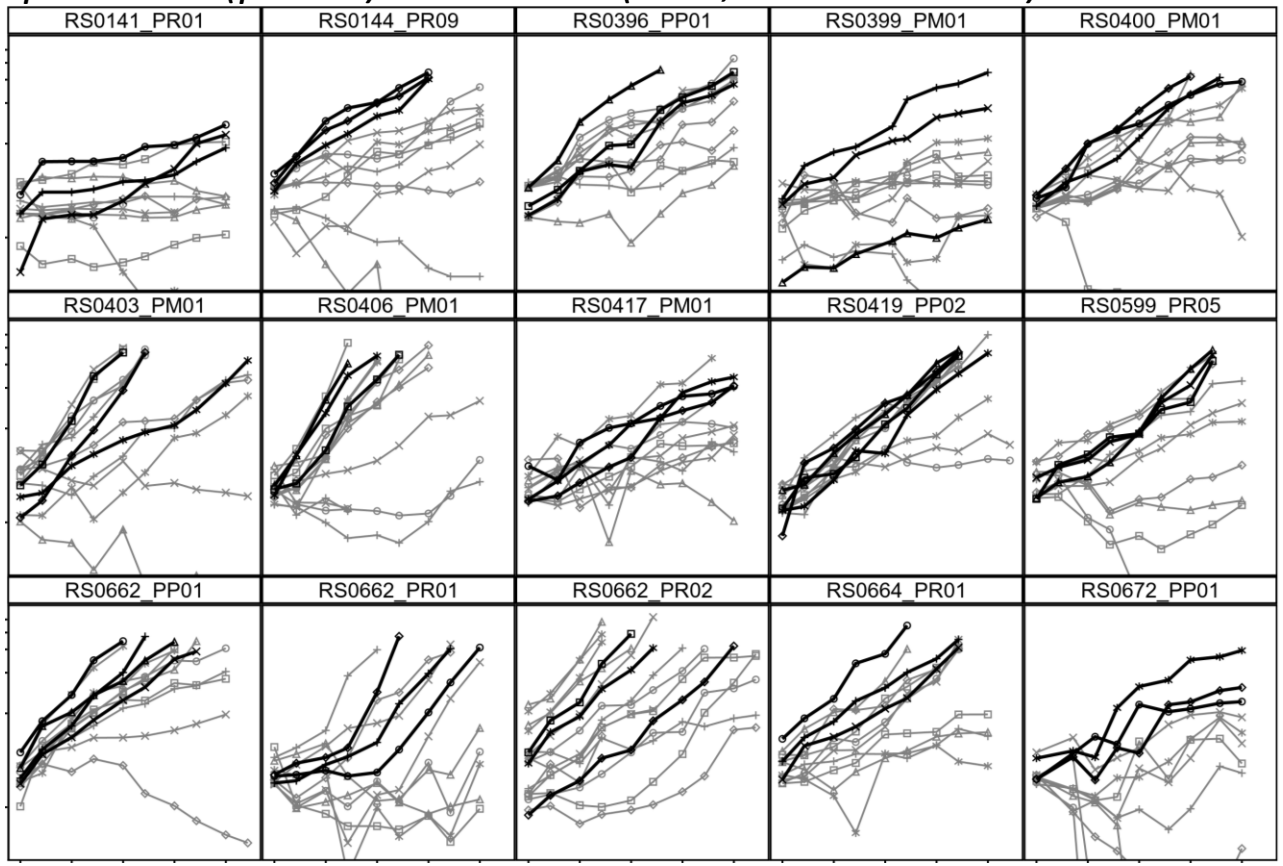
$$X_{tijk} = \alpha + \left(\gamma_j^{(3)} + \varepsilon_k^{(4\beta)} + \varepsilon_{ijk}^{(2\beta)}\right) * t + \varepsilon_{tijk}^{(1)}$$

- noise $\varepsilon_{tijk}^{(1)} \sim N(0, \sigma^2_{(1)})$ with $Cov\left(\varepsilon^{(1)}_{t_1 ijk}, \varepsilon^{(1)}_{t_2 ijk}\right) = \sigma^2_{(1)} \rho_{(1)}^{|t_1 - t_2|}$ within animal

ITCC-P4 use case

Specific modelling situation

tumor volume by time
per donor (panels) and animal (lines, vehicle in dark)



- separate analyses for several tumor types
- about 15-20 donors required per analysis

- model development
  - likelihood / goodness-of-fit criteria
  - convergence behavior

Estimation

```
PROC MIXED ... ;
  MODEL logtumorvol = time * group ;
  REPEATED / SUBJECT = animal(donor) TYPE = AR(1) ;
  RANDOM time / SUBJECT = animal(donor) TYPE = VC ;
  RANDOM time / SUBJECT = donor TYPE = VC ;
RUN;
```

- four (co-)variance parameters estimated ($\sigma^2_{(1)}$, $\rho_{(1)}$, $\sigma^2_{(2\beta)}$, $\sigma^2_{(4\beta)}$)
- 11 fixed effects estimated (intercept α, slopes $\gamma_j^{(3)}$ per treatment)

Conclusions

- feasible design requires adequate randomization and evaluation strategies
- both statisticians and animal experimentation CROs gained experience
- exponential tumor growth successfully modelled
  - growth rates significantly differ between treated and vehicle animals
- modelling approach could be extended (Cov-structure; GLMM)

Thank you!