Title: Multilevel mixed models for single-animal designs

Suggested topics: Quantitative Decision-making; Experimental design

Authors:

- Jonathan Rathjens
    - Early Development Statistics, Chrestos Concept GmbH & Co. KG, Essen, Germany
    - Short bio: Jonathan Rathjens is a research statistician working in the pharmaceutical industry in the field of preclinical statistics, with a focus on oncology and toxicology, among others.
- Hannes-Friedrich Ulbrich *(presenting)*
    - Research & Pre-Clinical Statistics, Bayer AG, Berlin, Germany
    - Short bio: Hannes-Friedrich Ulbrich is a certified biostatistician leading the Research and Pre-Clinical Statistics group at Bayer Pharmaceuticals.

Summary:

Background: The *Paediatric Preclinical Proof of Concept Platform* (*ITCC-P4*) was an EU (*IMI*) funded cancer research collaboration whose projects were completed by 2023. The statistical methods developed are intended to be applied in future studies with similar experimental designs.
In-vivo drug testing was performed in xenograft models. Tumor tissue samples from a donor were randomized over all treatment groups, such that there was one animal per donor and drug (single-animal design).

Data: Tumor volumes in mice have been measured over time. Data for several types of tumors have been provided by collaborating laboratories, obtained using grafts from about twenty donors per tumor type. The single-mouse design is completed by three vehicle mice per donor.

Methods: Donor, treatment group, animal and measurement are considered as four levels in a hierarchy. A linear model is applied for the log-transformed tumor volume depending on time, to reflect both the assumed log-normal distribution of measurements and exponential tumor growth. In a multilevel mixed model, the parameters (intercept and slope) from the level below are modeled as dependent variables on the next level, such that fixed effects and noise terms can be included regarding intercept or slope on any level where appropriate. A covariance structure depending on temporal distance is applied for the noise terms on measurement level.

Results: A mixed model comprising of a global intercept, a fixed treatment effect on the slope, and random effects of donor and animal on the slope turns out to be sufficiently feasible and provides reliable and stable results. The treatment effect is significant for most of the drugs compared to the vehicle, and especially strong for combination therapies.

Discussion: For the measurements' covariance structure, using continuous time has some advantages over a homo- or heterogeneous AR(1) structure. Another option is a treatment-wise heterogeneous variance of the animal random slope, as, e.g., vehicle animals are typically more similar in their tumor growth than treated animals, but this would render the model more complex.