Non-Clinical Statistics
Conference
2012

Non-Clinical Statistics Conference
Potsdam, Germany
September 24-26, 2012

Organizing Committee

Local Organizer and Chair of the NCSC 2012

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German Region of the International Biometric Society.

Head of Global Drug Discovery Statistics & Experimental Medicine Statistics
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Katja Remlinger (GSK, USA)
Bruno Boulanger (Arlenda, Belgium)
Emmanuel Pham (IPSEN, France)
Luc Bijnens (Janssen Pharmaceutica, Belgium)

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Welcome to the Non-Clinical Statistics Conference 2012!

Dear colleagues,

It is a pleasure to welcome you to the 3rd biannual non-clinical statistics conference. After almost exactly 6 years, we are back to where it started, in Potsdam. After the first in our series in 2006, successful events were organized in 2008 in Leuven, and 2010 in Lyon. In the meantime, our US colleagues organized US events in Boston in 2009 and 2011.

We are looking at a program with many diverse topics, all related to non-clinical statistics. As already observed in Lyon, the field is steadily increasing, and special attention is given to the translational efforts between pre-clinical research and early clinical research. The traditional ½ day tutorial on the first day is devoted to the application of Bayesian Statistics in the area of non-clinical statistics. The increase in Bayesian methodology in our field is also reflected in the scientific program.

An important part of such conference is, of course, the conversations between the sessions, and the input that the presentations give to your own work. We have organized social events in the evenings, so that you can take the opportunity to interact with your colleagues and perhaps learn about fields in biostatistics that you are not familiar with.

We hope you'll enjoy the conference!

Richard Vonk

On behalf of the organizing committee

Scientific Program

Monday, September 24, 2012		
12:00 – 13:30	**Registration**	
13:30 – 17:00	**Tutorial: Bayesian Statistics in the Non-Clinical Area**	Bruno Boulanger and Pierre Lebrun (material together with Emmanuel Lesaffre)
17:00 – 19:00	Establishment of European Working Group Statistical approaches for Anti-Drug-Antibody (ADA) identification	Ludwig Hothorn
19:00 – 21:00	**Poster Session & Welcome Reception**	
	Variability and transfer of analytical methods	Misbah Ahmed
	Mixed effect modeling for the identification of pharmacodynamic markers for cancer treatment in a multi-technology approach	Sabine Bader, C.-H. Ooi, B. Bossenmaier, T. Friess, M. Thomas
	Discussion Forum: European working groups in non-clinical statistics	**Catherine Hessler**

Tuesday, September 25, 2012		
08:45 – 09:00	**Welcome**	**Richardus Vonk**
09:00 – 10:30	**Session: Dose Response**	**Christian Ritz**
	State of the art in dose-response modelling	Jens Streibig, Christian Ritz
	Dose-response curves in industrial biotechnology	Thomas Agersten Poulsen
	Automating non-clinical statistics using the R Service Bus. The case of dose response modeling	Tobias Verbeke
10:30 – 11:00	*Break*	
11:00 – 12:30	**Session Bioassays**	**Emmanuel Pham**
	An evaluation of methods for determining confirmatory cut-points in enzyme-linked immunosorbent assays (ELISA)	Thomas Jaki
	Assessing the performance of statistical methods for estimating a common relative potency in bioassays	Thembile Mzolo, Edwin van den Heuvel, Marga Hendricks
	Comparison of Hemacytometer and HiRes cell counters used in potency assays	Priya Kulkarni, Bill Forrest
	Which method to estimate Kd and Ki in fluorescence polarization?	Dorothée Tamarelle, Armel Salangros
12:30 – 14:00	*Lunch Break*	
14:00 – 15:30	**Session Bayesian Approaches**	**Bruno Boulanger**
	Use of a 3 steps bayesian approach for the Behrens-Fisher problem in pre-clinical experiments	Karine Florin, Jean Michel Marin, Antoine Barbieri, Marouane Seffal
	Bayesian approach to risk assessment for dissolution testing of a marketed drug product	Coppenolle Hans, Altan Stan, Chen Juan, Manola Areti, Shen Yan, Shoung Jyh-Ming
	Bayesian analysis of analyte data	John Whittaker, Linda Warnock
	A Design Space to guarantee the long-term stability of a new formulation given production constraints: a Bayesian perspective.	Bruno Boulanger, Pierre Lebrun
	A Bayesian model for filling of a product to reduce risk of being OOS in presence of uncertainty.	Bruno Boulanger, Pierre Lebrun
15:30 – 16:00	*Break*	

Tuesday, September 25, 2012		
16:00 – 18:00	**Session QSTAR**	**Luc Bijnens**
	Quantitative Structure Transcriptional Activity Relationship (QSTAR)	Luc Bijnens, Willem Talloen
	Development of predictive models and feature selection using LASSO and elastic net	Pushpike Thilakarathne, Martion Otava, Nolen Joy Perualila, Tatsiana Khamiakova, Adetayo Kasim, Ziv Shkedy
	Analoging in large databases with structural fingerprint features	Martin Heusel, Andreas Mayr, Günter Klambauer, Andreas Mitterecker, Ulrich Bodenhofer, Djork-Arné Clevert, Sepp Hochreiter
	Integrated analysis of microarray data, biological data and chemical data in drug development	Nolen Joy Perualila, Pushpike Thilakarathne, Martion Otava, Tatsiana Khamiakova, Adetayo Kasim, Ziv Shkedy
	Biclustering in drug design	Djork-Arné Clevert, Günter Klambauer, Andreas Mayr, Andreas Mitterecker, Ulrich Bodenhofer, Martin Heusel, Sepp Hochreiter
	Integrated statistical analysis of three data sources for the detection of chemical fingerprint features that are simultaneously associated with bio-assay and transcriptomics data.	Alain Visscher, Federico Mattiello, Olivier Thas
18:00 – 22:00	**Social Event**	

Wednesday, September 26, 2012		
08:30 – 09:00	**Session Manufacturing**	**Bruno Boulanger**
	The use of multiple linear regression to find acceptable ranges for manufacturing materials	Mike Aylott
	Practical applications of statistical process control	Katharina Schiffl
09:00 – 10:30	**Session Toxicology**	**Ludwig Hothorn**
	Identification of the LOAEL by a model selection procedure	Ludwig Hothorn, Kuiper, RM, Gerhard, D
	Evaluation of statistical methods to construct confidence intervals for the lowest observed effect concentration	Xiaoqi Jiang
	A novel method to estimate the minimum effective dose for monotone and non-monotone dose-response relationships	Martin Wolfsegger
	Normalizing to control measurements in dose-response studies: What is the effect on parameter estimates?	Annette Kopp-Schneider, Xiaoqi Jiang, Oriana Ponta, Sven Stanzel, Alexius Freyberger, Marc Weimer
	Statistical planning and analysis of the HET-MN Assay for genotoxicity testing	Ralph Pirow, Dagmar Fieblinger, Manfred Liebsch, Albrecht Poth, Kerstin Reisinger, Thorsten Wolf, Daniel Gerhard, Ludwig Hothorn
	Stochastic models for brain aggregate cell cultures: Quantitative description of neurotoxicity	Maria Renner, Annette Kopp-Schneider
10:30 – 11:00	*Break*	
11:00 – 12:30	**Session Animal to Human Translation**	**Katja Remlinger**
	Trends in translational sciences: Role of industrial-academic collaboration	Khusru Asadullah
	Difficulties in Translation from Animal to Man - Some Examples from Neuroscience Research	David Willé
	Translation of Pre-Clinical Pharmocokinetic Parameters in the Determination of Dosing in First Time in Human Studies	Alun Bedding
	Panel / Audience Discussion	Katja Remlinger
12:30 – 13:30	*Break*	

Wednesday, September 26, 2012		
13:30 – 15:30	**Session Various**	**Richardus Vonk**
	Estimation of in-vitro dose response curves in the presence of drug resistant viral mutations	Doreen Abainenamar, T. Jacobs, H. Ceulemans, O. Lenz, G. Molenberghs, T. Verbinnen.
	Detecting pharmacodynamic drug-drug interactions – a pharmacometric success story	Tom Jacobs, C. Mackie, W. Talloen, T. Steckler, A. Megens
	Quantitative assessment of drug interactions by linear mixed effects modeling	Sven Stanzel, Helene Bayer, Annette Kopp-Schneider
	Normal ranges determination with quantile regression	Luc Esserméant
	Logistic regression re-modelled	Tina Müller, Hannes-Friedrich Ulbrich
	Quasi-species identification with model based clustering	Bie Verbist , Koen Van der Borght, Yves Wetzels , Kim Thys, Joke Reumers, Tobias Verbeke, Willem Talloen, Joris Meys, Carl Van Hove, Jeroen Aerssens, Luc Bijnens, Olivier Thas
	Biophysical rationale and quantitative benefits of using linear mixed effect models to summarize transitions of peptides to protein abundances in SRM	Jens Lamerz, Paul Cutler, Anton Belousov, G. Duchateau-Nguyen
15:30 – 15:45	**Close**	**Richardus Vonk**

Poster Session

Monday, September 24, 2012
19:00 – 21:00

Variability and transfer of analytical methods

(POSTER)

Misbah Ahmed (GSK)

Method variability assessments and method transfers are widely performed within the Pharmaceutical arena. The purpose of this poster is to outline and propose a possible strategy for the transfer of Analytical methods. With limited resourcing and money and time constraints, we advocate different approaches for low and high risk methods, mainly based on staggered measurement systems analysis (MSA) designs. The examples will illustrate the steps involved, including risk assessment, specification of an acceptance criterion and the experimental design involving a two step approach for high risk methods and a combined variability and transfer assessment for low risk methods. We will also discuss determination of the sample size in the presence of random effects, using the mixed procedure within SAS 9.2.

Mixed effect modeling for the identification of pharmacodynamic markers for cancer treatment in a multi-technology approach

S. Bader [1], C.-H. Ooi [1], B. Bossenmaier[2], T. Friess[2], M. Thomas [3]

[1] Biostatistics & Bioinformatics, [2] Oncology Discovery, [3] Translational Medicine
Pharma Research and Early Development, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg

The implementation of pharmacodynamic (PD) markers in clinical drug development is becoming a key element for guiding the optimal biological dose during Phase 1 dose escalation. PD markers are used to monitor drug target inhibition and the modulation of associated pathways, in particular with well-tolerated antibody treatments which often do not show any dose-limiting toxicity. To be able to observe PD effects during dose escalation, markers should be modulated robustly over time and assessed by a methodology which allows quantification of individual markers (e.g. mRNA expression).

The described study is based on a hypothesis free approach using three different technology platforms to identify potential PD markers for HER3 targeted treatment in pre-clinical models. The technology platforms were selected to enable the evaluation of RNA, miRNA and protein expression. Five different mouse xenograft models were treated once with 10 mg/kg of a glyco-engineered anti-HER3 antibody and tumors were explanted after 1 h, 24 h and 168 h post-treatment. In addition, tumors from vehicle-treated control animals were taken after 1 h and 168 h. Three out of five animal models are known to respond to anti-HER3 therapy whereas the other two do not respond.

The focus of this study is to identify global PD effects by statistical means of mixed effect modeling. The detected markers should show a significant and relevant modulation over time across xenograft models and not be observed in the matching vehicle controls. For this purpose, a statistical model including the factors time and response status as well as the time-response interaction is applied considering the xenograft model as a random factor. The incorporation of the time-response interaction allows us to also investigate PD markers which are dependent on the response status.

Overall we saw that, independent of the technology used, the samples of each individual animal model cluster together. Only few global PD markers could be found whereas more response-dependent PD markers were identified. Although there was no overlap of identified markers amongst the different technology platforms, the markers were found to belong to similar pathways in bioinformatics analysis. Nevertheless, it needs to be demonstrated that the molecular changes, which have been identified in the pre-clinical models, can be translated to clinics.

Dose Response

Session Chair: Christian Ritz

Tuesday, September 25, 2012
09:00 – 10:30

State of the art in dose-response modelling

Jens C. Streibig & Christian Ritz
University of Copenhagen

Dose-response curves are used to summarize experiments from biological assays with putative biologically active compounds. Modern science generates countless potentially biologically active compounds that for one reason or another need to be screened to assess their effects in animals, humans, or the environment at varying doses or exposure levels. In many areas of industry and science, the use of dose-response curves is essential in order to assess toxicity of various substances.

Consequently, proper experimental design and evaluation of usually nonlinear dose-response models have become pivotal for many areas in modern society. More than 35 years ago, Finney stated "Although a few statisticians have worked in it intensively, to the majority it appears as a topic that can be neglected, either because of it difficulties or became it is trivial." However, the difficulties or trivialities notwithstanding, biological assays illustrate the challenges encountered in applied statistics as to experimental design, use of significance test and the intimate link between appropriate statistical modelling and delicate elicitation of biological understanding from regression parameters.

In the past nonlinear regression required guesstimates of initial parameter values and in general it was difficult to apply for inexperienced experimenters and scientists. On the basis of my work in pesticide selectivity and toxicology for more than 20 years, we developed an add-on package *drc* for the open source environment **R**. This project started back in 2004. The key function is the function drm() with a built-in self starter facility that makes nonlinear regression almost as easy to carry out as linear regression. The package *drc* includes numerous dose-response models; the most important ones are the sigmoid symmetric and asymmetric curves but also non-monotonous dose-response models as well as some related models also used in biochemistry, e.g., Michaelis-Menten and the exponential decay model. The package allows the same model equations to be used regardless of the data type (e.g., continuous or binomially distributed) and there are also functions available for modelling joint action of mixtures of biologically active compounds on the basis of the commonly used ray design. The package relies on the after-fitting principle meaning that usually only a single model fit is needed per data set and, subsequently, various biologically meaningful parameters are derived on the basis of the single fit by propagating errors from model parameters to derived parameters.

The statistical problems facing experimenters and scientists include, among others, the use of transformations such as Box-Cox transformations and transform-both-sides approaches and the evaluation the adequacy of the obtained fit in general in view of statistical and biological considerations.

Dose-response curves in Industrial Biotechnology

Thomas A Poulsen
Novozymes A/S

Novozymes is the world leader in industrial enzymes with products in detergency, food, feed, bio-fuel and more. In all these applications a non-linear response to dosage of enzyme, substrate, inhibitor or other ingredients is the norm. The easy fitting of non-linear models in R makes it relatively easy to model the non-linearities directly.

The talk will give examples of non-linear dose-response in enzyme applications as

- Laundry: Remission response to enzyme dosage
- Enzyme kinetics: Activity response to inhibitor dosage (Michaelis-Menten experiment)
- Substrate depletion: activity response to change in substrate concentration (continuous dose-response)

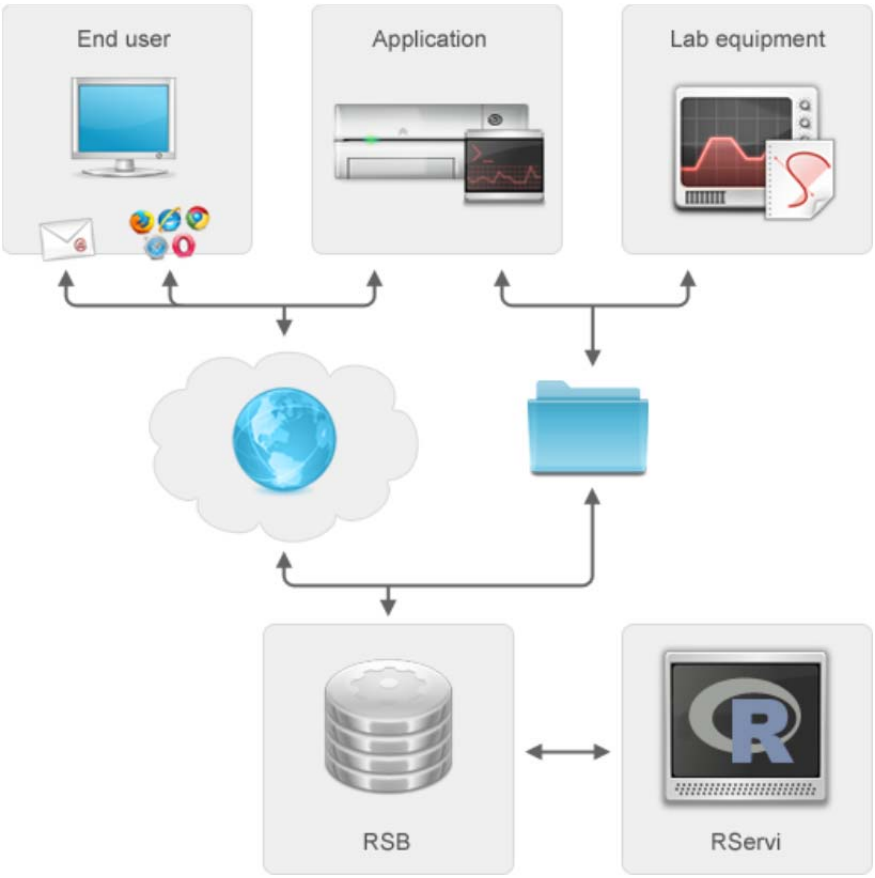
We'll also see how proper modeling can simultaneously improve and simplify on the experimental setup.

Automating Non-clinical Statistics using the R Service Bus. The Case of Dose Response Modeling.

Tobias Verbeke
OpenAnalytics

Non-clinical statisticians often develop analyses for experiments that can eventually be automated for usage in systematic screening campaigns. As R has become the lingua franca for non-clinical statistics, an open source framework has been developed for such automated deployment: the R Service Bus[1].

In this presentation we will demonstrate how a major pharmaceutical company turned to R and the R Service Bus to automate all of its company wide compound activity screening. We will highlight the strengths of R for dose-response analyses and show how the R Service Bus allowed to integrate such statistical functionality with a diverse set of legacy applications.



[1]Cf. http://www.openanalytics.eu/r-service-bus

Bioassays

Session Chair: Emmanuel Pham

Tuesday, September 25, 2012
11:00 – 12:30

An evaluation of methods for determining confirmatory cut-points in enzyme-linked immunosorbent assays (ELISA)

Thomas Jaki
University of Lancaster

Biotechnology derived therapeutics may induce an unwanted immune response resulting in the formation of anti-drug antibodies (ADA). As a result the efficacy and safety of the therapeutic protein could be impaired. For example, neutralizing antibodies may affect pharmacokinetics of the therapeutic protein or induce autoimmunity. Therefore a drug induced immune response is a major concern and needs to be assessed during drug development.

Appropriate assays need to be developed for the detection and characterization of ADAs. Those assays include screening assays for identifying a positive sample, confirmatory assays to proof the screening results and a functional assay for assessment of the neutralizing capacity of antibodies. A critical step during assay development and validation is to define an appropriate cut off that enables to distinguish between positive and negative samples.

In this talk we will discuss and compare several statistical and heuristic methods for cut-point determination in confirmatory enzyme-linked immunosorbent assays (ELISA) which are commonly used. We find that using a simple difference works well if different runs follow the same distribution while the approach based on the percent inhibition offers a more robust alternative when operator or run characteristics are different.

Assessing the performance of statistical methods for estimating a common relative potency in bioassays

Thembile Mzolo[1], Edwin van den Heuvel[1], and Marga Hendricks[2]*
[1]University Medical Center Groningen, Netherlands, [2]Schering-Plough, Netherlands
[*]email: t.v.mzolo@umcg.nl

Several methods have been proposed for estimating a common relative potency in parallel line bioassays. These include ordinary unweighted methods, average weighted methods, and maximum likelihood methods including the random effects models and in this study we focus on these approaches. Among the average weighted methods the focus will be on those methods proposed by Cochran 1954, Bliss 1952, and Morse and Bickle 1967. Furthermore, maximum likelihood methods that will be reviewed are those by Armitage et al. 1976, Williams 1978, Meisner et al. 1986 and lastly random effects model. A simulation study is performed to assess the performance of these various methods under varying assumptions and the results are compared across all methods. The assessment is based on the coverage probability and the length of the confidence interval.

Keywords: Parallel line bioassays, ordinary unweighted, average weighted, maximum likelihood methods, and coverage probability

References

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Comparison of Hemacytometer and HiRes Cell Counters Used in Potency Assays

Priya Kulkarni, Ph.D and Bill Forrest, Ph.D
Genentech, Inc.

Biological assays group traditionally used Hemacytometer for counting cells in potency assays. This method was highly labor-intensive and time-consuming. They acquired a new automatic Cedex HiRes cell counter which could potentially reduce the time and labor considerably. A series of experiments was performed using different cell lines and analysts at two target concentrations to evaluate the performance of the new HiRes cell counter. The goals were – 1) to assess the agreement between two methods and 2) to assess whether the number of replicates can be reduced from 8 to 2 or 3. Bivariate density plot and a random effects model were used to evaluate the data. The agreement between two methods was good and the number of replicates could be reduced to as low as 3. We shall discuss the data and results of this analysis in the talk.

Keywords: cell counter, potency

Which method to estimate Kd and Ki in fluorescence polarization?

Dorothée Tamarelle, Armel Salangros
Keyrus Biopharma

The use of fluorescence polarization (FP) is one alternative to traditional Binding radioligand assays. FP is used to study binding between small ligands and larger proteins using plan-polarized light to detect the change in molecular volume. To measure binding affinity in FP, classical methodology needs to be adapted. An overview of Kd estimation models in case of FP experiments will be presented. Examples of Kd estimations and fits will be compared.

Then, the situation when an inhibitor is added will be considered. The displacement assay allows to calculate the Ki in order to describe the affinity between receptor and inhibitors. In case of competitive binding assay of small molecule interaction, several publications propose alternative methods to calculate the inhibition constant (Ki). A concrete case of Ki estimation with various approaches will be presented and results compared.

Bayesian Approaches

Session Chair: Bruno Boulanger

Tuesday, September 25, 2012
14:00 – 15:30

Use of a 3 steps bayesian approach for the Behrens-Fisher problem in pre-clinical experiments

Karine Florin (*), Jean Michel Marin (**), Antoine Barbieri (***), Marouane Seffal (***)

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(**) Institute of Mathematics and Mathematical Modelling (I3M), University Montpellier 2, France
(***) Biostatistics Master Student, University Montpellier 2

Some important specificities of in vivo experiments research are the small sample sizes and the availability of historical data. Indeed, in numerous contexts, similar experiments are routinely performed for the same protocol. The Bayesian paradigm is typically well-suited for such a context: bayesian estimators are known to have good properties in case of small sizes and prior informations are naturally included. These methods improve the estimation quality and the power of the analyses, compared to the frequentist approaches currently used.

Here, we propose a specific sequential Bayesian method for the Behrens-Fisher question. That is the problem of hypothesis testing concerning the difference between the means of two normally distributed populations when the variances of the two populations are not assumed to be equal. Our method is based on a robust choice of combined objective and subjective priors. We consider that we have two historical dataset and a dataset on which the inference should be made.

In a first step, a non informative Jeffrey's priors on the parameters are used. In a second step, Normal-Gamma informative priors on the parameters are calibrated using the posterior distribution obtained in the previous step. On the two hypotheses, a uniform prior is considered with updated Normal-Gamma informative priors on the parameters. Three steps have been necessary to calculate the posterior probability of the two hypotheses as the Jeffrey's prior is improper.

Frequentist and Bayesian methods (including an approach based on Bayes Factor calibration) have been compared on real and simulated data. According the FDA recommendation (Guidance for the use of Bayesian statistics), control of the type 1 error rate and type 2 error rate have been realized.

Bayesian approach to risk assessment for dissolution testing of a marketed drug product

Coppenolle Hans, Altan Stan, Chen Juan, Manola Areti, Shen Yan, Shoung Jyh-Ming
Non-Clinical Statistics, Janssen Research & Development, Johnson & Johnson

Release of the active ingredient from the drug product and dissolution of the drug under physiological conditions is an important critical quality attribute. The rate and extent of drug release depends mainly on manufacturing process parameters and material attributes. An in vitro test method is used to determine drug release over dissolution time. The rate of release is believed to be linked to in vivo performance and useful for assessing manufacturing quality over shelf life defined by in vitro dissolution specification limits typically comprised of multiple stages.

A Bayesian simulation approach based on a linear mixed model analysis of the stability profile at different dissolution times is proposed to estimate the probability of pass/fail stage testing at time of lot manufacture and end of shelf life given calculated limits applied at time of manufacture and end of shelf life specification limits. The Bayesian approach leads to a natural calculation of both consumer and producer risk. The risks are expressed via a two-way contingency table displaying joint time of manufacture and end of shelf life outcomes. Costs associated with risks can then be easily calculated. The Bayesian methodology allows the effective use of prior information in the analysis.

The linear mixed model framework accounts for the typical restricted randomization of the analytical design for dissolution testing routinely used in practice and the effect of manufacturing parameters on dissolution. An alternative and more optimal analytical design strategy to characterize the time profile for dissolution stability studies and the ‘Quality by Design’ aspect of the study will also be discussed.

Bayesian analysis of analyte data

John Whittaker and Linda Warnock
GlaxoSmithKline, Stevenage, UK

Assays to determine the concentration of analytes such as proteins are often based on immunoassays. The analyte concentrations in test samples are read off a reference curve, often based on a non-linear logistic model which has been produced by a designed experiment using samples containing known concentrations of analyte. It is believed that reading the concentrations off the extremes of the reference curve will be unreliable and if the concentration falls below the lower limit of quantification (LLoQ) the value may be discarded or set to a threshold (eg, LLoQ or LLoQ/2). Here we argue that these values which fall under the LLoQ provide useful information, though they carry more uncertainty than values read from the central part of the reference curve. We discuss previous related work and present a Bayes method which appropriately allows for all sources of uncertainty in the model fitting, including that related to the reference curve itself. We discuss the properties of the method, with application to several real examples, and also discuss the value of modelling on the signal scale (eg fluorescence signal) but interpreting on the biologically meaningful concentration scale: the Bayes framework allows us to move naturally between the two whilst properly accounting for sources of uncertainty in the analysis. We implement the model using MCMC in WinBUGs which has some additional advantages, for instance in allowing for robustification of the analysis to deal with outliers.

A Design Space to guarantee the long-term stability of a new formulation given production constraints: a Bayesian perspective.

Pierre Lebrun, Bruno Boulanger

Arlenda S.A.

When entering in the stage of late formulation during the development of new product, it may also become important to find the formulation that also provides guarantees that the product will remain stable over the long term in expected normal stress conditions (temperature, freeze thaw, ….). To find such a stable formulation various components (X1 to X8) of the formulation have been explored using a response surface Design of experiment around the current nominal condition. The products have then been put into long term stability study under three stress conditions (S1, S2 and S3). The stability of the product was then assessed using an assay (Y). Change to initial values at t0 was used a measure of stability of the product.

In this presentation we'll present how the data have been modeled using a Bayesian linear model. The way to obtain, for this model, the predictions of new measures will also be explained using either the MCMC sampler and an analytical form of the predictive distribution under non-informative and informative priors will be presented.

Then the way to derive and obtain the Design Space under constraint in this multidimensional space will be explained. The results will be shown for various scenarios and under different assumptions.

A Bayesian model for filling of a product to reduce risk of being OOS in presence of uncertainty.

Bruno Boulanger and Pierre Lebrun

Arlenda S.A.

When it is envisaged to proceed to the filling of syringes, the bulk material must usually be diluted to achieve a desired concentration. Also the release of the final product also requires that the average content or result being greater or equal to a lower specification limit (LSL).

Given the estimate of the content of the bulk material is obtained with uncertainty, there is a risk that the dilution factor derived from a point estimate lead to under dose the final product. To avoid such an issue, the usual practice is to take a margin of safety and to slightly under-dilute the sample to ensure specifications will be met. But in this case there is a risk to over-dose the final product. In addition, the final release of the product is evaluated using the same assay, ie the release also is made with an uncertainty. Finally, the precision of the assay is itself determined with some uncertainty.

In this presentation we'll present a Bayesian model and simulation technique that take into account the three uncertainties to optimally diluted the bulk: the estimate of content of the bulk to derive the dilution factor, the estimate of the content of the final product and the estimate of the precision of the assay (repeatability and intermediate precision).

The results will be presented to minimize the risk of rejecting a lot while minimizing the risk of over dosing the final product. In addition various sample size [number of runs and number of replicates per run] will be examined to propose a practical sample size given time constraints existing in the laboratories.

QSTAR

Session Chair: Luc Bijnens

Tuesday, September 25, 2012
16:00 – 18:00

Quantitative Structure Transcriptional Activity Relationship (QSTAR) Introduction

Luc Bijnens and Willem Talloen

Janssen Pharmaceutical companies of Johnson and Johnson, Beerse, Belgium

The general aim of the QSTAR project is to tackle a major issue in the current drug discovery and development process of medicines, namely that relevant biological data can often only be acquired too late in the research process. This large time gap between selection of compounds to start medicinal chemistry on and the identification of potential side effects in toxicity studies has a highly negative impact on the productivity of the R&D process. There are two major aims in the project: 1) Selection of compounds 2) Signatures of activity for directing chemistry. These topics are relevant for the development of surrogates for cellular assays and of biomarkers. This sessions reports on the statistical methods used to combine three types of information namely bioassay data, structural chemistry data and transcriptomics data. The project is sponsored by the Flemish government (IWT) to encourage the collaboration between industry and academic institutes.

Development of Predictive Models and Feature Selection Using LASSO and Elastic Net

Pushpike Thilakarathne(1), Martion Otava(1), Nolen Joy Perualila(1), Tatsiana Khamiakova(1), Adetayo Kasim(2) and Ziv Shkedy(1)

(1)Interuniversity Institute for Biostatistics and Statistical Bioinformatics (I-BioStat), Center for Statistics, Universiteit Hasselt, Belgium.
(2)The Wolfson Research Institute, Durham University, UK

In this paper, we consider the problem of fitting linear models to data for which the number of features p greatly exceeds the number of samples n. However, only a small percentage of predictors are truly informative in predicting the response. This issue is regularly present in genomics. We focus on a genomic experiment in which selecting the biologically relevant features is one of the important goals. Direct application of statistical techniques results in ill-posed and often computationally infeasible problems due to the high-dimensionality of the feature space. We apply two well known regularization methods, Lasso and Elastic Net. The later is mixture of lasso and ridge regression. These methods can be used to construct linear predictive models in the high dimensional setting with strongly collinear features. Both methods are evaluated using the leave one out cross validations as well as three fold cross validations that is repeated for larger number of times. We show that both methods tend to select more or less the same features and can handle large problems and can also deal with sparse features. These methods are compared and validated based on their ability to select biologically relevant features and prediction accuracy.

Analoging in large databases with structural fingerprint features

Martin Heusel, Andreas Mayr, Günter Klambauer, Andreas Mitterecker, Ulrich Bodenhofer, Djork-Arné Clevert and Sepp Hochreiter

Institute of Bioinformatics, Johannes Kepler University, Linz, Austria

Analogs share a similar bioactivity with a given lead compound and are vital for drug design helping to improve the final product in terms of effectivity, toxicity, side effects, bacterial resistance and other limitations or optimizations. Structure–Activity Relationship (SAR) is the principle that structural similar molecules have similar activities. Here we propose a method which exploits gene expression data to derive a subset of structural fingerprint features indicative for a gene of interest. Using these fingerprint features a Support Vector Machine is trained and afterwards used to identify analogs in a large database. To get reasonable results two points are crucial: Select the relevant fingerprint features indicative for the bioactivity and the method has to be fast enough to scale with the data e.g. ChEMBL. Both requirements are fulfilled by the Potential Support Vector Machine (P-SVM). To avoid selecting features stemming from possible compound outliers we have defined a robust feature selection protocol based on Leave-One-Out Cross-validation and feature ranking. We briefly introduce the P-SVM focusing on the feature selection capabilities and characteristics of the P-SVM and present the robust feature selection protocol.

Based on an example from a gene expression study with 62 compounds with 3200 structural fingerprint features the results of a ChEMBL analog search are shown.

Integrated analysis of microarray data, biological data and chemical data in drug development

Nolen Joy Perualila (1), Pushpike Thilakarathne (1), Martion Otava (1), Tatsiana Khamiakova (1), +Adetayo Kasim (2), Ziv shkedy (1)

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(2) The Wolfson Research Institute, Durham University, UK

Pharmaceutical research and development processes are related to different data sources which can provide information about the properties of a new set of compounds or about its mechanism of action. Typically, the data sources are of high dimension. Within this framework, we focus our analysis on a set of compounds which aims in modeling the association between the compounds' chemical structure and its biological activities, as represented by the gene expression and bioassay data. In the current study, we present a method for data integration in which the association is modeled via latent variables which represent pathways. Each pathway consists of subset of genes, subset of biological variables and subset of compounds. Using this integrated analysis we are able to identify chemical structures that can be associated with elevated gene expression level and bioassay measurements.

Biclustering in drug design

Djork-Arné Clevert, Günter Klambauer, Andreas Mayr, Andreas Mitterecker, Ulrich Bodenhofer, Martin Heusel, Sepp Hochreiter

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Unsupervised bicluster analysis is a hot topic in Bioinformatics and has become an invaluable tool for extracting knowledge from high-dimensional -omics data. Biclustering simultaneously organizes a data matrix into subsets of rows and columns in which the entities of each row subset are similar to each other on the column subset and vice versa. This simultaneous grouping of rows (e.g. genes, bioassays, or chemical fingerprints) and columns (e.g. conditions or compounds) allows identifying new subgroups within the conditions, e.g. in drug design where researchers want to reveal how compounds affect gene expression (the effects of compounds may only be similar on a subgroup of genes) or for identifying chemical substructures that are shared by bioactive compounds. Standard clustering methods are not suited to tackle these kinds of problems. We therefore present a new biclustering approach, called FABIA, which goes far beyond the usually clustering concept. FABIA is a multiplicative latent variable model that extracts linear dependencies between column and row subsets by forcing both the hidden factors and the loading matrix to be sparse.

FABIA is a mathematical well-founded analysis technique that allows exploring high-dimensional data in an unsupervised manner and thereby shedding new light on the dark matter of many biological problems. In this talk, we will present:

a) the FABIA model for extracting biclusters and their ranking according to information content;

b) results from a high-throughput compound screening;

c) biclustering ChEMBL's bioactive small molecules (16 million chemical fingerprints times 1 million compounds

Integrated statistical analysis of three data sources for the detection of chemical fingerprint features that are simultaneously associated with bio-assay and transcriptomics data.

Alain Visscher (1), Federico Mattiello (2) and Olivier Thas (1,2,,3)

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In modern drug discovery pipelines, a multitude of data is collected, including three important data sources: (1) chemical properties of the compounds being investigated, (2) bio-assay data for targets of interest, and more recently (3) microarray gene expression data. The major objective is the identification of compound fingerprint features that are associated with bio-assay outcomes and/or gene expression.

A particular challenge is the integrated statistical analysis of these multiple data sources.

Such integrated analysis can reveal patterns or features of interest that may not be detected when analyzing the data sources two-by-two. However, no established methods exist for this type of data integration. We investigated the applicability of both an existing and a novel statistical methodology for this purpose.

With sparse Canonical Correlation Analysis (Witten & Tibshirani, 2009) we could identify sets of compound features and genes that are clustered together. When in addition we used the bio-assay data as a supervising variable, this allowed the identification within a single statistical framework of patterns of interest that were previously only detected in the combined interpretation of a series of statistical analyses of the data sets two-by-two.

We have developed a new method that allows "boosting" p-values of the association between chemistry and bioassay data, by incorporating the gene expression data obtained on the same compounds. This increases the power for detecting compound fingerprint features that are associated with the bio-assay outcome for which the pathway goes through the transcriptomics.

Both techniques are demonstrated on a real data set.

References: Witten, D. and Tibshirani, R. (2009). Extensions of Sparse Canonical Correlation Analysis with Applications to Genomic Data. Statistical Applications in Genetics and Molecular Biology, 8(1), article 28.

Manufacturing

Session Chair: Bruno Boulanger

Wednesday, September 26, 2012
08:30 – 09:00

The Use of Multiple Linear Regression to find acceptable ranges for manufacturing materials

Mike Aylott
GSK

In recent years regulators have become increasingly keen for input manufacturing materials, as well as for the end-product tests, to have acceptance criteria, and so a Linear Mixed Effect Model was applied to data from an inhaled product which involved specifying the required ranges of particle size, for both the active ingredient and the excipient (lactose). This model has the advantage of allowing for any collinearity between the various particle size summary statistics.

When correlations exist between input material and end-product quality attributes, these are often simply accounted for by a line of best-fit. However, with this proposed approach a prediction interval is calculated providing a multi-dimensional acceptance region, thus maximising the chances of the product being of acceptable quality to the patient, and ensuring manufacturing quality is maintained.

Practical Applications of Statistical Process Control

Katharina Schiffl

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Statistical Process Control has become a very important tool to ensure robust manufacturing processes and high quality products. Especially due to the rollout of Lean Six Sigma Strategy Statistical Process Control became more and more present in the manufacturing of drugs. In applications, operators are often interested in the correct use of common control charts and in non-standard solutions for specific questions. We introduce the state of the art statistical process control tools used in Roche manufacturing and give recommendations for their use.

Toxicology

Session Chair: Ludwig Hothorn

Wednesday, September 26, 2012
09:00 – 10:30

Identification of the LOAEL by a model selection procedure

Hothorn, LA[1], Kuiper, RM[2], Gerhard, D[1]

[1]Leibniz University Hannover, [2]Utrecht University

The LOEAL (lowest observed adverse event level) concept in toxicological risk assessment was seriously criticized, particularly its dependence on sample size. But the alternative benchmark concept for normal distributed endpoints lacks on a clear threshold definition.

Already Yanagawa and Kikuchi (2001) proposed an AIC criterion-based model selection approach for LOEAL identification. However, they did not take into account the monotone dose-response relationship, typically in toxicological assays. Recently, Kuiper et al. (2011) proposed a model selection using a generalized order restricted information criterion. Here, this approach is used specifically for LOEAL identification for normal distributed endpoints, common in repeated toxicity studies.

Simulation results reveal the advantages over the Dunnett/Williams-procedure for LOAEL identification, particularly for small sample sizes- common in regulatory toxicological assays An example with liver weight data of a chronic study on dogs is used to demonstrate this new approach using the R package goric.

References.

Yanagawa, T ; Kikuchi, Y: Statistical issues on the determination of the no-observed-adverse-effect levels in toxicology. ENVIRONMETRICS 12 (2001), 319-325.

Kuiper, R. M. ; Hoijtink, H. ; Silvapulle, M. J.: An Akaike-type Information Criterion for Model Selection under Inequality Constraints. In: Biometrika 98 (2) (2011), 495-501.

Evaluation of statistical methods to construct confidence intervals for the lowest observed effect concentration

Xiaoqi Jiang

Division Biostatistics, German Cancer Research Center (DKFZ)

The lowest observed effect concentration (LOEC) is usually determined by hypothesis testing. Such LOEC is the lowest concentration in the study at which the observed mean response is statistically different from the mean response observed for the control group, regardless of whether such effect is biologically relevant or not. This hypothesis approach to determine the LOEC has been severely criticized over the past decade for the main reason that the LOEC value depends on the choice of statistical test and level of significance, variability in the data, sample size and tested concentrations. Therefore, regression-based inverse modeling techniques were proposed to estimate either low toxic effective concentrations such as the quantiles EC10 or the EC20 or the concentration corresponding to a pre-specified low effect. In many practical situations the log-logistic model is applied to fit the observed concentration-response data. In case of increasing concentration-response curves the estimated EC10 or EC20 values tend to be biased towards extremely large values and may also be imprecise due to problems with estimating the upper asymptote of the curve, while the model-based LOEC estimate is less biased and more precise. Confidence intervals for the model-based LOEC can be constructed by application of delta method (Bishop & Fienberg, 2007), the profile likelihood method (Bates & Watts, 1988) or bootstrap resampling techniques (Efron & Tibshirani, 1993). We will present how these methods and their extended approaches can be utilized to calculate confidence intervals for the LOEC based on the log-logistic model. The performance of the various methods (in terms of coverage probability and confidence interval length) is evaluated by a simulation study. Some recommendations are given regarding which of the confidence interval construction methods should be preferred depending on the quality of the experimental data.

References

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Efron, B., & Tibshirani, R. (1993). *An introduction to the bootstrap.* Chapman & Hall/CRC.

A novel method to estimate the minimum effective dose for monotone and non-monotone dose-response relationships

Wolfsegger MJ,

Baxter Innovations GmbH, Vienna, Austria

In many clinical and non-clinical dose-finding trials estimation of the minimum effective dose (MED) is one of the main objectives. These evaluations can be addressed either by using a multiple comparison procedure or by using a modeling approach. Clinical dose-finding trials usually employ a rather narrow dose range that often allows specific assumptions on the dose response shape, e.g. monotonicity. In contrast, pre-clinical efficacy studies in early stages of drug development commonly use wide dose ranges where specific assumptions about the dose response shape are difficult to elicit and are hard to justify.

On assumption of a monotone dose-response shape, the step-down application of two-sample t-tests is a natural choice for evaluation of a dose-response data allowing estimation of an effective dose range of consecutive ordered dose levels starting from the highest dose investigated. If a monotone dose-response shape cannot be assumed a-priori, Dunnett's procedure which does not require that the highest dose investigated is superior to the zero dose can be utilized.

Here we present a new method for estimation of the MED as a combination of Dunnett's procedure and the step-down application of two-sample t-tests. We show that this approach combines the advantages of these two methods in a simulation study and show that the type-I error rate of underestimating the true minimum effective dose is controlled.

Normalizing to control measurements in dose-response studies: What is the effect on parameter estimates?

Annette Kopp-Schneider(1), Xiaoqi Jiang(1), Oriana Ponta(1), Sven Stanzel(1), Alexius Freyberger(2), Marc Weimer(1)

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(2) Bayer Pharma AG, GGD GED Toxicology, Department of Pathology and Clinical Pathology, Aprather Weg 18, 42096 Wuppertal, Germany

Dose-response studies are performed to investigate the potency of the substance under investigation. Data are typically evaluated using non-linear regression techniques, a common model is the log-logistic model. Often, response values and/or dose levels are transformed before proceeding with the analysis of their relationship. This is motivated by various practical reasons, including comparability of results across different assays. Data transformation is problematic in practice if its consequences are not taken well into account. For instance, data transformation may erroneously lead to a reduced number of parameters to be estimated. We observe this procedure in particular when response data are corrected for background signal and then normalized by background corrected solvent control. It will be shown that transformed data should nevertheless be fitted with a four-parameter log-logistic model to include parameters for lower and upper boundary of mean response, EC50 and Hill slope. We further shed light on the impact of general data transformations and on common problems arising during the analysis of dose-response data. Computer simulations and a real data example are used to illustrate the impact of data transformations on parameter estimation.

Statistical planning and analysis of the HET-MN Assay for genotoxicity testing

Ralph Pirow[1], Dagmar Fieblinger[1], Manfred Liebsch[2], Albrecht Poth[3], Kerstin Reisinger[4], Thorsten Wolf[5], Daniel Gerhard[6], Ludwig A. Hothorn[6]

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2 BfR/ZEBET, Berlin, Germany
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Given the requirements of the European chemicals legislation REACH and the deadlines for a complete ban on the use of cosmetic ingredients tested on animals (EU Cosmetics Regulation 1223/2009), there is a need for validated alternatives to animal testing. One of the promising follow-up methods for enhancing the predictive power of existing *in-vitro* test batteries for genotoxicity testing (Pfuhler et al., 2010) is the hen's egg test for micronucleus induction (HET-MN; Wolf et al., 2008). The HET-MN is distinguished from established *in-vitro* genotoxicity assays by the ability to cover important toxicological processes such as metabolic activation, elimination, and excretion. The performance of the HET-MN in terms of transferability, reproducibility and predictivity is currently analyzed in a BMBF-supported pre-validation study with three participating laboratories including the Henkel AG & Co. KGaA, the research contract organization Harlan, and the Federal Institute for Risk Assessment (BfR). The present paper provides a thorough evaluation of the experimental design and statistical analysis of this assay. The design of the HET-MN is a randomized one-way layout, including three doses of the test compound, a negative control (NC), and a positive control. Six eggs are randomized to each group. The primary endpoint is the number of micronucleated erythrocytes (MN) per 1000 scored definite (i.e. polychromatic and normochromatic) erythrocytes, suggesting a binomial model for proportions or a poisson model for count data. Critical features are the near-to-zero counts in the NC and the effect-dependent heterogeneity of variance (between-egg variability), which suggests accounting for extra variance. Methods of choice for detecting statistically significant increases of the MN in the treatment groups are Dunnett-type and downturn-protected Williams-type comparisons with the NC (Hothorn & Gerhard, 2009). In an attempt to find most appropriate procedure, we compared the applicability, robustness and power of different analytical approaches comprising extra-binomial and extra-poisson models, normal (parametric) models with and without Freeman-Tukey (FT) square-root transformation and variance heterogeneity consideration, and non-parametric models. As a result, we arrived at the following recommendations: (i) pre-test of the concurrent NC values using quality control charts of the historical NCs, (ii) FT transformation of the near-to-zero counts, (iii) use of the downturn-protected Williams-type procedure based on FT-transformed data without variance heterogeneity modification, and (iv) use of the mean of the historical NCs instead of the concurrent NC in case the latter is outside the normal range of the historical NCs. Simulation studies finally revealed that the chosen experimental design is adequate to detect different

types of expected value profiles (linear increase, hockey stick, saturation, umbrella) of moderate magnitude with sufficient power.

References

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Pfuhler S *et al.* (2010). A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: genotoxicity. A COLIPA analysis. *Regul Toxicol Pharmacol* 57, 315–324.

Wolf T, Niehaus-Rolf C, Banduhn N, Eschrich D, Scheel J, Luepke NP (2008). The hen's egg test for micronucleus induction (HET-MN): Novel analyses with a series of well-characterized substances support the further evaluation of the test system. *Mutat Res* 650, 150–164.

Stochastic models for brain aggregate cell cultures: Quantitative description of neurotoxicity

Maria Renner and Annette Kopp-Schneider

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Brain aggregates are three-dimensional *in vitro* cell cultures which offer a unique system for central nervous system toxicity testing. They contain the different brain cell types (neurons, astrocytes, oligodendrocytes, microglial cells and stem cells) which are able to interact in a physiological manner. The populations are present in proportions similar to those in the brain of an adult rat *in vivo*.

For toxicity testing the cultures are exposed to different concentrations of the test substances at several time points. Cell type specific toxicity of the selected drugs is assessed by routine protocols for qRT-PCR analyses and enzyme activity measurements. The selected genes and enzymes code for the different cell types. The cell cultures combine organ-specific traits with robustness and are highly available and reproducible.

We model a subsystem of this *in vitro* brain culture system as a stochastic process. We have developed a Markov model for the size and behavior of a single cell population which incorporates cellular stress and death in continuous time. The instantaneous transition rates may depend linearly on the concentration of the tested compound and have a biological meaning. The cell population model is linked to LDH activity measurements. We obtain stochastic models for time and concentration dependent LDH activity.

The parameters of the models are estimated via maximum likelihood and least squares techniques from simulated data. Thus the neurotoxic effect of a compound on brain aggregate cell cultures can be captured in a mechanistic and quantitative way.

Reference:

M.-G. Zurich, P. Honegger, B. Schilter, L.G. Costa and F. Monnet-Tschudi. Use of aggregating brain cell cultures to study developmental effects of organophosphorus insecticides. NeuroToxicology 21 (2000) 599-606.

Animal To Human Translation

Session Chair: Katja Remlinger

Wednesday, September 26, 2012
11:00 – 12:30

Trends in Translational Sciences: Role of industrial-academic collaboration

Khusru Asadullah
VP and Head of Global Biomarker at Bayer Healthcare,
Professor of Medicine at the Medical School Charité

Progress with novel technologies as well as the increasing molecular understanding of diseases led to high expectations for better drugs. Despite these developments and increasing investments, however, the number of new drug launches has not increased. Introduction of translational research is expected to improve this situation. This requires close collaboration between industry and academia, which creates both opportunities and challenges.

Difficulties in Translation from Animal to Man - Some Examples from Neuroscience Research

David Willé,
Applied Statistician, Statistical Consulting, GlaxosmithKline

Translation from animal studies to man remains a key challenge in applied pharmaceutical research but why is it so hard? Why do early signals often not translate? This talk discusses a number of possible causes, exploring difficulties in both statistical and the underlying biological models used to find new drugs taking examples from neuroscience research.

In particular, our discussion is motivated by contributions to a multinational EU and EFPIA lead collaboration on translation from animals in man in Alzheimer research. Areas of statistical support are described as well as the advantages and potential of working together in such a consortium.

Translation of Pre-Clinical Pharmocokinetic Parameters in the Determination of Dosing in First time in human studies

Alun Bedding,
Director, Statistical Consulting, GlaxoSmithKline
(on behalf of the GSK PK Predictions Initiative)

Decisions based on human pharmacokinetic predictions have a significant impact on the candidate selection of a drug, as well as on the number of animal studies triggered to support it. The prediction of the human PK data is therefore key, but how well is it done?

There is much debate within the pharmaceutical industry about which method to predict human PK parameters (e.g. clearance, half-life, volume of distribution, bioavailability, dose...) from in vitro or in vivo animal data should be used, and the reliability of those predictions. The accuracy of such predictions is important given the doses for first time in human studies are based on them. Recently the PhRMA CPCDC Initiative on Predictive Models of Human PK published its recommendations on this issue[1-5] .

A working group within GSK was formed to look at the predictability of the animal models. The work of this group focuses on a subset of GSK Phase I data and looking at how well it was predicted from the relevant pre-clinical PK. Emphasis is placed on statistical comparisons and model prediction.

It is envisaged that the findings of this working party will help to raise awareness of the methodologies and their limitations, and provide the GSK scientific community with easy access to the finally recommended decision process, via computational tools.

References

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2. Rhys Do Jones, et al., "PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 2: Comparative assessment of prediction methods of human volume of distribution," J. Pharm. Sci. 100(10), 4074 (2011).
3. Barbara J. Ring, et al., "PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 3: Comparative assessement of prediction methods of human clearance," J. Pharm. Sci. 100(10), 4090 (2011).
4. Ragini Vuppugalla, et al., "PhRMA CPCDC initiative on predictive models of human pharmacokinetics, part 4: Prediction of plasma concentration□time profiles in human from in vivo preclinical data by using the Wajima approach," J. Pharm. Sci. 100(10), 4111 (2011).
5. Patrick Poulin, et al., "PHRMA CPCDC initiative on predictive models of human pharmacokinetics, part 5: Prediction of plasma concentration□time profiles in human by using the physiologically-based pharmacokinetic modeling approach," J. Pharm. Sci. 100(10), 4127 (2011).

Various

Session Chair: Richardus Vonk

Wednesday, September 26, 2012
13:30 – 15:00

Estimation of in-vitro dose response curves in the presence of drug resistant viral mutations

D. Abainenamar, T. Jacobs, H. Ceulemans, O. Lenz, G. Molenberghs, T. Verbinnen.

Inhibition of viral replication with direct acting antiviral agents can result in selection of viral variants with reduced susceptibility to these antiviral agents (Sarrazin and Zeuzem, 2010). Therefore, a thorough understanding of the factors determining the emergence of treatment resistant viral strains is important in the development of new compounds.

Our case study consists of in-vitro inhibition experiments using single wild type and mutant replicons of HCV, and different proportional mixtures of both (Verbinnen et al 2012). The in-vitro exposure-response of the replication of the different replicon mixtures is modeled using a dual dose response function, i.e., the dose response of the (replicon) mixture is described as a combination of the individual viral strains. The obtained model is subsequently used in a simulation study for the optimization of the experimental set up.

References

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Verbinnen T., Jacobs T., Vijgen L., Ceulemans H., Neyts J., Fanning G., Lenz O. (2012) Replication capacity of minority mutants in mixtures can affect the assessment of resistance in HCV chimeric replicon phenotype assays. Journal of Antimicrobial Chemotherapy, 00:00-00.

Detecting pharmacodynamic drug-drug interactions – a pharmacometric success story

T. Jacobs, C. Mackie, W. Talloen, T. Steckler, A. Megens

Synergy is a phenomenon describing the potency shift of one or more compounds under combined administration. Synergy between compounds is typically assessed in an in-vitro experiment, see for example Tallarida (2007). However, synergy-like behavior can also be observed – deliberately or unexpectedly - in in-vivo experiments.

Our case study was designed to assess the potential for future co-administration as a new therapeutic option of a new molecule with a marketed product. When both compounds were co-administered in a first experiment, the observed effect on the pharmacodynamic outcome of interest was more pronounced than expected. As such, a turnover model (Gabrielson and Weiner, 2001) estimating the impact on the production and elimination rate of the response was combined with a latent pk-profile (Jacqmin et al, 2007). This has led to the design of additional studies and an in-depth model-based drug development. Synergy-like behavior was concluded at high doses, however, anticipated therapeutic doses remained unaffected.

References

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Tallarida, RJ, Pharmacol. Ther, 113(1), 2007, 197-209.

Quantitative assessment of drug interactions by linear mixed effects modeling

Stanzel, Sven[1] ; Bayer, Helene[2]; Kopp-Schneider, Annette[1]

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Studies of drug interactions have become increasingly important in biomedical research. The interaction index can be used to quantitatively assess drug interactions present at a specific combination of the doses of two drugs as additive, synergistic or antagonistic (Chou and Talalay, 1984). Lee and Kong (2009) proposed a procedure to estimate the interaction index from the individual dose-response curves that were obtained for the two drugs. This estimation procedure is based on fitting a simple linear regression model to data points (log(x), log(y/1-y)), where x stands for the dose and y for the normalized response. This approach is numerically almost identical to fitting a two-parameter log-logistic model to normalized concentration-response data (x,y).

One major drawback of the suggested approach is that it assumes that only a single response measurement is taken at every concentration level. However, this assumption does not reflect the typical situation present in *in vitro* studies, where usually several experiments are conducted for each test substance, with two or more technical replicates measured at every concentration level. Variability between experiments can be large and therefore have a great impact on the estimated interaction index. To account adequately for this variability and thus to yield more reliable estimates of the interaction index, we propose to replace the simple linear regression model used in the Lee and Kong estimation procedure by a suitably chosen linear mixed effects model.

We will present the modified estimation procedure in detail and illustrate it by application to a study from cancer research.

References

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Normal ranges determination with Quantile regression

Luc Esserméant

PreClinical Development Biostatistics, sanofi

Reference values, or normal ranges, are more and more requested as a help to interpret biological measurements. Calculated from historical control data, they are classically defined as a pair of numbers that bound the central 95% of a collection of values. Several methods, parametric or not, are classically proposed for their computation.

In some cases, the normal ranges could depend from covariate(s), for example the age; in this case, more advanced methods could be useful to determine accurately the normal ranges. Quantile regression is a very convenient tool to obtain polynomial model of the percentiles, with no restrictive hypotheses on residuals.

In this presentation, quantile regression approach is explained by analogy with OLS regression, and applications with the SAS system are presented.

Logistic Regression Re-Modelled

Tina Müller and Hannes-Friedrich Ulbrich
Bayer Pharma AG

In preclinical research, some target variables are measurements of percentages. One example that we have been involved with concerns the neovascularization of mice's cornea. The measurement of interest is the percentages of cornea area that is vascularized compared to the complete cornea area. Two or more groups of mice should be compared to each other with respect to their vascularized percentages.

Percentages in this case are bound downwards and upwards (lying within the closed interval [0%, 100%]). Thus, all analysis should take these restrictions into account. Analyses based on the classical linear model (like t-tests) do not fulfill the requirements as e.g., confidence intervals can include values outside the specified meaningful range. Non-parametric alternatives were not favored as effect sizes and confidence intervals were needed for a proper analysis.

A well-known method especially designed for dealing with values between 0 and 1 (or 0% and 100%) is logistic regression. However, it is based on the assumption that the response of interest is a dichotomous variable of either 0 or 1. Then, the respective probability of a success (= 1) is modeled within the logistic framework, and the error term is assumed to be binomially distributed. The binomial assumption cannot be applied to the percentage data, meaning that logistic regression cannot readily be adapted for our purposes.

Using a slight adjustment links out problem and logistic regression: We model the log odds of the percentages as we would model the probability and add a normally distributed error term. All confidence intervals are transformed back to the original scale and now lie within the limits of 0% and 100%. Statements about significant differences are based on tests on odds ratios and can be interpreted in the given context.

We will show the application, also in contrast to results from a classical t-test, and open the discussion about alternative approaches to similar problems.

Quasi-species Identification with Model Based Clustering.

Bie Verbist[1] , Koen Van der Borght[2] , Yves Wetzels[3], Kim Thys[2], Joke Reumers[2], Tobias Verbeke[4] , Willem Talloen[5] , Joris Meys[6], Carl Van Hove[2], Jeroen Aerssens[2], Luc Bijnens[5], Olivier Thas[6]

*[1]IWT Baekeland Mandatory with Janssen nv**Fehler! Textmarke nicht definiert.**, 5 as industrial partner and Ugent6 as academic partner.*
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[5]Janssen Pharmaceutica, Biostatistics and Programming COE, Turnhousteweg 30, 2340 Beerse, Belgium.
[6]UGent, Mathematical Modeling, Statistics and Bioinformatics, Coupure Links 653, 9000 Gent, Belgium
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Deep-sequencing is one of the applications of the new massively parallel sequencing (MPS) technologies allowing for an in-depth characterization of sequence variation in more complex populations, including low-frequency viral strains. However, MPS technology-associated errors in the resulting DNA sequences may occur up to equal or even higher frequency than the truly present mutations in the biological sample, impeding a powerful assessment of low-frequency virus mutations. Our focus will primarily lie on the Illumina platform to study viral quasi-species. As there are no obvious solutions to reduce the technical noise by further improvements of the technology platform, we believe that the search for statistical algorithms that can better correct the technical noise can be pivotal. Therefore algorithms that increase detection power in presence of technical noise and quantify base-call reliability are required.

Phred-like quality scores, provided with the base-calls are such a quantification of the base-call reliability. However the translation of these quality scores to error probabilities does not always reflect the true error rate and furthermore they are rather discrete. Therefore different measures based on the raw intensity values, from which the quality scores are derived, are also taken into account together with some other parameters to get a more accurate quantification of the reliability. These covariates determine the multinomial model structure in a model-based clustering approach which will allow identification of viral quasi-species. The parameters are estimated using the EM algorithm and confidence levels are constructed.

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Biophysical Rationale and Quantitative Benefits of using Linear Mixed Effect Models to summarize Transitions of Peptides to Protein abundances in SRM

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INTRODUCTION

Selected Reaction Monitoring (SRM, aka MRM) is a quantitative mass spectrometry method for targeted proteomics. It is increasingly useful when a lack of suitable antibodies excludes conventional immunoassay of specific proteins of interest. Such a situation is common with preclinical animal models. SRM's positive quantitative characteristics in plasma and serum have already been shown (1). In the SRM process, proteins are first trypsinated to peptides. Following introduction into the mass spectrometer, typically via chromatography, specific targeted peptides are selected by the first quadrupole mass filter, and fragmented to transitions with specific masses in a collision cell. The distinguishing feature of SRM is that the third quadrupole analyses only a small number of sequence-specific fragments, as compared with the full scan in conventional MS where all the fragment ions are analyzed. This targeted analysis allows a significant (up to 100 fold) enhancement of LOD.

The quantitative readout is the peak area of a transition detected in the third quadrupole. To quantitate individual transitions in terms of peptides abundance, taking the sum, median or mean of the peak areas of the fragments of an involved protein are widely accepted. The utility of using linear mixed effect models (LMEM) to infer protein abundance from transitions has been previously discussed (2). In this abstract, we will derive the biophysical rationale and demonstrate the quantitative advantages of using LMEM to summarize peak areas of transitions to protein abundances in SRM in comparison to sum, mean and median.

METHOD

Based on the learnings of a recent preclinical study, an example data set has been simulated. The parameters for simulation have been chosen to exaggerate custom problematic circumstances, in order to simplify the understanding of the problem. These include severe heterogeneity in the ionization efficiencies between peptides and in the fragmentation efficiencies between transitions. Additional usual complication arises when the read out value is missing given the peak area of a transition is below a given threshold. All the above mentioned artefacts occur in real data, yet typically to a lower degree that reflected in the simulated data set.

Data Simulation: The nominal abundance of the simulated protein ranges from 0 to 10 arbitrary concentration units. This protein was targeted with 3 peptides, each quantified with 3 transitions. The transition areas (A) are computed from imaginary ionization and fragmentation factors multiplied with nominal abundance using Formula 4 (see below). Transition peak areas A which were below an arbitrary selected limit of quantitation ($A < 10$ arbitrary peak area units) were set to Non Available in order to simulate below LLoQ behaviour).

RESULT I: Derivation of the statistical model from biophysical considerations.

After trypsination, a peptide's concentration should be function of its progenitor protein`s concentration:

$c(Peptide) = c(Protein) * f_{Tryps,\,p} * \varepsilon_1$ Formula 1

The number of ionized peptides after Electron Spray Ionization is a function of the peptides concentration:

$n(Peptide) = c(Peptide) * f_{Ion,\,p} * \varepsilon_2$ Formula 2

The AUC of transitions in MS2 and the degree of fragmentation is a function of the number of electrospray–ionized peptides passing the mass selection in MS1 and the fragmentation factor f_{frag}.

$A(Transition) = n(Peptide) * f_{frag,\,p} * \varepsilon_3$ Formula 3

Combining the three terms forms a relation between A and protein abundance:

$A(Transition) = c(Protein) * f_{Tryps,\,p} * f_{Ion,\,p} * f_{frag}\ \varepsilon_*$ Formula 4

After log-transformation, factors become addends:

$\log(A(Transition)) = \log(c(Protein)) + \log(f_{Tryps,\,p} * f_{Ion,\,p}) + \log(f_{frag}) + \log(\varepsilon_*)$ Formula 5

$\log(A(Transition)) = RelAbundanceProtein + PeptideEffect + FragEffect + \varepsilon^*$ Formula 6

The hierarchical nature of transitions, being nested in a peptide, which are themselves nested in a protein, can be translated into the syntax of R using the package lme4.

$\log(A(Transition)) \sim 0 + SampleID + (1|\ PeptideID/TransitionID)$ Formula 7

As a result, the effect sizes of factor SampleID are estimates of the relative abundance the protein in a given sample.

RESULT II: Comparing Inferred protein abundances based on the four summarization methods to nominal protein abundance.

"Observed" protein abundances were inferred by using either the sum, mean, median or LMEM (Formula 7) methods and compared with the originating, nominal protein abundances. Plotting the observed abundances, inferred from these four methods, against nominal abundances and examining the low nominal abundance range already reveals that the "mean" and "median" summarization methods overestimate, while the "sum" method underestimates the nominal abundance. Only the "LMEM" method delivers a good representation of the nominal abundance. Bland-Altman plot facilitates consideration of these effects with regard to linearity, precision and accuracy: Method "sum" results in the highest deviation from linearity followed by methods "median" and "mean". The pattern of the residuals after summarizing by LMEM, as compared to that for the other methods indicates that LMEM provides better accuracy. In the range of low nominal abundance, the precision derived from the sum, mean and median methods are comparable, but lower than that achieved by LMEM.

DISCUSSION & CONCLUSION

We hope that the acceptance of using a LMEM will increase among researchers as we have shown its formula can easily be derived from biophysical considerations. It was demonstrated that inferring protein abundance from transition peak areas using LMEM is superior in terms of linearity, precision and accuracy, especially when the read-out can be censored due to low protein abundance.