Design of preclinical combination studies

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Talk Outline

What is a drug combination?

Definition of bioassay

Scientific experiment for determining the effect of a compound or other substance.

Drug combination

Medications which contain two, or more, different compounds.

Combinations of drugs

Combinations of drugs may be useful when:

- using one drug is not sufficient to control a disease;
- the required dose is too high and has undesirable side effects;

Pre-clinical experiments

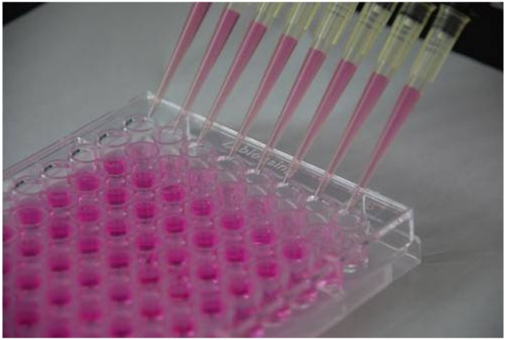
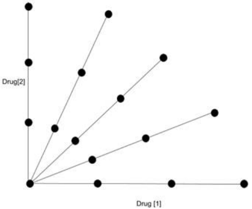


Figure: Typical 96-wells plate

Data structures

- Doses of drugs: d_{ij1} and d_{ij2},in constant ratios $R_i = d_{ij1}/d_{ij2}$, $i, i = 3, \ldots, r$, $j, j = 1, \ldots, c$;
- Response of interest Y_{ij} measured at the doses $x_{ij} = d_{ij1} + d_{ij2}$.

Ray design



Ray-Designs

The name come from!


Radiating style

Methods for evaluating drug interactions

Combination index

The drug interactions at combination of two drugs can be characterized as

$$\frac{d_1}{D_{y_0,1}} + \cdots + \frac{d_2}{D_{y_0,2}} \begin{cases} < 1, & \text{Synergy;} \\ = 1, & \text{Additivity;} \\ > 1, & \text{Antagonism,} \end{cases}$$

- d_i are doses of each drug in the combination of the k drugs resulting in effect y_0;
- $D_{y_0,i}$ are the doses of drugs that result in the effect y_0 for each respective drug given alone.

Sources of variability

- occasions
- batches of cultures
- plates
- . . .

Statistical models

Distribution of response

$Y \Longrightarrow$ Exponential family (EFD)

Nonlinear predictor for each ray

$$\eta(x_{ij}, \boldsymbol{\theta}) = \gamma + \frac{\delta - \gamma}{\left(1 + 10^{\left(x_{ij} - \alpha_i\right)\beta_i}\right)^{\lambda_i}},$$

where $\boldsymbol{\theta} = (\alpha_i \ \beta_i \ \gamma \ \delta \ \lambda_i)$, $i = 1, \ldots, r$.

Link function...

between $\eta(x_{ij}, \boldsymbol{\theta})$ and $E[Y] = \mu_{ij} \Longrightarrow g(\mu_{ij}) = \eta(x_{ij})$.

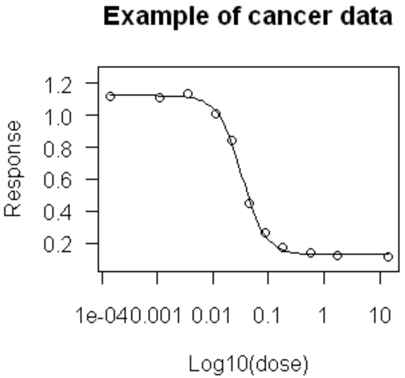
Hill model (1913)

4 parameters

$$Y_{ij} = \gamma + \frac{\delta - \gamma}{1 + 10^{\left(x_{ij} - \alpha_i\right)\beta_i}} + \varepsilon_{ij}.$$

where:

- γ Minimum response
- δ Maximum response
- α LogIC50, or logEC50)
- β Hill slope
- ϵ Experimental error, $\varepsilon \sim N(0, \sigma^2)$



Alternative models

Special cases

- Minimum response = 0

$$Y_{ij} = \frac{\delta}{1 + 10^{\left(x_{ij} - \alpha_i\right)\beta_i}} + \epsilon_{ij}.$$

- Minimum response = 0 and Maximum response = 1

$$Y_{ij} = \frac{1}{1 + 10^{\left(x_{ij} - \alpha_i\right)\beta_i}} + \epsilon_{ij}.$$

- Asymmetric

$$Y_{ij} = \gamma + \frac{\delta - \gamma}{\left(1 + 10^{\left(x_{ij} - \alpha_i,\right)\beta_i}\right)^{\lambda_i}} + \epsilon_{ij},$$

Nonlinear mixed effects models

Example - all parameters random

- $$Y_{ij} = (\gamma + \varepsilon_\gamma) + \frac{(\delta + \varepsilon_\delta) - (\gamma + \varepsilon_\gamma)}{\left(1 + 10^{\left(x_{ij} - (\alpha + \varepsilon_\alpha)\right)(\beta + \varepsilon_\beta)}\right)^{(\lambda + \varepsilon_\lambda)}} + \epsilon_{ij},$$

- where $\boldsymbol{\theta}_{p_i = \alpha_i, \beta_i, \gamma_i, \delta_i, \lambda_i} \sim EFD(\boldsymbol{\theta}_p, \phi_p)$, and $\epsilon_{ij} \sim EFD(0, \phi)$.

- Related work include: Mentré, Mallet, Baccar (1997), Fedorov, Gagnon, Leonov (2002), Han and Chaloner (2004), Gagnon and Leonov (2005) and Atkinson, A.C. (2008).

Analysis of data

- There are many examples of successful combination studies in the literature, e.g.:
  - Loewe, S. 1957;
  - Chou and Talalay, 1984;
  - Berenbaum MC, 1985;
  - Faessel,H.M. et. al. 1998;
  - Straetemans R. et al. 2005.
- Extensive discussions of the scientific background of such studies, include:
  - Berenbaum MC, 1989;
  - Chou, T. C 1991;
  - Greco et. al. 1995;
  - Tallarida, R. J. 2000.
  - Chou, T.C et. al. 2006.

Analysis of data

- Recently, there has been substantial interest in the appropriate statistical methods for analysis of data obtained in combination studies including:
    - Brun YF et al. 2010;
    - Chou, T. C 2010;
    - Donev, A. N. 2010;
    - Fujimoto Junya et. al. 2010;
    - Kong M et al. 2010;
    - Lee JJ et al. 2010;
    - Palomares et al. 2010;
    - Peterson JJ 2010;
    - Straetemans R. et al. 2010;
    - Yan Han et al. 2010;

Analysis of data

- Some of these statistical methods of combination studies have been implemented as statistical packages in the free statistical software R include:
  - *drc* $\Longrightarrow$ Analysis of dose-response curves
  - *grofit* $\Longrightarrow$ Fitting biological growth curves with R
  - *dosefinding* $\Longrightarrow$ Planning and Analyzing Dose Finding experiments
  - *mixlow* $\Longrightarrow$ An R Package for Assessing Drug Synergism/Antagonism
- These packages are regularly improved and updated!

Design challenges

- Less attention has been given to the choice of suitable experimental designs for such studies;
- Need to develop the necessary methodology;
- **Need to develop an R package;**

Design challenges

Types of studies

- Single compounds;
- Screeing compounds library for combinations - using one design - *population studies*;
- Confirmatory experiments, safety studies, etc;
- Variety of error distributions (normal, binary, Poisson, etc).

Types of models

- Nonlinear (NL);
- Generalized nonlinear (GNL);
- Nonlinear mixed effects (NLME);
- Generalized nonlinear mixed effects (GNLME).

Optimality criteria

- The information matrix

$$\mathbf{M} = \mathbf{X}^{\mathsf{T}}\mathbf{W}\mathbf{X}$$

depends on:

the unknown parameters;

the covariance matrix of the observations $\mathbf{W}$.

- A common approach is to linearize:

Locally D-optimum design ξ^*:

$$\min |\mathbf{M}^{-1}(\xi, \boldsymbol{\theta})||_{\boldsymbol{\theta}=\boldsymbol{\theta}_0}$$

Pseudo-Bayesian D-optimum design ξ^*:

$$\min |\mathbf{M}^{-1}(\xi, \boldsymbol{\theta})||_{\boldsymbol{\theta}\in\boldsymbol{\theta}_0}$$

R package

State

- Early stage of preparation;
- Still developing code;

Search for D-optimum designs for models:

- Nonlinear (NL);
- Generalized nonlinear (GNL);
- Nonlinear mixed effects (NLME);
- Generalized nonlinear mixed effects (GNLME).

Example 1. D-optimum designs for NL and GNL models

Table: Parameters values

Design	α	β	γ	δ	λ	σ^2	κ	υ	m
Local	1.92	0.94	0.1	1.1	1.5	1	0.5	1.5	$2 \times p$
Pseudo-Bayesian	[1.31,3.01]	[0.50,1.15]	0.1	1.1	1.5	1	0.5	1.5	$2 \times p$

where

- $\Longrightarrow$ α, β, γ, δ and λ are models parameters;
- $\Longrightarrow$ κ and υ are parameters of gamma and inverse Gaussian distribution;
- $\Longrightarrow$ m is the number of trials in binomial distribution.

Example 1: Locally D-optimum designs

Table: Locally D-optimum designs for Ray 4

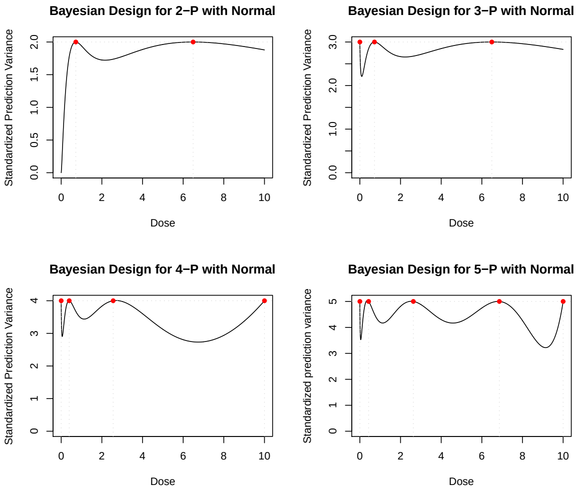
Model parameter	Design point	Error: Normal	Poisson	Binomial	Gamma	I-Gaussian
2-P	1	0.45	0.24	0.67	1.17	0.94
	2	5.55	2.71	7.19	10 (*max*)	10 (*max*)
3-P	1	0	0	0	0	0
	2	0.45	0.22	0.71	1.17	0.94
	3	5.56	2.52	7.51	10 (*max*)	10 (*max*)
4-P	1	0	0	0	0	0
	2	0.24	0.15	0.25	0.41	0.36
	3	2.07	1.41	2.17	3.39	3.03
	4	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)
5-P	1	0	0	0	0	0
	2	0.24	0.14	0.10	0.16	0.13
	3	2.05	1.48	0.71	1.28	1.17
	4	6.39	5.96	2.21	5.04	4.93
	5	10 (*max*)	10 (*max*)	5.10	10 (*max*)	10 (*max*)

Example 2: Pseudo-Bayesian D-optimum designs

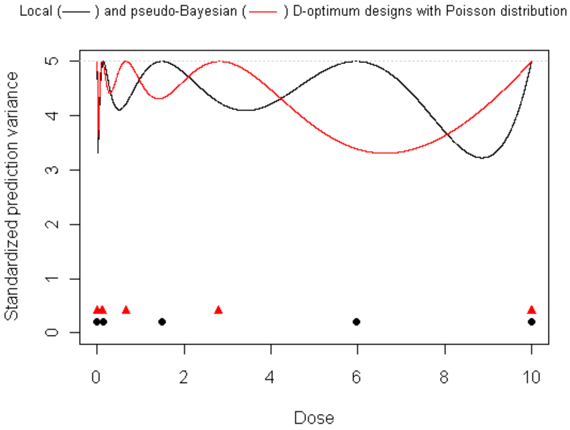
Table: Pseudo-Bayesian D-optimal designs for Ray 4

Model parameter	Design point	Error				
		Normal	Poisson	Binomial	Gamma	I-Gaussian
2-P	1	0.72	0.41	1.02	1.58	1.31
	2	6.49	3.47	8.13	10 (*max*)	10 (*max*)
3-P	1	0	0	0	0	0
	2	0.72	0.40	1.08	1.58	1.31
	3	6.49	3.27	8.45	10 (*max*)	10 (*max*)
4-P	1	0	0	0	0	0
	2	0.40	0.27	0.42	0.65	0.57
	3	2.56	1.85	2.66	3.88	3.52
	4	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)
5-P	1	0	0	0	0	0
	2	0.43	0.15	0.18	0.28	0.25
	3	2.63	0.87	1.02	1.61	1.42
	4	6.86	3.56	3.79	5.15	4.81
	5	10 (*max*)	10 (*max*)	10 **(max)**	10 (*max*)	10 (*max*)

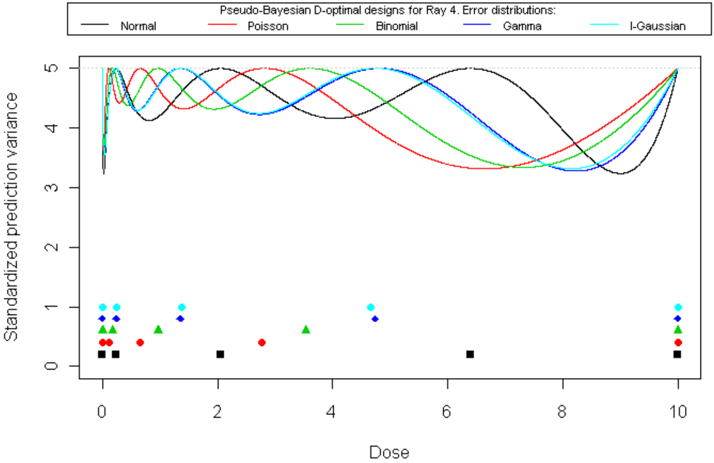
Verifying D-optimal designs for NLMs using GET



Optimum designs for GNLM with Poisson errors



Optimum designs for GNLM models



Example 2. D-optimum designs for NLME and GNLME models

Model assumption:

- the model can be given as

$$Y_{ij} = (\gamma + \varepsilon_\gamma) + \frac{(\delta + \varepsilon_\delta) - (\gamma + \varepsilon_\gamma)}{\left(1 + 10^{\left(x_{ij} - (\alpha + \varepsilon_\alpha)\right)(\beta + \varepsilon_\beta)}\right)^{(\lambda + \varepsilon_\lambda)}} + \epsilon_{ij}, \qquad (1)$$

- where $\boldsymbol{\theta}_{p_i = \alpha_i, \beta_i, \gamma_i, \delta_i, \lambda_i} \sim EFD(\boldsymbol{\theta}_p, \phi_p)$, and $\epsilon_{ij} \sim EFD(0, \phi)$.

Optimum designs for GNLME models

5 cases:

1. Case 1:
   $\boldsymbol{\theta}_{p_i} \sim N(\boldsymbol{\theta}_p, \sigma_p^2) = ((1.69, 0.84, 2.90, 0.20, 1.5), (0.26, 0.03, 0.76, 0.01, 0.02))$
   $\epsilon_{ij} \sim N(0, \sigma^2) \quad = (0, 0.25)$
2. Case 2:
   $\boldsymbol{\theta}_{p_i} \sim \ln N(\boldsymbol{\theta}_p, \sigma_p^2) = ((1.69, 0.84, 2.90, 0.20, 1.5), (0.26, 0.03, 0.76, 0.01, 0.02))$
   $\epsilon_{ij} \sim \ln N(0, \sigma^2) \quad = (0, 0.25)$
3. Case 3:
   $\boldsymbol{\theta}_{p_i} \sim \Gamma(\kappa_{\boldsymbol{\theta}_p}, \vartheta_{\boldsymbol{\theta}_p}) = ((1, 0.9, 1, 1, 1), (0.2, 0.3, 1, 0.02, 0.5))$
   $\epsilon_{ij} \sim \Gamma(\kappa_\epsilon, \vartheta_\epsilon) \quad = (1, 0.5)$
4. Case 4:
   $\boldsymbol{\theta}_{p_i} \sim \text{I-G}(\mu_{\boldsymbol{\theta}_p}, \kappa_{\boldsymbol{\theta}_p}) = ((1, 0.9, 1, 0.1, 0.5), (0.1, 0.3, 1, 0.01, 0.02))$
   $\epsilon_{ij} \sim \text{I-G}(\mu_\epsilon, \kappa_\epsilon) \quad = (0.1, 0.3)$
5. Case 5:
   $\boldsymbol{\theta}_{p_i} \sim \ln N(\boldsymbol{\theta}_p, \sigma_p^2)$
   $\boldsymbol{\theta}_\alpha \sim Exp(\lambda_\alpha) = (3.2)$

Example 3: Locally D-optimum designs for NLMEM

Table: Locally D-optimal designs for ray 4

Model parameter	Design point	Case 1	Case 2	Case 3	Case 4	Case 5
4-P	1	0	0	0	0	0
	2	0.98	1.01	0.99	1.00	1.01
	3	5.36	5.45	5.40	5.43	5.44
	4	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)
5-P	1	0	0	0	0	0
	2	0.24	0.29	0.24	0.33	0.29
	3	1.67	1.92	1.68	2.31	1.92
	4	5.47	5.92	5.28	6.67	5.95
	5	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)	10 (*max*)

Discussion

- Challenges ahead!

Note: This work is part of Bader's PhD thesis and is currently been written up for submission to a journal.

References

- 96-well picture was taken from:
  http://www.sz-wholesaler.com/p/893/905-1/96-well-cell-culture-plate-406392.html