



Biostatistics

Basic Designs for BE Studies

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BEBAC

To bear in Remembrance...

Whenever a theory appears to you as the only possible one, take this as a sign that you have neither understood the theory nor the problem which it was intended to solve.



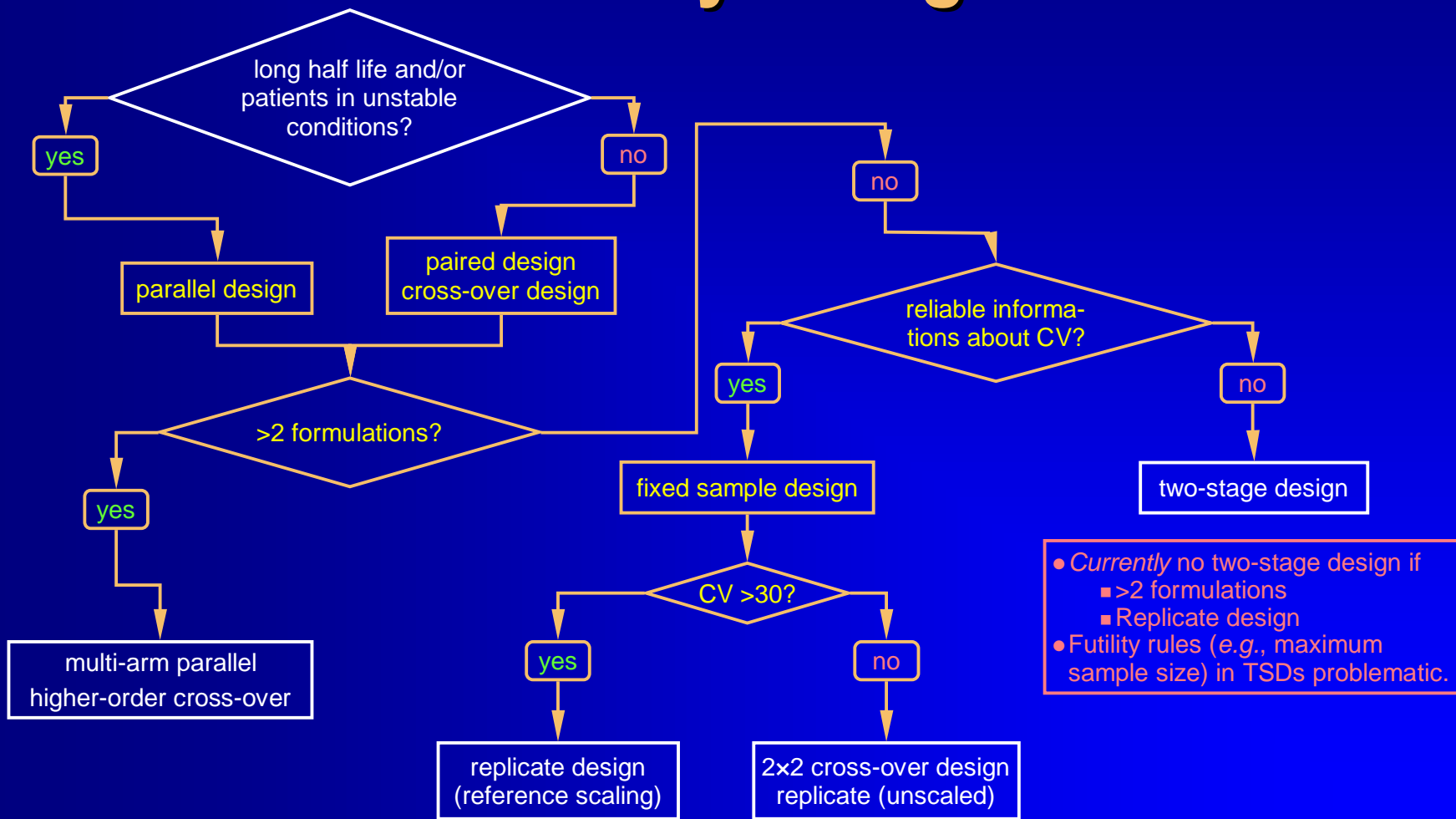
Karl R. Popper

Even though it's *applied* science we're dealin' with, it still is – *science!*



Leslie Z. Benet

BE Study Designs



BE Study Designs

- The more 'sophisticated' a design is, the more information can be extracted

- Hierarchy of designs:

Full replicate (TRTR | RTRT or TRT | RTR), ↗

Partial replicate (TRR | RTR | RRT) ↗

Standard 2×2 cross-over (RT | RT) ↗

Parallel (R | T)

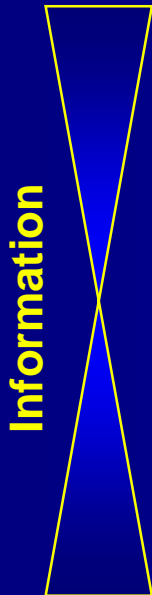
- Variances which can be estimated:

Parallel: total variance (between + within)

2×2 Xover: + between, within subjects ↗

Partial replicate: + within subjects (reference) ↗

Full replicate: + within subjects (reference, test) ↗



Data Transformation?

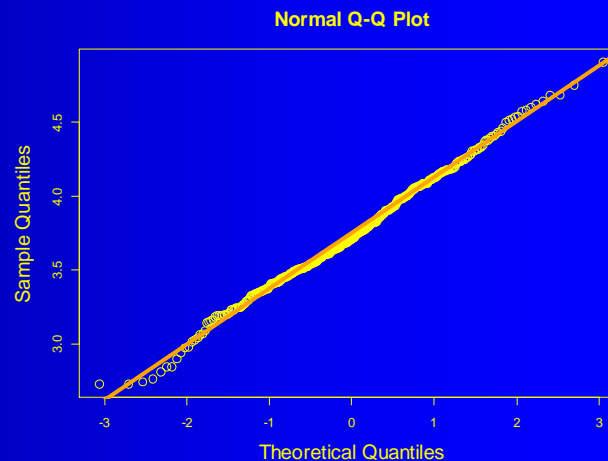
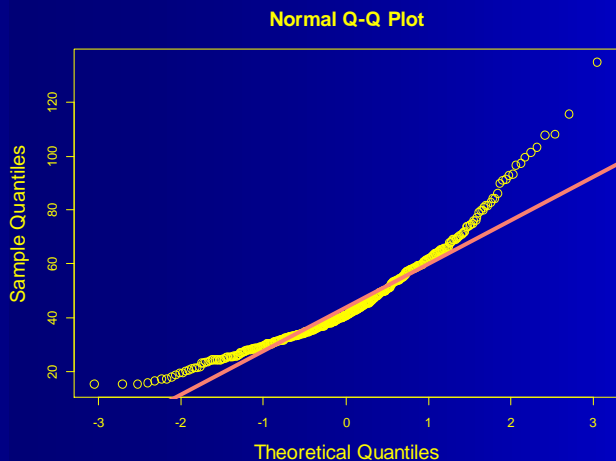
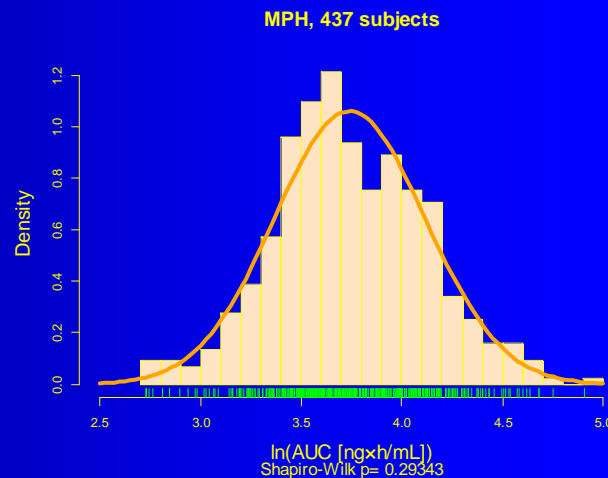
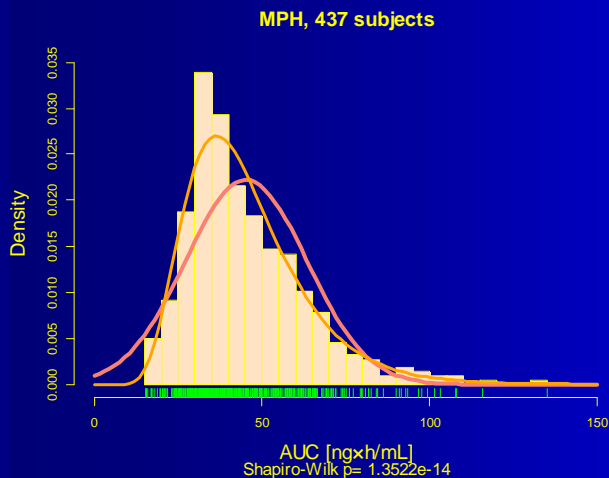
- BE testing started in the early 1980s with an acceptance range of 80% – 120% of the reference based on the *normal* distribution
- Was questioned in the mid 1980s
 - Like many biological variables AUC and C_{max} do not follow a normal distribution
 - Negative values are impossible
 - The distribution is skewed to the right
 - Might follow a *lognormal* distribution
 - Serial dilutions in bioanalytics lead to multiplicative errors

Data Transformation?

Pooled data from real studies.

Clearly in favor of a lognormal distribution.

Shapiro-Wilk test highly significant for normal distribution (assumption rejected).



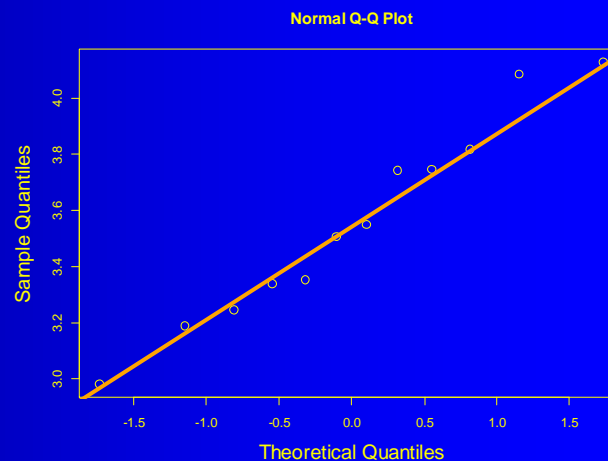
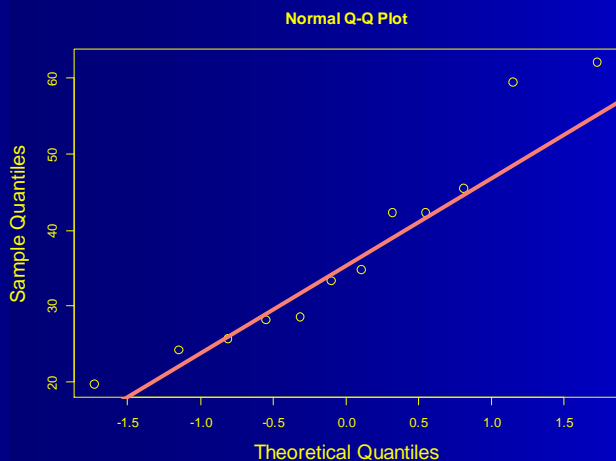
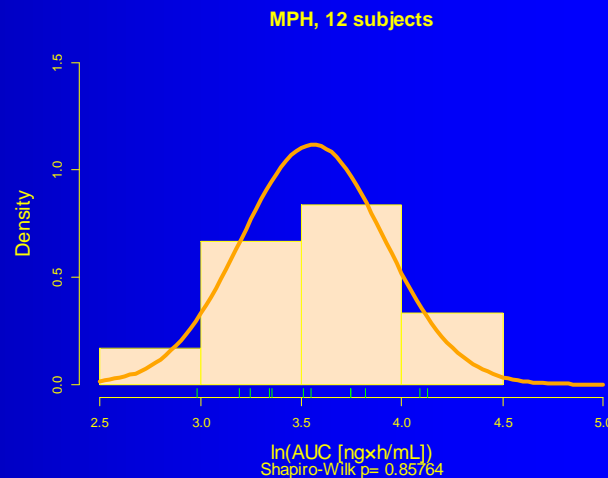
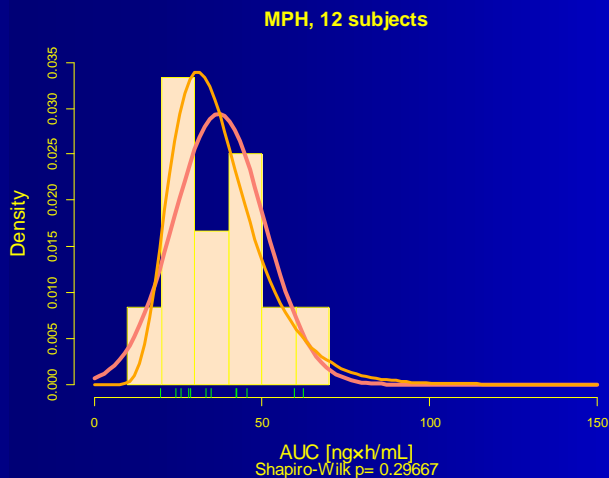
Data Transformation!

Data of a real study.

Both tests *not* significant (assumptions accepted).

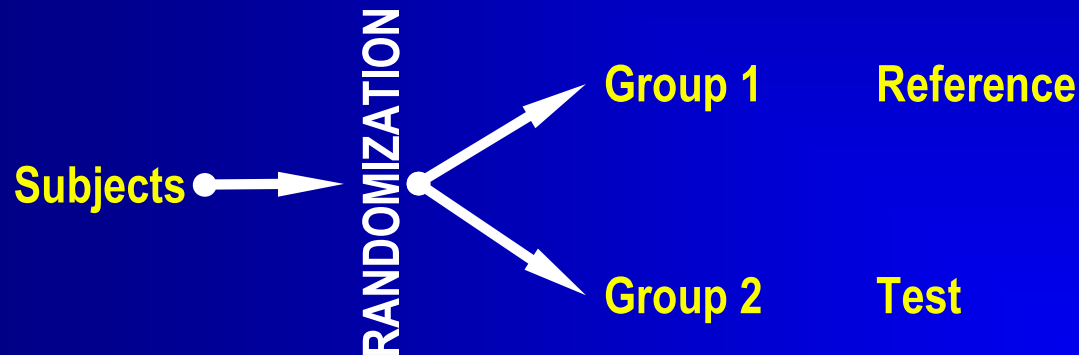
Tests not acceptable according to GLs.

Transformation based on prior knowledge (PK)!



Parallel designs

● Two-Group Parallel Design



Parallel designs (cont'd)

● Two-group parallel design

■ Advantages

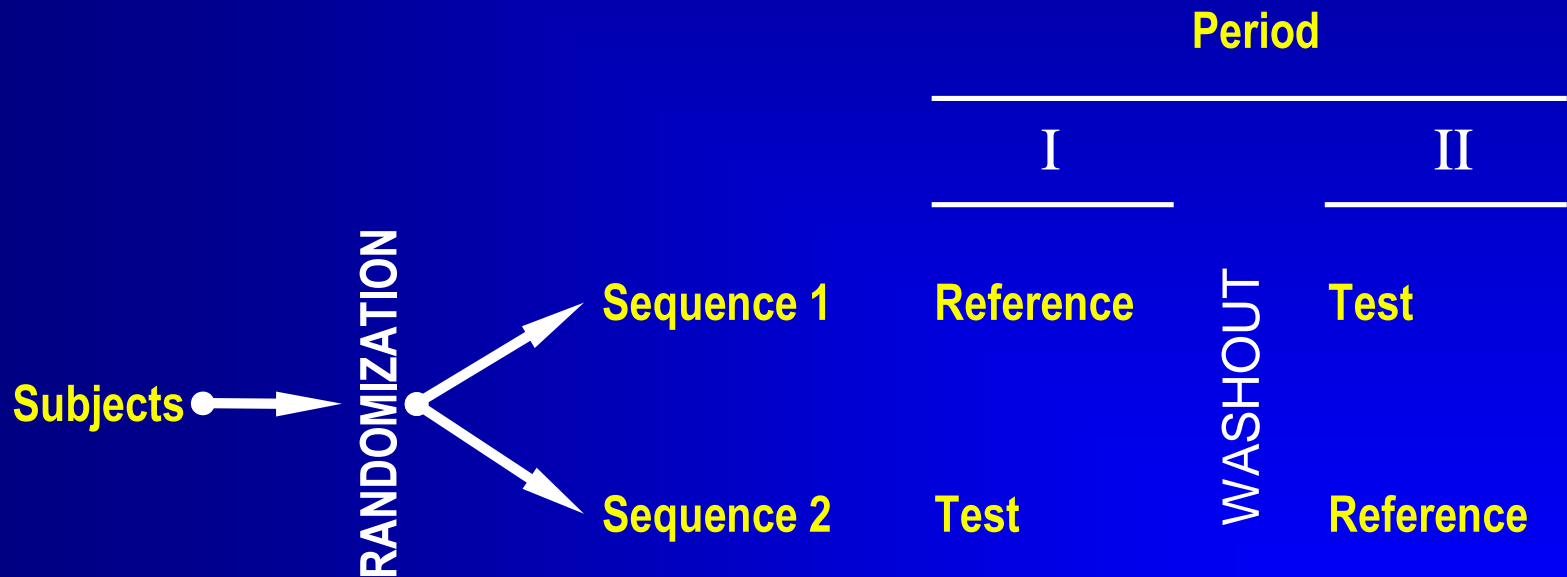
- Clinical part – *sometimes* – faster than X-over.
- Straightforward statistical analysis.
- Drugs with long half life.
- Potentially toxic drugs or effect and/or AEs unacceptable in healthy subjects.
- Studies in patients, where the condition of the disease irreversibly changes.

■ Disadvantages

- Lower statistical power than X-over
- Phenotyping mandatory for drugs showing polymorphism.

Cross-over designs

● Standard 2×2×2 Design



Cross-over designs (cont'd)

- Every subject is treated both with test and reference
- Subjects are randomized into two groups; one is receiving the formulations in the order RT and the other one in the order TR.
These two orders are called 'sequences'.
- Whilst in a paired design we must rely on the assumption that no external influences affect the periods, a cross-over design will account for that.

Cross-over design: Model

Multiplicative Model (X-over without carryover)

$$\ln(X_{ijk}) = \ln(\mu) + \ln(\pi_k) + \ln(\Phi_l) + \ln(s_{ik}) + \ln(e_{ijk})$$

$$X_{ijk} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ik} \cdot e_{ijk}$$

X_{ijk} : response of j -th subject ($j=1, \dots, n_i$) in i -th sequence ($i=1, 2$) and k -th period ($k=1, 2$), μ : global mean, μ_l : expected formulation means ($l=1, 2$: $\mu_1 = \mu_{test}$, $\mu_2 = \mu_{ref.}$), π_k : fixed period effects, Φ_l : fixed formulation effects ($l=1, 2$: $\Phi_1 = \Phi_{test}$, $\Phi_2 = \Phi_{ref.}$)

Cross-over design: Assumptions

Multiplicative Model (X-over without carryover)

$$X_{ijk} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ik} \cdot e_{ijk}$$

- All $\ln\{s_{ik}\}$ and $\ln\{e_{ijk}\}$ are independently and normally distributed about unity with variances σ_s^2 and σ_e^2 .
 - This assumption may not hold true for all formulations; if the reference formulation shows *higher* variability than the test formulation, a 'good' test will be penalized for the 'bad' reference.
- All observations made on different subjects are independent.
 - This assumption should not be a problem, unless you plan to include twins or triplets in your study...

Cross-over designs (cont'd)

● Standard 2×2×2 design

■ Advantages

- Globally applied standard protocol for bioequivalence, PK interaction, food studies
- Straightforward statistical analysis

■ Disadvantages

- Not suitable for drugs with long half life
→ parallel design
- Not optimal for studies in patients with instable diseases
→ parallel design
- Not optimal for HVDs/HVDPs
→ replicate designs with reference-scaling

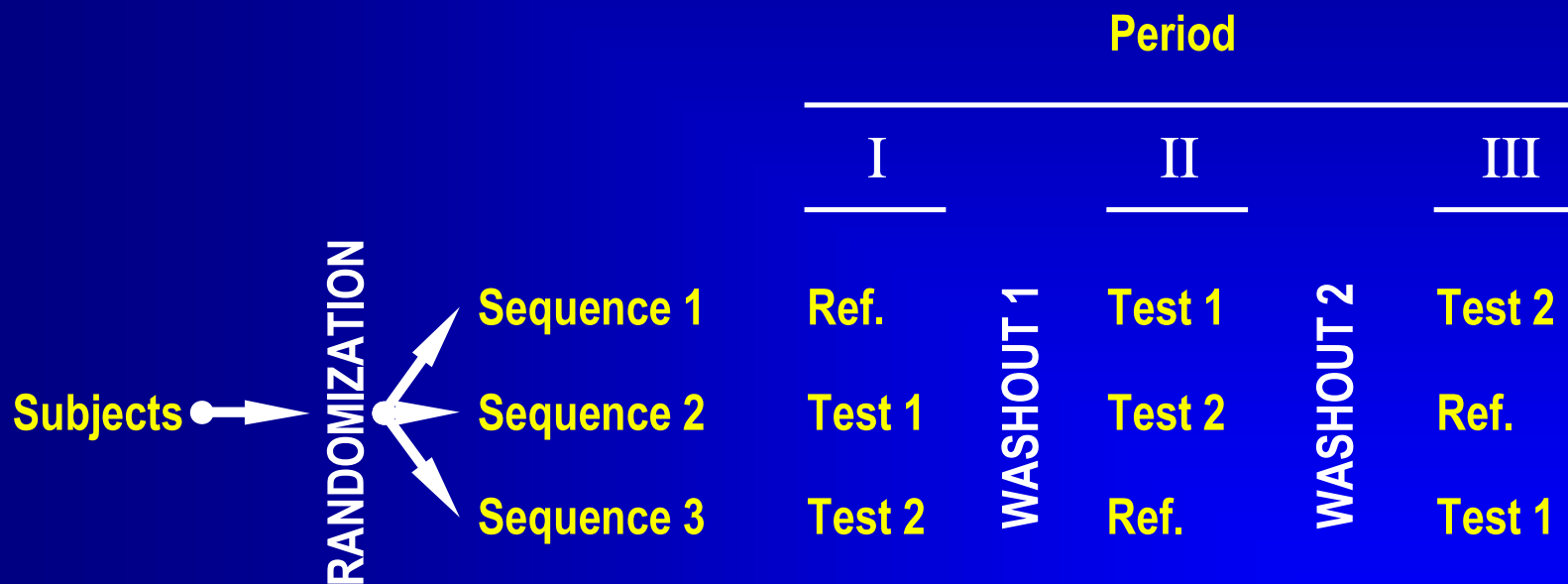
Cross-over designs (cont'd)

- **Higher Order Designs (for more than two treatments)**
 - **Latin Squares**

Each subject is randomly assigned to sequences, where number of treatments = number of sequences = number of periods.
 - **Variance Balanced Designs**

Cross-over designs (cont'd)

- 3x3x3 Latin Square design



Cross-over designs (cont'd)

● 3×3×3 Latin Square design

■ Advantages

- Allows to choose between two candidate test formulations or comparison of one test formulation with two references.
- Easy to adapt.
- Number of subjects in the study is a multiplicative of three.
- Design for establishment of Dose Proportionality.

■ Disadvantages

- Statistical analysis more complicated – not available in all software.
- Pairwise comparisons are imbalanced.
- May need measures against multiplicity (increasing the sample size).
- Not mentioned in any guideline.

Cross-over designs (cont'd)

- **Higher Order Designs (for more than two treatments)**
 - **Variance Balanced Designs (Williams' Designs)**
 - For e.g., three formulations there are three possible pairwise differences among formulation means (*i.e.*, form. 1 vs. form. 2., form 2 vs. form. 3, and form. 1 vs. form. 3).
 - It is desirable to estimate these pairwise effects with the same degree of precision (there is a common variance for each pair).
 - Each formulation occurs only once with each subject.
 - Each formulation occurs the same number of times in each period.
 - The number of subjects who receive formulation i in some period followed by formulation j in the next period is the same for all $i \neq j$.
 - Such a design for three formulations is the three-treatment six-sequence three-period Williams' Design.

Cross-over designs (cont'd)

- Williams' Design for three treatments

Sequence	Period		
	I	II	III
1	R	T ₂	T ₁
2	T ₁	R	T ₂
3	T ₂	T ₁	R
4	T ₁	T ₂	R
5	T ₂	R	T ₁
6	R	T ₁	T ₂

Cross-over designs (cont'd)

- Williams' Design for four treatments

Sequence	Period			
	I	II	III	IV
1	R	T ₃	T ₁	T ₂
2	T ₁	R	T ₂	T ₃
3	T ₂	T ₁	T ₃	R
4	T ₃	T ₂	R	T ₁

Cross-over designs (cont'd)

● Williams' Designs

■ Advantages

- Allows to choose between two candidate test formulations or comparison of a test formulation with two references.
- Design for establishment of Dose Proportionality.
- Paired comparisons are balanced.
- Mentioned in Brazil's (ANVISA) and EMA guidelines.

■ Disadvantages

- More sequences for an *odd* number of treatment needed than in a Latin Squares design (but equal for even number).
- Statistical analysis more complicated – not available in all software.
- May need measures against multiplicity (increasing the sample size).

Cross-over designs (cont'd)

● Higher Order Designs (cont'd)

■ Bonferroni-correction needed (sample size!)

■ *If more than one formulation will be marketed (for three simultaneous comparisons without correction patients' risk increases from 5 to 14%).*

■ *Sometimes requested by regulators in dose proportionality.*

k	$P_{\alpha=0.05}$	$P_{\alpha=0.10}$	$\alpha_{adj.}$	$P_{\alpha_{adj.}}$	$\alpha_{adj.}$	$P_{\alpha_{adj.}}$
1	5.00%	10.00%	0.0500	5.00%	0.100	10.00%
2	9.75%	19.00%	0.0250	4.94%	0.050	9.75%
3	14.26%	27.10%	0.0167	4.92%	0.033	6.67%
4	18.55%	34.39%	0.0125	4.91%	0.025	9.63%
5	22.62%	40.95%	0.0100	4.90%	0.020	9.61%
6	26.49%	46.86%	0.0083	4.90%	0.017	9.59%

Cross-over designs (cont'd)

● Higher Order Designs (cont'd)

■ Effect of α -adjustment on sample size

(expected T/R 95%, CV_{intra} 20%, power 80%)

CV%	2×2 α 0.05	6×3 $\alpha_{adj.}$ 0.025	comp. 2×2	4×4 $\alpha_{adj.}$ 0.0167	comp. 2×2
10.0	8	12	+50%	16	+100%
12.5	10	12	+20%	16	+60%
15.0	12	18	+50%	16	+33%
17.5	16	24	+50%	24	+50%
20.0	20	24	+20%	28	+40%
22.5	24	30	+25%	36	+50%
25.0	28	36	+29%	40	+49%
27.5	34	42	+24%	48	+41%
30.0	40	54	+35%	56	+40%

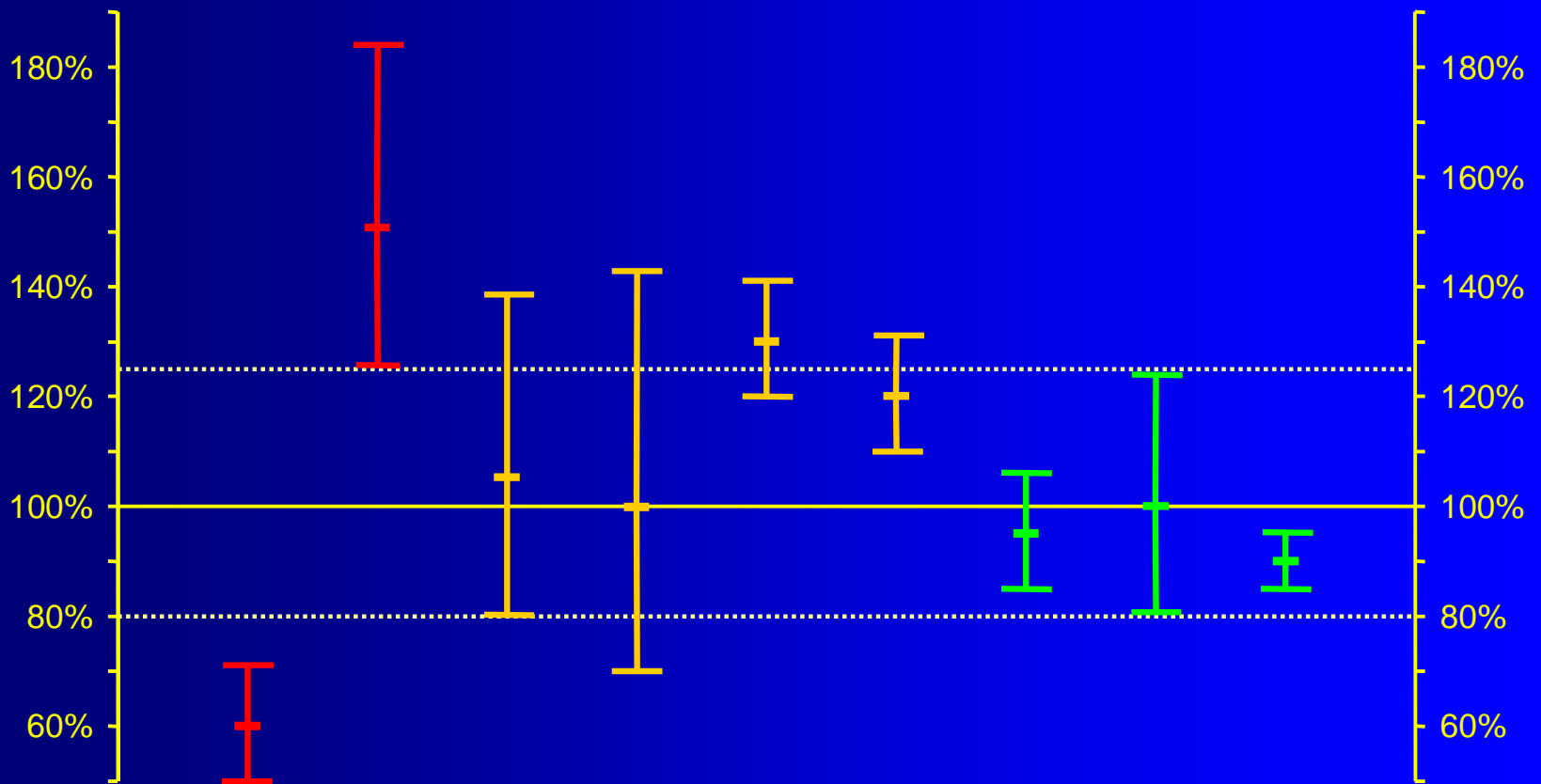
BE Evaluation

- Based on the design set up a statistical model.
- Calculate the test/reference ratio.
- Calculate a (generally 90%) confidence interval (CI) around the ratio.
- The *width* of the CI depends on the variability observed in the study.
- The *location* of the CI depends on the observed test/reference-ratio.

BE Assessment

- Decision based on the CI and the Acceptance Range (AR)
 - CI *entirely outside* the AR:
Bioinequivalence proven
 - CI *overlaps* the AR (lies *not entirely within* the AR):
Bioequivalence not proven – indecisive
 - CI lies *entirely within* the AR:
Bioequivalence proven

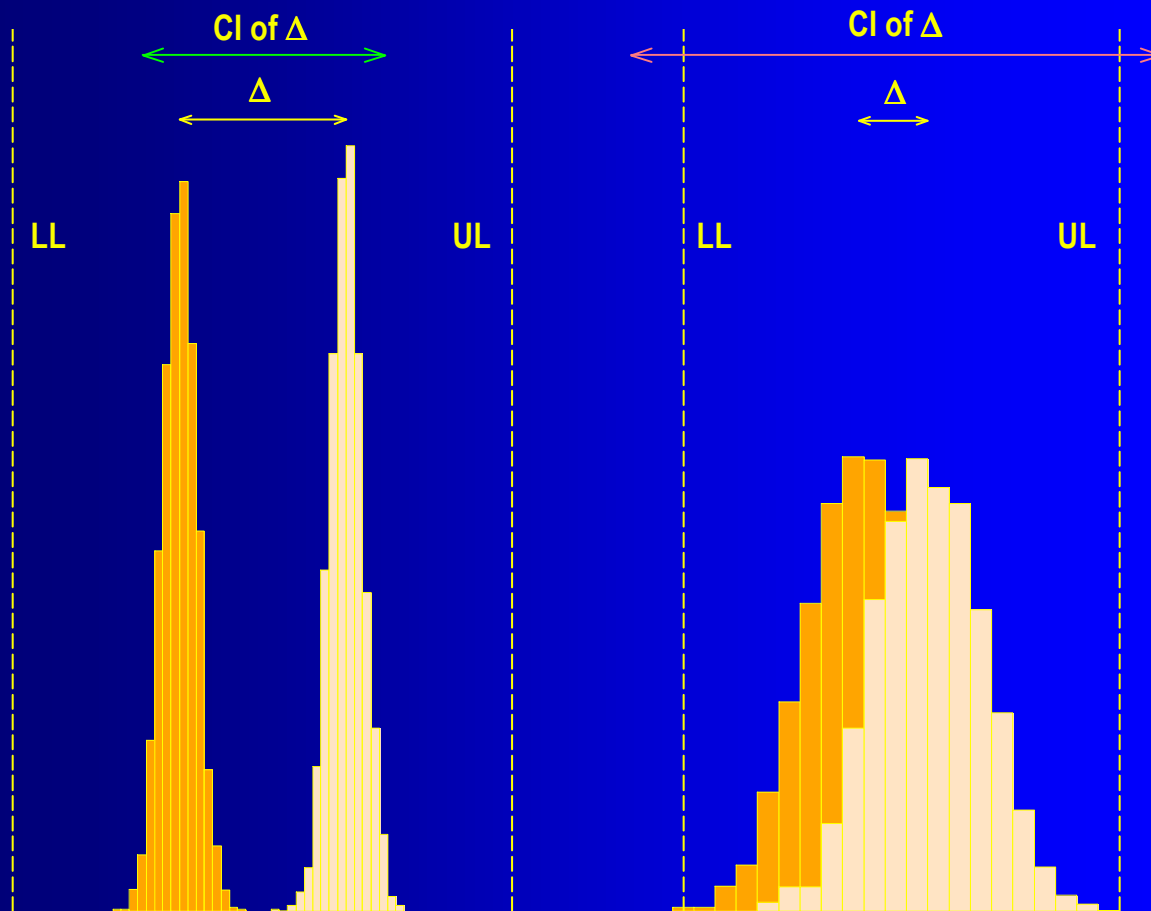
BE Assessment



Add-on / Two-Stage Designs

- Sometimes properly designed and executed studies fail due to
 - 'true' bioinequivalence,
 - poor study conduct (increasing variability),
 - pure chance (producer's risk hit),
 - false (over-optimistic) assumptions about variability and/or T/R-ratio.
- The patient's risk must be preserved
 - Already noticed at Bio-International Conferences (1989, 1992) and guidelines from the 1990s.

High variability...



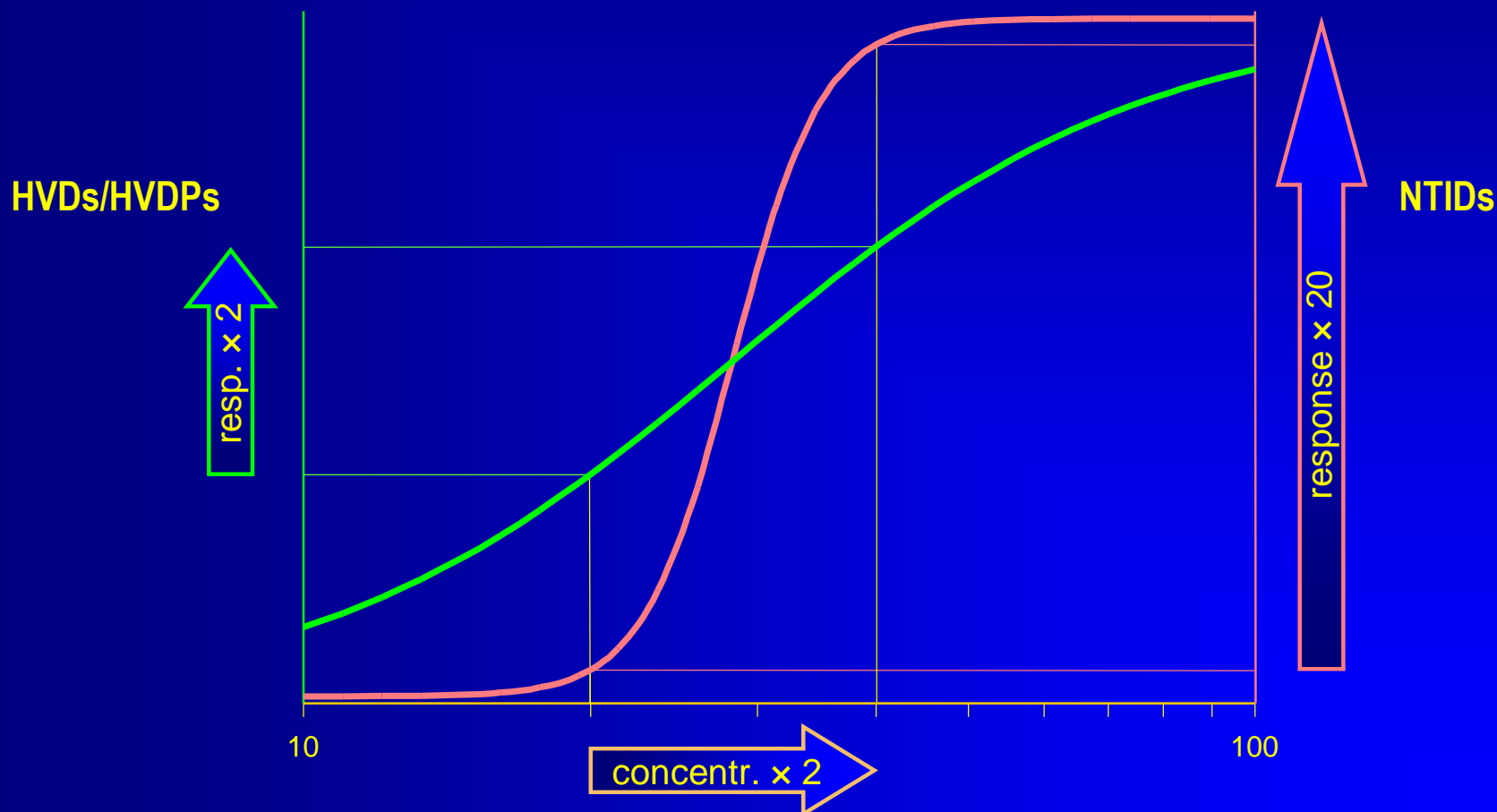
Modified from Fig. 1
Tothfaluasi *et al.* (2009)

Counterintuitive
concept of BE:

Two formulations with
a large difference in
means are declared
bioequivalent if vari-
ances are low, but not
bioequivalent – even if
the difference is quite
small – due to high
variability.

HVDs/HVDPs are safe

flat & steep PK/PD-curves



High variability

- For Highly Variable Drugs / Drug Products (HVDs/HVDPs) it may be almost impossible to show BE with a reasonable sample size.
- The common 2×2 cross-over design over assumes Independent Identically Distributions (IID), which may not hold. If e.g., the variability of the reference is higher than the one of the test, one obtains a high common (pooled) variance and the test will be penalized for the ‘bad’ reference.

Replicate designs

- Each subject is randomly assigned to sequences, where *at least one* of the treatments (generally the reference) is administered *at least twice*
 - Not only the *global within-subject variability*, but also the *within-subject variability per treatment* may be estimated.
 - *Smaller* subject numbers compared to a standard $2 \times 2 \times 2$ design – but outweighed by an increased number of periods.
 - *Same* overall number of individual treatments (biosamples to be analyzed)!

Replicate designs

- Any replicate design can be evaluated according to 'classical' (unscaled) Average Bioequivalence (ABE)
- ABE mandatory if scaling not allowed
 - FDA: $S_{WR} < 0.294$ ($CV_{WR} < 30\%$); different models depend on design (*i.e.*, SAS PROC MIXED for full replicate and PROC GLM for partial replicate).
 - EMA: $CV_{WR} \leq 30\%$; all fixed effects model according to 2011's Q&A-document preferred (*e.g.*, SAS PROC GLM).
 - Even if scaling is not intended or applicable, replicate designs give more information about formulation(s).

Application: HVDs/HVDPs

● $CV_{WR} > 30\%$

✓ USA Recommended in API specific guidances. Scaling for AUC and/or C_{max} acceptable, GMR 0.80 – 1.25; ≥ 24 subjects enrolled.

± EU Widening of acceptance range (only C_{max}) to maximum of 69.84 – 143.19%), GMR 0.80 – 1.25. Demonstration that $CV_{WR} > 30\%$ is not caused by outliers. Justification that the widened acceptance range is clinically not relevant.

Replicate designs

- Two-sequence three-period

T R T

R T R

- Two-sequence four-period

T R T R

R T R T

- and many others...

(FDA: TRR | RTR | RRT, aka 'partial replicate')

- The statistical model is complicated and depends on the actual design!

$$X_{ijkl} = \mu \cdot \pi_k \cdot \Phi_l \cdot s_{ij} \cdot e_{ijkl}$$

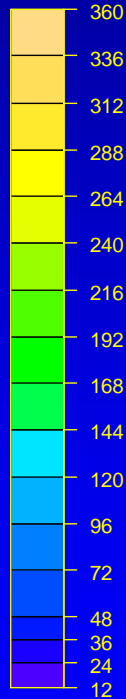
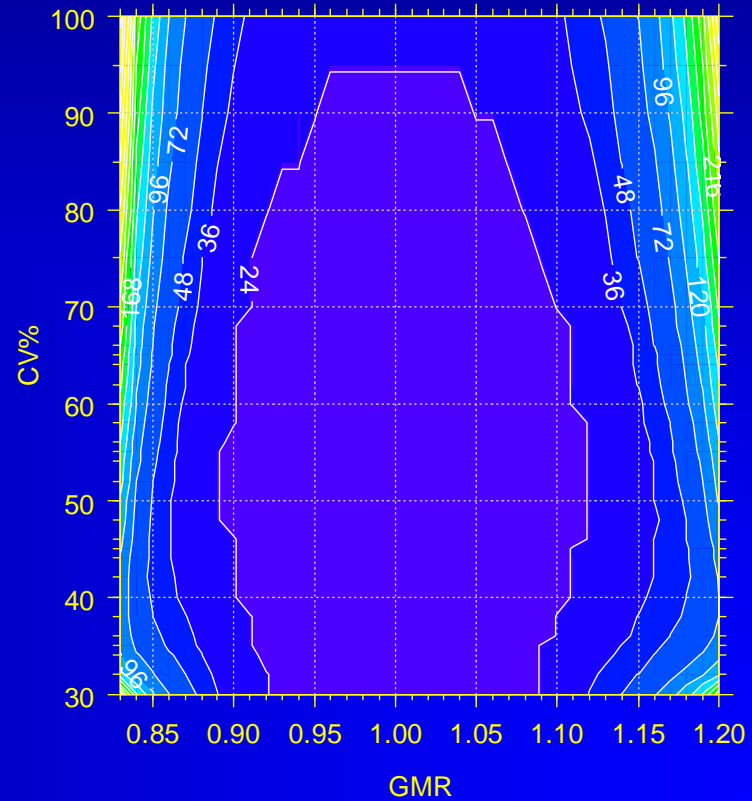
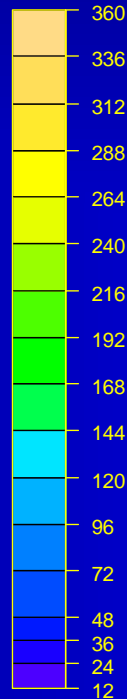
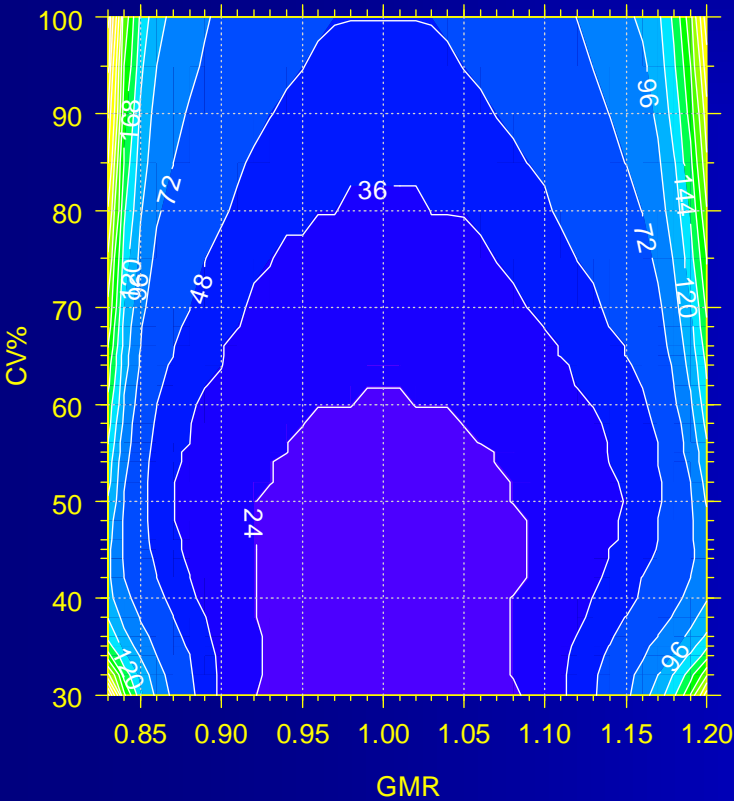
HVDPs (EMA/FDA; sample sizes)

RTTR | TRTR, 80% power, EMA

sample size

RTTR | TRTR, 80% power, FDA

sample size



HVDPs (EMA)

● EU GL on BE (2010)

■ Average Bioequivalence (ABE) with Expanding Limits (ABEL)

- Based on σ_{WR} (the *intra*-subject standard deviation of the reference formulation) calculate the scaled acceptance range based on the regulatory constant k ($\theta_s = 0.760$); limited at CV_{WR} 50%.

$$[L - U] = e^{\mp k \cdot \sigma_{WR}}$$

CV_{WR}	$L - U$
≤ 30	80.00 – 125.00
35	77.23 – 129.48
40	74.62 – 143.02
45	72.15 – 138.59
≥ 50	69.84 – 143.19

HVDPs (EMA)

- Q&A document (March 2011)
 - Two methods proposed (Method A preferred)
 - **Method A:** All effects fixed; assumes equal variances of test and reference, and no subject-by-formulation interaction; only a common within (*intra-*) subject variance is estimated.
 - **Method B:** Similar to A, but random effects for subjects. Common within (*intra-*) subject variance and between (*inter-*) subject variance are estimated.
 - **Outliers:** Boxplots (of model residuals?) suggested.

*Questions & Answers on the Revised EMA Bioequivalence Guideline
Summary of the discussions held at the 3rd EGA Symposium on Bioequivalence
June 2010, London
http://www.egagenerics.com/doc/EGA_BEQ_Q&A_WEB_QA_1_32.pdf*

Example datasets (EMA)

- Q&A document (March 2011)

- Data set I: Full replicate (RTRT | TRTR), 77 subjects, imbalanced, incomplete

- FDA

$s_{WR} 0.446 \geq 0.294 \rightarrow$ apply RSABE ($CV_{WR} 46.96\%$)

a. critbound $-0.0921 \leq 0$ and

b. PE $115.46\% \subset 80.00-125.00\%$ ✓

- EMA

➤ $CV_{WR} 46.96\% \rightarrow$ apply ABEL ($> 30\%$)

➤ Scaled Acceptance Range: 71.23–140.40%

➤ Method A: 90% CI 107.11–124.89% \subset AR; PE 115.66% ✓

➤ Method B: 90% CI 107.17–124.97% \subset AR; PE 115.73% ✓

Example datasets (EMA)

- Q&A document (March 2011)

- Data set II: Partial replicate (TRR | RTR | RRT), 24 subjects, balanced, complete

- FDA

$s_{WR} = 0.114 < 0.294 \rightarrow$ apply ABE ($CV_{WR} = 11.43\%$)
 90% CI 97.05–107.76% \subset AR ($CV_{intra} = 11.55\%$)



- EMA

➤ $CV_{WR} = 11.17\% \rightarrow$ apply ABE ($\leq 30\%$)

➤ Method A: 90% CI 97.32–107.46% \subset AR; PE 102.26%



➤ Method B: 90% CI 97.32–107.46% \subset AR; PE 102.26%



➤ A/B: $CV_{intra} = 11.86\%$

Thank You!

Basic Designs for BE Studies

Open Questions?



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To bear in Remembrance...

To call the statistician after the experiment is done may be no more than asking him to perform a *post-mortem* examination: he may be able to say what the experiment died of.

Ronald A. Fisher



[The] impatience with ambiguity can be criticized in the phrase:
absence of evidence is not evidence of absence.

Carl Sagan

[...] our greatest mistake would be to forget that data is used for serious decisions in the very real world, and bad information causes suffering and death.

Ben Goldacre

