SocraTec R&D
We make it work

Validation of pharmacodynamic methods and endpoints

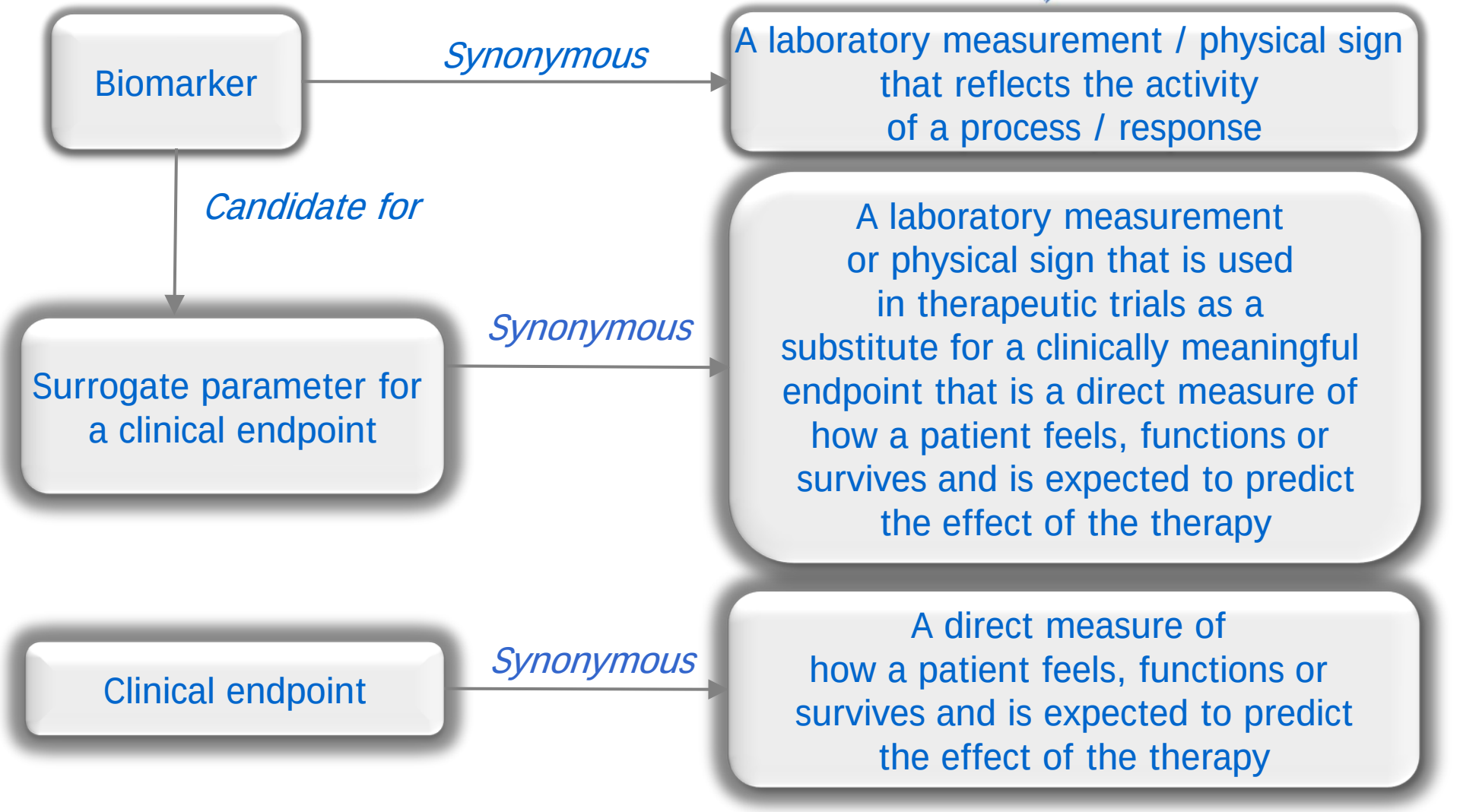
Challenges, strategies and solutions depending on the type of endpoint

Dr. Barbara Schug
SocraTec R&D, Oberursel , Germany
www.socratec-pharma.de

AGAH Workshop: Bad Homburg, May 16th, 2013

Definitions

SocraTec R&D



Katz. R., Am Soc Exp NeuroTherapeutics, 2004, 189-195

Biomarker qualification - NCEs

SocraTec R&D

Questions to be answered for biomarkers used in new drug development

- How can biomarker evidence help demonstrate that a candidate product is not too toxic to test in humans?
- How can biomarkers be used to select dose ranges for human initial testing?
- How can biomarkers be used most effectively to evaluate dose response in later trials?
- What biomarker evidence is appropriate to guide selection of patients for clinical testing?
- What types and levels of evidence are needed to accept a biomarker as a surrogate endpoint for product efficacy?

FDA Critical Path Opportunity List, 2006

Surrogate in NCEs

SocraTec R&D

Relevance of the surrogate in NCE development

- shortens the development time
- is expected to allow prediction of clinical effect even though the drugs (and not only the exposure) are different
- often useful for Me-Toos
- difficult for “first-in-class” drugs

“ *The difference between a surrogate and a true endpoint is like the difference between a cheque and cash. You can often get the cheque earlier, but then, of course, it may bounce*.”

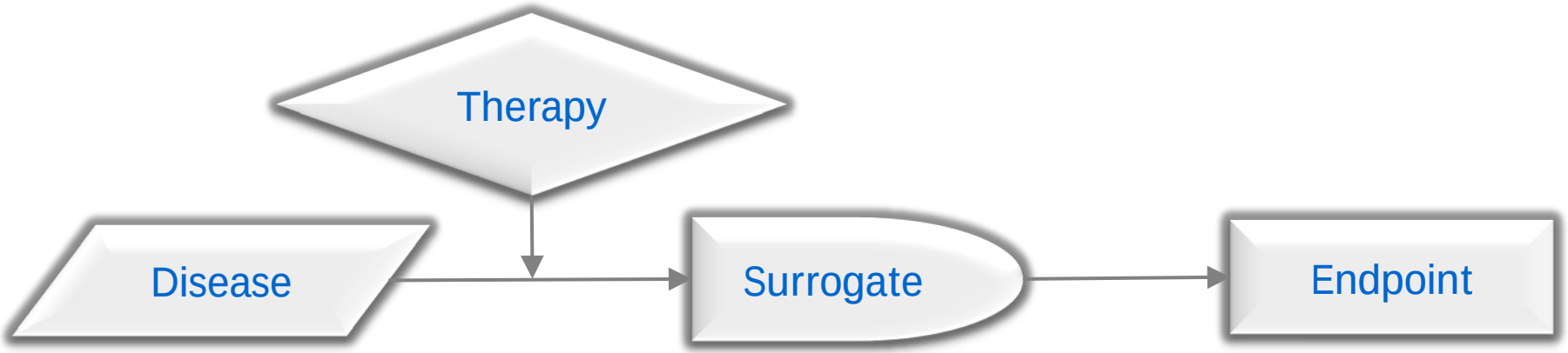
Stephen Senn, 1997

Surrogate for clinical endpoint

SocraTec R&D

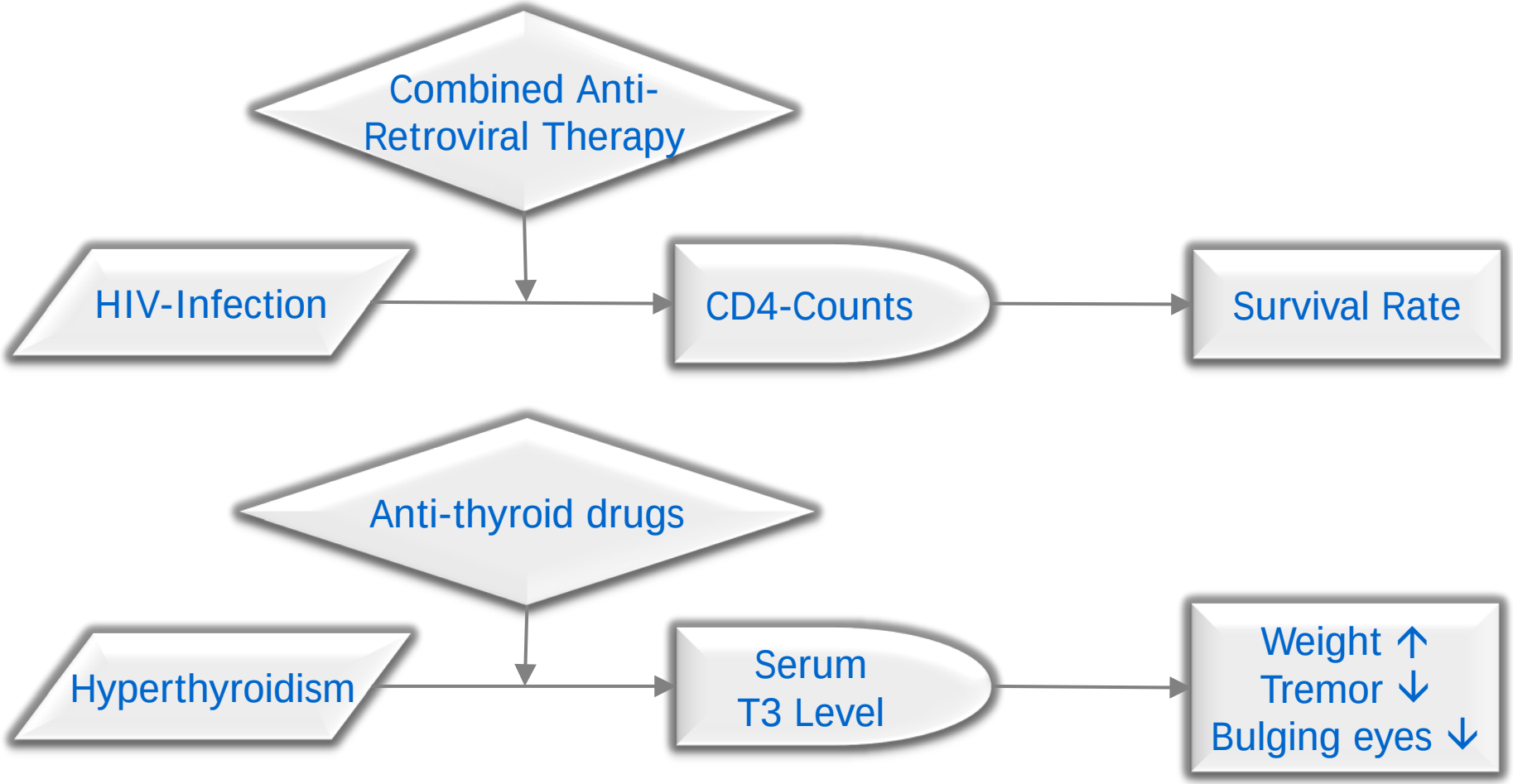
Validated surrogate parameters

- a biomarker for which evidence has been established that a drug–induced effect on the surrogate predicts or results in the desired effect on the clinical outcome of interest



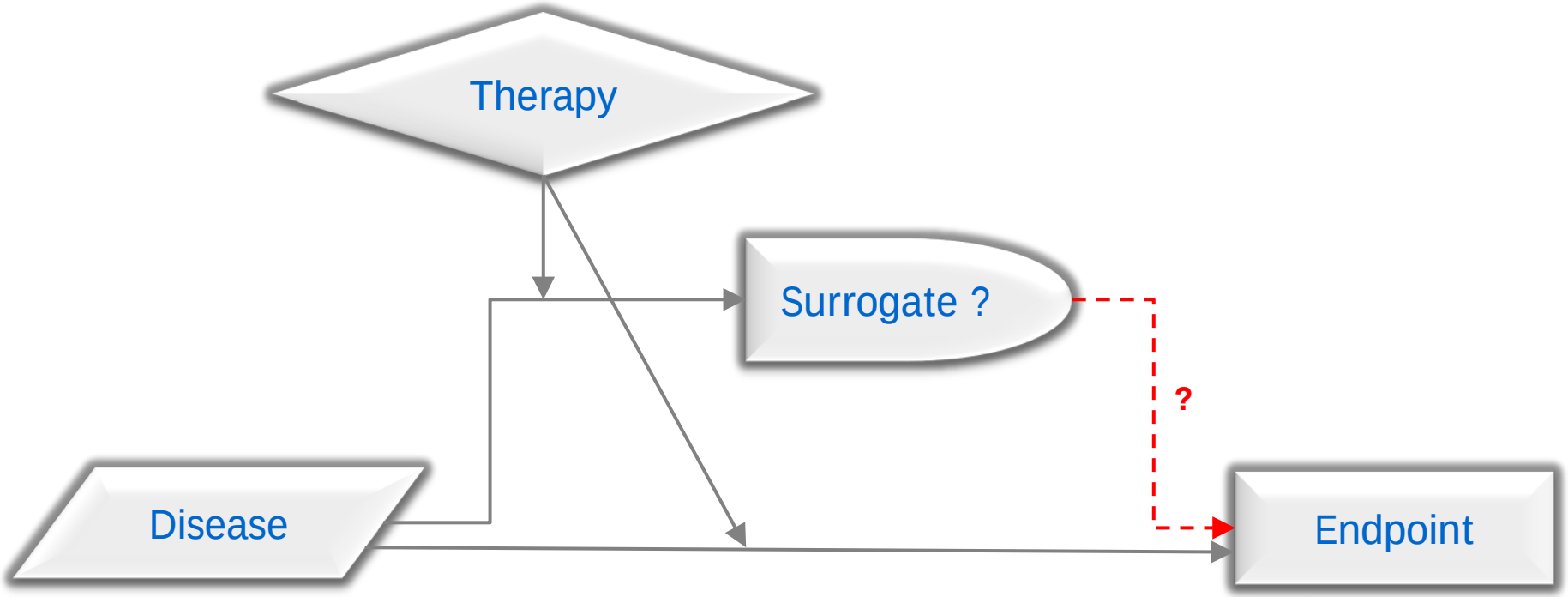
Examples: surrogate parameters

SocraTec R&D



Biomarker-to-Surrogate failure I

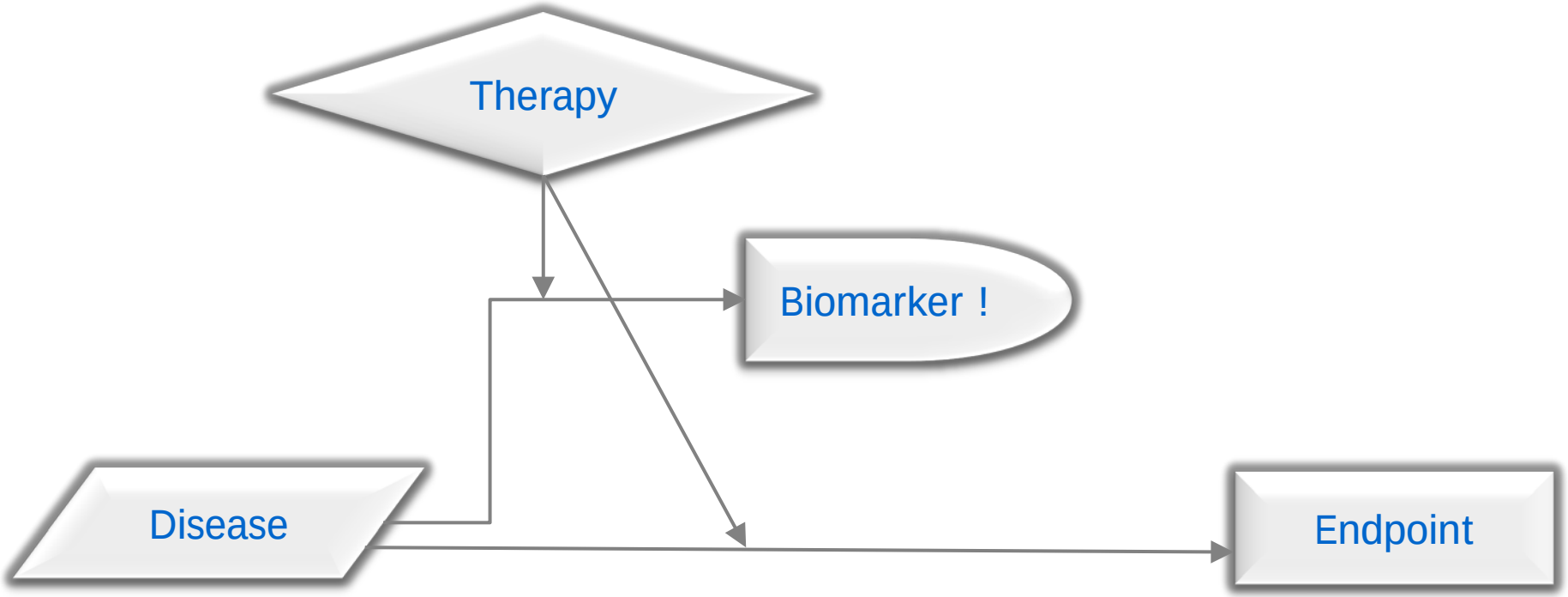
SocraTec R&D



The surrogate parameter is not on the pathway

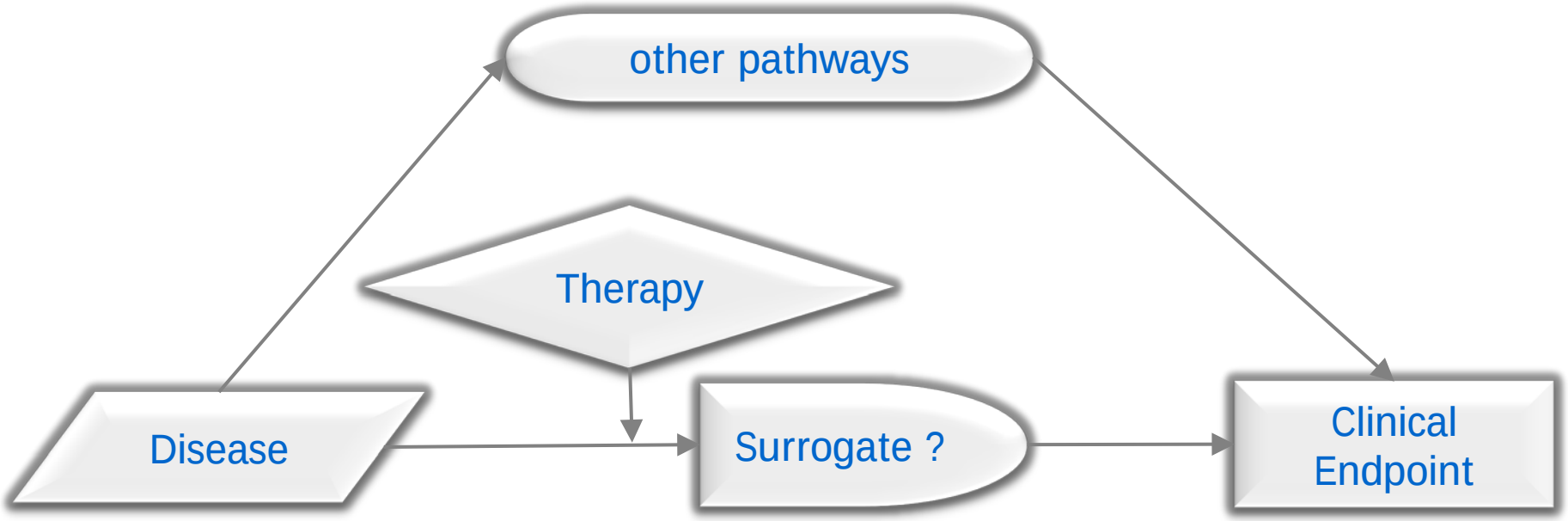
Biomarker-to-Surrogate failure I

SocraTec R&D



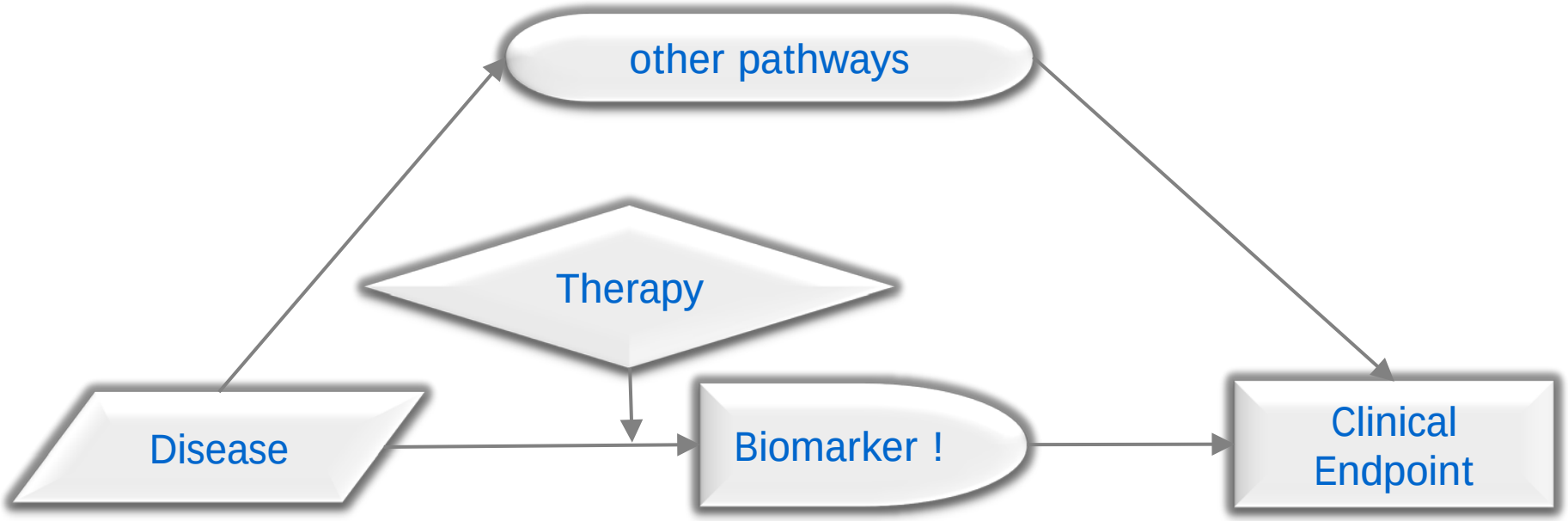
Biomarker-to-Surrogate failure II

SocraTec R&D



Biomarker-to-Surrogate failure II

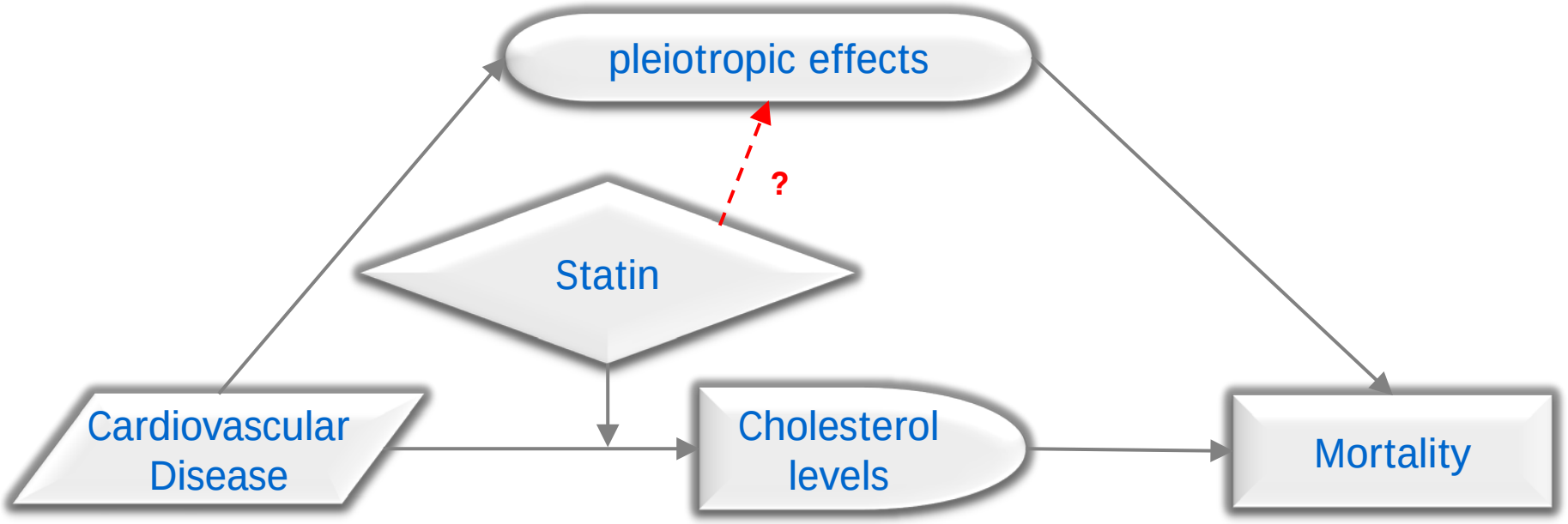
SocraTec R&D



There are other relevant pathway(s) beside

Surrogate parameters: Examples

SocraTec R&D



Lowering cholesterol levels reduces mortality but other effects of statins may also contribute

Biomarker qualification: generics

SocraTec R&D

Questions to be answered for biomarkers to be used as surrogate for bioequivalence assessment

- Is the analytical / clinical method to be used for quantification adequately validated?
- Is the biomarker sensitive to drug concentration
  - at the site of action for locally acting drugs
  - in the systemic circulation for systemically acting drugs
- How to justify acceptance limits?

Note: The questions to be answered are completely different compared to the use as surrogate for the clinical endpoint

Biomarker: the mathematicians view

SocraTec R&D

- **binary (dichotomous):** biomarker value below or above a certain threshold (e.g., CD4þ counts over 500=mL) or clinical 'success' (e.g., tumor shrinkage);
- **categorical (polychotomous):** biomarker value falling in successive, ordered classes (e.g., cholesterol levels <200mg=dL, 200–299mg=dL, 300þ mg=dL) or clinical response (e.g., complete response, partial response, stable disease, progressive disease);
- **continuous (Gaussian):** biomarker (e.g., log PSA level) or clinical measurement (e.g., diastolic blood pressure);
- **censored continuous:** time to biomarker below or above a certain threshold (e.g., time to undetectable viral load) or time to clinical event (e.g., time to cardiovascular death);
- **longitudinal or repeated measures:** biomarker (e.g., CD4 counts over time) or clinical outcome (e.g., blood pressure over time);
- **multivariate longitudinal:** several biomarkers (e.g., CD4 and viral load over time) or several clinical measurements (e.g., dimensions of quality of life over time).

G. Molenberghs et al., 2004

Preference cascade for BE assessment

SocraTec R&D

1	Concentration of the active ingredient/ active moiety in systemic circulation
2	In-vitro test with proven correlation to concentration in systemic circulation
3	Urinary excretion of active moiety/ metabolite over time
4	Appropriate acute pharmacological effect of the active moiety as a function of time
5	Controlled clinical trial to establish safety and effectiveness

FDA-CFR 21 Sec 320.24

General requirements

SocraTec R&D

All testing procedures regarding BE need to be

- sufficiently accurate
- sufficiently sensitive
- sufficiently reproducible

Sensitivity regarding product differences is expected to decrease

- from PK ...
- ... over PD ...
- ... to clinical endpoints

The approach of performing well-controlled clinical trials that establish the safety and effectiveness of a drug product is the least accurate, sensitive and reproducible one (FDA-position)

Validation – analytical method

SocraTec R&D

(Bio-)analytically determined parameters

- Guideline on bioanalytical method validation (EMEA/ CHMP/ EWP/ 192217/2009)
- Workshop/ Conference Report – Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assay (AAPS Journal 2007:9; Viswanathan et al), i. E. “White Paper”
- Guidance for Industry: Bioanalytical method validation, US-FDA 2001

Well-established principles for assessment of selectivity accuracy, precision, recovery, calibration curves, stability and – meanwhile – incurred sample analysis

Locally acting/ locally applied

SocraTec R&D

Drug substance	Indication	Mechanism of action	BE-Surrogate
Colestilan, aluminiumhydroxide, calcium carbonate, etc.	Hyperphosphat-emia in patients with renal impairment	Phosphate binder (ion exchange resin or formation of insoluble salts)	Plasma/ serum phosphate levels
Cholestyramin	Hypercholesterin-emia	Ion exchange resin for binding of anionic bile acids	Plasma/ Serum cholesterol levels
Acarbose	Diabetes mellitus type 2	Inhibition of α-glucosidase	Plasma / serum glucose and insulin levels
Orlistat	Obesity	Inhibition of pancreatic lipases	Faecal fat excretion

Measurable biomarkers

SocraTec R&D

Non-PK analytical methods

- in the past it was very common to use clinical laboratory methods with a validation level applied to clinical chemistry
- meanwhile the requirements for such methods / laboratories have been extended to a GLP level based on the EMA-Reflection Paper
- method validation and quality control during measurements have to fulfil the criteria commonly applied to bioanalytical methods (sometimes difficult)

Examples

- cholesterol, fat in faeces, phosphate, insulin, glucose, NO, CD4 counts etc.

Specificities of endogenous substances often rise problems

Validation – others than PK-samples

SocraTec R&D

New regulations for all clinical trial samples

- requirements for the lab itself
  - organisation
  - personnel
  - contracts
  - trial conduct
  - patient/ subject protection and informed consent
  - chain of custody
- requirements regarding the analysis
  - method validation
  - repeat analysis
  - quality control

Reflection paper on guidance for laboratories (EMA/ INS/ GCP/ 532 137/ 2010)

Analysis of clinical trial samples

SocraTec R&D

"Clinical trial samples means any biological sample collected from a participant in a clinical as required by the protocol. Samples may include but are not limited to: blood, plasma, serum, urine, faeces, tissues and cells."

"The analysis of samples collected from subjects participating in clinical trials forms a key part of the clinical trials process. Sample analysis or evaluation provides important data on a range of endpoints which is used, for example, to assess the pharmacokinetic profile of investigational Medicinal products and to monitor their safety and efficacy. Consequently, it is essential that sample analysis or evaluation is performed to an acceptable standard which will ensure patient safety is not compromised and that data is reliable and accurately reported."

Reflection paper on guidance for laboratories (EMA/ INS/ GCP/ 532 137/ 2010)

Non-analytical determination

SocraTec R&D

Example: skin blanching test for topical steroids

- systemic availability not relevant as product characteristic
- topical effects: correlation between potency/ local availability and vasoconstriction
- first published by Roger Williams (FDA) in 1992 proposing Stoughton-McKenzie vasoconstrictor assay

Stoughton – McKenzie test

SocraTec R&D

General principles of the test

- pharmacology of (topical) corticosteroids
  - modification of function of dermal cells and leukocytes
  - interference with phospholipase A2 (arachidonic acid ↓, interleukin ↓)
  - anti-inflammatory, immunosuppressive, anti-mitogenic
  - and corticosteroids produce blanching/ vasoconstriction
- biomarker characteristic vasoconstriction depends on
  - potency of the drug substance
  - amount of drug delivered to the site of action
  - duration of exposure

But how to realise measurements?

FDA – Guidance for Industry (1997)

SocraTec R&D

Accuracy, precision and sensitivity

- to be determined in the study population
  - analogously to bioanalytical assay
  - use of untreated controls as well as calibrators
  - different potency classes / modification of dose duration

➡ Effect is population dependent (responders !)

- use of human observer: technically “old-fashioned”
- commercially available chromameter/ colorimeter
- nowadays: digital imaging techniques considered

➡ EMA still recommends visual or chromameter - based methods

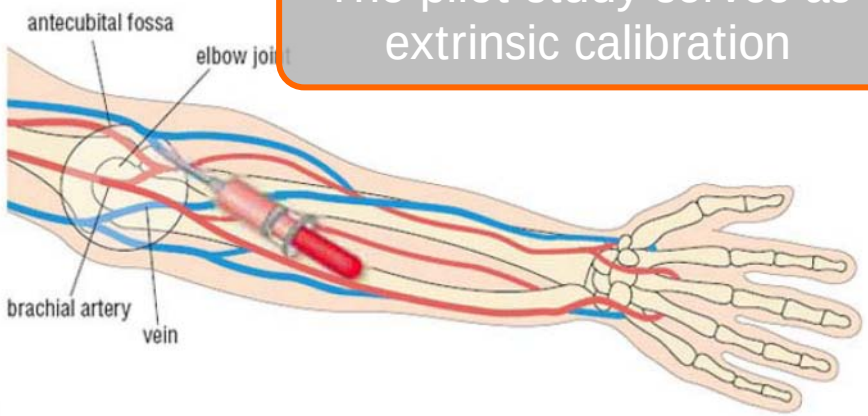
Pivotal study

SocraTec R&D

Design depends on data from pilot study

- duration commonly 0,25 to 6 hours
- $D_1 \approx 0{,}5 * ED_{50}$; $D_2 \approx 2 * ED_{50}$

The pilot study serves as extrinsic calibration



General procedures

- exposure to the arm, skin sites not closer than 3-4 cm to the antecubital fossa or to the wrist
- untreated control sites on each arm
- measurements at various times over 24h
- dose duration – response data should be modelled (either nonlinear mixed effect modelling method or a naive pooled data method) ⇨ ED_{50}
- ED_{50} value serves as approximate dose duration for BE comparison

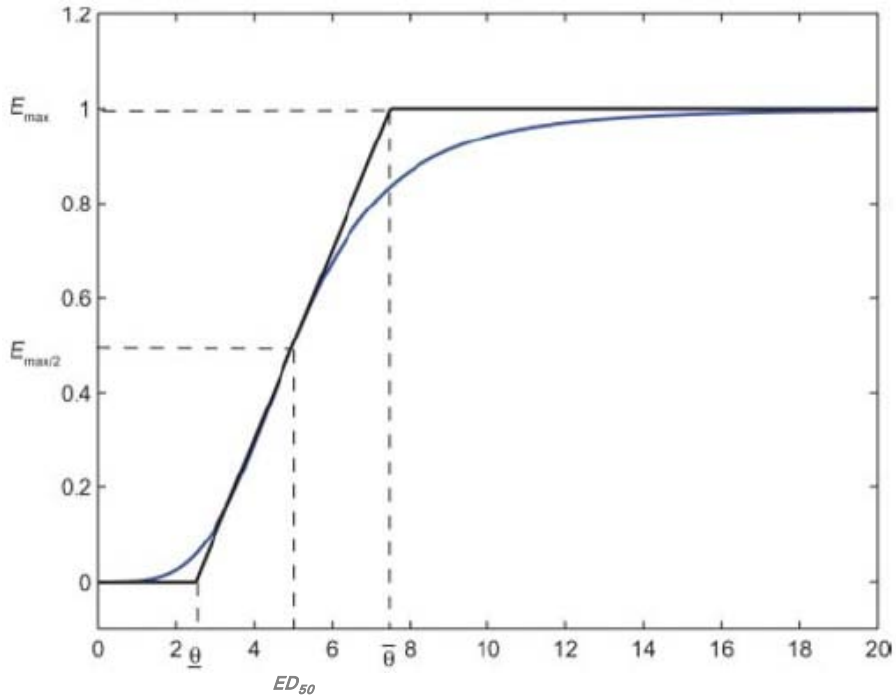
FDA – Guidance for Industry (1997!)

SocraTec R&D

Study design for validation

- pilot dose duration – response study
  - conducted with reference only
  - application of E_{max}-model
    $E = E_0 + E_{max} * d/(ED_{50} + d)$

    E_0 baseline effect
    E_{max} maximal effect
    D dose at ED_{50}
    ED_{50} half-maximal effect



Intention: The drugs shall be tested in the steep (≙ linear) part of the sigmoidal E_{max} – model in order to end-up with a good discriminatory potency ⇨ linearity of the assay

Li et al. Cancer Inform 2012

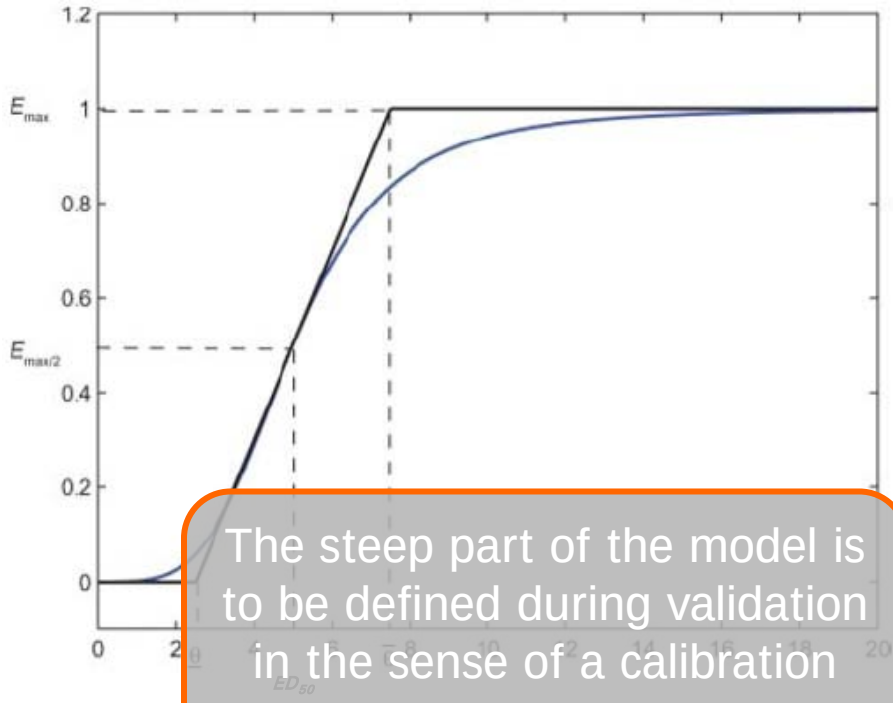
FDA – Guidance for Industry (1997!)

SocraTec R&D

Study design for validation

- pilot dose duration – response study
  - conducted with reference only
  - application of E_{max}-model
    $E = E_0 + E_{max}*d/(ED_{50} + d)$

    E_0 baseline effect
    E_{max} maximal effect
    D dose at ED_{50}
    ED_{50} half-maximal effect



The steep part of the model is to be defined during validation in the sense of a calibration range

Intention: The drugs shall be tested in the steep (≙ linear) part of the sigmoidal E_{max} – model in order to end-up with a good discriminatory potency ⇨ linearity of the assay

Li et al. Cancer Inform 2012

Statistical analysis

SocraTec R&D

Data structure

- baseline adjusted data have to be used…
- …resulting in both positive and negative data

Statistical method for evaluation

- Locke s method for determination of the exact confidence interval from untransformed data
- details are given in Guidance for Industry

"The office of generic drugs has not determined at this time equivalence interval for bioequivalence. The Office recognizes that an equivalence interval wider than 80-125 % as a public standard, may be necessary pending evaluation of data submitted to the agency" (1997)

Locke CS, J Pharmacokinet Biopharm 1984, 649-655

Statistical analysis

SocraTec R&D

Data structure

- baseline adjusted data have to be used…
- …resulting in both positive and negative data

Statistical method for evaluation

- Locke s method for determination of the exact confidence interval from untransformed data
- details are given in Guidance for Industry

Later data show that 80-125 % can be met with acceptable effort

"The office of generic drugs has not determined at this time equivalence interval for bioequivalence. The Office recognizes that an equivalence interval wider than 80-125 % as a public standard, may be necessary pending evaluation of data submitted to the agency" (1997)

Locke CS, J Pharmacokinet Biopharm 1984, 649-655

Subjective method

SocraTec R&D

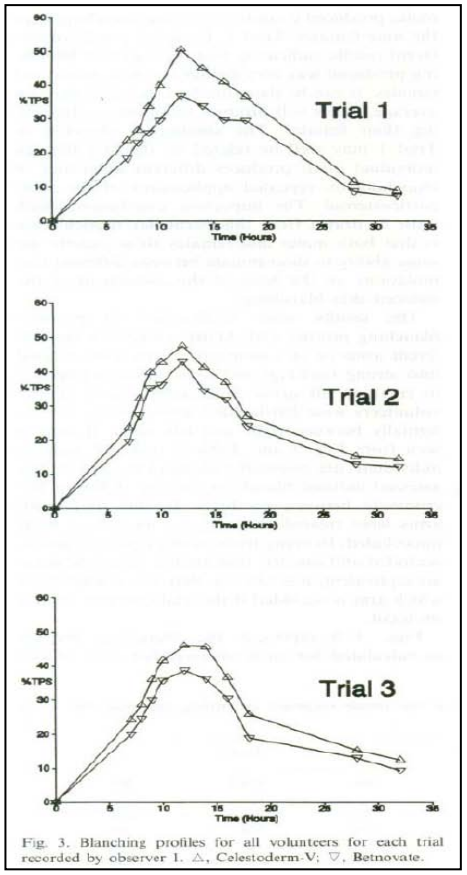


Fig. 3. Blanching profiles for all volunteers for each trial recorded by observer 1. △, Celestoderm-V; ▽, Betnovate.

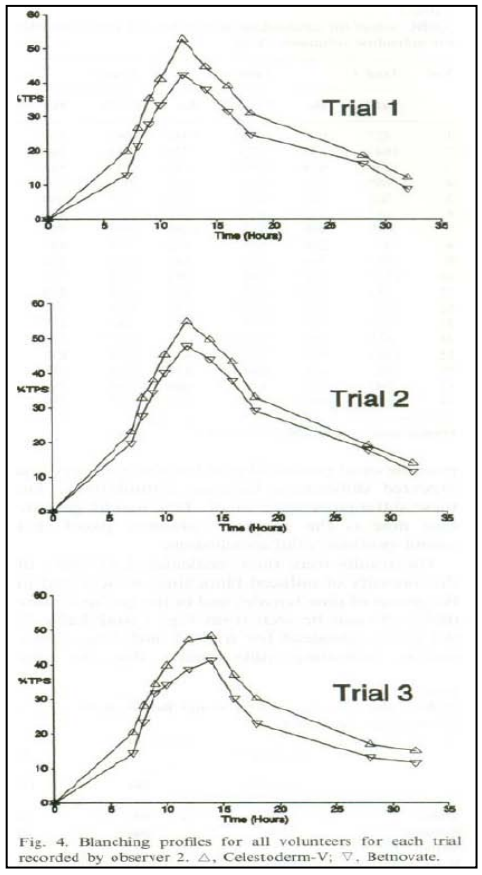


Fig. 4. Blanching profiles for all volunteers for each trial recorded by observer 2. △, Celestoderm-V; ▽, Betnovate.

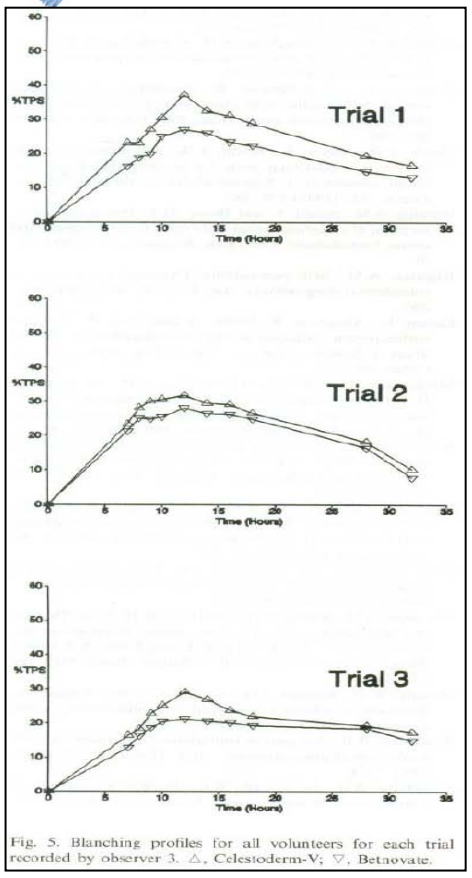


Fig. 5. Blanching profiles for all volunteers for each trial recorded by observer 3. △, Celestoderm-V; ▽, Betnovate.

Blanching profiles for all volunteers for each trial recorded by three different observers with identical test and reference products, identical subjects and identical application sites (visual assessment)

John M. Haigh et al., „The human skin blanching assay for in vivo topical corticosteroid assessment II. Subject- and observer-dependent variation in blanching responses"

Subjective method

SocraTec R&D

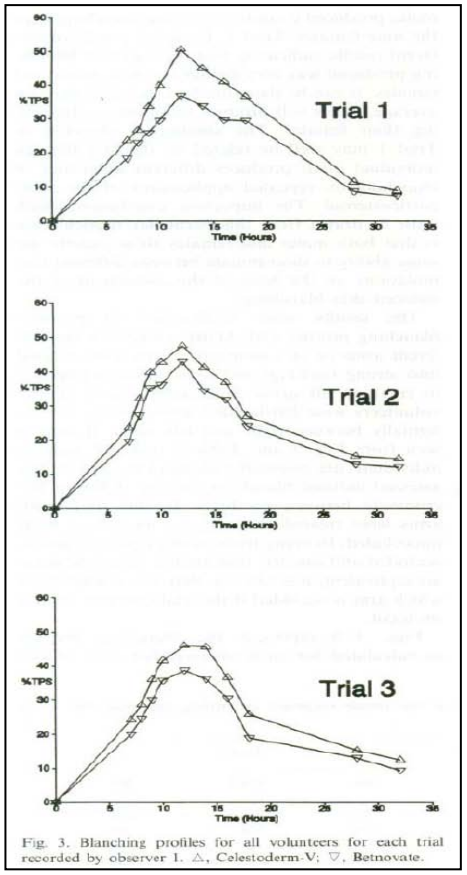


Fig. 3. Blanching profiles for all volunteers for each trial recorded by observer 1. △, Celestoderm-V; ▽, Betnovate.

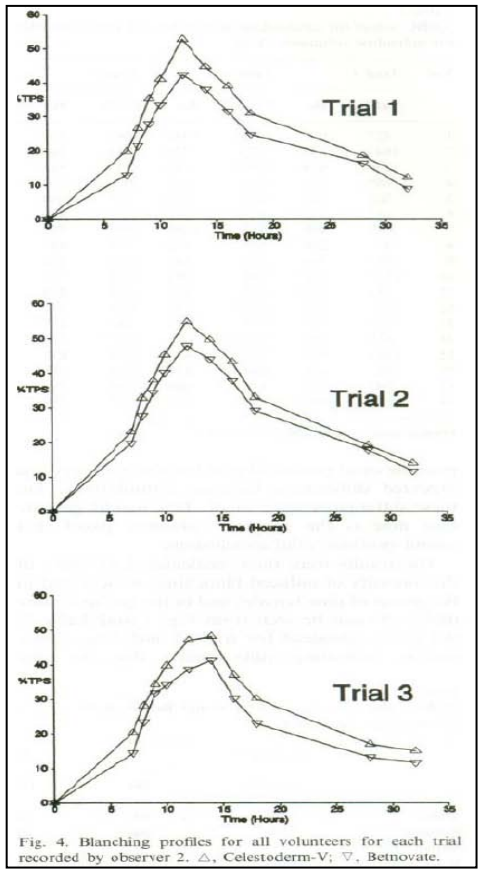
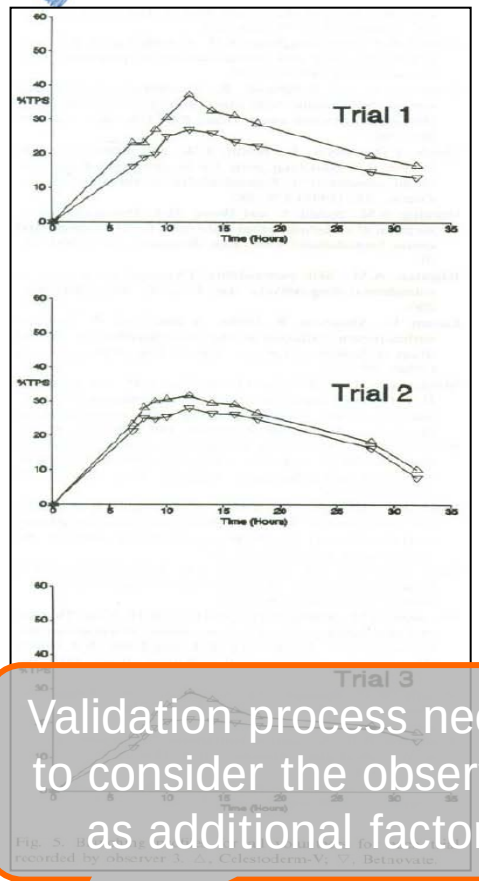


Fig. 4. Blanching profiles for all volunteers for each trial recorded by observer 2. △, Celestoderm-V; ▽, Betnovate.



Validation process needs to consider the observer as additional factor

Blanching profiles for all volunteers for each trial recorded by three different observers with identical test and reference products, identical subjects and identical application sites (visual assessment)

John M. Haigh et al., „The human skin blanching assay for in vivo topical corticosteroid assessment II. Subject- and observer-dependent variation in blanching responses"

Data are highly method dependent II

SocraTec R&D

Comparison of visual assessment and chromameter

	Confidence Interval Visual assessment	**Confidence Interval Chromameter**
"detectors" acc to FDA definition (n=23)	99.3-111.6 %	86.5-129.3 %
all subjects (n=34)	97.9-109.2 %	90.2-120.7 %

Validation includes the population characteristics

Validation includes the method of quantitation

Au et al, J Pharm Sci, 2008, 11, 160-166

Equivalence surrogate safety / efficacy

SocraTec R&D

Inhaled corticosteroids result in

- reduced inflammation of the airways
  - to be determined by e.g. exhaled NO
- hypothalamic-pituitary axis depression
  - to be determined by quantitation of cortisol suppression

Both methods require different types of “validation”

- validation of the sensitivity of the assays regarding adequate detection of product differences
- validation of the sample handling for NO
- validation of the analytical methods for quantitation of NO and cortisol

Validation of the biomarker FENO

SocraTec R&D

FENO ≙ Fractional Concentration of Exthaled Nitric Oxide

- inducible NOsynthase (iNOS) is increased in airway inflammation
- FENO is increased in (some!) asthma patients
- iNOS is expressed in the respiratory epithelium as response to cytokines from macrophages and lymphocytes
- corticosteroids modulate expression of iNOS

FENO measures mainly inflammation whereas other parameters as e.g. FEV_1 characterise obstruction

Questions to be answered

- population characteristics?
- dose – response?
- reproducibility?
- acceptable Δ?

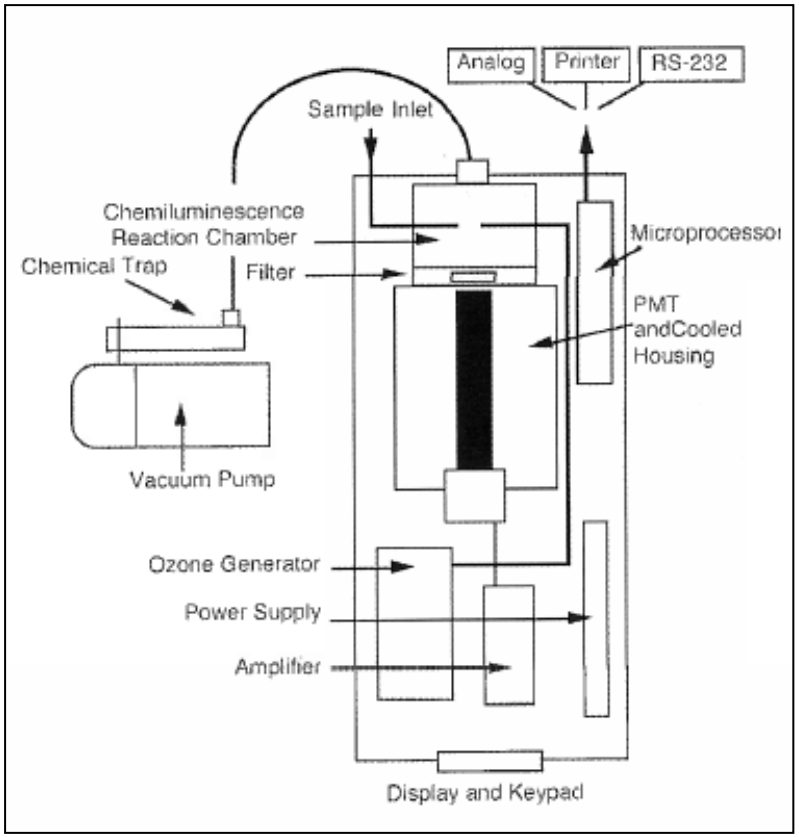
Analytical determination of NO

SocraTec R&D

Gas-based chemi-luminescent reaction

$$NO + O_3 \rightarrow NO_2^* + O2$$
$$NO_2^* \rightarrow NO_2 + h\upsilon$$

Detection by a red-sensitive photo-multiplier tube



Schematic presentation of Sievers Nutric Oxide Analyzer NOA 280i for research purposes

Validation of the analytical method

SocraTec R&D

Specifications according to operation manual

- sensitivity: 1 ppb
- range: < 1 – 500,000 ppb
- response time: 67 msecs (lag time 1 sec.)
- repeatability: 5 %
- sample size: 10 – 300 ml/ min

Calibration for measurements

- recommended: once daily, as dependent on ambient temperature, humidity, flow, etc.
  - measurement of “zero air” (background signal to be subtracted)
  - measurement of known concentration typically a gas containing 10 to 100 ppm
  - ⇨ analyzer automatically calculates the response for the ppm-range

The steps to be realised according to the manual do not cover the requirements of the EMA-guideline for bioanalytical measurements!

Adequate validation?

SocraTec R&D

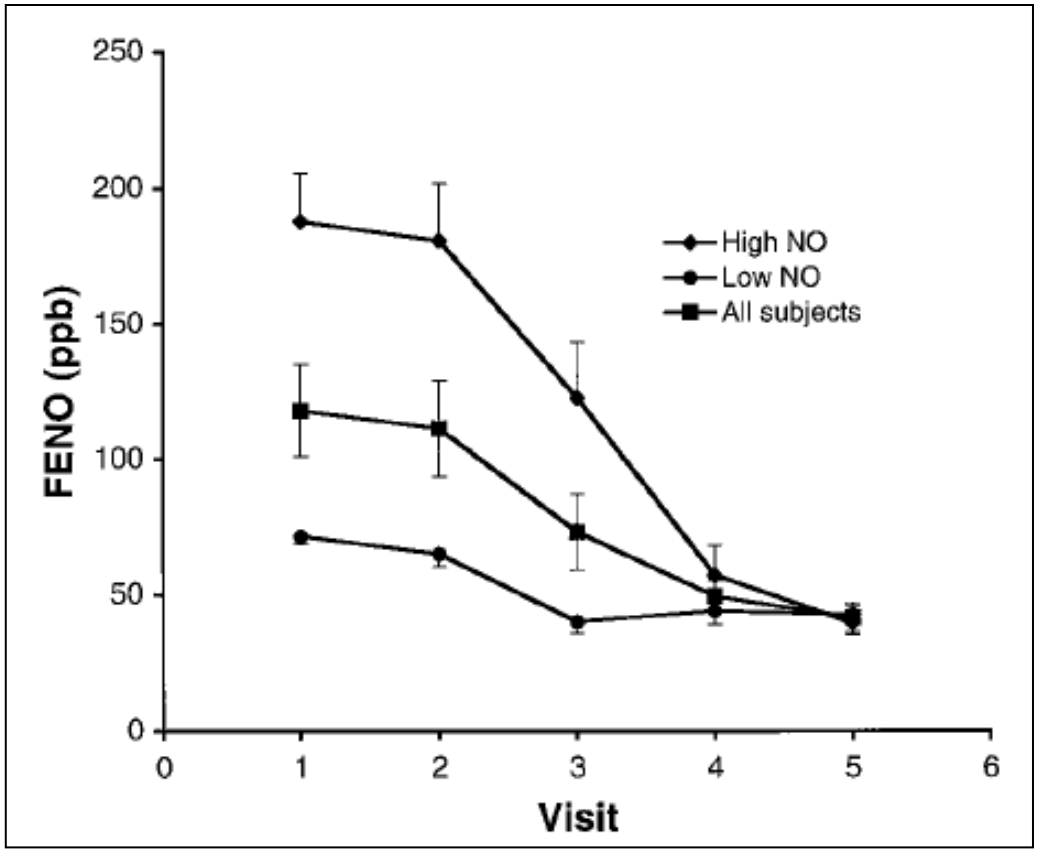
Additional measurements needed

- further data to be provided by manufacturer ?
- additional pre-study validation using calibrator gases covering the analytical range ?
- additional daily re-calibrations ?

Meaningful validation procedure needs to be established and justified!

Population characteristics

SocraTec R&D

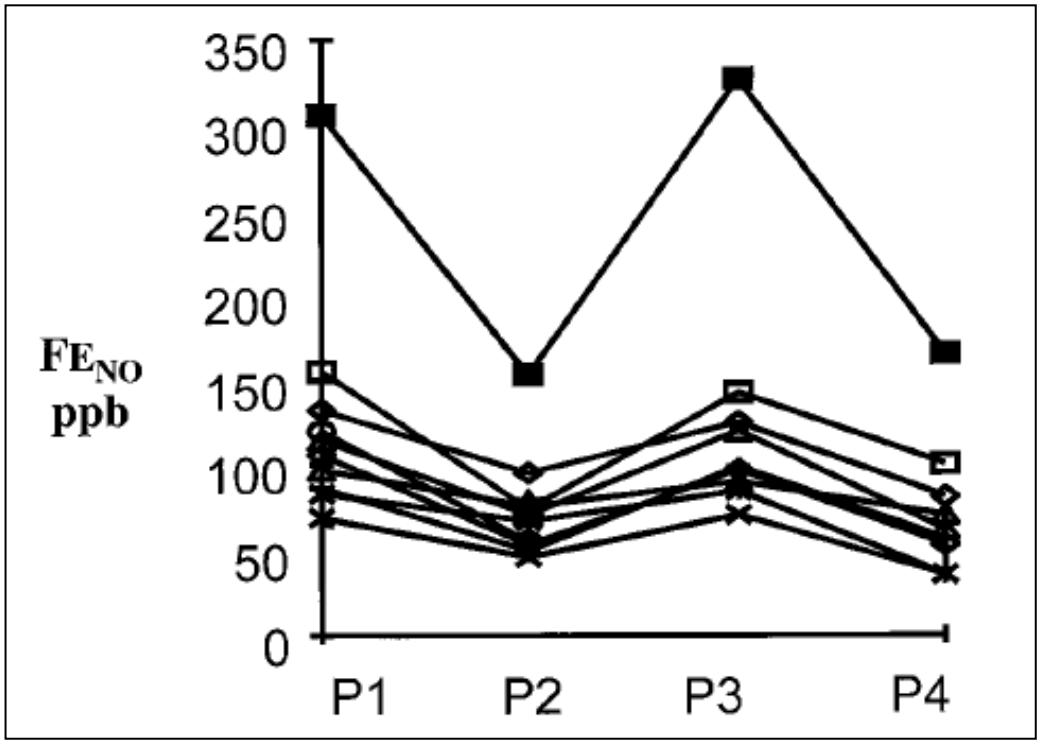


Change in FENO at each visit corresponding to baseline, placebo treatment and increasing doses of inhaled budesonide proprionate (100 µg/d; 400 µg/d; 800 µg/d)

Silkoff et al. 2001 Chest 119: 1322-1328

Reproducibility

SocraTec R&D



Change of FENO after 2 days treatment in 4 periods separated by wash-out periods (200 µg/d)

Open question: acceptance criteria for equivalence assessment?

Silkoff et al. 2001 Chest 119: 1322-1328

Conclusion

SocraTec R&D

Selection and validation of BE surrogates

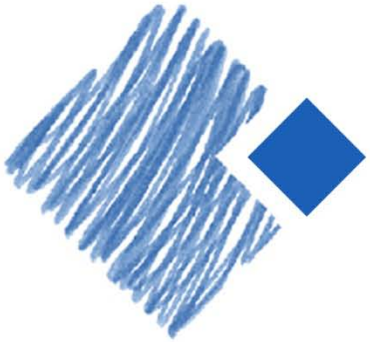
- general suitability of the parameter:
  - correlation with concentration at the site of action
  - reproducible
- selection of adequate range of the method
  - ability to discriminate between products
- validation of the method for determination
  - “classical” bioanalytical method validation or
  - validation of all critical parameters of the method

Adequate quality control during measurements

- adequate calibration
- adequate QC-samples

Acceptance criteria

- 80.00 – 125.00 %
- or clinically justified margins



SocraTec R&D

We make it work

Concepts in Drug Research and Development

Contact:

Prof. Dr. Henning Blume
Phone: +49-6171-5857-120
henning.blume@socratec-pharma.de

Dr. Barbara Schug
Phone: +49-6171-5857-111
barbara.schug@socratec-pharma.de

André Warnke
Phone: +49-6171-5857-122
andre.warnke@socratec-pharma.de

Fax +49-6171-5857-25; Postal Address: Im Setzling 35, 61440 Oberursel (Taunus), Germany