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

Biomarker

Synonymous

Candidate for

Surrogate parameter for a clinical endpoint

Synonymous

Clinical endpoint

Synonymous

A laboratory measurement / physical sign that reflects the activity of a process / response

A laboratory measurement or physical sign that is used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions or survives and is expected to predict the effect of the therapy

A direct measure of how a patient feels, functions or survives and is expected to predict the effect of the therapy

Biomarker qualification - NCEs

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?
Surrogate in NCEs

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
**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

- HIV-Infection
- CD4-Counts
- Survival Rate

- Hyperthyroidism
- Serum T3 Level
- Weight ↑
- Tremor ↓
- Bulging eyes ↓
Biomarker-to-Surrogate failure 1

The surrogate parameter is not on the pathway
Biomarker-to-Surrogate failure I
Biomarker-to-Surrogate failure II

- Disease
- Surrogate?
- Clinical Endpoint
- Therapy
- Other pathways
Biomarker-to-Surrogate failure II

There are other relevant pathway(s) beside
Lowering cholesterol levels reduces mortality but other effects of statins may also contribute.
Biomarker qualification: generics

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

- **binary (dichotomous):** biomarker value below or above a certain threshold (e.g., CD4⁺ 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, 300⁺ 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

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

*FDA-CFR 21 Sec 320.24*
General requirements

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

(Bio-)analytically determined parameters

- Guideline on bioanalytical method validation (EMEA/ CHMP/ EWP/ 192217/2009)

Well-established principles for assessment of selectivity, accuracy, precision, recovery, calibration curves, stability and – meanwhile – incurred sample analysis
<table>
<thead>
<tr>
<th>Drug substance</th>
<th>Indication</th>
<th>Mechanism of action</th>
<th>BE-Surrogate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colestilan, aluminiumhydroxide, calcium carbonate, etc.</td>
<td>Hyperphosphatemia in patients with renal impairment</td>
<td>Phosphate binder (ion exchange resin or formation of insoluble salts)</td>
<td>Plasma/ serum phosphate levels</td>
</tr>
<tr>
<td>Cholestyramin</td>
<td>Hypercholesterinemia</td>
<td>Ion exchange resin for binding of anionic bile acids</td>
<td>Plasma/ Serum cholesterol levels</td>
</tr>
<tr>
<td>Acarbose</td>
<td>Diabetes mellitus type 2</td>
<td>Inhibition of α-glucosidase</td>
<td>Plasma / serum glucose and insulin levels</td>
</tr>
<tr>
<td>Orlistat</td>
<td>Obesity</td>
<td>Inhibition of pancreatic lipases</td>
<td>Faecal fat excretion</td>
</tr>
</tbody>
</table>
Measurable biomarkers

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

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

“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.”
Non-analytical determination

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

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?
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

Design depends on data from pilot study
- duration commonly 0.25 to 6 hours
- $D_1 \approx 0.5 \times ED_{50}$; $D_2 \approx 2 \times ED_{50}$

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) $\Rightarrow ED_{50}$
- $ED_{50}$ value serves as approximate dose duration for BE comparison
FDA – Guidance for Industry (1997!)

Study design for validation
- pilot dose duration - response study
  - conducted with reference only
  - application of $E_{\text{max}}$-model
    \[ E = E_0 + E_{\text{max}} \cdot \frac{d}{(ED_{50} + d)} \]

  - $E_0$ baseline effect
  - $E_{\text{max}}$ 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 $E_{\text{max}}$ – model in order to end-up with a good discriminatory potency ⇔ linearity of the assay

Li et al. Cancer Inform 2012
Study design for validation

- pilot dose duration - response study
  - conducted with reference only
  - application of $E_{\text{max}}$-model
    \[ E = E_0 + E_{\text{max}} \cdot \frac{d}{(ED_{50} + d)} \]

  - $E_0$: baseline effect
  - $E_{\text{max}}$: maximal effect
  - $D$: dose at $ED_{50}$
  - $ED_{50}$: half-maximal effect

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

Li et al. Cancer Inform 2012
**Statistical analysis**

**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)
Statistical analysis

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)

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

Locke CS, J Pharmacokinet Biopharm 1984, 649-655
Subjective method

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

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)

Validation process needs to consider the observer as additional factor

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

Comparison of visual assessment and chromameter

<table>
<thead>
<tr>
<th></th>
<th>Confidence Interval</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visual assessment</td>
<td>Chromameter</td>
</tr>
<tr>
<td>“detectors” acc to FDA definition (n=23)</td>
<td>99.3-111.6 %</td>
<td>86.5-129.3 %</td>
</tr>
<tr>
<td>all subjects (n=34)</td>
<td>97.9-109.2 %</td>
<td>90.2-120.7 %</td>
</tr>
</tbody>
</table>

Validation includes the population characteristics

Validation includes the method of quantitation

Equivalence surrogate safety / efficacy

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

FENO ≜ Fractional Concentration of Exhaled Nitric Oxide

- inducible NO synthase (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. FEV₁ characterise obstruction

Questions to be answered

- population characteristics?
- dose – response?
- reproducibility?
- acceptable ∆?
Analytical determination of NO

Gas-based chemi-luminescent reaction

\[ \text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2 \]

\[ \text{NO}_2^* \rightarrow \text{NO}_2 + h\nu \]

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

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?

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

Silkoff et al. 2001 Chest 119: 1322-1328
Reproducibility

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

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
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