Relevance of G(C)LP and validation procedures in the clinical setting of early phase trials in healthy subjects and patients

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Where we come from...

... when we talk about validation

- history: pre-clinical toxicology (GLP)
  - to ensure reliability
  - but also to avoid fraud
  - and to make sure all data are reported correctly

High standards for validation and trackability

- adapted for bioanalytical assessments of drug concentrations in body fluids and tissues
  - first-in human studies
  - (early) PK studies
  - bioequivalence trials for generics

Comparable standards are applied!
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

Pillars of bioanalytical method validation

Full validation requested

- to demonstrate reliability of the method for determination of an analyte concentration in a biological matrix
- using an adequate reference standard (purity!) to prepare calibration standards, quality control samples and stability samples
- in addition an internal standard may be used
- for mass spectrometry: a stable isotope – labelled standard is recommended (no isotope exchange!)

Source: Guideline on bioanalytical method validation EMEA/CHMP/192217/2009 Rev. 1 Corr. 2
Pillars of bioanalytical method validation

**Selectivity**
- to ensure differentiation of analyte / IS against endogenous compounds or against other components in the sample
- *against endogenous compounds*: at least 6 individual sources of blank matrix
  - maximum 20% response of LLOQ
  - 5% for internal standard
- *against exogenous compounds*:
  - metabolites of the drug(s) of interest
  - co-medications normally used in study population
  - retrospective consideration of medication used in the study
  - possibility of back-conversion of metabolite into parent should be evaluated

Source: Guideline on bioanalytical method validation EMEA/CHMP/192217/2009 Rev. 1 Corr. 2

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**Carry-over**
- should be addressed and minimised
  - injection of blank sample after a high concentration sample / ULQ
  - limited to 20% of LLOQ (5% for IS)
- in case of unavoidable carry-over...
  - no randomisation of samples (!)

**Lower Limit of Quantitation LLOQ**
- lowest concentration with
  - reliable quantification
  - acceptable accuracy and precision
  - 5 times the signal of a blank sample
  - adapted to aim of the study, e. q. for BE trials < 5% of $C_{\text{max}}$

Not to be confused with Limit of Detection LoD

Source: Guideline on bioanalytical method validation EMEA/CHMP/192217/2009 Rev. 1 Corr. 2
Pillars of bioanalytical method validation

Calibration Curve
- same matrix as samples to be measured
- curve should cover expected concentration range
- minimum of 6 concentration levels plus blank (no IS) and zero sample
- slope and intercept should be reported
- acceptance limit: back calculated concentrations ± 15% of nominal values (±20% at LLOQ)
  - 75% of all data have to fit

Accuracy
- closeness of the determined value to the nominal concentration
- within-run accuracy
  - 5 samples at 4 concentration levels each (one level at LLOQ)
  - acceptance criteria: ± 15% (±20% at LLOQ)
- between-run accuracy
  - at least 2 different days
  - acceptance criteria: mean conc ± 15% of nominal value (± 20% at LLOQ)

Source: Guideline on bioanalytical method validation EMEA/CHMP/192217/2009 Rev. 1 Corr. 2
Pillars of bioanalytical method validation

**Precision**
- describes the closeness of repeated individual measures expressed as CV%
- to be demonstrated for LLOQ, low, medium and high QCs
- within-run precision
  - 5 samples per concentration levels
  - should not exceed ± 15% (± 20% at LLOQ)
- between-run precision
  - at least 3 runs
  - should not exceed ± 15% (± 20% at LLOQ)

**Further parameters**
- dilution integrity and matrix effects
- stability (stock solution, freeze/thaw, short term, long term, room temperature, auto sampler ... *full blood?*)

Source: Guideline on bioanalytical method validation EMEA/CHMP/192217/2009 Rev. 1 Corr. 2

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Analysis of study samples

**Definition of analytical run/ batch**
- blank + zero sample
- calibration curve
- QC samples
- samples to be analysed

**Acceptance criteria**
- accuracy
  - ± 15% (± 20% at LLOQ)
  - at least 75% of calibration samples
  - at least 67% of all QC samples and 50% of each concentration level

Incurred samples reanalysis: \( \% \text{ difference} = \frac{\text{repeat value} - \text{initial value}}{\text{mean value}} \times 100 \)

Not greater than 20% for at least 67%!
Transfer of standards?

Relevance of adequate validation for other types of analytical assessments?
- increasing relevance of biomarkers in early drug development
- increasing relevance of surrogate parameters when systemic concentration is not predictive for efficacy/safety
  - e.g. LALAs (locally acting locally applied)
  - e.g. drug targeting

Definitions

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

**Surrogate parameter for a clinical endpoint**
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

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

The magic term: GC(L)P

Reflection paper from 2010 gives some insight into current regulatory discussion

"The analysis of samples collected from subjects participating in clinical trials from a key part 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."

"To date no detailed guidance has been produced which outlines the expectations of national monitoring authorities with respect to the analysis or evaluation of samples collected as part of a clinical trial. In the absence of guidance, some laboratories apply the principles of good laboratory practice when conducting clinical analysis."

Source: Reflection Paper on guidance for laboratories...EMA/INS/GCP/532137/2010

Organisation of laboratories

Requirements for laboratories analysing clinical trials samples

- definition of roles and responsibility
- trainings and job descriptions (incl. GCP training!)
- prospective definition of communication lines with demarcation of responsibilities
- adequate contracts: process SOP-based with periodic review for sponsor contracts
- protocol needs to be known (or parts of it)

Processes need to ensure that informed consent and withdrawal of consent are adequately reflected!

Source: Reflection Paper on guidance for laboratories...EMA/INS/GCP/532137/2010
Sub-contracting laboratory analysis

Selection process / sub-contractor qualification
- the ability of the sub-contractor to perform the work must be assessed prior its initiation
- “Particular attention should be paid to staff training”

Patients/subject safety / Informed Consent
- reporting process of unexpected / out-of-range values should be in place
- different time zones / holidays should be considered

A mechanism should be in place to ensure that any relevant amendments is communicated

Source: Reflection Paper on guidance for laboratories - EUA/IM/INS/GCP/532/137/2010

Further requirements

Of relevance for practical work
- chain of custody is to be ensured
- method requirements
  - appropriately validated method
  - storage stability
  - system suitability tests
- acceptance criteria for analytical runs and criteria for repeat analysis need to be defined
- data recording need to ensure trackability
- reporting needs to be adequate

Very often reference is made to requirements of bioanalytical method validation and quality control – at least partly and adapted!
Further requirements

Of relevance from a structural perspective
- facilities need to be adequate
- equipment maintenance needs to be ensured
- computerised systems need to reflect current standard
- training of all staff is of highest relevance
- QA processes (independent!) in place
  - systematic audits
  - review of QA system

“...A mechanism for informing the sponsor and the concerned investigator or coordinating investigator (as appropriate) of significant deviations (those that may impact on data integrity, patient safety etc.) should be agreed prior to the initiation of laboratory work. Quality assurance personnel will normally require the underlying cause of a deficiency to be addressed as well as the specific deficiency itself. The most effective quality assurance programmes will include a documented CAPA procedure. All routine quality assurance activities should be documented in standard operating procedures or laboratory policies. A system should be implemented to ensure that the quality assurance personnel are working in accordance with their own procedures and in compliance with the principles of GCP”

Source: Reflection Paper on guidance for laboratories...EUA/INS/GCP/532137/2010
Locally acting / applied: ciclesonide

Metered dose inhaler for treatment of asthma

Structural formula of ciclesonide

A. Weinbrenner: J Clin Endo 87(5): 2160-2163, 2002

Surrogate for safety / efficacy

Inhaled corticosteroids result in
- hypothalamic-pituitary axis depression (safety)
  - to be determined by quantitation of cortisol suppression
- reduced inflammation of the airways (efficacy)
  - to be determined by e.g. exhaled NO

Representative examples for one clear and relatively simple and for one nearly impossible task when method validation becomes an issue!
Surrogate for safety

Cortisol suppression as safety marker

- HPA-axis suppression determined in adults
- 12 healthy male volunteers
- 4-period-changeover design over 7 days each
  - placebo
  - 800 µg morning administration
  - 800 µg evening administration
  - 400 µg BID
- comparison with placebo

HPA-axis suppression also assessed in asthmatic children

A. Weinbrenner: J Clin Endo 87(5): 2160-2163, 2002

Influence on HPA-axis

Cortisol suppression as safety marker

- primary endpoints (serum)
  - 24h mesor \( (\text{AUC}_{0-24h}) / 24h \)
  - 24h amplitude \( (1/2 \Delta \text{max} / \text{min}) \)
  - acceptance criterion: 80 – 125%
  - assay: fluorescence polarisation immunoassay

- study results (PE in comparison to placebo)

<table>
<thead>
<tr>
<th>Dose</th>
<th>Ratio / Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>morning dose 800µg</td>
<td>0.94 / 0.86 – 1.02</td>
</tr>
<tr>
<td>evening dose 800µg</td>
<td>0.98 / 0.90 – 1.07</td>
</tr>
<tr>
<td>BID 400µg</td>
<td>0.93 / 0.86 – 1.02</td>
</tr>
</tbody>
</table>

A. Weinbrenner: J Clin Endo 87(5): 2160-2163, 2002
Cortisol suppression: Safety

![Graph showing cortisol profiles](image)

Fig 2. Mean 24-h profiles of serum cortisol of 12 healthy volunteers treated for 7 d with (A) placebo twice daily, (B) 800 µg ciclesonide in the morning and placebo in the evening, (C) 800 µg ciclesonide in evening and placebo in the morning, and (D) 400 µg ciclesonide in the morning and 400 µg ciclesonide in the evening in a randomized, double-blind, change-over, equivalence study with at least 7 d wash-out.

Deficiency letter for HPA-axis in children

Comments to sponsor of ciclesonide asthma spray

- study in children with mild persistent asthma
  - more than 40% of the subjects included had a 24h-urine volume < 250ml
  - analytical method for cortisol in urine showed no adequate validation
  - patient compliance could not be assured

- new study requested with children aged 4-11 y
  - 24h urine cortisol and
  - 24h serum cortisol
Lesson to be learned

Whenever standard laboratory parameters become biomarker / surrogate parameter / endpoint

- method should be validated following the principles of bioanalytical method validation / GLP
- standards comparable to GLP are to be applied to the analysing laboratory

Example: biomarker in asthma

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

Attractive biomarker for anti-inflammatory effect

- non-invasive method
- highly sensitive analytical determination
- rapid assessment with direct measurement
- sophisticated technical equipment available on the market
Analytical determination of NO

Gas-based chemi-luminescent reaction

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

Detection by a red-sensitive photo-multiplier tube

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

How to assess the “pillars” of bioanalytical validation?
- selectivity ?
- accuracy and precision ?
- calibration curve and LLOQ ?
- carry-over ?
Quality control during measurement

Calibration for measurements acc. to manual

- 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

How to control acceptability of the measurements?

- accuracy?
- calibration curve?

Mission impossible!

Adequate validation and QC?

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 during qualification!
Lesson to be learned

Adequate validation and quality control during measurement
- often (nearly) impossible
- prospective risk assessment needed
- qualification of manufacturer and apparatus become part of the justification of adequacy
- intelligent and creative but also pragmatic solutions needed
- one size fits all approach not possible

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