Feedback from AAPS Workshop on Crystal City VI: Bioanalytical Method Validation on Biomarkers

Sept 28–29, 2015, Baltimore

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on behalf of EBF

8th Open symposium

18-Nov-2015, Barcelona

Crystal City VI; BMV on Biomarkers

Workshop agenda

- Keynote presentation Brian Booth

- Session 1A: Creating a discussion framework
- Session 1B: Challenges in making LBA and LC/MS biomarker assays meet PK assay criteria

- Session 2: LC–MS approaches (traditional and immunocapture) and assay criteria

- Session 3: LBA approaches and assay criteria: surrogate matrix/buffer, parallelism and stability

- Session 4: Perspectives and recommendation for assay criteria

BMV = Bioanalytical method validation

Theme

- **Biomarker Assays are not PK Assays**
- Nor are they the sons/daughters of PK assays
- Or their distant cousins
- Or even same species

- **Biomarker assays are not PK assays**
- And they don't want to grow up to be PK assays either

Biomarkers & Biology

- Biomarker data are used to make important decisions in your project
  - Assay must be reliable in order to be confident in these decisions
  - Assay validation plays a pivotal role

- Do you know what you are measuring?
- What is the purpose of the assay?
- Assay design – reagents ?
- What are the limitations of the assay?
- What is the precision of the measurement?
- How do sample handling conditions affect the measurement?

Biomarker categories Consensus

- Category 1
  - Internal decision making
  - Extent of assay validation is up to you!
- Category 2
  - Biomarker to support pivotal decision & label claim
  - Assay validation in scope of FDA

Reliability of biomarker data depends on their measurement

Focus on Category 2 Biomarker for regulatory review

Consensus for Biomarker assay Validation

- Surrogate matrix
- Precision
- Parallelism
- Sensitivity / Range (LLOQ-ULOQ)
- Endogenous analyte Stability
- Relative accuracy
- In study assay controls

Biomarker Assays ≠ PK assays

- **Standard**
  - No reference material; recombinant protein may not resemble endogenous protein

- **Matrix;**
  - Endogenous analyte present in the matrix
  - Use of surrogate matrix

Analyte to be measured is not identical to the calibrator – it is not absolute quantification!

Biomarker Assays ≠ PK assays

- **Accuracy**

  Most biomarker assays are relative accurate

  – Spiking recombinant/purified material into endogenous analyte containing matrix; A+B ≠ C

  – Even if A+B = C, combinations of recombinant and endogenous not representative of samples and do not inform about endogenous analyte accuracy

- Relative to calibrator quantitation = relative accuracy

  – In contrast to absolute quantification;
  Reference material = analyte; standard curve

Biomarker Assays ≠ PK assays

- **Parallelism**
- The use of incurred samples with endogenous analyte
- Sample dilution curve should be parallel to the calibrator curve
  - Validate the use of surrogate matrix for calibrators preparation
- Identify MRD to achieve acceptable %RE and %CV
- Done in multiple individuals = **Selectivity**
- LLOQ estimated for the endogenous analyte

Challenge:
Informed consent: US versus Europe

MRD= Minimum required dilution

Biomarker Assays ≠ PK assays

- **Stability**

  Stability of spiked material does not resemble endogenous analyte

  – No true reference material => not possible to spike samples in order to represent real samples => challenge in establish a true nominal value

  – Challenges in getting a ‘fresh’ sample with endogenous analyte => how to establish a t=0

- Stability evaluations should focus on endogenous analyte – best efforts

  – Consider ISS (incurred sample stability)

Biomarker Assays ≠ PK assays

- **Validation report**
  - Document the assay performance
  - Should state how data can be used
    - Relative quantitative
    - Quasi quantitative / Qualitative

- **ISR – not relevant**
  - Prefer to use another approach to demonstrate that assay is in control
    - e.g. endogenous QC-pool analysed in each run

If Biomarker Assays ≠ PK assays….

- Then how do we approach biomarker assay development and validation?
  - Apply scientific principles and start with questions
- Level of Assay validation
  - Depend upon the intended use of the data

- Assay criteria
  - Not as prescriptive as for PK assays
  - Should take the biology into the account
  - To detect changes in analyte levels that are meaningful
  - Assay should have a precision in which a biological change can measured
    - disease versus healthy state
    - between treatment groups

Take home message

- Biomarker Assays ≠ PK assays
- Category 2 Biomarkers are in scope
- Rethink accuracy
- Emphasis on precision
- Parallelism is the key analytical validation experiment
- Endogenous analyte to define performance and manage change
- Establish reference range for assay
- Understand the biology!

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THANK
YOU !