EBF Recommendation on method establishment and bioanalysis of Biomarkers in support of drug development.

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on behalf of the EBF

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Reconnect with the EBF presentation from the 4th Open Symposium in 2011
2 examples

All measured with 4-6-15 assay, but was this necessary?
EBF reflections on **biomarker classification**

1. Observed or anticipated biomarker levels
2. Development Phase in which a biomarker is measured
3. Decisions taken from the biomarker data
   - efficacy decisions
   - safety decisions
4. Fit of assay with Regulated Bioanalysis Guidelines

Above classification systems are superimposable and should be applied together to tailor an individual bioanalytical strategy in support of a biomarker request.
Inform and be informed

In order to apply aforementioned classification systems successfully, the EBF also included a 5th principle upon which the overall recommendation is built:

- Ensure regular, cross functional and iterative communication with the investigator requesting the biomarker concentration data (e.g. the pharmacologist, PK/TK, Tox-path, clinician or others)
2012: our recommendation

- Team continued discussions after BCN-2011 and developed 2 flowcharts

- Analysis of BM using a novel assay.
- Analysis of BM using an existing assay.
Analysis of biomarkers using a novel assay.

- Understand biology of BM
- Translate BM biology and science into Bioanalysis
- Qualify assumptions

Success:
- Agree on final assay requirements
- Set up the assay
- Analyze samples

Fail:
Understand biology of BM

Translate BM biology and science into Bioanalysis

Qualify assumptions

Agree on final assay requirements

Set up the assay

Analyze samples
1. Understand the biology and science of BM

- Connect with PK, PD or TK person requesting (=‘requester’) the BM data, and get informed on:
  - PK of BM and PD effect of drug on PK
  - Target species-population and matrix

- **Deliverable** of step 1 = understand why assay is needed and what is expected outcome
Type of information from step 1: 4 examples

- BM is a safety marker amenable to LC-MS. We need assay in human CSF. BM has a basal level of a few ng/ml with natural circadian concentration changes of 2-3 fold from basal. (Expected) PD effect of the drug on the BM (= PK of the BM) is an up regulation to 5-fold of basal. Up regulation maximum concentration exceeds the natural fluctuations.

- BM is a compound amenable to LC-MS. We need assay comparing plasma ratios of 2 BMs. Basal value BM1/BM2 ratio is 0.01. BMs can interconvert ex-vivo. (Expected) PD effect of the drug on the BM ratio is expected to see a 2-fold increase of BM1/BM2-ratio.

- BM is a compound amenable to LBA. We need assay in serum, BM has a basal level of a few hundreds ng/ml with limited natural concentration changes. (Expected) PD effect of the drug on the BM (= PK of the BM) is a 10 fold down regulation.

- Etc…. 

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Understand biology of BM → Translate BM biology and science into Bioanalysis → Qualify assumptions → Agree on final assay requirements → Set up the assay → Analyze samples

Fail →

Success —

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2. Translate BM biology and science into Bioanalytical strategy

- Based on outcome of interactions with requester, decide on analytical platform that is best suited to answer the questions
- **Deliverable**: proposed bioanalytical strategy and assay format
  - Preferred analytical platform, e.g.:
    - MS based, Chromatography based
    - Cell based, Enzymatic, Ligand Binding Assay
    - Hyphenated
  - Target analyte: availability of reference material
  - Target matrix: availability of (surrogate) matrix
  - Target concentration range: Desired LOQ/ULOQ
  - Desired analytical performance of the assay based on PK behavior of BM

Note: An assay may be available for the BM. However, this assay may not be suited to provide the required answers in the specified situation (accuracy, precision, range,…). In this case, the assay may serve as a basis on which the actually needed assay is build.
Understand biology of BM

Translate BM biology and science into Bioanalysis

Qualify assumptions

Fail

Success

Agree on final assay requirements

Set up the assay

Analyze samples

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Qualify the PK/(TK)/PD assumptions made in “1” using the assay format proposed in “2”
- Develop and set up assay for qualification of assumptions
  - Be careful not to take assay development to far…stop at stage (or investment) where assay can answer the assumptions defined in “1”
- Measure actual samples

Deliverable:

Assumptions not confirmed
→ Reconnect with “1” or “2”

Assumptions confirmed
→ move to 4

1. Understand the biology and science of BM
2. Translate BM biology/science into BA strategy

4. Agree on final assay requirements

Note: For some decisions or stages of development, BM assay development may stop here when data / assay performance suffice for internal decision making.
Reconnect with requester

- Provide feedback on bioanalytical pre-work, assumption testing and qualification
- Only now agree on assay requirements based on
  - Decisions to be taken from the data
  - Potential of assay format
    - Consider change if/when assay is used in later phases of development (species change may require a assay deliverables to be re-assessed)
  - Known Regulatory requirements
    - Consider change if/when assay is used in later phases of development
Understand biology of BM → Translate BM biology and science into Bioanalysis → Qualify assumptions → Fail

Success → Agree on final assay requirements → Set up the assay → Analyze samples
5. Set up the assay

- Write ‘bioanalytical protocol’
  - A priori document the BM assay requirements
  - maybe include more detail on purpose and scope

- Develop assay
  - Build on ‘assumption testing experience’
  - Include assay requirement parameters (screening → validation)

- Set up the assay
  - Screening – qualification - validation
  - Include all required “validation” parameters
  - Note: assay format may not fit preset regulated BA requirements or expectations, but assay data are anticipated to fit regulatory standard. In this case, it is advisable to flexibly pre-define what elements of regulated BA can be applicable for the assay or where it is not possible (e.g. wider or more narrow acceptance criteria, matrix choice, reference standards, etc…). This approach should be favored above trying to fit the assay into a standard regulated BA format in absence of scientific rationale to do so.
Analysis of biomarkers using an existing assay.
Flowchart: “New Biomarker”

Existing BM platform

Overlay BM assay performance on BM request

BM assay performance and BM request fits

Agree on final assay requirements

Set up assay and analyze samples

Analyze samples

Yes

No

Close fit

Flowchart:

- New Biomarker
- Overlay BM assay performance on BM request
- BM assay performance and BM request fits
- Agree on final assay requirements
- Set up assay and analyze samples
- Analyze samples

Yes

No

Existing BM platform
1. Overlay BM assay performance on BM request

- Existing assay may not have been established for new BM question:
  - Other species
  - Other calibration range
  - Other isomer
  - Established as qualified assay but now validated assay is required or vice versa
  - etc...

- Understand convergence of divergence of assay performance and new assay requirements
Flowchart: “New Biomarker”

- Overlay BM assay performance on BM request
- BM assay performance and BM request fits
- Agree on final assay requirements
- Set up assay and analyze samples
- Analyze samples
- Yes
- No

Existing BM platform?

Yes

Close fit

- Agree on final assay requirements
- Set up assay and analyze samples
- Analyze samples

No

- Overlay BM assay performance on BM request
- BM assay performance and BM request fits
- Agree on final assay requirements
- Set up assay and analyze samples
- Analyze samples
2. BM assay performance and BM request fits

- The existing assay fits with the request:
  - Additional BM data within an ongoing project
    - Copy established and earlier agreed acceptance criteria
    - Run samples using existing (screening, qualified or validated) method and follow regulated bioanalysis standards as needed.

- The existing assay does not fit the request:
  - New request is in support of a different study design or a new project, and therefore requires different endpoints compared to the performance of the established assay
  - Agree on new assay requirements
    - Small changes needed → adapt method and move forward
    - Major changes needed → use flowchart “novel biomarker”
Flowchart: “New Biomarker”

1. Existing BM platform
   - Overlay BM assay performance on BM request
   - BM assay performance and BM request fits
   - Agree on final assay requirements
   - Set up assay and analyze samples
       - Analyze samples
       - Yes

   - Close fit
       - Yes

2. Existing BM platform
   - No
   - Overlay BM assay performance on BM request
   - BM assay performance and BM request fits
   - Agree on final assay requirements
   - Set up assay and analyze samples

   - Close fit
       - Yes

3. New Biomarker
   - Close fit
       - Yes

4. New Biomarker
   - Overlay BM assay performance on BM request
   - BM assay performance and BM request fits
   - Agree on final assay requirements
   - Set up assay and analyze samples

   - Close fit
       - Yes
Combined flowchart

1. New BM platform → Understand biology of BM → Translate BM biology and science into Bioanalysis
2. Qualify assumptions
3. Existing BM platform
   - Overlay BM assay performance on BM request
4. BM assay performance and BM request fits
   - Agree on final assay requirements
   - Set up the assay
   - Analyze samples
5. No
   - Close fit
6. Yes
   - Agree on final assay requirements

Success/Fail
Additional reflections

With more BM analysis amenable to LC-MS, industry may say/think “we can easily reach 4-6-15 quality”, stimulating regulated bioanalysis standards for BM analysis, but:

- A significant number of biomarkers are novel and analysis involves the use of cutting edge or developing science
  - High resolution MS
  - novel hyphenated techniques
  - novel LBA/cell based assay formats
  - assays combining LBA and MS technology,…

- “Yes, can do!” shouldn’t mean “Yes, let’s do!” if there is no scientific driver.
  - “Yes, let’s do!” may increase cost with no added value for the patient.
  - “Yes, let’s do!” may jeopardize science to progress.

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Summary and overall recommendation

➢ Consider 4 classifications and use them in concert to decide on the assay requirements.
  – Anticipated concentration changes (PD effect)
  – Development phase
  – Decisions taken from the data
  – Fit of assay format with Regulated Bioanalysis Guidelines.

➢ Ensure effective/iterative communication between the BA team and the investigator, also when a method is available.

➢ Apply tiered approach principles when defining assay performance.
  – If assay performance needs to comply with regulated bioanalysis requirements are needed, allow and document flexible and scientific adaptation of these Guidelines.

➢ Follow EBF flowcharts when developing your assay
  – start from understanding the biology of BM, confirm the scientific assumption (a preliminary assay), prior to embarking on final method establishment and sample analysis.
EBF Recommendation on method establishment and bioanalysis of Biomarkers in support of drug development.

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