EBF Focus Workshop

Discussion points for Breakout

Philip Timmerman, on behalf of EBF

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Introduction

The dual challenge:
- Metabolite profiling
- Metabolite quantification
EBF position (2010) on Metabolite quantification

Use Validated methods to quantify all metabolites qualifying for ICH M3(R2)

Milestone:
Available data after MAD facilitates decision on which metabolite to characterize with validated methods (including in which studies)

Metabolites with unknown activity or toxicity

Use Screening methods to assess abundance of metabolites

Use Qualified methods to confirm the abundance of metabolites

Use Validated methods to quantify all metabolites qualifying for ICH M3(R2)

Milestone:
Available data after MAD facilitates decision on which metabolite to characterize with validated methods (including in which studies)

Metabolites with known activity or toxicity, relevant for the overall understanding of PK/TK and/or PK/PD relationship

Use Qualified methods to confirm the abundance of metabolites

Use Validated methods to quantify all metabolites pre-qualifying for ICH M3(R2)

Continue characterization of all metabolites qualifying ICH M3(R2) using Validated methods

EBF position (2010) on regulations for BA

non GLP

Screening

or

Qualified Methods

Clinical, GLP and non GLP

Validated Methods
(non) rodent plasma (unchanged drug)
Other matrices - metabolites as appropriate

Human plasma
Other matrices - metabolites as appropriate

Additional preclinical species
Other matrices - metabolites as appropriate

Qualified Methods
• Samples from:
  • *In vitro* (e.g. PPB),
  • some mechanistic PK, PK/PD
  • most non standard matrices (e.g. tissues)
• Quantification of metabolites

Although we gathered some data in our first survey in 2009, this subject was out of scope.
A lot happened since → new insights increased experience

Surveys

2010: 1
2011: 2
2012: 3
2013: 4
2014: 5
2015: 6

Publications

2010: a
2011: b
2012: c
2013: d
2014: etc
2015: today

Meetings

2010: α
2011: β
2012: γ
2013: δ
2014: ε
2015: today
And we want to use them.....
Updated EBF position on regulations for BA

Revised definition of validated methods and proposed timing/usage into:
- **Regulatory validation:**
  - Late stage studies
- **Scientific validation:**
  - 5 areas: dosed/unchanged drug/metabolites in tissue homogenates, urine, early clinical (e.g. SAD/MAD), early preclinical and ‘pre-ICH(M3)’ metabolites in plasma
  - Provided guidance on pre and in study validation criteria, study acceptance and reporting for regulatory validation

Ref:
- Tiered approach into practice - scientific validation for chromatography-based assays: a recommendation from the European Bioanalysis Forum. Bioanalysis, in press
Starting proposal Metabolite profiling – when and how?

- Discovery
- Pre phase 1
- Phase 1
- 2a/2b
- 3
- 4

AMS?

In vitro

In vivo

$^{14}$C in man

Sound mixture of (HR)MS, UV and RAD

Relative or absolute RAD estimation

Absolute quantification in partnership with BA team

MAD milestone

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Starting proposal Metabolite profiling – what and when?

<table>
<thead>
<tr>
<th>In vitro</th>
<th>In vitro</th>
<th>In vitro – major metabolites</th>
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<tbody>
<tr>
<td><strong>discovery</strong></td>
<td><strong>pre phase 1</strong></td>
<td><strong>phase 1</strong></td>
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<td><strong>Metabolic clearance</strong></td>
<td><strong>Radiolabel</strong></td>
<td><strong>DDI potential</strong></td>
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<tr>
<td><strong>Met ID (hotspots)</strong></td>
<td><strong>Met ID+quantification</strong></td>
<td>(enzymes/transporters)</td>
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<td><strong>Enzymes involved</strong></td>
<td><strong>Met. activity screen</strong></td>
<td><strong>PPB (human only)</strong></td>
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<td><strong>Other matrices/organs</strong></td>
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<td><strong>Trapping exp.</strong></td>
<td><strong>Metabolic pathways</strong></td>
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<td><strong>In vivo</strong></td>
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<td><strong>Clearance pathways</strong></td>
<td><strong>SAD/MAD metabolite profile</strong></td>
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<td><strong>Metabolite profile in plasma/excreta</strong></td>
<td><strong>and quantification (BA)</strong></td>
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<td><strong>IVIVC</strong></td>
<td><strong>Definitive Met ID</strong></td>
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<td><strong>In vivo</strong></td>
<td><strong>Define MIST and BA strategy</strong></td>
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<td><strong>Radiolabel</strong></td>
<td><strong>Prepare for human AME</strong></td>
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<td><strong>Metabolite profile + ID in plasma/excreta</strong></td>
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<td><strong>Evaluate microtracer/ microdose FIH</strong></td>
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</table>
Starting proposal Metabolite quantification – what when and how (in vivo)?

**Metabolites with (un)known PD activity or toxicity**

- Focus on in vitro - don’t get pulled into metabolite quantification too early for no reason
- Use screening and scientifically validated methods to document the PK and ICH M3 coverage of metabolites
- Consider quantification in special studies (e.g. DDI) of other metabolites using scientifically validated methods

**Milestone: around MAD**

- Document ICH(M3) coverage of metabolites (MIST perspective)
- Ensure coverage of human disproportionate metabolites in animal studies (may require separate Tox study)
- Ensure documentation of PD activity profile (collaborative work with clinical/pharmacology partners)

**Clinical studies only - Quantify only those metabolites contributing to >25% activity (based on activity and not only on AUC) relative to UD using regulatory validated methods (consider selection of studies and/or selection of samples)**

- Consider AMS as guide
- Consider quantification in special studies (e.g. DDI) of other metabolites using scientifically validated methods

**14C Does not involve regulatory BA**

- Focus on in vitro - don’t get pulled into metabolite quantification too early for no reason
- Use screening and scientifically validated methods to document the PK and ICH M3 coverage of metabolites

@ FiH: Consider AMS as guide

@ POC: Consider AMS as confirmation

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Afternoon breakout

➢ Metabolite profiling subteam
  – Move quickly in back of room
  – Discuss starting proposal, consider early morning presentation, peer reviewed literature and your experience
  – 5 moderators (Geert, Graeme, Martha, Stefan and Claude) will capture thoughts and plan forward and present @ 15.00

➢ Metabolite quantification subteam
  – Stay or come a bit closer
  – Discuss starting proposal, consider early morning presentation, peer reviewed literature and your experience
  – 4 moderators (John, Steve, Stuart and Philip) will capture thoughts and plan forward and present @ 15.30

➢ Plan forward will become the basis of updated EBF recommendation on Metabolite Profiling and Quantification to be published in 2016

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