DMPK

Development Germany

Current practices on metabolite profiling and quantification in Drug Development at Boehringer-Ingelheim

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EBF – Focus Workshop:
Metabolite Profiling and Quantification Strategies in Drug R&D
Sep 25, 2015
Brussels, Belgium

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Agenda:

- Regulatory Background

  → already covered

- Implementation in Drug Development @ BI

  → Details

- Practice:

  → Case Study

Metabolite Profiling and Quantification Strategies at BI
Rules and Terms

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➢Disproportionate Human Drug Metabolites (DHMs):

- Metabolites exposed to humans at higher plasma levels (at highest therapeutic dose) than in any of the relevant animal species used in non-clinical safety testing (at NOAEL)
- Only DHMs accounting for >10% of total drug-related exposure (according to ICH, confirmed by FDA) in plasma can raise a safety concern
- For drugs with a low daily dose (<10 mg) a higher threshold (than >10% of total) might be justified
- Phase II metabolites will generally not need non-clinical safety testing*
- Metabolite exposures are generally based on steady state conditions - based on AUCss

➢This means that a human metabolite is of no safety concern when:

- Exposure is <10% of total drug-related exposure or
- Adequate exposure is established in at least one relevant animal species of safety testing or
- Metabolite structure was identified as phase II conjugate*

➢Timelines: all data is needed until EoPhII

* Exception: Acylglucuronides

Metabolite Profiling and Quantification Strategies at BI
'MIST Milestone Meetings'

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Objective:
Identification of DM-differences in humans and animals as early as feasible
-> Risk minimization of late identification of 'Disproportionate Human Metabolites'

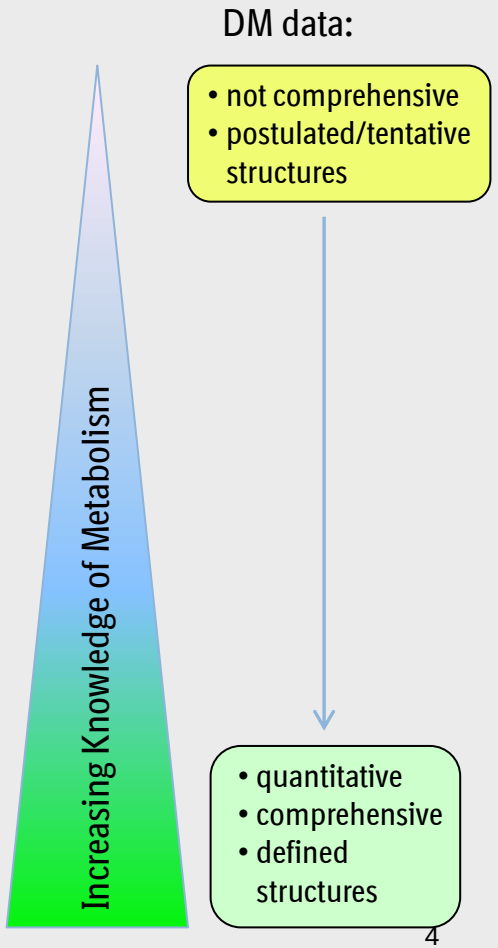
MIST Milestone Work packages until EoPhII:

- MM1: - Human DM data: 14C DM study in human hepatocytes
  vs.
  - Rat DM data: 14C ADME study

- MM2 : - Human DM data: SRD study
  vs.
  - DM data of 4 week Tox studies: Rat and 2nd tox species

- MM3: - Human DM data: MRD study
  vs.
  - DM data of 4 week + 13 week Tox studies: Rat and 2nd tox species,
  - optional if needed: - DM data of 13 week MTD mouse
    - Segment II and Carcinogenicity Studies

- MM4: - Human DM data: 14C ADME + MRD study:
  vs.
  - DM data of all relevant animal species including 14C DM studies



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MIST milestone meetings – topics to be discussed:

- Risk assessment: what is the likelihood a DHM exists?
- Initiate structural elucidation and/or synthesis of metabolites?
- Should pharmacological activity be tested?
- Develop bioanalytical assay and determine exposure in clinical or non-clinical studies?
- Is toxicological qualification of DHM necessary?
- Is the project-specific MIST strategy documented for information or approval by management?

Metabolite Profiling and Quantification Strategies at BI Clinical Samples

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⇨ **Key step within metabolite profiling strategy:**

1. **Metabolite screening in SRD- and MRD-studies**
2. **Comparison of metabolite exposures in humans and animals of Tox species**

Samples for clinical SRD/MRD-Trials:

Default 'Drug Metabolism section' is included in the Clinical Trial Protocols

- Dose groups: - 'Anticipated' therapeutic human dose only,
  - 'Formulation only-samples' (placebo) are usually implemented within dose groups

  → Sampling of dedicated plasma for Drug Metabolism
- Sampling times: - Same as for PK sampling
  - MRD: Plasma sampling only at steady state conditions (day 1 not needed)
- Additional urine will be provided for optional analysis - not required according to guidelines
- Metabolite screening studies are non GxP studies
- 'Informed consent'/'subject information' (= 'Probandeninformation') is adjusted accordingly

Metabolite Profiling and Quantification Strategies at BI Preclinical Samples

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Sample material from animal species:

- **Plasma; e.g. 4wk tox, GLP:**
  - Default 'Drug Metabolism section' is included in Test Protocols
  - Rodent: Plasma from satellite animals
  - Non rodent: Plasma from additional aliquots
  - Dose group: Anticipated NOAEL group, Active treatment and Placebo
  - Sampling times: - Same as for TK sampling
    - Plasma sampling only at steady state conditions (day 1 not needed)
  - Metabolite screening studies are non GxP studies

Metabolite Profiling and Quantification Strategies at BI
Quantitative Analysis – after MetID*

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1. **Plasma samples: Preparation of AUCpools**

   - Human plasma: - Anticipated therapeutic dose, Active treatment and Placebo

   - Animal plasma: - NOAEL group, Active treatment and Placebo

2. **Cross Supplementation of AUC(0-24)pools:**

   - Human **verum** plasma + Animal **blank** plasma (1:1)
   - Animal **verum** plasma + Human**blank** plasma (1:1)

   → Compensation of matrix effects with respect to differences of species and formulations

3. **Addition of internal standard**
4. **Sample preparation** e.g. SPE, Protein precipitation
5. **MS-Analysis:** UPLC/high res. MS, full 'scan' mode
6. **Data analysis:** - Assessment of metabolite exposures by direct – head to head – comparison of XIC-areas

   - Calculation of individual metabolite exposure multiples: EM_i

*Metabolite identification:
- SRD/MRD samples, Cmax or AUCpools
- untargeted head to head analysis, active treatment vs. placebo
- UPLC/TWIMS/highres MS^E (Synapt G2Si) or Orbitraps
- Background info from ^{14}C invitro studies and ADME rat

Metabolite Profiling and Quantification Strategies at BI Analytics

(S. Ma, S. K. Chowdhury, Anal. Chem. 2011, 83, 5028-5036)

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$$\text{Exposure Multiple} = \frac{\text{Exposure in Animals}}{\text{Exposure in Humans}}$$

$$EM_i = \frac{A_i^a / A_{IS}^a}{A_i^h / A_{IS}^h}$$

A_i^a: peak area metabolite (i) in animal

A_i^h: peak area metabolite (i) in human

A_{IS}^a: peak area internal standard in animal

A_{IS}^h: peak area internal standard in human

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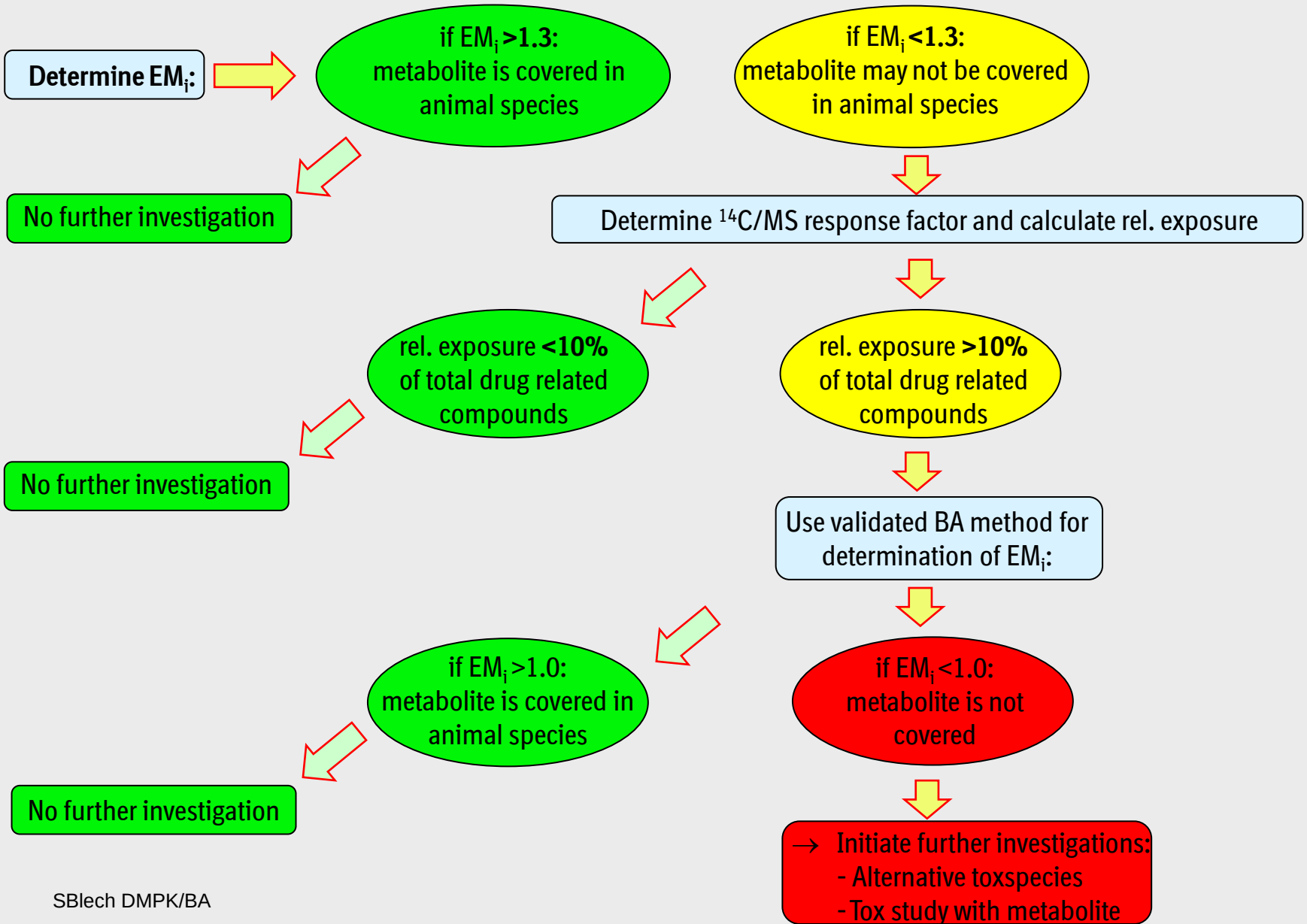

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Case study:

BI XYZ: - SRD study

- MRD study

MIST Strategies, Analytical Approaches for Discussion

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- Analytical 'MIST'-approaches and procedures are - and will be - in a continuous flow and will be more and more improved
- Adequate analytical tools for the determination of exposure multiples and rel. exposures are in the toolbox of drug metabolism
- Validated bioanalytical data is needed for human metabolites
  - with >10% (...)
  - which are not adequately covered by tox species

  → Earliest point in time: after drug metabolism investigations of FIM-Studies

  → Followed by selected clin. studies as appropriate, e.g. hepatic/renal impaired patients ...
- Quantification of metabolites in early Tox studies might be reasonable in case of:
  - On target or off target pharmacology data
  - Not due to 'high' concentrations in plasma ...

  → To be discussed in project teams