Outcomes from large molecule MS Task force team

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Outlines

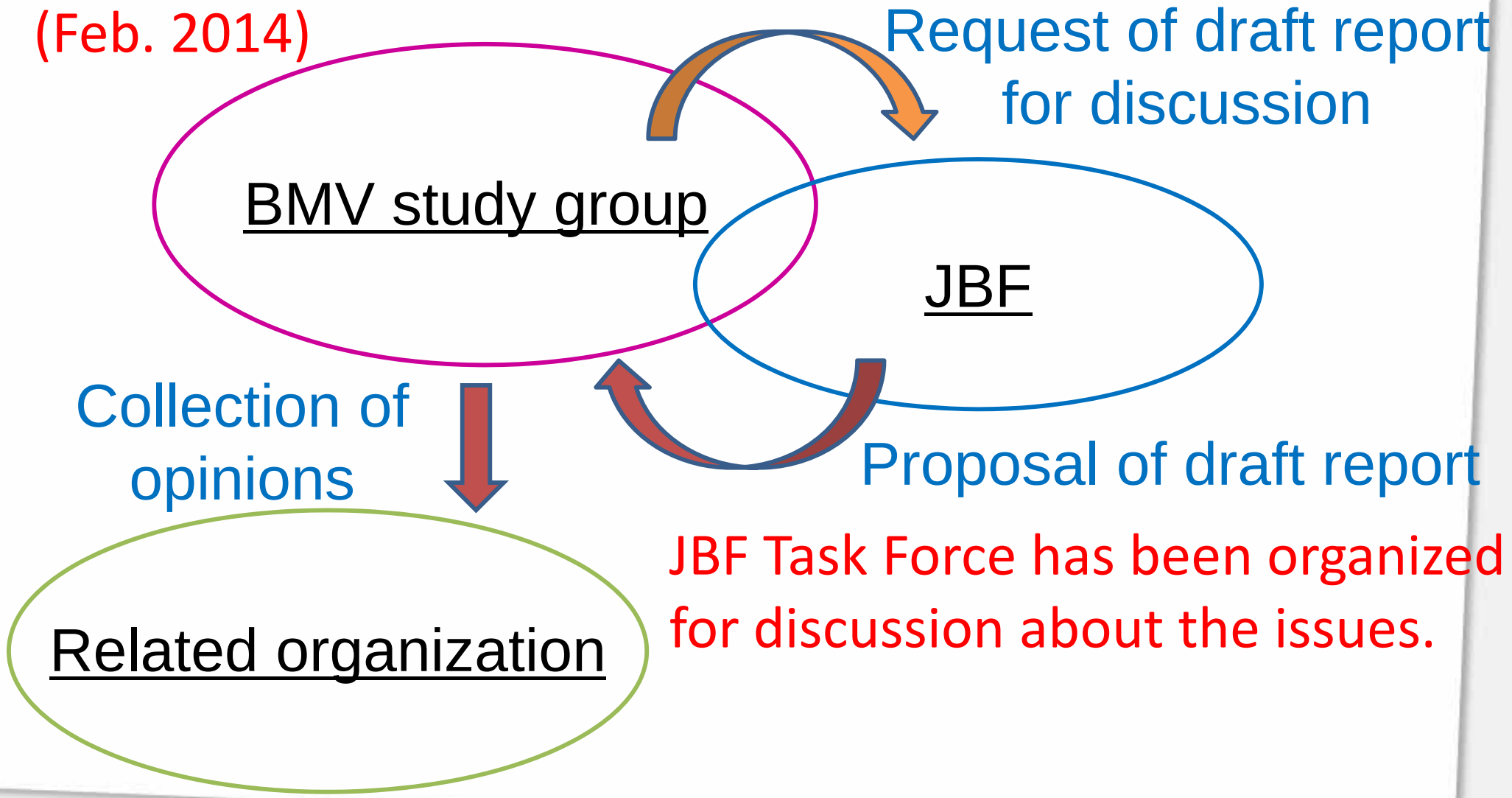
- Background
- Scope of application
- Outcomes as Q&A format
- Future perspective

Background

- Recently, large molecules sometimes should be analyzed by LC/MS instead of LBA.
- “Guideline on Bioanalytical Method Validation in Pharmaceutical Development” (LC guideline) notified in July 2013 by the Ministry of Health, Labour and Welfare
- There are some difficulties to apply the LC guideline directly to the bioanalysis of large molecules using LC methods with pretreatment of enzymatic digestions or ligand bindings.

BMV study group and JBF

Kick off Large molecule LC WG
(Feb. 2014)



JBF Task Force has been organized for discussion about the issues.

Activity of JBF Task Force

- Discussion about…
  - ✓ a basic point of view
  - ✓ scope of application
  - ✓ extraction of the point for discussion
  - ✓ format of work product

- Preparation of the work product

Scope of application

- JBF Task Force focused on “Drug”, not endogenous large molecules.

#	Intact	Digested	
	Oligonucleotide, peptide and protein	Peptide	Protein
SIL-IS	Available	Available	Whole protein Digested fragment Flanking peptide
Timing of IS addition	No need for discussion	No need for discussion	Case by case
Pretreatment	・Physical-chemical approach ・Use of ligand binding technique	・Physical-chemical approach ・Enzymatic digestion ・Use of ligand binding technique	
Others	Adsorption, aggregation, use of ion-pair reagents, peak through a column, etc.		

The issues about the pretreatment with enzymatic digestion (including IS matter) or ligand binding technique were extracted for discussion

Work product

- JBF Task Force has aimed to compile a Q&A document on LC guideline for some considerations or recommendations regarding the bioanalysis of large molecules by LC/MS.

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Reference standard

- Biotechnology-derived pharmaceuticals are generally heterogenous.
- Careful attention to the quality of reference standard is required for the change of its lot.
- It is recommended to follow “Guideline on Bioanalytical Method Validation in Pharmaceutical Development” (LBA guideline).

Critical reagents

In cases where the ligand binding (immunoaffinity capturing) is used for sample pretreatment,

- Expiration or retest date would not be required for critical reagents.
- The evaluation of data from calibration standards and QC samples would be sufficient to confirm the quality of critical reagents throughout the period of use.
- It is recommended to use a single lot of reagent for a batch analysis to avoid the effects of lot-to-lot variation .

Number of measurement for sample analysis

- One measurement (n=1) for each sample analysis is usually acceptable.

- The number of measurement should be defined based on the evaluation of precision during assay development and confirmed in validation study.

Internal standard (IS)

- Stable isotope-labeled (SIL) protein is ideal to compensate for variations in sample preparation with enzymatic digestion or/and ligand binding technique. However, its synthesis is generally difficult and expensive at this time.

- SIL-peptide (digested fragment) and non-labeled analog proteins can be used for IS. The difficulty of the correction of digestion efficiency variations by these IS requires the optimization of enzymatic digestion.
- SIL-flanking peptide IS could resolve this correction issue.

- It is recommended to add SIL-peptide IS after the pretreatment by ligand binding technique. Because the binding activity for ligand would be different between protein and peptide.

Considerations: Enzymatic digestion

- Other (non-target) protein concentrations and types are different between individuals, and change under pathological condition.

  ✓ Sufficient amount of digestive enzymes should be used in the pretreatment theoretically.

  ✓However, that leads to the production of a large amount of impurity peptides from non-target proteins and the further digestion of the target peptide by a small amount of impurity enzymes.

Considerations: Enzymatic digestion

Method development

- ✓ Selection of target peptide(s)
- ✓ Selection of IS type
- ✓ Optimization of digestion conditions
  - Effects of non-target proteins/peptides on digestion efficiency
  - Stabilization of the target peptide(s)

Method validation

- ✓ Use of QC samples with individual matrices
- ✓ Increase of number of individual matrix to confirm the selectivity

Discussion on the criterion for accuracy and precision

- Consensus was reached that there are several cases where the acceptance criteria of LC guideline are difficult to apply for the bioanalysis of large molecule by LC/MS due to the complexity of the assay (enzymatic digestion or affinity capturing etc.).

- There are many discussion about the acceptance criteria for bioanalysis of large molecule by LC/MS. One recommendation was to apply the acceptance criteria of LBA guideline. However, the way to express the criteria is very important not to confuse readers.

Accuracy and precision

- The acceptance criteria should be established in advance based on scientific justification.

- Basic acceptance criteria proposed:
  - ✓ Accuracy: ≤ ±20% (at LLOQ; ≤ ±25%)
  - ✓ Precision: ≤ 20% (at LLOQ; ≤ 25%)

- A wider range is thought to be acceptable when an alternative method is not available.

Selectivity

- Selectivity should be evaluated in accordance with LC guidelines.
  - ✓ At least 6 individual sources

- Specificity stated in LBA guidelines is unnecessary in principle.
  - ✓ LC/MS possess considerably higher selectivity based on the mass-to-charge (*m*/*z*) ratios in addition to LC separation.
  - ✓ Complete separation of the target from analogues in pretreatment is not necessarily required.

ISR and Cross validation

- In principle, ISR and cross validation should be conducted in accordance with LC guidelines.

- However, the appropriate criteria (assay variability) should be defined based on the criteria in **the validation study**.

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Future Perspective

- JBF TF has prepared the draft Q&A format regarding the bioanalysis of large molecule by LC/MS.

- However, further discussion with the related organizations is needed to finalize the work product.