Bioanalysis of peptides and proteins in drug research and development: from strategy into practice.

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Presentation outline

- Some definitions
- Our strategy
- Some examples
  - From discovery
  - From development
- Conclusions
Peptide and Proteins
NDA vs BLA
drug or biologic?

• FDA Draft Biosimilars Guidance: Feb 2012

• Manufacturing Method and Peptide Size matter

| Small: ≤ 40 amino acids molecule is a drug | Medium: 41 to 99 amino acids – designation depends on method of manufacturing | Large: ≥ 100 amino acids molecule is a biologic |

• 12 year (biologic) vs 5 (drug) year data exclusivity
Organisation @Janssen

Small molecule development
• LC-MS/MS based BA support

Biologics Development
• Antibodies
• Recombinant proteins
• Immunoassay based BA support

Strategy for peptides? Where does it get supported?
• Teams eager to tackle the challenge of BA peptide method development on both LBA and MS platforms

• Towards integration of LBA and MS strategies

• Limited experience with peptides in both small and large molecule bioanalytical teams
Strategy proposal for BA support of peptides/proteins @ Janssen

Preclinical: Use multiple platforms

Discovery

LBA = primary assay > compound selection, LC-MS in support (orthogonal assay)

Majority of projects: LBA, because of IR potential

Some projects (i.e. smaller peptides): LC/MS, because of > selectivity/sensitivity

Apply validated LBA assay(s) in studies

@ next milestone: evaluate assay

Assay selective enough?

Assay sensitive enough?

Consider other data, e.g. IR?

Optimize and revalidate

@ NME: Define assay requirements?

Appropriate level of scientific method rigor to allow documented decision making on i.e. PK, PD, metabolism.

Evaluate technologies in consultation with BA core team and CDT to build BA strategy.

Intense use of complementary assays should extend into early preclinical

Optimized communication between BA and CDT to ensure timely input and appropriate design of BA strategy.

Phases

Ph 1

Ph 2

Ph 3

PM

Potential redefine assay requirements

Preclinical

* Majority of projects: LBA = primary assay > compound selection, LC-MS in support (orthogonal assay)

No

No

No

No

No

Yes

Continue with LBA assay (until next milestone)

Continue with LC/MS assay (until next milestone)

Optimize and revalidate

Optimize and revalidate

Confirm/check selectivity, sensitivity, IR, @ next development phase, (pre)defined (bioanalytical) milestone or based on data

Janssen

PHARMACEUTICAL COMPANIES
OF JOHNSON & JOHNSON
Let’s dissect this...
Strategy proposal for BA support of peptides/proteins

**Discovery**

Use multiple platforms

**Pre-NME:**

- Complementary use of LBA, cell based and MS-based assays in support of compound selection.
- Apply appropriate level of scientific method rigor to allow documented decision making on i.e. PK, PD, metabolism.
- Evaluate technologies in consultation with BA core team and CDT to build BA strategy.
- Intense use of complementary assays should extend into early preclinical.
Preclinical

**LBA**

@ **NME**: Define assay requirements?

- **Majority of projects**: **LBA** = primary assay after compound is selected for development
  - IR potential requires reagent preparation
  - qualified LC-MS(/MS) in support (as confirmatory or orthogonal assay)
  - Some projects (i.e. smaller peptides): **LC/MS**, because of > selectivity/sensitivity

- **Method establishment** LBA and LC/MS assay(s)
  - build on existing experience from discovery or previous phase for both types

Discovery

Ph1 Ph2 Ph3 PM
LBA → (Develop and) validate LBA assay(s)
(Develop and) validate LC-MS(/MS) – orthogonal
(build on existing experience)

@ next milestone: evaluate assay

Y/N? No → Apply validated LBA assay(s) in studies
+ (qualified) LC-MS(/MS) as supporting orthogonal assay

Yes → (Develop and) validate LC-MS(/MS) assay:

assay sensitive enough?
assay selective enough?
consider other data

Yes → Continue with LBA assay (until next milestone)

No → Potentially redefine assay requirements

LC-MS

Ph1 Ph2 Ph3 PM

Discovery Preclinical

Optimize and revalidate

Potential redefine assay requirements

Optimize and revalidate

Continue with LC/MS assay (until next milestone)

Re-Define assay requirements?

Continue with LBA assay (until next milestone)

Optimize and revalidate

Continue with LBA assay (until next milestone)

Optimize and revalidate
Or...in simple words

Apply validated LBA assay(s) in studies + (qualified) LC-MS(/MS) supporting orthogonal assay

@next milestone:

evaluate assay performance against new situation and (if needed) re-define assay requirements:

- Confirm/check selectivity, sensitivity, IR,.. @ next development phase, (pre)defined (bioanalytical) milestone or based on data

- Assay (still) sensitive enough
- Assay (still) selective enough
- Include all information from previous phases/methods
Optimized communication between BA and teams to ensure timely input and appropriate design of BA strategy.
An example ...

- Integrated in vitro/in vivo workflow to support extensive lead optimization effort
- In vivo PK studies currently serve as the major PK screening tool during lead optimization
  - Challenge: significant inter-species differences in PK (e.g. CL, absorption) had been noted for some compounds—Which species is relevant?

In vivo optimisation of clearance
All supported by LC-MS/MS
Short cycle times
# peptides
BAN approach

- **Step 1:** Request for LC-MS/MS method development and stability evaluation @ 1 conc in human and rat plasma

- **Sequel:** *in vivo* administration in rats (1 or 2 mg/kg IV, SC, IM)

- **BA:** SRM-based approaches
  - Multiply charged ions
  - combination/summation of SRM
  - Sample prep – adapted/optimized protein precipitation
  - LLOQs 1-10 ng/mL
Another example ...

• Pegylated protein (non-pegylated protein ~100 AA)
• IA for protein: non- and pegylated protein
• pegylated protein also quantified
• Desire to analyse non-pegylated protein in presence of pegylated protein
POC: pull down of protein containing Histag

protein with His tag

PEG-protein with His tag

T₇ = free protein

elute

wash

magnets

Ni

wash

elute

digestion

T₇

Discovery
Chromatograms of TD after HisTag

pegylated protein

non-pegylated protein

EIC of 5+ charged ion
An example of orthogonal assays

- 40 AA in-licensed peptide
- Development program (phase I and II program): validated electrochemiluminescent immunoassay
- Trigger: FDA question on metabolite identification
The validated assay - 1

• Validated and used for Phase I and II analysis
• Sandwich assay format
  ✓ biotinylated anti-peptide polyclonal antibody for capture
  ✓ Ru++ labeled anti-peptide monoclonal antibody for detection
• LLOQ 30 pg/mL
The validated assay – **peptide challenges**

- Possible factors affecting behavior:
  - Stability (-70°C, 4°C, RT), container adsorption, protein binding, self-association, matrix degradation, etc.
  - Contribute to higher dilution variability than in typical mAb assays
- Use of protease inhibitors necessary during sample collection
  - Assess immunoassay performance in PIC plasma
The validated assay – **immunoassay challenges**

- Indirect measurement – is immunoassay response proportional to “bioactive” peptide concentration?

- Unable to differentiate between intact vs. modified peptide
  - No derived information on *in vivo* metabolite profile

- Cross-reactivity with endogenous peptides?
The different assays

1. Quantitative – PPT/triple quad LC-MS
2. Qualitative – PPT/QTOF LC-MS
3. Quantitative – Electrochemiluminescent immunoassay
4. Quantitative/Qualitative – IAP/RPLC – TOF-MS
## The different assays: results of dog study

<table>
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<tr>
<th>Results (ng/mL)</th>
<th>Assay 1 (LC-MS/MS)</th>
<th>Assay 3 (LBA)</th>
<th>Assay 4 (IA-LC-MS)</th>
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Metabolite analysis

- LC-MS/MS detection of low concentrations of peptide metabolites (N-truncated peptides synthesized) – both biased and unbiased approach used
- Individual metabolite was < 1% of intact peptide
- Total metabolite concentration: 2-3% of intact peptide measurement
- Results are in agreement with immunoassay and IAP-LC-MS analyses
Conclusions platform comparison

• 3 different methods show identical results for UD

• MS-based (unbiased) methods positively identified peptide metabolites in plasma (< 3% of total peptide in vivo concentration)

• Neither IAP/LC-MS nor LC-MS/MS reached required sensitivity for clinical bioanalysis

• validity of immunoassay method for ongoing clinical bioanalysis confirmed
Finally

• Presented current thinking @ Janssen
• Cross fertilize expertise from different groups
• Immunogenicity always assessed with immunoassay
• What are the current approaches in your company?
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