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

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Drug Safety Sciences

Analytical Sciences, Non-regulated Bioanalysis

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

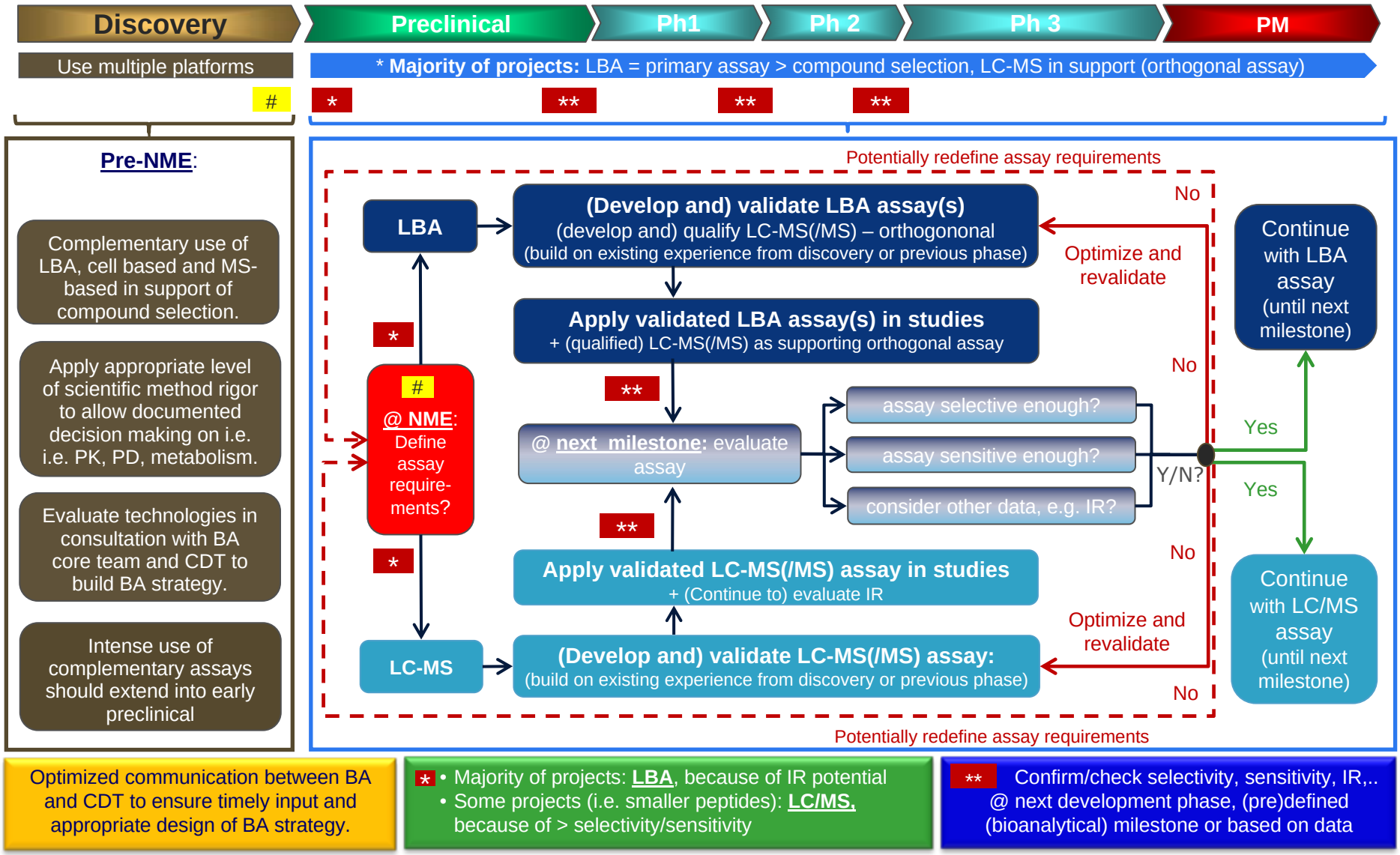
- Antibodies
- Recombinant proteins
- Immunoassay based BA support

Strategy for peptides? Where does it get supported?

And…

- 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



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

* • Majority of projects: **LBA**, because of IR potential
• Some projects (i.e. smaller peptides): **LC/MS,** because of > selectivity/sensitivity

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

Let's dissect this…

Strategy proposal for BA support of peptides / proteins

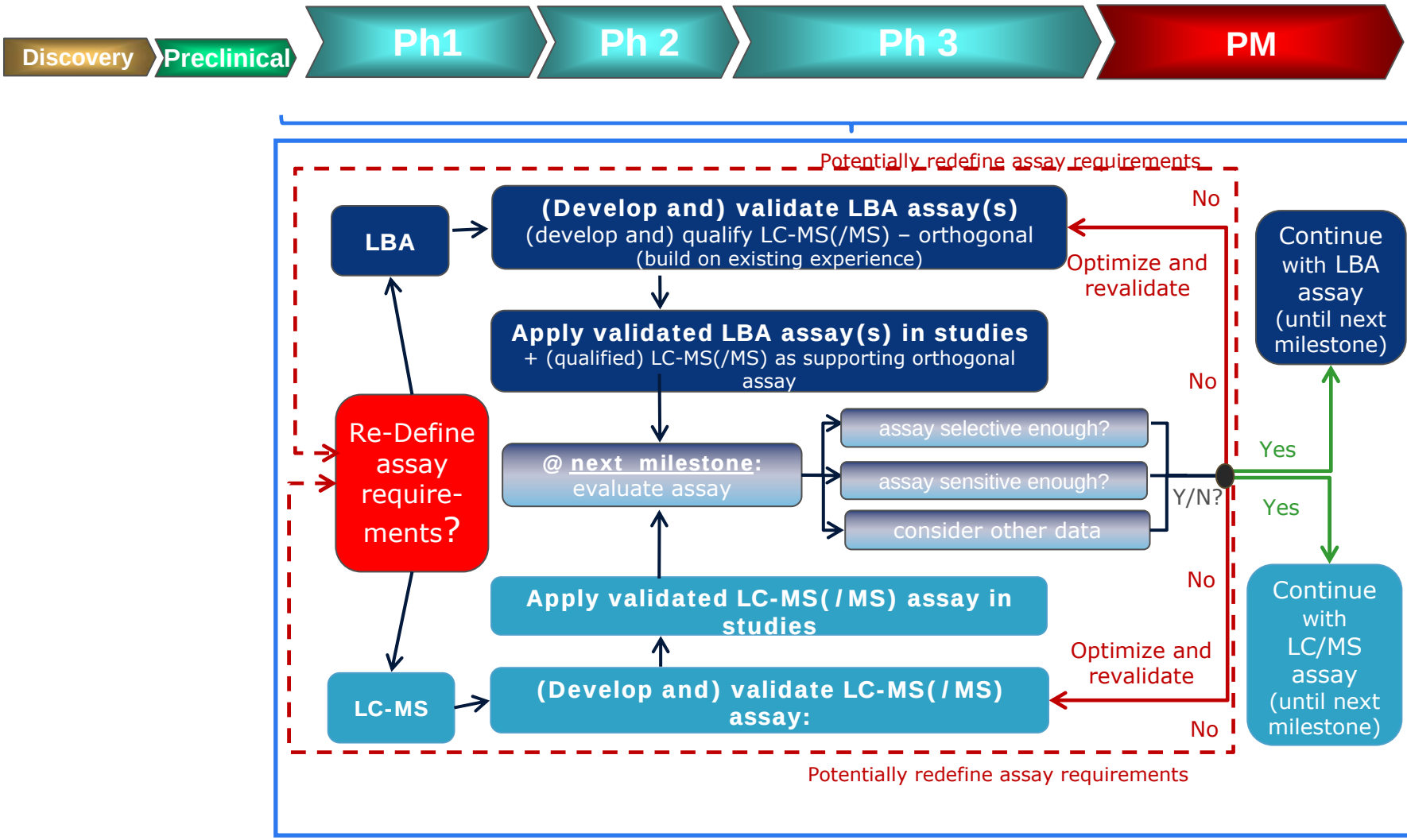
Use multiple platforms

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

Discovery | **Preclinical** | Ph1 | Ph 2 | Ph 3 | PM

- 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
Preclinical
Ph1
Ph 2
Ph 3
PM
Potentially redefine assay requirements
LBA
(Develop and) validate LBA assay(s)
(develop and) qualify LC-MS(/MS) – orthogonal
(build on existing experience)
No
Optimize and revalidate
Continue with LBA assay (until next milestone)
Apply validated LBA assay(s) in studies
+ (qualified) LC-MS(/MS) as supporting orthogonal assay
No
Re-Define assay require-ments?
@ next milestone:
evaluate assay
assay selective enough?
assay sensitive enough?
consider other data
Y/N?
Yes
Yes
Apply validated LC-MS(/MS) assay in studies
No
Optimize and revalidate
Continue with LC/MS assay (until next milestone)
LC-MS
(Develop and) validate LC-MS(/MS) assay:
No
Potentially redefine assay requirements

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:

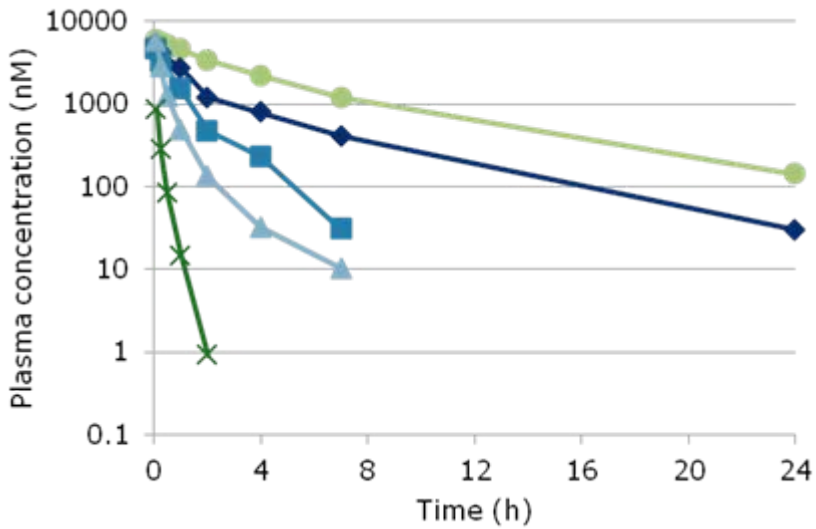
•Confirm/check selectivity, sensitivity, IR,.. @ next development phase, (pre)defined (bioanalytical) milestone or based on data

- 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
  - o 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

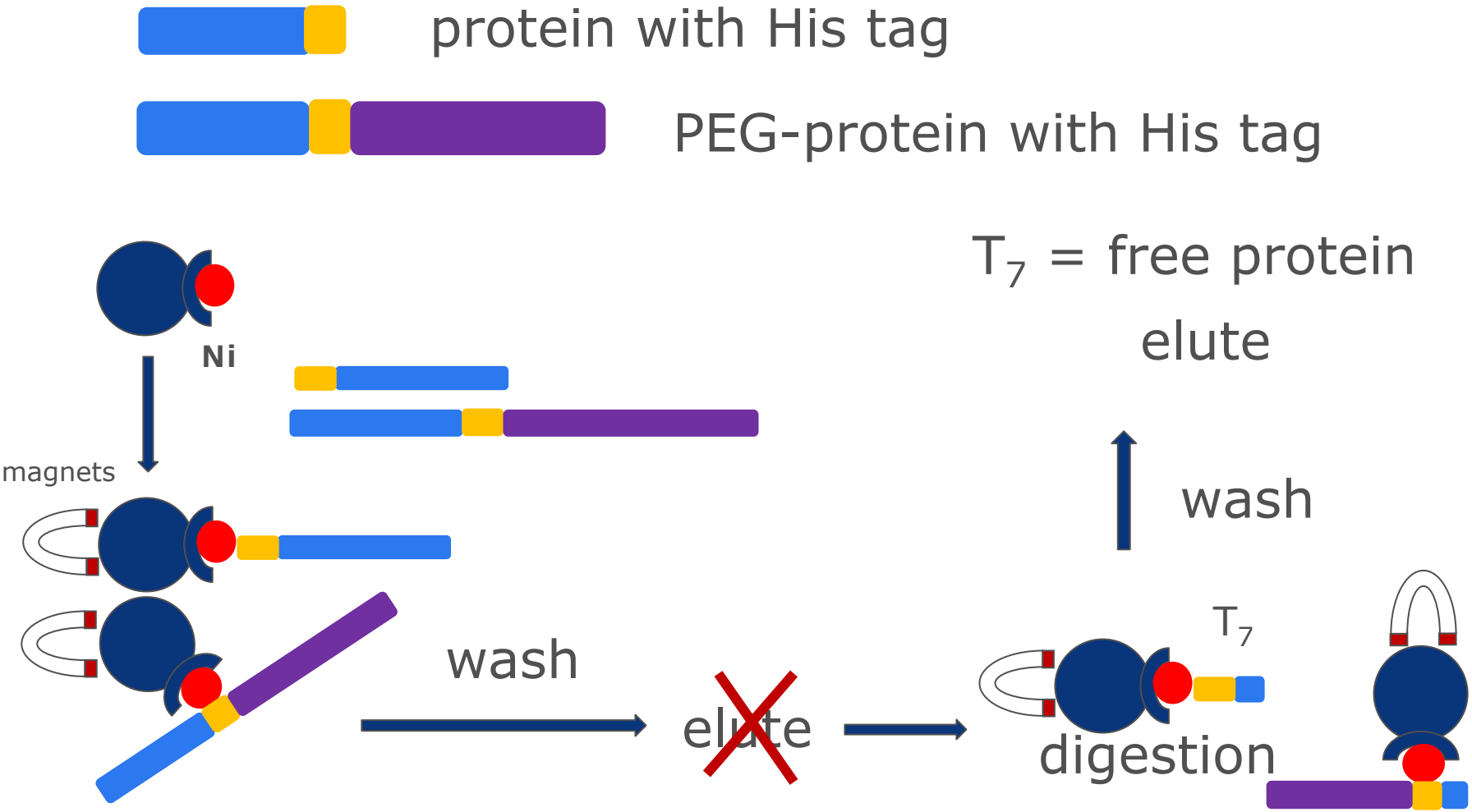
BAN approach

- **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 …

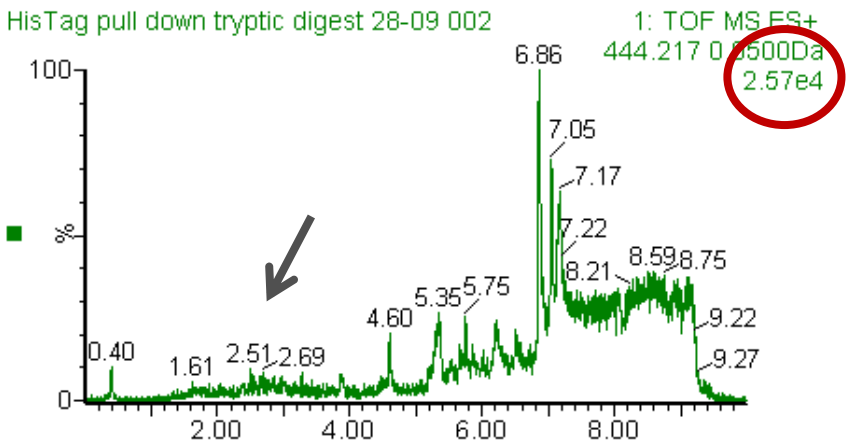
- Pegylated protein (non-pegylated protein ~100 AA)
- IA for protein: non- and pegylated protein
- pegylated protein also quantified
- Desire to analyse non-pegylated protein in presence of pegylated protein

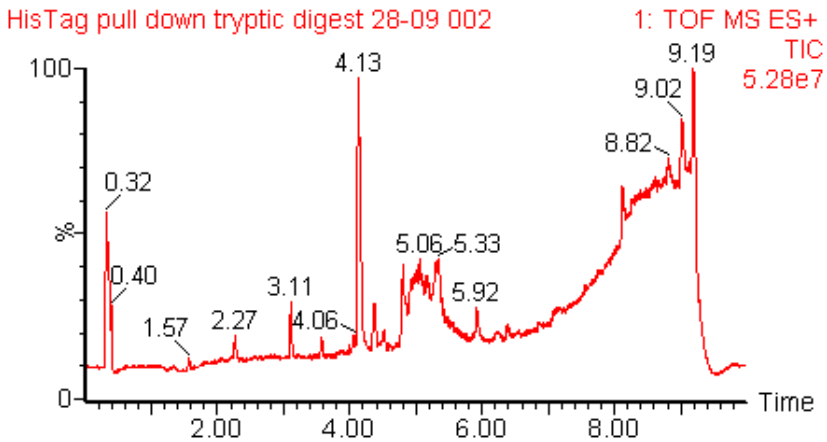
POC: pull down of protein containing Histag



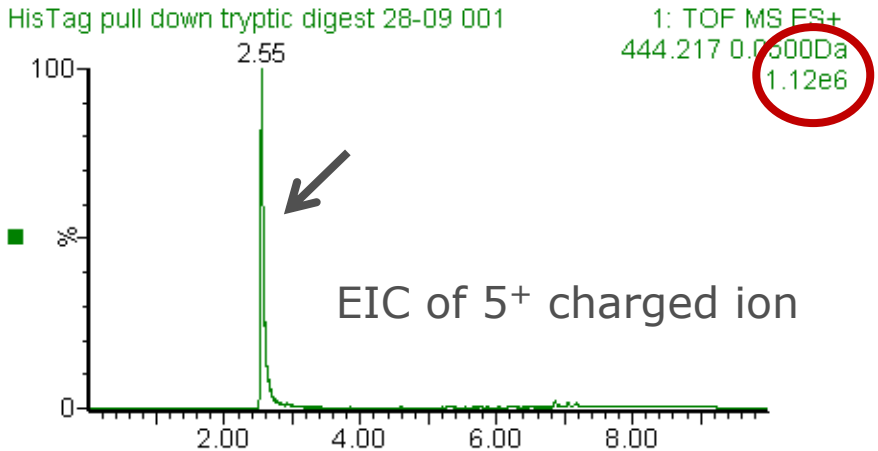
Chromatograms of TD after HisTag

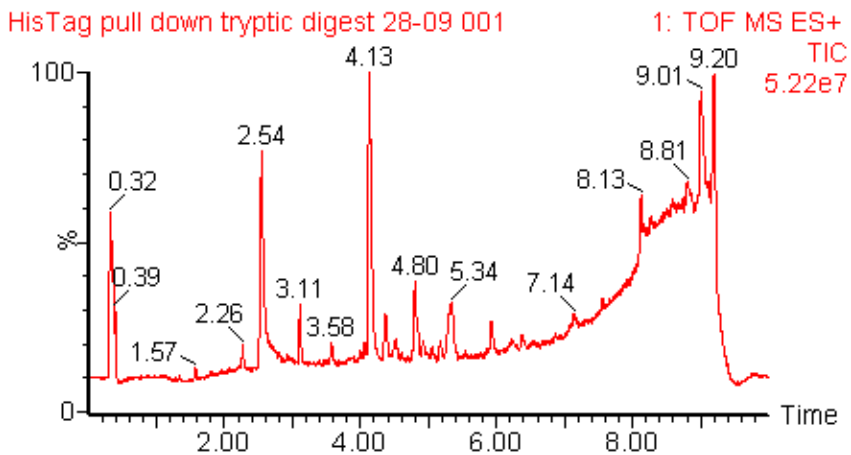
pegylated protein





non-pegylated protein



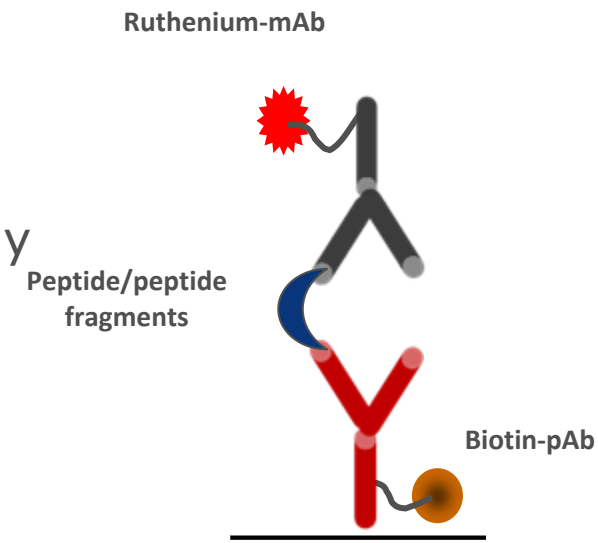


An example of orthogonal assays

- 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

Results (ng/mL)	Assay 1 (LC-MS/MS)			Assay 3 (LBA)		Assay 4 (IA-LC-MS)	
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 1	Test 2
1 - timepoint 1	2380	2320	2640	2317	1765	2031	2039
1 - timepoint 2	316	320	331	431	315	225	222
2 - timepoint 1	4020	3920	3740	3901	3773	3619	3028
2 - timepoint 2	598	552	619	702	654	513	436
3 - timepoint 1	3040	3060	3520	2249	2110	2291	2307
3 - timepoint 2	442	440	442	525	527	343	360

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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