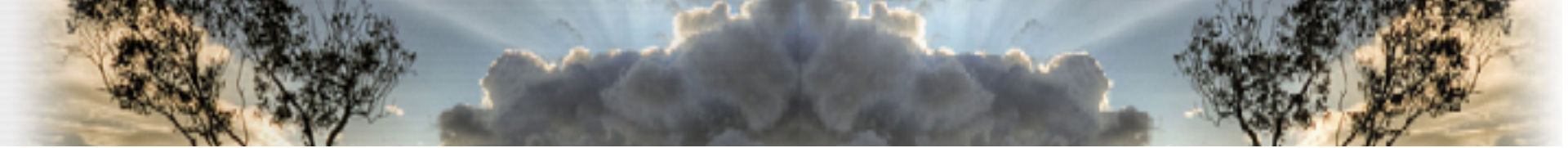

EBF

EBF Cyberconnect Events

Focus Workshop: Peptide/Protein Analysis with LC-MS/MS

17-18 June 2021

Questions to Consider when Building a Bioanalytical Strategy for Proteins

Iain Love, on behalf of the EBF

Introduction

- 3 part pre-meeting survey
  - Capture the major drivers behind platform selection
  - Understand factors that may influence platform selection
  - Detailed data to provide a starting point for a fantastic discussion
- 18 respondents from the community
  - 11 CRO, 7 Pharma
- Significant amount of information generated
  - Key trends surfaced in anticipation of a vibrant panel discussion

Part 1-2 – Preclinical Development Phase

	CRO (%)	Phrm (%)	Total (%)
Availability of reagents for LBA (e.g. from earlier programs)	45	**100**	**67**
Availability of diagnostic kits (repurpose for PK)	27	29	28
Availability of reagents for LC/MS	45	29	39
Anticipated LOQs needed	**64**	57	61
Experience from discovery (as a driver to change of platform)	45	57	50
Experience from discovery (as a driver to continue of platform)	36	71	50
Timelines & cost required to develop and validate the assay	9	57	28
Cost of running the assay in routine analysis	18	14	17
Throughput of running the assay in routine analysis	18	0	11
Outsourcing possibility	0	86	33
Compliance issues associated with the platform, e.g. e-environment	0	14	6

Part 1-2 – Clinical Development Phase

	CRO (%)	Phrm (%)	Total (%)
Availability of assays from preclinical	45	71	56
Availability of reagents for LBA	27	**86**	56
Availability of reagents for LC/MS (e.g. Stable isotope IS)	27	71	44
Experience from preclinical (as a driver to change of platform)	36	57	44
Experience from preclinical (as a driver to continue of platform)	45	57	50
Anticipated LOQs needed	**64**	57	**61**
Timelines & cost required to develop and validate the assay	18	57	33
Cost of running the assay in routine analysis	18	14	17
Throughput of running the assay in routine analysis	18	14	17
Outsourcing possibility	0	**86**	33
Compliance issues associated with the platform, e.g. e-environment	0	0	0

Part 1-2 – Data comparison across platforms

- *What is the difference you accept as inter assay platform variability?*
- *If there is a difference, how do you decide which is the true value?*
- *If there is a difference, how do you mitigate legacy (assumed incorrect) data?*

Tendency towards cross-validation/ISR-like criteria of 20-30% where comparisons between platforms were performed

Strong indication from respondents that difference itself matters less than an understanding of the reason for the difference

Suggestion that the results should be evaluated by stakeholders within the context of use

CRO responses often deferred to Sponsor preferences

Part 3 – The Survey

	Discovery	Preclinical	Clinical
What is your first choice bioanalytical platforms for your PK assay	• …	• …	• …
Validation strategy: what level of validation do you apply for the assay	• …	• …	• …
What acceptance criteria do you use	• …	• …	• …
What are the Analytical platforms you use as confirmatory assay (i.e. orthogonal assay to confirm specificity of PK assay)	• …	• …	• …
Which of ADA/Nab assays do you include in your bioanalytical and project strategy	• …	• …	• …

Part 3 – The Survey: Peptides

Peptides: up to 40 AAs, MW with substituent(s) less than 5kDa, no pegylation

- Good agreement between Pharma and CRO – LC-MS/MS broadly dominates
- Validation for development phases follows BMV guidance
- Acceptance criteria follow 4/6/15 BMV guidance
  - One comment highlighted no SIL availability as default reason for 4/6/20
- Discovery phase supported with fit for purpose assays
  - Little or no assay qualification, expanded acceptance criteria
- Though rare, aberrant PK may be a driver for ADA/NAb assays
  - Strategy commonly driven by Sponsor

Part 3 – The Survey: Proteins

Proteins/Mabs: > 40 AAs, MW with substituent(s) >10kDa

- Good agreement between Pharma and CRO – LBA broadly dominates
  - Follows BMV for development, FFP for discovery phases
- Where hybrid LC-MS/MS was mentioned acceptance criteria of 4/6/20 were suggested but the dominant position of respondents was: ‘it depends’
- Confirmatory assay strategies vary from none to a dependency on selectivity/interference data
- Mixed perspectives in responses for immunogenicity strategy
- CRO responses trend heavily towards Sponsor requests

Take Aways

- Sensitivity and Selectivity remain main drivers when selecting a bioanalytical platform though practical considerations also key
- Data generated on two platforms are both “true”
  - An understanding of why results are different is more important than any numerical difference. How is the communicated?
- Acceptance criteria for LC-MS assays of proteins: “it depends”
  - Depends on what? Can we standardise?
- Does not seem to be a clear strategy surrounding confirmatory analyses
  - When is there value?
- No consensus on immunogenicity testing

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