LC-MS/MS Biomarker Assay Validation Considerations from the New Guidance: Discussion of the Upcoming AAPS Whitepaper Recommendations and Context of Use

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Outline

- AAPS BFG working group
- Scope of the project
- Working Group Perspective
- Outline of WG Recommendations for validation of biomarker assays by LC-MS
- Context of Use and Biomarker Assay Validation
- Assay Life Cycle

AAPS APQ Bioanalytical Focus Group Sub-Group on LC/MS Biomarker Method Validation

- Beginning in the early 2000's, pharma started evaluating the use of novel biomarkers in drug development leading, to now more than a dozen white papers, mention of biomarkers in the latest FDA guidance, and perhaps a more prescriptive guideline on biomarkers in drug development.
  - The large collection of literature on the topic created a need for a pharma-centric description of best practices from industry practitioners.

- Following the AAPS Crystal City VI Workshop in 2015 on biomarker measurements, the BFG Biomarker Topic Group was formed to continue discussions on the validation of biomarker assays based on LC-MS/MS technology.
  - The group is comprised of industry leaders in the area of biomarker analysis by mass spectrometry
  - The group discussed the application of LC-MS to quantification of small molecule, peptide, and protein biomarkers; compiled best practices; built on the discussions from Crystal City VI; and is providing practical approaches to biomarker validation.

- This group was tasked with *providing clarity* on the validation of LC/MS assays for biomarkers used in drug development.

AAPS APQ Bioanalytical Focus Group Sub-Group on LC/MS Biomarker Method Validation

- Goals and Objectives
  - Establish an expert resource within AAPS
  - Collect best practices from across the industry
  - Present a consensus view of minimum required best practices for LC/MS validation of biomarkers used in drug development

- Membership
  - Brad Ackermann, Mark Arnold, Binodh deSilva, Carmen Fernández-Metzler, Fabio Garofolo, Huidong Gu, Vinita Gupta, Omar Laterza, Yan Mao, Mark Rose, Rick Steenwyk, Faye Vazvaei

Scope of the Project

- In Scope
  - Validation of LC/MS assays measuring endogenous substances used in studies providing evidence of drug safety and/or efficacy
  - LC/MS assays measuring endogenous substances supporting studies intended to be submitted to a regulatory agency (includes assays used to prove an endogenous substance is a clinically useful biomarker)

- Out of Scope
  - Established clinical assays used for the diagnosis and treatment of patients by physicians (regulated under CAP/CLIA)
  - Assay kits sold as in vitro diagnostics used without further validation

What We All Agree On

- We all know PK assays
- We all know what an assay validatio
- We recognize that the elements of a are the same whether in Pharma, Cl Environmental, Petrochemical, Fooc
- Ultimately, it's all about selectivity, a reproducibility

Accuracy
Precision
- Within run
- Between runs
- Between days
- Between operators
- Between lots

Sensitivity
- Analytical Measurement Range
- Lower and Upper limit of quantitation

Selectivity
- Blanks, Interference testing, Matrix effects
- Parallelism
  - Minimum Required Dilution
- Recovery
  - Extraction, Ionization, Overall Method

Dilutional Linearity/Integrity
Stability
- Reference material, reagents, samples
- Autosampler, Bench top, Extract, Freeze-thaw, Short term, Long term, Stock Solution

Robustness
Ruggedness

Universal Tenets of Assay Validation

- Clear definition of the MEASURAND
- Proof of SELECTIVE measurement of the target measurand
- Complete characterization of the assay REPRODUCIBILITY
- Best available assessment of the ACCURACY of the measurements
- Characterization of the MEASUREMENT RANGE of the assay
- SAFETY precautions and hazards associated with conducting the assay

Biomarker Assays Are Special

- We don't always know the relationship between the molecular species we are detecting by LC-MS and what the body is producing
  - Hydrolysis of sterol and steroid esters
  - Peptides as a surrogate for proteins
  - Proteins as a surrogate for systems

- Authentic reference materials are not always available
  - Certified reference standards
  - Traceable reference material
  - Fully characterized reference material
  - Uncharacterized reference material
  - Normal sample controls without known concentration

- Sample Matched, Analyte-Free Matrix is rarely available

➢ *All lead to additional experiments and perhaps additional validation elements*

Working Group Perspective

- Biomarker Assay Validation using LC-MS should be
  - Fit-For- Purpose whose elements depend on
    - the availability and characterization of reference material,
    - the context of use of the biomarker,
    - the assay life cycle
      - an assay evolves without the need to call out a new assay, just the next stage in the assay development
  - Based on a validation plan that
    - Describes the scientific justification for approaches used in the validation of a given biomarker
    - Is known by stakeholders

Working Group Methods to Determine Best Practices

- Monthly teleconference meetings focused on topics identified as relevant from the Crystal City VI summaries
- Published literature describing biomarker assay development, use and validation
- Best practices from each of the represented pharmaceutical companies
- Summaries and surveys from group participants

Methods to Determine Best Practices:

Use of Team Surveys to Collect Best Practices

Example: Biomarker Assay QC Preparation

1. How well characterized do QCs have to be, e.g., establish linearity and ranges, assign nominal value, determine stability, etc.?
2. How is the concentration of the biological matrix pool used to assign nominal value QC?
3. When do you use multiple level QCs? How many levels? Under what conditions are 2 level QCs (high and low) enough?
4. How do you prepare QC's when endogenous levels are very high?
5. How do you prepare a low-level QC when endogenous levels are high?

Recommendations

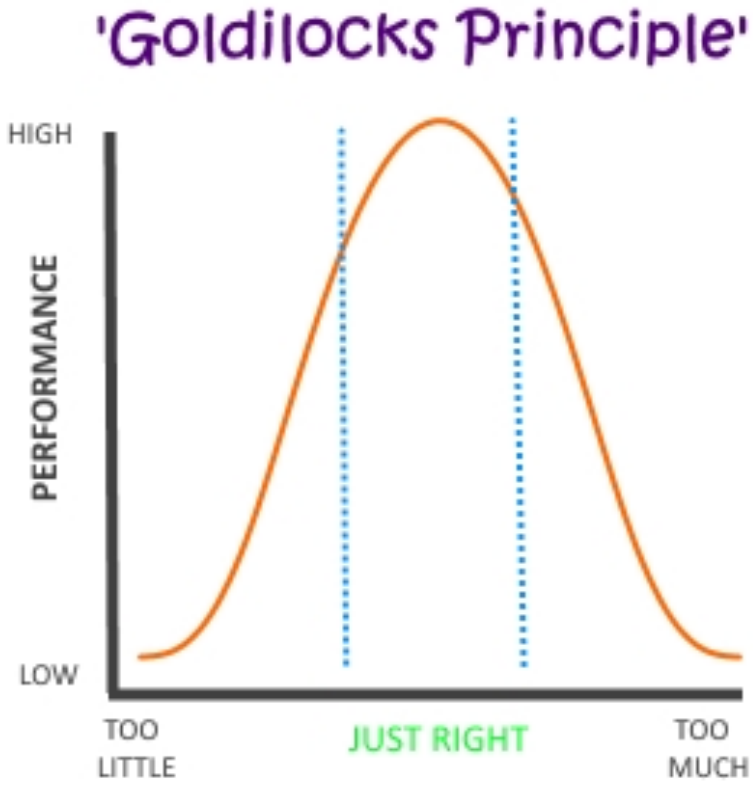
- Create and follow a Validation Plan shared with stakeholders
- Outline all performance characteristics of the validated assay that will ensure appropriate data is collected to drive decisions in drug development, including safety and efficacy
- Develop the validation plan to provide proof of the claims made regarding the performance of the assay
- Provide proven scientific justification when changing, adding or deleting elements of the assay validation from the FDA Guidance on Bioanalytical Method Validation

Context of Use and Assay Validation

Not IF but WHEN

- Meant to convey the relationship between how the data is intended to be used, that is, the decisions that will be informed by the data, and the level of rigor to which the assay is validated
- The problem comes when different stakeholders control the assay and the experiment/project. There is near certainty that how the data is used will not match with how the assay was validated.
- Therefore, pre-defined validation requirements that can support a changing context of use are needed.

Fit for Purpose is an Everyday Concept



How do we link Context of Use and Assay Validation?

Selectivity

- Changes in assay selectivity requirements are driven by:
  - Sample matrix
  - Analyte
  - Analytical technique

Accuracy

- Changes in the requirements for accuracy of the results are driven by:
  - Use of the data
  - Readout + Experiment
  - Reference material

Precision

- Changes in the requirements for assay precision are driven by:
  - Use of the data
  - Readout + Experiment

Assay Readout

The actual **measurement** reported from the analysis of samples is the readout

Qualitative
What?

Quantitative
How much?

Binary
Yes/No

Pattern
Yes/No

Concentration
or Amount
(absolute)

Change
(relative)

Requires positive/negative controls

Requires reference material

Targeted

Untargeted
(Omics)

Targeted

In practical terms, it is difficult to separate the Readout from the Experiment

Practical aspects that support a particular use of the data

- Readout + Experiment
- Reference material
- Matrix

Assay Life Cycle

An IT concept applied to BA methods

- The traditional thinking is that an assay is developed one time for a compound and used to measure the analyte in different experiments. Very much like a computer program.
- In practice, we rarely have a single assay for a compound, even if we stay with the same analytical instrumentation. Computer apps change as the needs change.
- We can communicate the need to change the assay in a similar fashion to the development of the drug itself, using the same gates to allow for assay changes in an expected and definable way. A proactive rather than reactive approach.
- Assay Life Cycle is a way to communicate that the assay is due for a change.
- Context of Use
  - drives when that change happens and
  - defines the particulars of the change required to meet the need.
- If we are thoughtful, we can stage the Assay Life Cycle to match our company's pipeline stages.

Fit for Purpose Biomarker Assay Validation Scheme

Type of Assay Work	Number of Samples	Acceptance Criteria	Exploratory (Cat 1)			Decision Making (Cat 2)	
			MoA/ PD, no TE	Predictive	Efficacy	Safety	Target Engagement
			Qualification	Qualification	Qualification	Full Validation	Full Validation
Sample sensitivity in relevant population	20 disease and 10 normal	>80% samples above LLOQ	Y	Y	Y	Y	Y
Reference range establishment, may be same as above	20 disease and 10 normal	NA	optional	optional	Y	Y	optional
Specificity at assay development stage, not qual	5--7 individuals	>90% inhibition upon blocking with cap	Y	Y	Y	Y	Y
LLOQ	2 samples or lowest std point	80-120% recovery and <20%CV	Y	Y	Y	Y	Y
Linearity of dilution - MRD verification	7--10 individuals	80-120% recovery	Y	Y	Y	Y	Y
Linearity of dilution - admixing/ endogenous spike recovery	3 sets, H and L	80-120% recovery	optional	optional	optional	optional	optional
Real sample/ matrix stability - freeze thaw and room temp	3-5 individuals	report as found, <20% diff considered stable	Y	Y	Y	Y	Y
Spike recovery	3-5 normals, 2 levels	80-120% recovery	optional	optional	optional	Y	Y
Precision using matrix based controls	3 levels, over all qual/ val runs	<20%CV	Y	Y	Y	Y	Y
Intra-subject variability	5 individuals, 3-4 time points	report as found	optional	Y, can help decide thresholds	Y, can help decide thresholds	Y, can help decide thresholds	Y, can help decide thresholds
Total analytical error		<30-40% std curve	optional	optional	optional	Yes?	Yes?
Long term reagent stability in real time	2-3 years, collect the data until alowable	report as found	only if it is longitudinal?	only if it is longitudinal?	only if it is longitudinal?	Y	Y

Context of Use Assay Validation Matrix

	QUALITATIVE			QUANTITATIVE	
VALIDATION ELEMENTS	**Binary**	**Pattern**		**Concentration**	**Relative Change**
SELECTIVITY					
matrix effect				x	
matrix equivalence				x	
blanks	x	x		x	x
SPECIFICITY					
interference testing		x		x	x
CARRYOVER	x	x		x	x
STABILITY					
stocks	x	x		x	x
reagents	x	x		x	x
samples (prepn, long-term)	x	x		x	x
freeze-thaw	x	x		x	x
TOTAL ERROR					
Systematic Error					
accuracy (relative)				x	
bias					
pos/neg control	x	x			
Random Error					
precision	x	x		x	x
ROBUSTNESS					
CRITICAL PROCEDURE STEPS					

pre-clin research | pre-clin safety | clin exploratory | clin safety | clin efficacy | clin diagnostic

Local Application of Global Principles

- Each company and each project team have their own way of structuring research and development
- The program changes that dictate changes in the assay are very likely to define a new Context of Use
- The elements of validation and the experiments to be performed as proof of meeting a claim about the performance of an assay are nearly universal.
- By creating a local validation matrix to support local processes using global validation principles, we can pre-define validation requirements where we know we will have a new Context of Use. The Assay Life Cycle ties the two together practically by providing a way to communicate the expectation that the assay will change as the use of the data, the sample matrix, and the assay readout change.

Acknowledgments

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Questions