A Survey of Pharmacodynamic Biomarkers Bioanalysis in 20 Biologics License Applications Approved for Enzyme Replacement Therapy Indications

Yunghon Lee, PharmD, ORISE Fellow ; Yow-Ming Wang, PhD ; Xiulian Du, PhD

Office of Clinical Pharmacology, Office of Translational Sciences, Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD, USA

FDA U.S. FOOD & DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION & RESEARCH

OFFICE OF CLINICAL PHARMACOLOGY

Objective

The objective is to survey the landscape of pharmacodynamic (PD) biomarkers in the regulatory submissions of enzyme replacement therapy (ERT) products and their method validation profiles. Evaluation of the current state of PD biomarkers bioanalysis can inform future best practice.

Methods

Twenty BLAs of ERT products (approved 1990-2023) were reviewed for their PD biomarkers and analyzed. PD biomarkers were stratified into groups (YES/YES, NO/YES, YES/NO, and NO/NO) dependent on the combination of two criteria they met:

- Listed in their product's label (USPI)
- Had a bioanalytical method validation report.

Whereas the YES/NO and NO/NO groups were not amenable for analysis due to the absence of a method validation report, the validation parameters for each biomarker of the YES/YES and NO/YES groups were analyzed for the following:

- The 7 key analytical parameters (accuracy, analytical measurement range (AMR), parallelism, precision, selectivity, specificity, stability) mentioned in the Critical Path (CP) Institute's Points to Consider Document (2019) and if they were evaluated in their validation reports.
- Concordance of method validation with the recommendations in "*Bioanalytical Method Validation Guidance for Industry*" (2018).
- Types of endpoints and context of use (COU) of PD biomarkers in clinical studies.
- Relationship between COU and evaluated parameters.
- Relationship between PD biomarkers, clinical studies, endpoints, COU, bioanalytical methods, and parameters.

Results

- From 20 BLAs, 22/35 PD biomarkers belonged to either the YES/YES and NO/YES groups for analysis, specifically 13/35 and 9/35 respectively **(Figure 1).**
- Each of the 22 biomarkers assessed a subset of the 7 key analytical parameters, ranging from 2 parameters for 1 biomarker to 7 parameters for 1 biomarker **(Figure 2).**
- In both groups, the most used endpoint type of PD biomarkers was secondary endpoint (12/13, 7/9) while the least used endpoint type was tertiary endpoint (4/13,4/9) **(Table 1).**
- The most used COU type in both groups were PD response (13/13,9/9), while the least used type was safety (4/13,2/9) and dosing (4/13, 3/9) **(Table 1).**
- Across all 22 biomarkers, the number of biomarkers evaluated for each of the 7 key parameters varied between the YES/YES and NO/YES groups, respectively: accuracy and precision (13,9) AMR (11,8), parallelism (9,5), selectivity (13,6), specificity (8,2), and stability (11,7) **(Table 1).**
- The relationship between the COU and assay parameters varied depending on the COU type. In the YES/YES group, the most evaluated parameters for all COU types was accuracy, precision, and selectivity, while the least evaluated was parallelism (PD response & efficacy) and specificity for all COU types. In the NO/YES group, the most evaluated parameters were precision and accuracy for 3/4 COU types while for dosing it was AMR and parallelism. The least evaluated parameter was specificity for all COU types **(Table 2).**
- Compared to the recommendations for validation parameters, the tested parameters demonstrated variability in adherence. The parameters with the highest adherence were as follows: YES/YES (accuracy, precision, selectivity) and NO/YES (accuracy, precision). The parameters with the lowest adherence were parallelism and specificity for both YES/YES and NO/YES group **(Figure 3).**
- The relationship between PD biomarkers, clinical studies, endpoints, COU, bioanalytical methods, and parameters conveyed heterogeneity **(Figure 4).**

Results (Continued)

Figure 1: Number of PD Biomarkers using (1) listed in USPI - YES or NO, (2) with a validation report - YES or NO, to divide into four groups

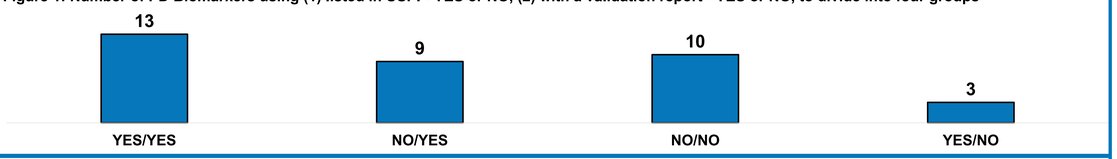

Figure 2: Average Number of Parameters Evaluated per PD Biomarker

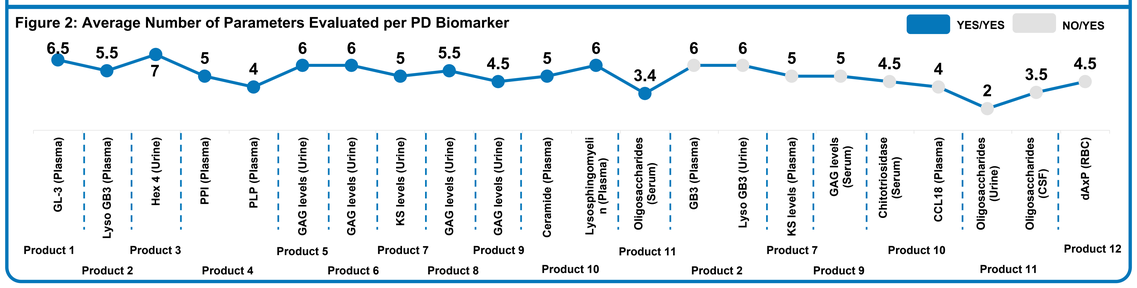

Figure 3: Adherence to BMV Guidance (2018) Recommendations by Parameter

DRUG INFORMATION						PRECISION					ACCURACY					SELECTIVITY				STABILITY	SPECIFICITY				PARALLELISM	AMR
Group	Product #	PD Biomarker	Method	Assay Type	VR Year	C1	C2	C3	C4	C/L1	C1	C2	C3	C4	C/L1	C5	C6	C7	C8	C/L2	C6	C7	C8	C/L 3		
YES/YES	1	GL-3 (Plasma)	HPLC-MS/MS	CA	2005																				✓	✓
	2	Lyso-GB3 (Plasma)	UPLC-MS/MS	CA	2021																					✓
	2	Lyso-GB3 (Plasma)	UPLC-MS/MS	CA	2019																				✓	✓
	3	Hex 4 (Urine)	UPLC-MS/MS	CA	2013																				✓	✓
	4	PLP (Plasma)	HPLC-MS/MS	CA	2014																					
	5	GAG levels (Urine)	GAG Analyzer	CA	2001																				✓	✓
	7	KS levels (Urine)	HPLC-MS/MS	CA	2009																					
	9	GAG levels (Urine)	UPLC-MS/MS	CA	2015																					✓
	9	GAG levels (Urine)	UPLC-MS/MS	CA	2014																					✓
	10	Ceramide (Plasma)	HPLC-MS/MS	CA	2011																					✓
	10	Ceramide (Plasma)	HPLC-MS/MS	CA	2019																					✓
	10	Lysosphingomyelin (Plasma)	UPLC-MS/MS	CA	2015																				✓	✓
	11	Oligosaccharides (Serum)	HPLC-MS/MS	CA	2012																				✓	✓
	11	Oligosaccharides (Serum)	HPLC-MS/MS	CA	2013																				✓	✓
	11	Oligosaccharides (Serum)	HPLC-MS/MS	CA	2015																					✓
	11	Oligosaccharides (Serum)	UPLC-MS/MS	CA	2017																					✓
	11	Oligosaccharides (Serum)	UPLC-MS/MS	CA	2021																					✓
NO/YES	2	GB3 (Plasma)	HPLC-MS/MS	CA	2019																				✓	✓
	2	Lyso GB3 (Urine)	UPLC-MS/MS	CA	2019																				✓	✓
	7	KS levels (Plasma)	HPLC-MS/MS	CA	2009																					
	9	GAG levels (Serum)	UPLC-MS/MS	CA	2014																					✓
	11	Oligosaccharides (Urine)	HPLC-MS/MS	CA	2012																				✓	✓
	11	Oligosaccharides (Urine)	HPLC-MS/MS	CA	2015																					
	11	Oligosaccharides (CSF)	HPLC-MS/MS	CA	2012																				✓	✓
	11	Oligosaccharides (CSF)	HPLC-MS/MS	CA	2013																				✓	✓
	12	dAxP (RBC)	HPLC-MS/MS	CA	2012																				✓	✓
	12	dAxP (RBC)	HPLC-MS/MS	CA	2016																				✓	✓

DRUG INFORMATION						PRECISION					ACCURACY					SELECTIVITY			STABILITY		SPECIFICITY			PARALLELISM	AMR
Group	Product #	PD Biomarker	Method	Assay Type	VR Year	L1	L2	L3	L4	C/L1	L1	L2	L3	L4	C/L1	L5	L6	L7	C/L2	L8	L9	L10	C/L 3		
YES/YES	1	GL-3 (Plasma)	ELISA	LBA	2000																			✓	✓
	4	PPI (Plasma)	Enzyme Coupling Assay	LBA	2012																				
	4	PLP (Plasma)	Enzyme Coupling Assay	LBA	2009																				✓
	5	GAG levels (Urine)	Alcian Blue	LBA	1999																				✓
	6	GAG levels (Urine)	DMMB Assay	LBA	2002																			✓	✓
	8	GAG levels (Urine)	DMMB Assay	LBA	2001																			✓	✓
	8	GAG levels (Urine)	DMMB Assay	LBA	2004																			✓	
	9	GAG levels (Urine)	DMMB Assay	LBA	2005																			✓	✓
	9	GAG levels (Urine)	DMMB Assay	LBA	2014																			✓	
NO/YES	10	Chitotriosidase (Serum)	4MU Standard Curve	LBA	2000																				✓
	10	Chitotriosidase (Serum)	4MU Standard Curve	LBA	2015																				✓
	10	CCL18 (Plasma)	ELISA	LBA	2012																				✓

- **Green:** Parameter tested with exact match to BMV recommendation
- **Yellow:** Parameter tested with partial match to BMV recommendation
- **Red:** Parameter tested but did not test BMV recommendation
- **White:** Parameter not tested for PD biomarker in validation report so can't assess adherence to BMV recommendations
- ✓: Parameter tested in validation report as recommended but no specific BMV recommendations to assess adherence.

Figure 4: Relationship between PD biomarkers, Clinical Studies, Endpoints, Context of Use (COU), Bioanalytical Methods, and Parameters

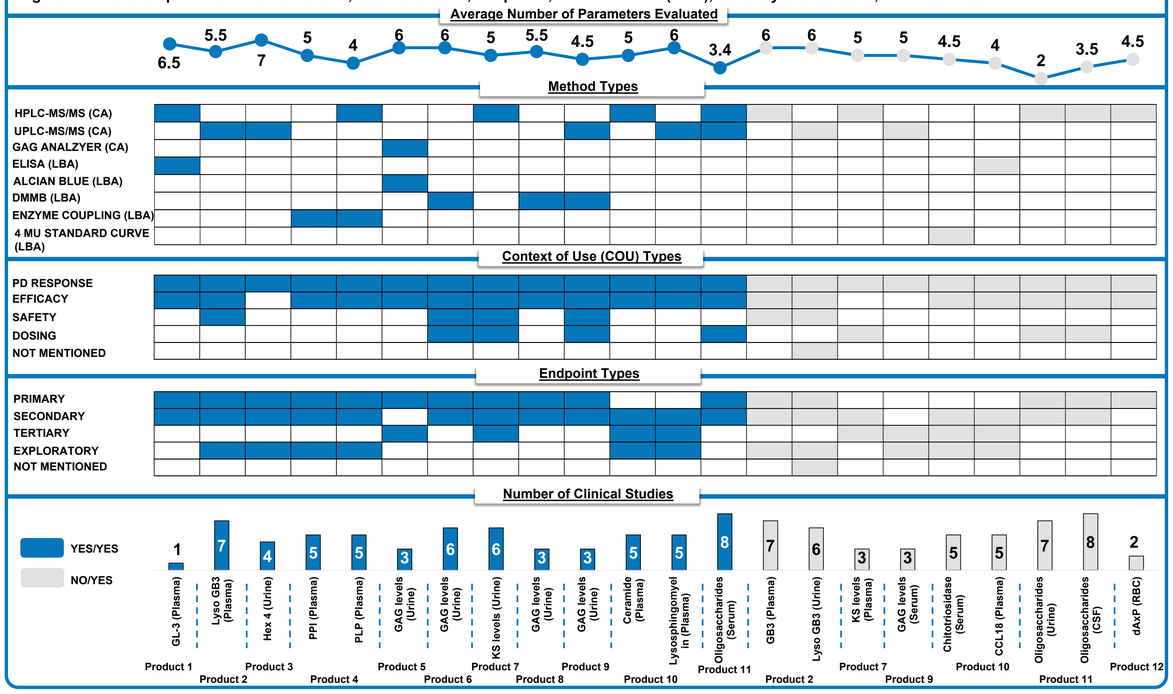

Table 1: Number of PD Biomarkers For Each Endpoint Type, COU Type, and Assay Parameter

Green: Most used endpoint type, COU type, or evaluated parameter

Endpoint Type	YES/YES (N=13)	NO/YES (N=9)
Primary	11	5
Secondary	12	7
Tertiary	4	4
Exploratory	6	5
Not Mentioned	0	1
Context of Use (COU) Type		
PD Response	13	9
Efficacy	12	7
Safety	4	2
Dosing	4	3
Not Mentioned	0	1
Assay Parameter		
Accuracy	13	9
Precision	13	9
AMR	11	8
Parallelism	9	5
Selectivity	13	6
Specifcity	8	2
Stability	11	7

Table 2: Context of Use (COU) and Assay Parameter Relationship

Red: Least used endpoint type, COU type, or evaluated parameter

YES/YES	N=13	N=12	N=4	N=4	N=0
Assay Parameter	PD Response	Efficacy	Safety	Dosing	Not Mentioned
Accuracy	13	12	4	3	0
Precision	13	12	4	3	0
AMR	11	10	3	3	0
Parallelism	8	8	3	3	0
Selectivity	13	12	4	3	0
Specificity	8	7	1	1	0
Stability	11	10	4	3	0
NO/YES	**N=9**	**N=7**	**N=2**	**N=3**	**N=1**
Accuracy	9	7	2	1	1
Precision	9	7	2	1	1
AMR	8	7	2	2	1
Parallelism	5	5	2	2	1
Selectivity	6	4	2	1	1
Specificity	2	1	0	1	0
Stability	7	5	2	1	1

Bioanalytical Method Validation Guidance (2018) Recommendations

*** Applicable for Chromatographic (CA) and Ligand Binding Assays (LBA)**

***C = Chromatographic Assay Recommendation**

***L = Ligand Binding Assay Recommendation**

***C/L = Chromatographic and Ligand Binding Assay Recommendation**

- **C1**: At least 3 independent runs
- **C2**: 4 QC levels/conc per run (LLOQ, L, M, HQC)
- **C3**: ≥ 5 replicates per QC level
- **C4**: ± 15% of nominal concentrations (Accuracy) or CV (Precision); except ± 20% at LLOQ.
- **L1**: At least 6 independent runs
- **L2**: 5 QC levels/conc per run (LLOQ, L, M,H, ULOQ)
- **L3:** ≥ 3 replicates per QC level
- **L4:** ± 20% of nominal concentrations (Accuracy) or CV (Precision); except ± 25% at LLOQ, ULOQ
- **C/L1**: Run should meet the calibration curve acceptance criteria and include the LLOQ calibrator
- **C5:** Analyze blank samples of the appropriate biological matrix from at least six individual sources.
- **C6:** Blank and zero calibrators should be free of interference at the retention times of the analyte(s) and the IS.
- **C7:** Spiked samples should be ± 20%LLOQ.
- **C8:** The IS response in the blank should not exceed 5% of the average IS responses of the calibrators and QCs.
- **L5:** Investigate parallelism (for endogenous products).
- **L6:** Conduct an analysis of blank samples in the matrix from ≥ 10 individual sources.
- **L7:** For ≥ 80% of sources, unspiked matrix should be BQL, and spiked samples should be ± 25% at LLOQ, and ± 20% at HQC.
- **L8:** The accuracy (% nominal) at each level should be ± 20%.
- **C/L2:** For auto-sampler, bench-top, extract, freeze-thaw, stock solution/reagent and long-term stability, perform at least three replicates at LQC and HQC concentrations.
- **L9:** Potential interfering materials should be added to calibration curves in buffer.
- **L10:** QCs should meet ± 20%, or 25% at the LLOQ and ULOQ.
- **C/L3:** The method specificity should be assessed for interference by cross-reacting molecules, concomitant medications, bio-transformed species, etc.

Conclusions

Though recommendations for PD biomarker bioanalytical method validation are available regarding which parameters should be evaluated and how they should be evaluated, the survey results conveyed heterogeneity in biomarker bioanalysis and further work is necessary to develop best practice.

Disclaimers & Funding

The findings and conclusions in this poster reflect the views of the authors and should not be construed to represent FDA's views or policies. Dr. Yunghon Lee's fellowship was supported through the Oak Ridge Institute for Science and Education (ORISE).

References

Available upon request. WWW.FDA.GOV