APPLIED PHARMACEUTICAL ANALYSIS

SHORT COURSE

SUNDAY, SEPTEMBER 17

OMNI PROVIDENCE HOTEL | PROVIDENCE, RI

Practical Considerations for Biomarker Bioanalysis: Scientific and Regulatory Perspective

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APA 2017 CONFERENCE SHORT COURSE AGENDA

SUNDAY, SEPTEMBER 17

Course Coordinator: Darshana Jani, Pfizer

8:30 am - 9:30 am
Registration

9:30 am - 9:40 am
"What are Biomarkers? The Role of Biomarkers in Drug Development"
Darshana Jani, Pfizer

9:40 am - 10:20 am
"Biomarker Reference Standard-White Paper"
Paul Rhyne, Biologics Development Services

10:20 am - 10:50 am
"Choosing the Right Assay Platform for Biomarker Quantitation"
Hans Ulrichts, UCB Pharma

10:50 am - 11:10 am
Break

11:10 am - 11:40 am
"LBA Biomarker Assay Performance - Bioanalytical Challenges and Solutions"
Stephanie Fraser, Pfizer

11:40 am - 12:10 pm
"Biomarker Analysis Using Flow Cytometry Focused on Preclinical Studies"
Martin Schwickart, Celgene

12:10 pm - 1:10 pm
Lunch

1:10 pm - 1:40 pm
"Practical Approaches to Protein Biomarker Quantification by LC-MS"
Tim Sikorski, GlaxoSmithKline

1:40 pm - 2:10 pm
"Biomarker Quantitation by LC-MS: Solutions to Challenges"
Fizal Nabbie, Bristol-Myers Squibb

2:10 pm - 2:20 pm
Break

2:20 pm - 3:00 pm
"A Regulatory Perspective on Biomarkers for Pivotal Studies: Method Validation, Sample Analysis and Inspections"
John Kadavil, FDA

3:00 pm - 3:30 pm
Q & A Session

3:30 pm - 4:30 pm
Cocktail Hour Sponsored by

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APA SHORT COURSE ABSTRACTS

Choosing Right Assay Platform for Biomarker Quantitation

Hans Ulrichts, UCB Pharma

- Biomarker Selection: from hypothesis to analysis. How to translate study objectives in analytical requirements
- Biomarker Assay Selection: selecting the right tool for the job. How to select the right assay format and platform for the study needs.

LBA Biomarker Assay Performance - Bioanalytical Challenges and Solutions

Stephanie Fraser, Pfizer

The inclusion of protein biomarkers to support preclinical and clinical studies has increased notably over the last 15 years. More often than not program decisions now include and/or depend on data from these biomarkers. The quality of this data relies equally upon an understanding of the biology of the system and robust assay performance. It is common for assay performance issues to arise throughout assay development, qualification and/or validation. Each performance issue provides a unique opportunity to better understand the assay and the data it is capable of delivering. Examples of analytical challenges presented include matrix selection for endogenous biomarkers, dealing with recombinant reference materials, reagent aggregation, and sample stability.

Practical Approaches to Protein Biomarker Quantification by LC-MS

Tim Sikorski, GSK

This lecture will provide an introduction to different LC-MS workflows available for targeted biomarker analysis, and discuss the tradeoffs that are often required when choosing one strategy over another. Also, there will be discussions around lessons learned during study sample analysis using different LC-MS platforms. Finally, this lecture will highlight some systematic method development strategies that Dr. Sikorski's group has developed to ensure measurement accuracy of endogenous protein and minimize unforeseen obstacles during study support.

Biomarker Quantitation by LC-MS: Solutions to Challenges

Fizal Nabbie, BMS

LC-MS application to challenging ligand-binding assays: Case studies showing how LC-MS was used in a hybrid format to resolve challenging issues in Biomarker assays.

A Regulatory Perspective on Biomarkers for Pivotal Studies: Method Validation, Sample Analysis and Inspections

John Kadavil, FDA

The supporting role of biomarker determinations continues to grow in pivotal pharmacokinetic (PK) and pharmacodynamics (PD) studies for drug applications submitted to the FDA. Because of the increased use of biomarker assays, the Office of Study Integrity and Surveillance (OSIS) has broadened its scope of bioanalytical inspections in order to evaluate data and methods associated with biomarker determination in pivotal studies. During an inspection of method validations and study sample analysis for biomarker measurements, OSIS may take into account special considerations when evaluating parameters such as precision, sensitivity, stability, the calibration curve, reproducibility, specificity and selectivity. These considerations may include the technology used, the biological nature of the analyte, the use of the assay, and the intended purpose of the biomarker determination. Although methods for biomarker determination present additional challenges compared to traditional PK assays, variables that are evaluated during inspections of PK studies may still apply to biomarker measurements for pre-study validation and in-study analysis.

Darshana Jani, Pfizer

What are Biomarkers? Role of Biomarkers in Drug Development

Course Moderator:

Darshana Jani, M.Sc.

Darshana.Jani@pfizer.com

Applied Pharmaceutical Analysis

Sep 17, 2017

Program at Glance

Objective

- To provide intensive and in-depth training in the field of Biomarkers-practical considerations
- To provide attendees with a convenient Biomarker references
- Introduce attendees to Biomarker Network

Agenda Overview

Top scientists of the world

- Current practices Future Horizons
- Technologies Upcoming platforms
- Regulatory Landscape Panel Discussion

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Disclaimer

- The contents of this presentation reflect the personal opinion of the author and may not represent the official perspectives of the affiliated organization.

What is Biomarker?

"Almost anything you can measure"

- **A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.**

- **Type 0: Markers of natural history of disease and that correlate longitudinally with known clinical indices.**
- **Type I: Markers that demonstrate mechanism of action of a drug.**
- **Type II: Markers that predict a clinical benefit (surrogates).**

Biomarkers Definitions Working Group, NIH, Clin Pharm&Thera 69(3):89-95

Few More Definitions…..

Source	Definition
National Cancer Institute	A biological molecule found in blood, other body fluids, or tissues that is sign of a normal or abnormal process or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. Also called molecular marker or signature molecule
Center for Biomarkers in Imaging (Mass General Hospital)	Anatomic, physiologic, biochemical or molecular parameters associated with the presence and severity of specific disease states
Medicine Net Dictionary	A biochemical feature that be used to measure the progress of disease or effect of treatment

Importance of Biomarkers

Biomarkers can be used clinically

- Screen, diagnose or monitor the activity of diseases

  E.g. Blood sugar to identify and monitor patients with diabetes

- Guide molecularly targeted therapy

  E.g. BRCA1/2 gene mutations to evaluate a patient's risk of developing certain cancers, including breast and ovarian cancer

- Assess therapeutic response

  E.g. Viral load counts to evaluate a patient's response to antiretroviral treatments

Importance of Biomarkers

In the biopharmaceutical industry

- Attrition rate high during drug development
- Provides new ways to measure disease activity and the impact of the medicines being studied
- Biomarkers define molecular taxonomies of patients and diseases
- Serve as surrogate endpoints in early-phase drug trials

Deep-Dive Role of Biomarkers in Pharmaceutical Research and Development

- Discovery/Preclinical
  - Increase/confirm understanding of the target/pathway biology
  - Establish POM in early stage of target evaluation/selection
  - Screening of lead compounds
  - Establish relevance of preclinical model
  - PD considerations and clinical projections
- Development/Clinical Studies
  - Bridging the mechanism to humans- May be exploratory in early phase 1
  - Bridging the mechanism to humans- Confirm the mechanism
  - Dose modulation based on PD
  - Potential surrogacy for efficacy
  - Linkage to possible diagnostic tool

Terminology

Exploratory Biomarkers

Internal decision making

They tend to be endpoints that help the sponsor understand the pharmacodynamics or mechanism of action of the compound

Hypothesis generation

Confirmatory Biomarkers

Support pivotal determinations of efficacy

It is critical to ensure highest standards are met to preserve the integrity of the data.

Biomarker Assay Flow from Research to Clinical Study

	Research/Discovery	Translational Lab	Development Lab	Clinical Study	Clinical Utility
Key activities and deliverables	Identification of Biomarkers **Generate a reasonable proposal** **Evaluate appropriate technology platforms**	Relevance to human subjects and target disease population In-house assay performance? Commercial Reagents? Method transfer from preclinical matrix to human matrix Assay optimization-Single assay vs multiplex format? Ensure operational feasibility-sample type, volume, throughput	**Method validation** **Data Generation** Control/sample Performance and trending **Review the challenges-Fit for purpose** **Successful data generation**	Confirm **assay utility** **Assay maintainence** **Establish or revise clinical utility based on intended use of the data**

Concept appears simple, however, biomarker analysis is a considerable challenge

- What are the biggest challenges - technical, clinical, regulatory?
- What are the common accepted approaches?
- What is the best matrix to utilize?
- And few more……
- Overall consensus is Biomarker assays are not PK assays

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- Let us begin…….

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Summary

- Study design and assay methodology - Comprehensive strategy needed
- Diverse assays, require multidisciplinary team execution
- Bioanalysis is only one piece of the puzzle; consider overall biology
- Biomarker assays are not PK assays – use scientific judgement to treat them accordingly.

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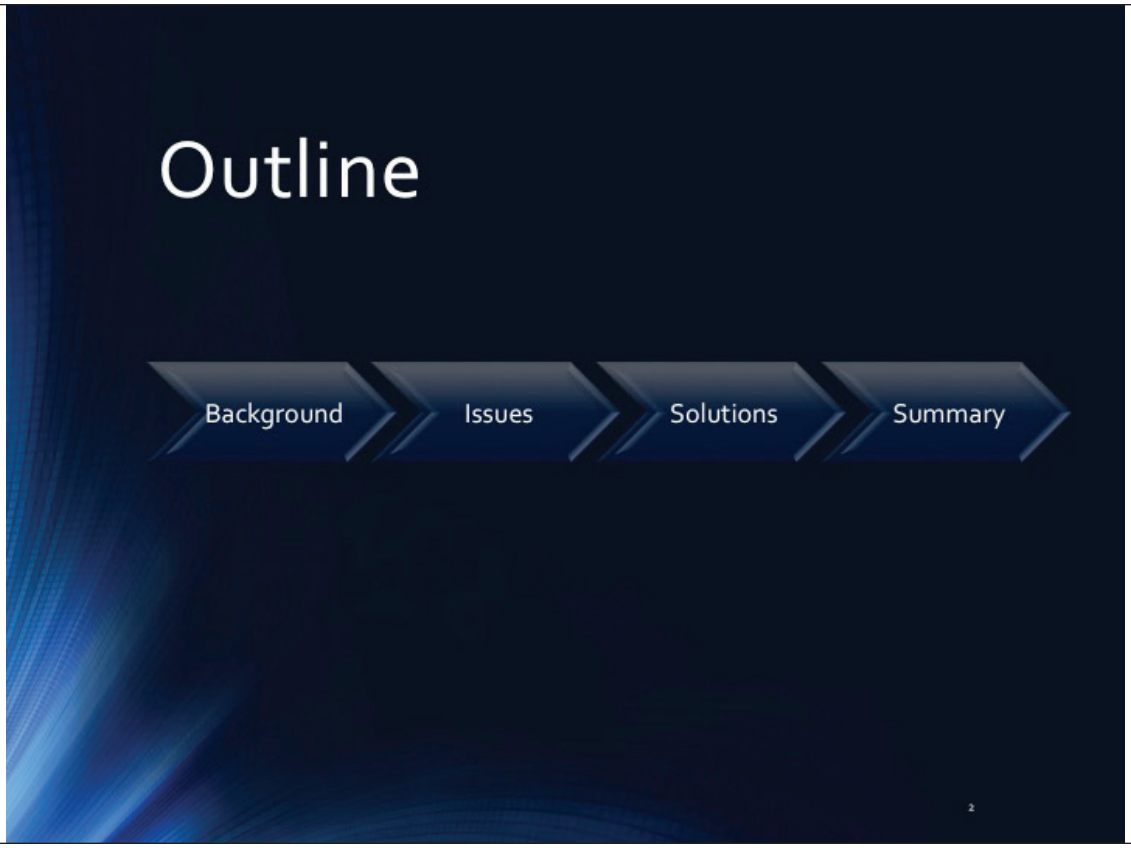
Recommendations to Challenges for Appropriate Selection and Characterization of Calibrator Material
PAUL RHYNE, PH.D
BIOLOGICS DEVELOPMENT SERVICES


Outline
Background
Issues
Solutions
Summary
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Building industry consensus at conferences

AAPS NBC 2014, 2015, 2016

- Biomarker themes, topics

Crystal City V 2013

- FDA draft guidance discussion

Crystal City VI 2015

- Biomarker assay discussion
- Lowes, Ackermann 2016
- 2nd Upcoming publication

WRIB 2014, 2015, 2016

- Biomarker assay discussions

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Consensus and industry white papers

Lee et al. 2005, 2006, 2009

- Biomarker assay validation

O'Hara et al. 2012

- Critical Reagents characterization

King et al. 2014

- GBC Harmonization white paper on critical reagents for LBAs

Bower et al. 2014

- Commentary paper on reference standards and reagents in BMV

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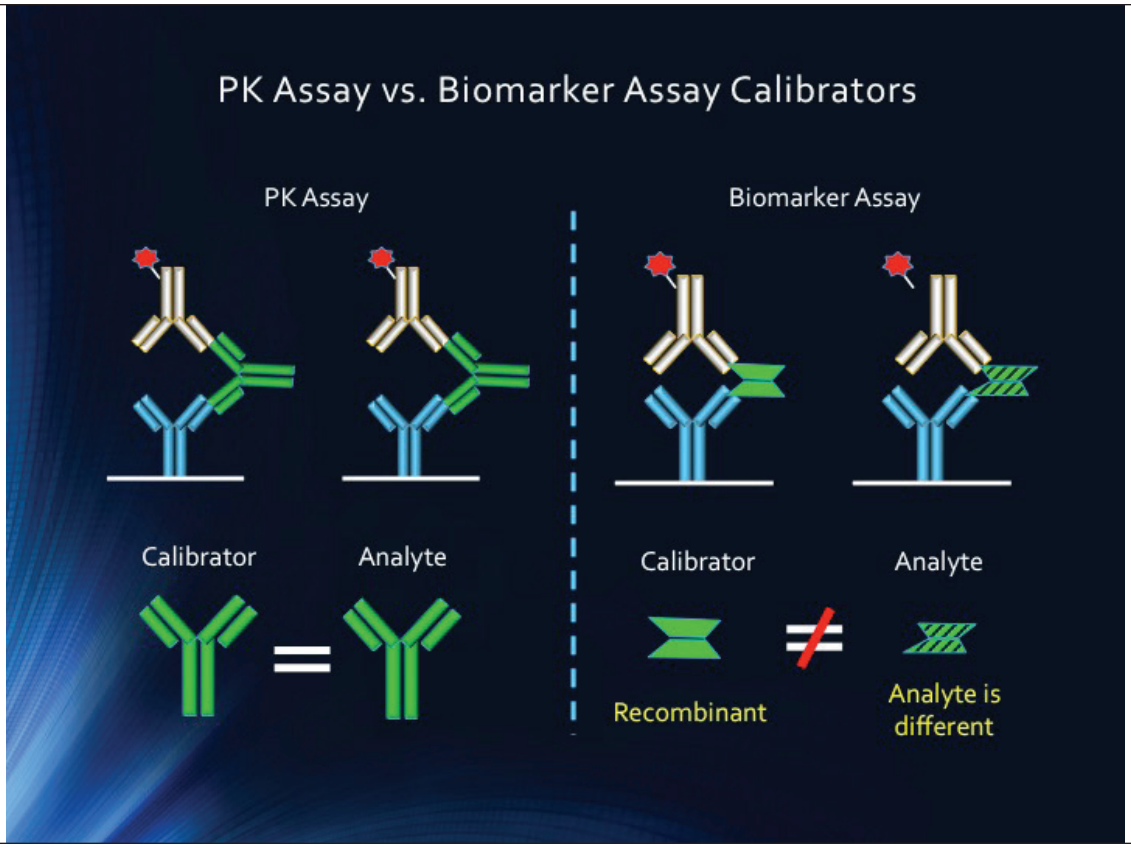
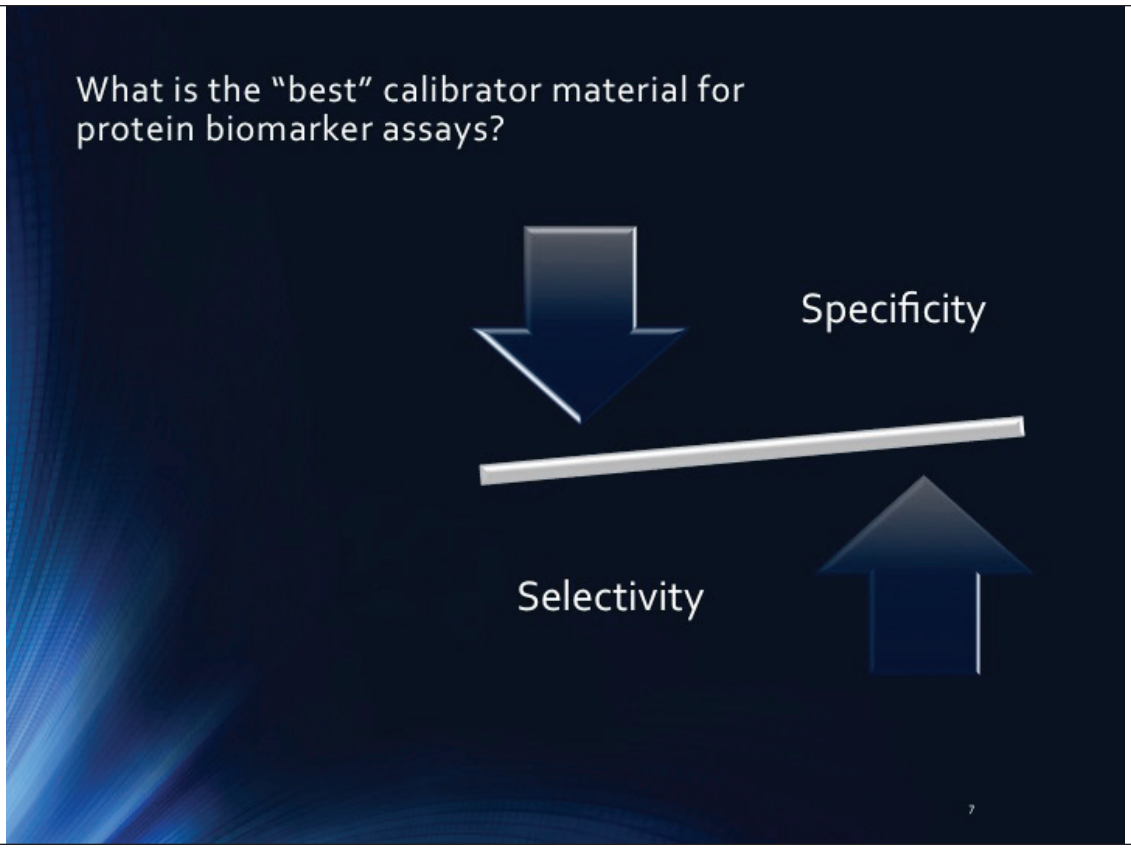
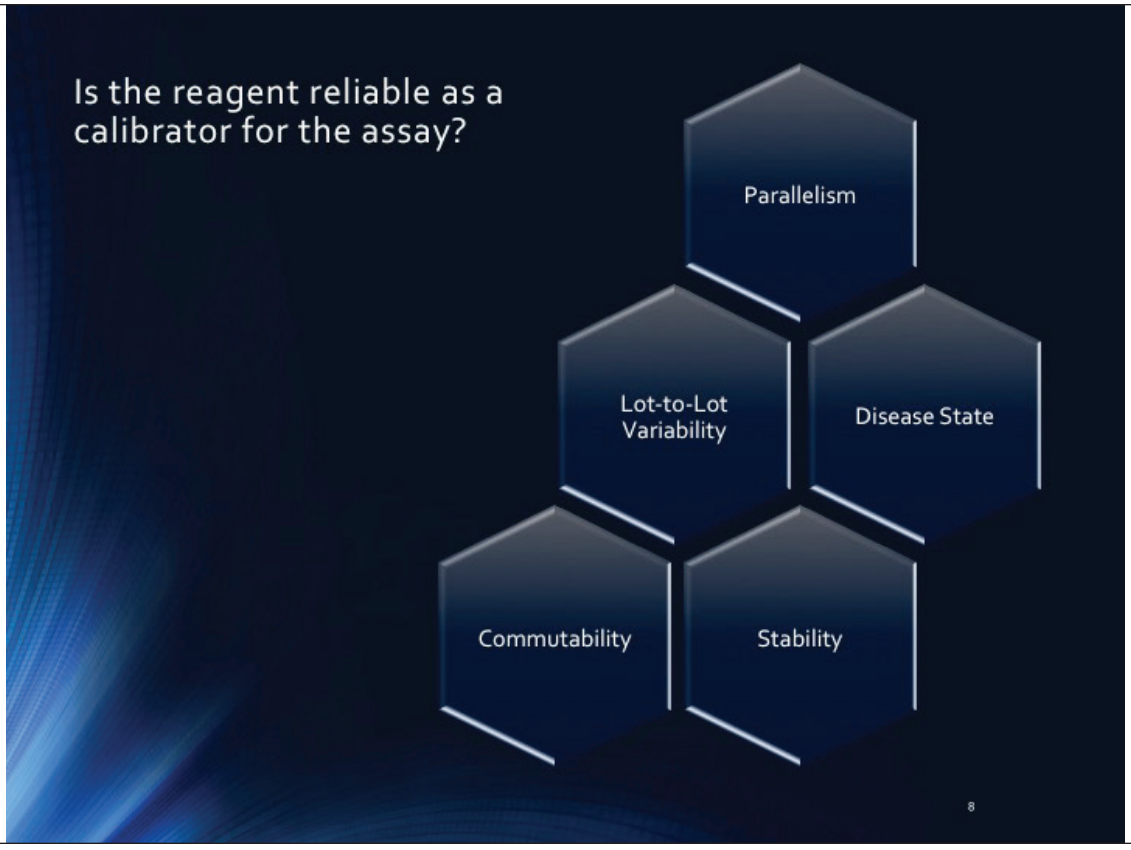

PK Assay vs. Biomarker Assay Calibrators
PK Assay
Biomarker Assay
Calibrator
Analyte
Calibrator
Analyte
Recombinant
Analyte is different


Challenges with recombinant calibrators
Recombinant
Endogenous
Post translational
Sequence
Folding
Expression vectors
Genetics


What is the "best" calibrator material for protein biomarker assays?
Specificity
Selectivity
7


Is the reagent reliable as a calibrator for the assay?
Parallelism
Lot-to-Lot Variability
Disease State
Commutability
Stability
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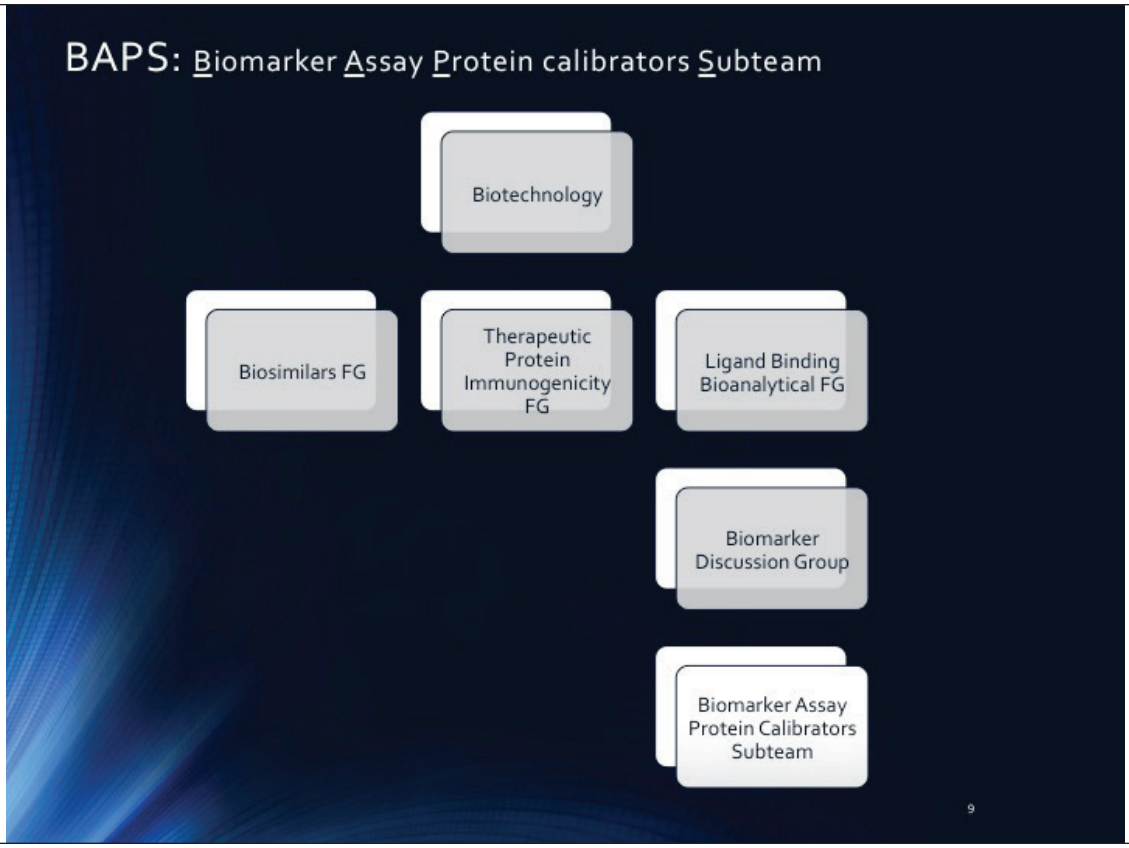
BAPS: Biomarker Assay Protein calibrators Subteam



Biomarker Assay Protein calibrators Subteam

- **Provide recommendations for biomarker calibrator selection, characterization, and to establish consensus to address challenges**
- **Recommend best practices for:**
  - **Fit-For-Purpose approach for selection, characterization, and assay acceptance.**
  - **Based on experience, challenges faced, case studies, and relevant literature.**
  - **Define the important characteristics that a scientist should look for in identifying a reliable recombinant material.**

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Approach

Define Challenges → **Fit-for-purpose** → **Commutability**

- Common to all protein biomarkers
- Widely used protein biomarker assays
- Novel targets/uncommon proteins

- Recommendations across proteins for characterization
- Risks and caveats

- Apply to recombinant material for biomarker assays

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Know your endogenous protein biomarker

How much must the exogenous protein have similarity to the endogenous protein?

- Amino acid sequence
- Secondary, tertiary, quaternary structure?
- Monomeric/dimeric/oligomeric in nature?
- Protein cleavage or alternative splicing?
- "Total" protein: How is the different forms represented?

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Know your endogenous protein biomarker

How much must the exogenous protein have similarity to the endogenous protein?

- Does it have a binding partner? Interaction or binding with the drug being tested?
- Are "misfolded" proteins reactive in the assay? Are they bioactive?
- Endogenous and exogenous comparison
  - Do the modifications or post-translational modifications match?
  - Glycosylation differences?
  - Isoforms?
  - Differences with His-tag, FLAG,-tag, GST fusion, etc.?

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Know your protein biomarker

- Protein Database Resources and published biology
- Calibrator material should be as close to the endogenous form of the protein as possible; improves confidence for quantitative measurement
- Understand your assay and what it can/ cannot detect.

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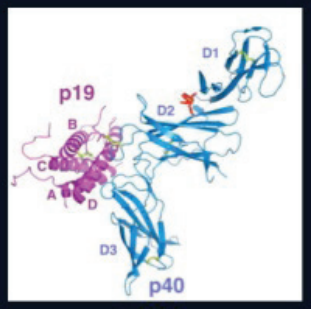
Case Study: IL-23 Luminex Commercial Kit

- IL-23 is a heterodimer protein: p19 and p40 subunit.
- Shares p40 subunit with IL-12 cytokine
- **Commercial IL-23 Assay:**
- Calibrator material is a p19 subunit fused to the p40 subunit.
- Capture Ab made to calibrator material and does not bind IL-12
- Sample data from assay yields higher than expected levels of IL-23 (based on previous publications and in-house data).



RJ Neely, BMS 15

Case Study: Comparison of IL-23 assays (values reported in pg/ml)

Sample	Luminex Assay pg/mL	BMS Assay	3rd Party Vendor
1	283	<9.6	0.047
2	44.5	<9.6	0.111
3	7004	13.4	0.084
4	OOR <	<9.6	0.033
5	102	<9.6	0.035
6	396	<9.6	0.065
7	60	<9.6	0.116
8	1529	<9.6	0.011
9	715	<9.6	0.247
10	OOR <	<9.6	0.071

Jennifer Postelnek, RJ Neely, BMS 16

Case Study: Comparison of IL-23 assays (values reported in pg/ml)

- Calibrator for Luminex assay is a fused p19/p40 heterodimer vs. calibrators for BMS and third party assay are formed ex vivo
- Capture antibodies are different
- Fusion protein may have p19/p40 heterodimers/aggregates
- Ex vivo material likely reflects endogenous more closely than fusion protein

Lessons Learned

•Calibrator material should be as close to the endogenous form of the protein as possible

•Make sure the analyte biology supports the results generated.

Jennifer Postelnek, RJ Neely, BMS

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Information from Protein Manufacturers?

- Concentration of the protein
- Purity (such as a percentage, based on a silver stain or HPLC analysis)
- Source or origin (*E. coli* derived, for example)
- An accession number (providing basic information about the protein)
- Formulation (phosphate buffered saline, for example)
  - Storage and stability information.

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Information from Protein Manufacturers?

Common method(s) used to assign a concentration to a protein product (*if provided*)

- Absorption spectroscopy
- Plate-based colorimetric assays or absorption spectroscopy
- Activity (units)

Recommendation: Contact technical support key to understand this information, including why the vendor selected a particular method over another.

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Recommendations for selection of calibrators

- Define the needed acceptance/rejection criteria (base on intended use of data)
- Determine if any, additional characterization should be done (Need vs nice to have)
- Identify the risks associated with the current characterization vs without additional characterization

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Calibrator assessment

Exploratory

Decision making

Certificate of Analysis

No additional characterization

Additional characterization

Performance in method

Orthogonal methods

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Concentration

Exploratory

- CoA from Manufacturer
- Absorbance (if carrier free)

Decision making

- Additional protein quantitation recommended
- Performance in assay
- Orthogonal methods

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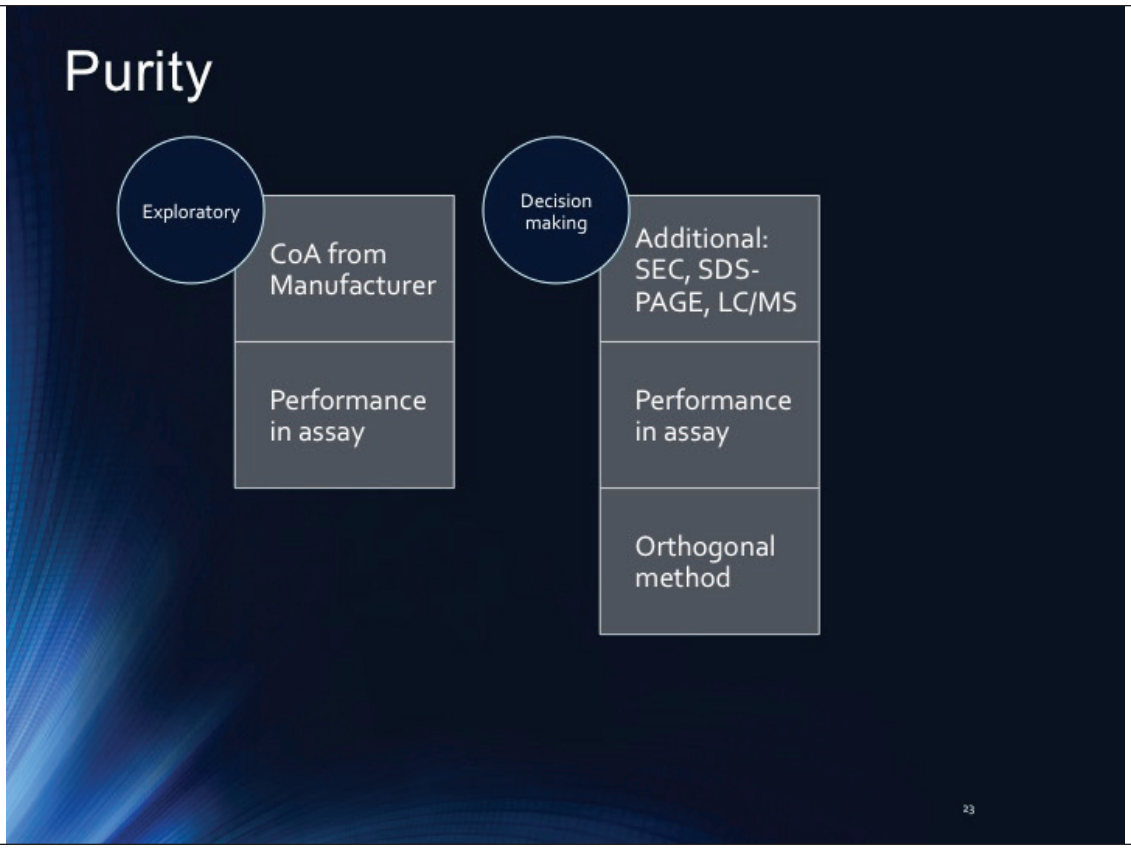
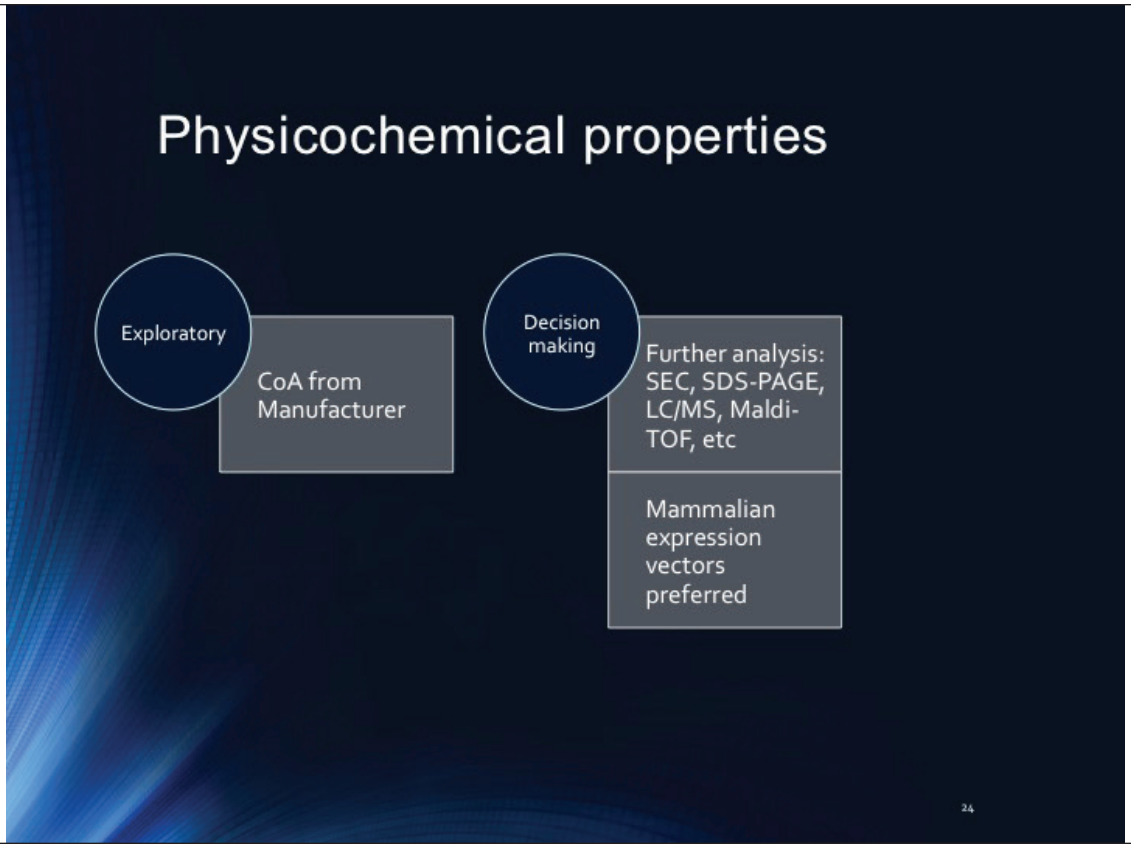
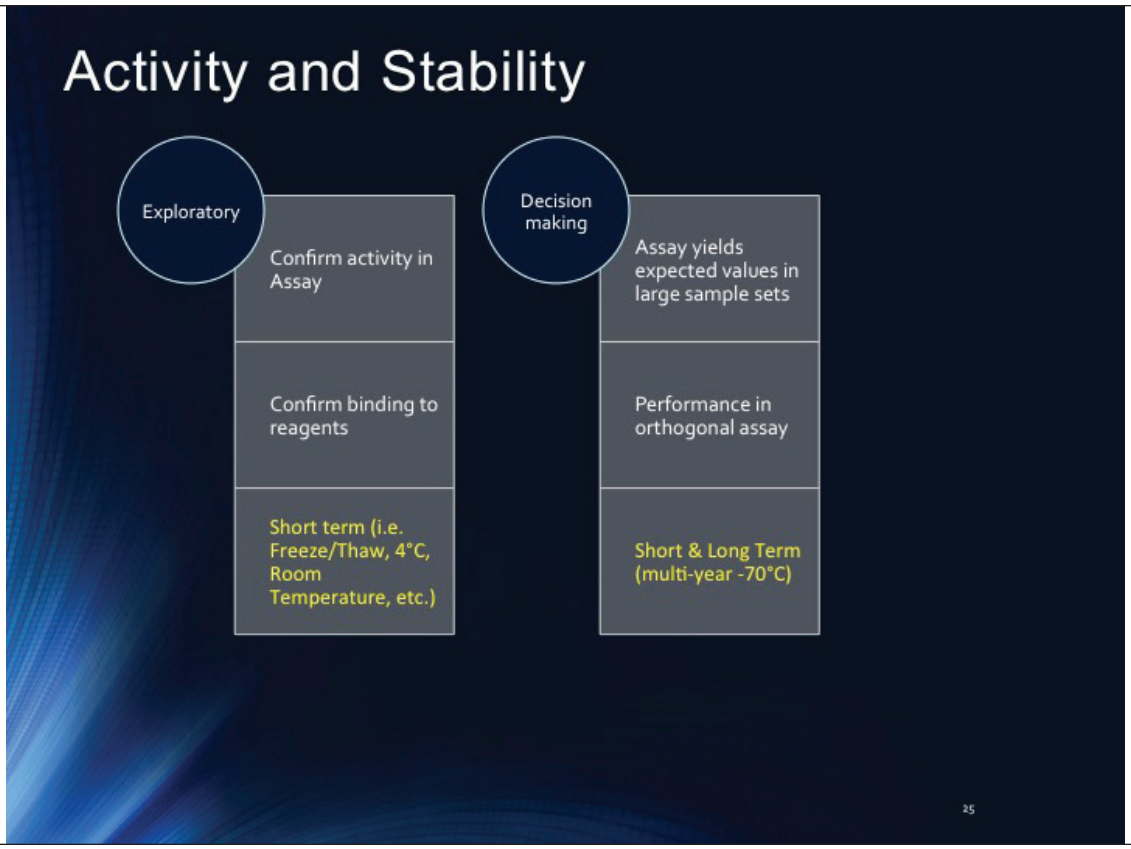
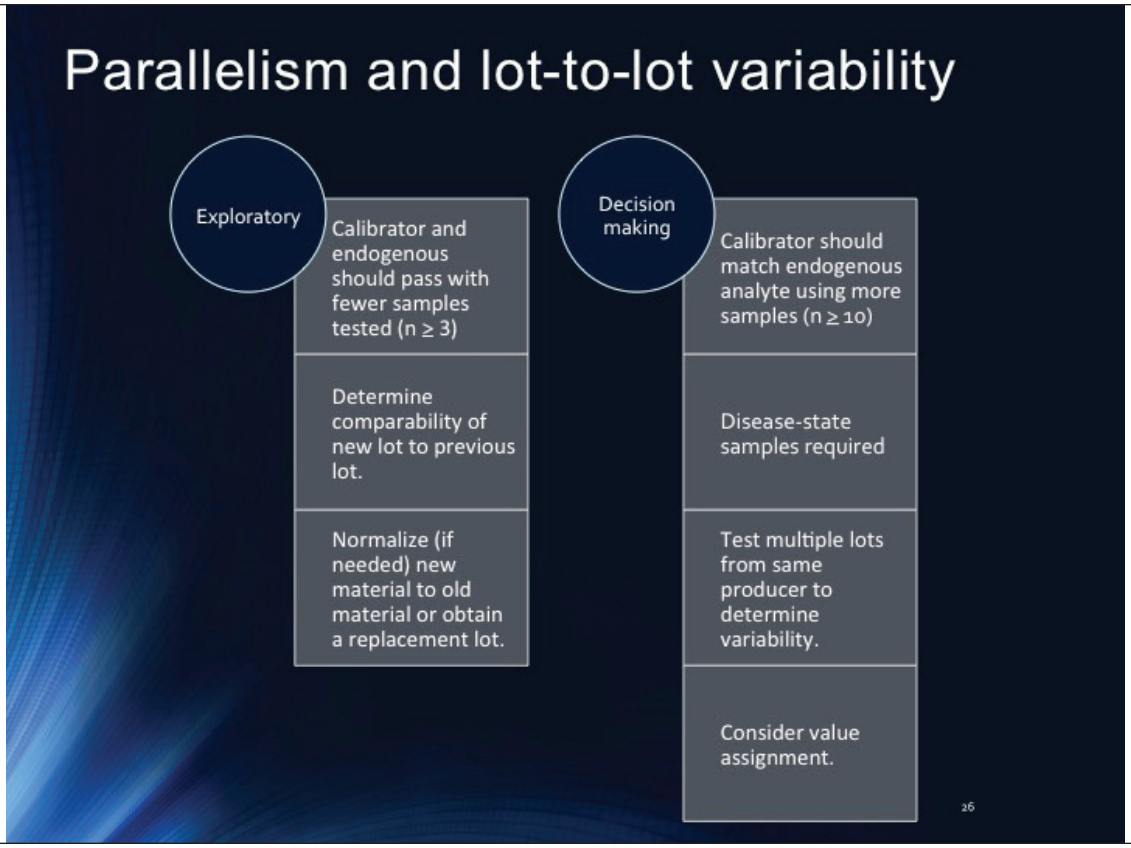

Purity
Exploratory
CoA from Manufacturer
Performance in assay
Decision making
Additional: SEC, SDS-PAGE, LC/MS
Performance in assay
Orthogonal method
23


Physicochemical properties
Exploratory
CoA from Manufacturer
Decision making
Further analysis: SEC, SDS-PAGE, LC/MS, Maldi-TOF, etc
Mammalian expression vectors preferred
24


Activity and Stability
Exploratory
Confirm activity in Assay
Confirm binding to reagents
Short term (i.e. Freeze/Thaw, 4°C, Room Temperature, etc.)
Decision making
Assay yields expected values in large sample sets
Performance in orthogonal assay
Short & Long Term (multi-year -70°C)
25


Parallelism and lot-to-lot variability
Exploratory
Calibrator and endogenous should pass with fewer samples tested (n ≥ 3)
Determine comparability of new lot to previous lot.
Normalize (if needed) new material to old material or obtain a replacement lot.
Decision making
Calibrator should match endogenous analyte using more samples (n ≥ 10)
Disease-state samples required
Test multiple lots from same producer to determine variability.
Consider value assignment.
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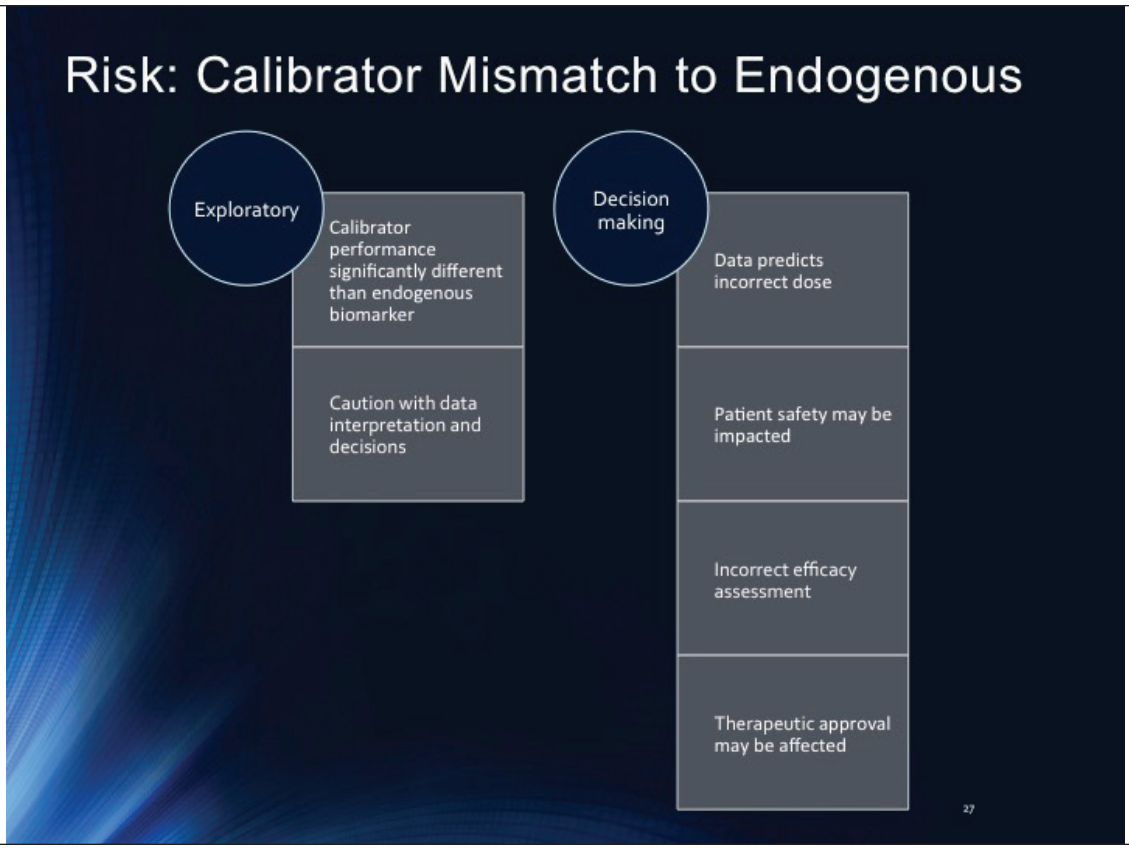
Risk: Calibrator Mismatch to Endogenous
Exploratory
Calibrator performance significantly different than endogenous biomarker
Caution with data interpretation and decisions
Decision making
Data predicts incorrect dose
Patient safety may be impacted
Incorrect efficacy assessment
Therapeutic approval may be affected
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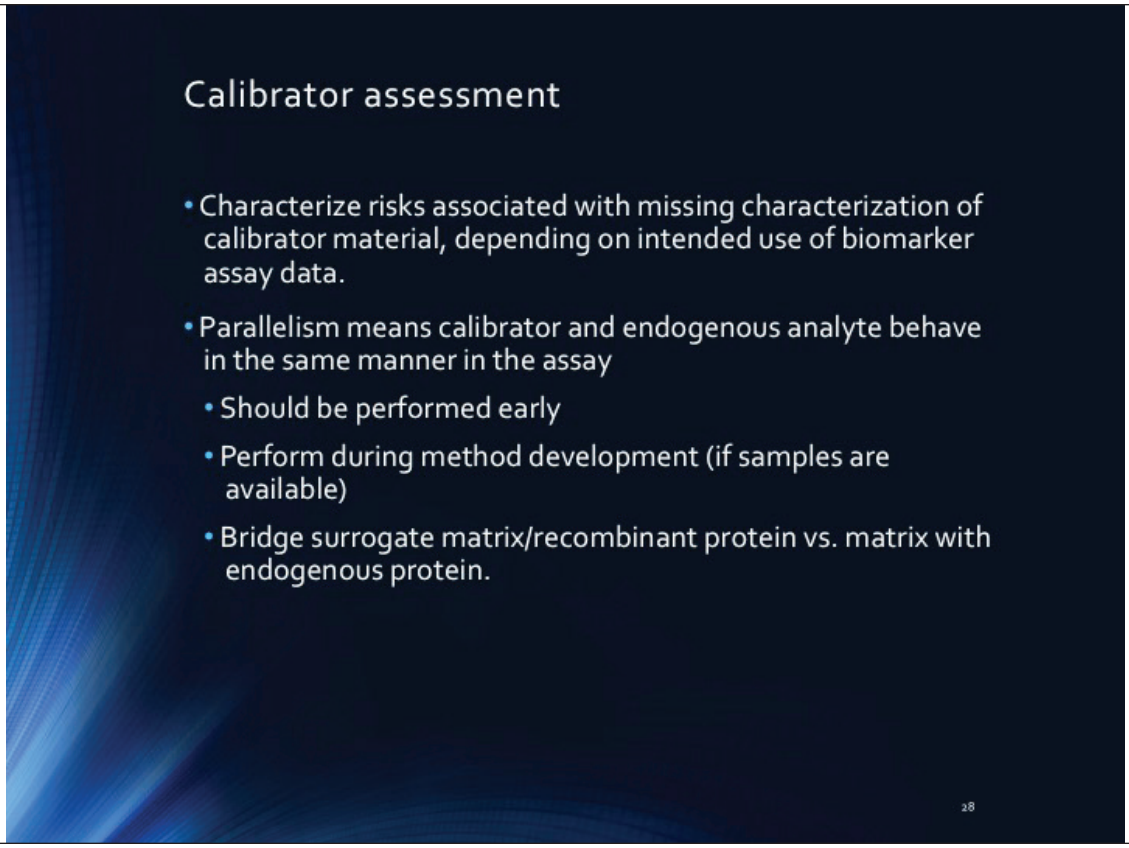
Calibrator assessment
• Characterize risks associated with missing characterization of calibrator material, depending on intended use of biomarker assay data.
• Parallelism means calibrator and endogenous analyte behave in the same manner in the assay
• Should be performed early
• Perform during method development (if samples are available)
• Bridge surrogate matrix/recombinant protein vs. matrix with endogenous protein.
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Commutability

- Commutability: Any mathematical relationship between the results of different assays for a biomarker calibrator and for representative samples.
- A property of the calibrator with respect to a defined set of assays and samples.
- Used in Clinical Chemistry settings to compare calibrators with samples.
- Any assay calibrator material from different sources, or even a different lot from same source, may not be commutable.
- Commutability enables the management of a biomarker assay over long periods of time (years) and to support the use of different platforms

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Summary

- Continue discussions on calibrators for protein biomarker assays
- Know your endogenous protein vs calibrator
    - Biology and binding partners
    - Structure, sequence, etc.
    - Stability and multi-merization
- Calibrator assessment
    - Base on intended use of data
    - Additional characterizations
    - Parallelism is recommended and perform early
- Commutability approaches recommended

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Acknowledgements

- Cross-Industry:
- Lakshmi Amaravadi
- Mark Cameron
- Damien Fink
- Darshana Jani
- Medha Kamat
- Lindsay King
- RJ Neely
- Yan Ni
- Paul Rhyne
- Renee Riffon
- Yuda Zhu

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Thanks!

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Hans Ulrichts, UCB Pharma

Choosing right assay platform for biomarker quantitation

Hans Ulrichts

17SEP2017

Inspired by patients.
Driven by science.

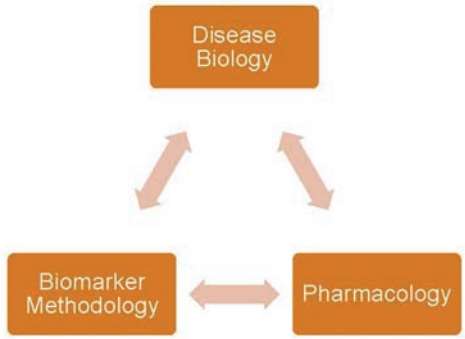
Agenda

- Introduction
- Criteria for biomarker selection
- Method selection
- Case Study

3

Biomarkers – central in drug development

- A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.
- Biomarkers are fundamental to the translational medicine plan, and can support demonstration of pharmacology, efficacy or safety of a new compound hereby allowing decision making or supporting label claims



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Biomarkers – what's in a name

- There exists no universal, standardized BM classification system
- Various 'unofficial' systems / classes are being used, that often differ fundamentally in their point-of-view

Build Appropriate Biomarker Class Lexicon

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Biomarker Lexicon

Target Engagement	Is the drug reaching the site of action ? **Target Occupancy** Is it having a downstream effect ? **Target Engagement**
Efficacy	Is the drug modulating a regulatory accepted endpoint ? **Clinical Response Biomarkers** Are pharmacodynamic changes consistent with its expected MoA ? **PD/Biological Effect Biomarkers**
Safety	Is the drug safe to use in patients ? **Safety Biomarkers**
Stratification	In which patient population(s) is the drug providing the greatest value to patients ? **Patient Stratification**

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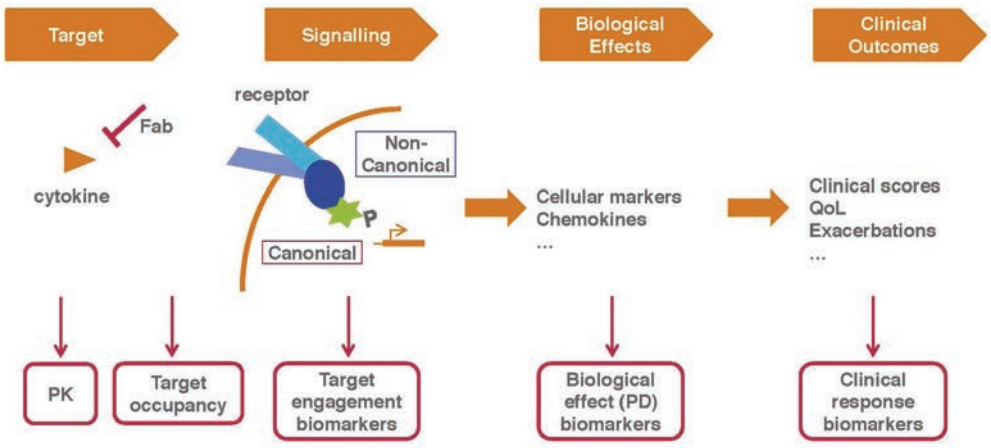
Rationale for Biomarker Hypothesis - Efficacy

Identify the 'golden thread' that links the mechanism to expected biological effects and subsequently clinical response



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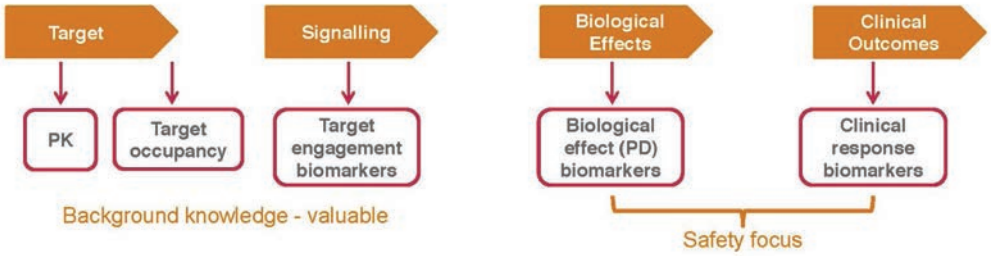
Rationale for Biomarker Hypothesis - Safety

Mechanistic Safety: Mechanism to *expected target* & *known off-target* biological effects and potential clinical responses



Structural-Class Safety: Structural alerts to target & *off-target mechanisms to* biological effects and potential clinical responses

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Biomarker Selection Criteria

Qualification
- Evidence linking the biomarker with the biological process of interest
- From discovery to surrogate biomarkers

Translationability
- The extent to which the same biomarker can be applied in different stages of drug development, across species, experimental models and disease indications

Feasibility
- The feasibility of a biomarker determines whether the marker can be technically / practically applied and analysed for its intended purpose.

Method Availability
- Availability (commercial or in-house) of techniques, equipment, expertise, materials and any other tools required to measure the biomarker

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Biomarker Selection Criteria: Bioanalytical Scientist

Qualification
- Evidence linking the biomarker with the biological process of interest
- From discovery to surrogate biomarkers

Translationability
- The extent to which the same biomarker can be applied in different stages of drug development, across species and across experimental models

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Feasibility – a checklist (1)

Parameter	Check	Example
Source or matrix	One source easier to procure than other	blood / plasma / serum > urine > sputum > synovial fluid (SF) and BALF
Sample size and volume	Depends on source Depends on species Depends on population	Smaller volumes for SF Paediatric development
Required/desired frequency of sampling	How soon are changes expected in BM levels	for a rapid onset, frequent sampling should be feasible for a very slow change in biomarker, study duration need to be very long

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Feasibility – a checklist (2)

Parameter	Check	Example
Stability of the biomarker	The sampling method, storage and shipment of the sample can affect the stability of the biomarker	MMPs are typically unstable and samples need to be handled on ice
'Inherent' biomarker variation	influence of gender, race, diet, stress, diurnal variation	anticipated acute effects can be confounded by diurnal variation

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Method availability – a checklist (1)

Parameter	Check	Example
Method performance characteristics	The sensitivity and dynamic range of a method	• expected changes in BM levels upon drug administration (decrease or increase) • healthy vs diseased
	Accuracy and precision of a method	What are the expected changes of a biomarker What is the inherent variation in a biomarker
	Method compatible for study matrix	Commercial kits for serum, translate to urine
	Specificity of method	
	Reproducibility of method	Inter-lab variation for global trials

13

Defining Preliminary Assay Performance Criteria

Biological Variation → **Acceptable Analytical Performance**

Within subject variation (Coefficient of Variation, CV_I)

Between subject variation (CV_G)

Analytical variation (CV_A) "required precision"

Analytical bias (B_A) "bias goals"

Small expected change in biomarker and/or the high biological variation

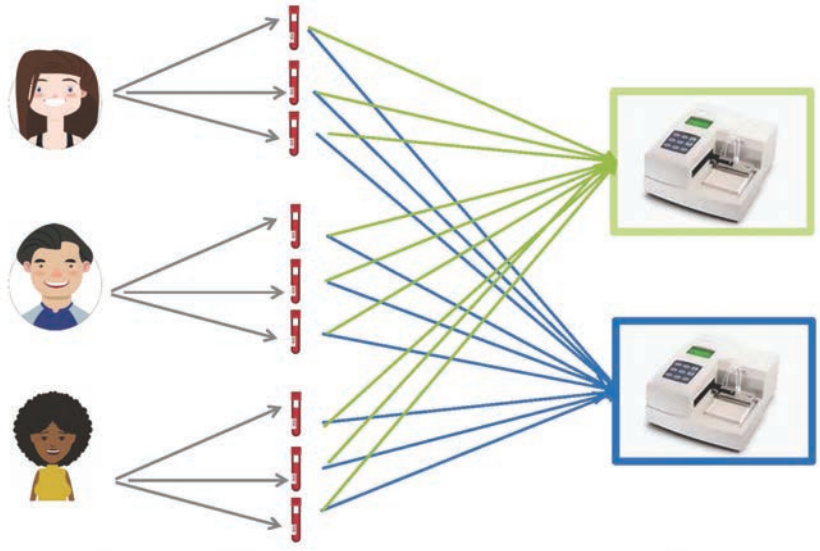
↓

High analytical Performance and/or more subjects required

Ichihara *et al.* Clin Chem Lab Med 2010 Nov;48(11):1537-51

14

Defining Preliminary Assay Performance Criteria



Between subject Variation

Within subject Variation

Analytical Variation

Ichihara *et al.* Clin Chem Lab Med 2010 Nov;48(11):1537-51

15

Defining Preliminary Assay Performance Criteria

CV_A guidance

Optimal	$CV_A < 0.25 * CV_I$
Desirable	$CV_A < 0.5 * CV_I$
Minimal	$CV_A < 0.75 * CV_I$

B_A guidance

Optimal $B_A < 0.125 * (CV_I^2 + CV_G^2)^{1/2}$

(falsely assign <3.3% subjects outside the 90% CI of the **reference limit**)

Desirable $B_A < 0.25 * (CV_I^2 + CV_G^2)^{1/2}$

(<4.4%...)

Minimal $B_A < 0.375 * (CV_I^2 + CV_G^2)^{1/2}$

(<5.74%...)

Mean +or- 1.645*SE
where (SE = SD/$N^{1/2}$)
And N = sample size

Ichihara *et al.* Clin Chem Lab Med 2010 Nov;48(11):1537-51

16

Method availability – a checklist (2)

Parameter	Check	Example
Method throughput	Number of samples that can be processed within certain periof of time	
Calibrator material	Resemblance to endogenous material	Plasma purified, recombinant, pooled matrix,…
Regulatory environment	Can the method be run in a GLP/GcLP-compliant environment	Biomarkers supporting label claims

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Assay formats

Ligand binding assays

platform	Sample volume	Sensitivity (ng/ml)	Matrix effect	Dynamic range	Cost
Elisa	≈ 10 ul	Moderate	Medium	2 logs	Low
MSD	≈ 10 ul	High	Low	3-4 logs	Medium
RIA	100- 500 ul	High	Low	2-3 logs	Low
AlphaLisa	5-100ul	High	High	2-3 logs	Medium
Gyrolab	≈ 5 ul	High	Low	3-4 logs	Medium
Singulex	5- 200 ul	Ultra	Low	3-4 logs	Medium
ImmunoPCR	≈ 2-10 ul	Ultra	High	3-4 logs	High
Luminex	≈ 10 ul	High	Medium	2-3 logs	Medium

Dudal et.al. AAPS J. 2014 Mar;16(2):194-205

18

Assay formats

Mass Spectrometric

platform	Sample volume	Sensitivity (ng/ml)	Matrix effect*	Dynamic range	Cost
LC/MS (conventional)	≈ 20ul	High	Moderate	3-4 logs	Low
LC/MS (protein digestion)	≈ 10-50ul	Moderate	Moderate	3-4 logs	Moderate
LC/MS (immunocapture)	≈ 10-50ul	High	Low	3-4 logs	High
Nano-LC/MS	≈ 5- 20ul	High	Moderate	3-4 logs	Moderate

* Depending on sample pre-treatment

The proces of biomarker selection

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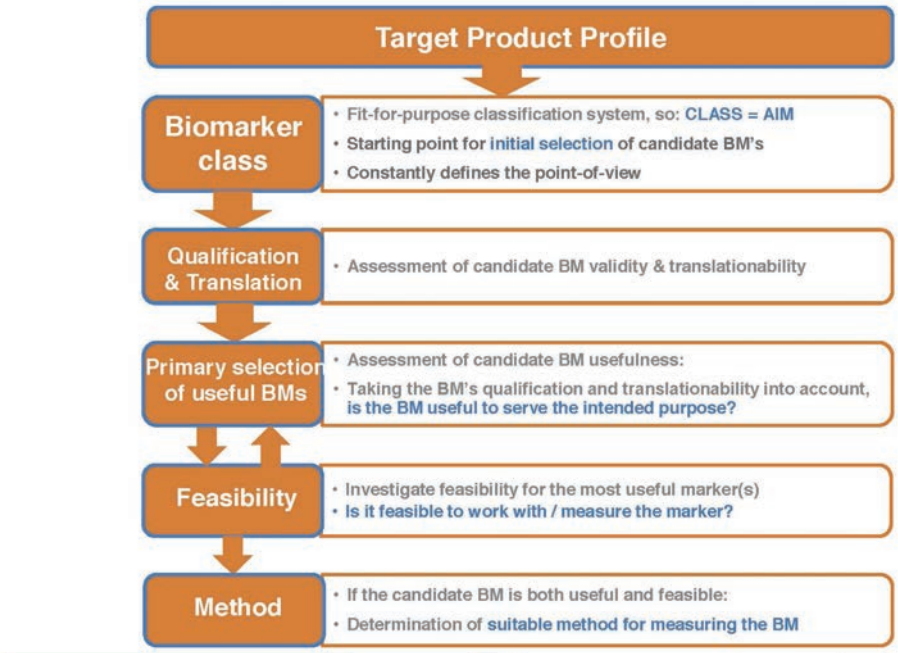


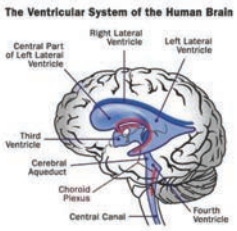
Case study: TTR as marker for choroid plexus function

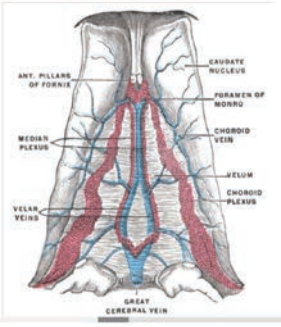
21

Choroid plexus

Structure and Function

- Layer of cuboid epithelial cells found in all four ventricles acts as a barrier between Blood and CSF (similar to BBB)
- Responsible for production of CSF
- Acts as a filtration system
  - Remove metabolic waste
  - Remove foreign substances
  - Balance of neurotransmitters, folate transport, TTR, ion transport and acid-base balance in CSF





' Laterra J, Keep R, Betz LA; et al. (1999). "Blood–Cerebrospinal Fluid Barrier". Basic Neurochemistry: Molecular, Cellular and Medical Aspects. (6th ed.). Philadelphia: Lippincott-Raven.
' Henry Gray (1918) Anatomy of the Human Body Figure 565

22

Case Study

Transthyretin as a biomarker of Choroid plexus function

Transthyretin (TTR)- (prealbumin)

- Functions as a transporter of thyroxine and retinol, which are essential in brain development
- 508 amino acids.
- Synthesized and secretion by CP (90%)
  - Approximately 10% of CSF protein is TTR
- CSF-Blood Barrier prevents passive diffusion of serum TTR in CSF
- Synthesis of serum TTR and CSF TTR is regulated independently

Plasma TTR / CSF TTR ratio as marker for unaffected CP function

Immuno-analytical platforms
Spector and Johanson Fluids and Barriers of the CNS 2013, 10:28

23

Case Study

Study Objective

- Evaluate changes to TTR plasma/CSF ratio's as marker for choroid plexus function in a juvenile 26week toxicity study

Feasibility

Parameter	Check
Source or matrix	Plasma CSF (similar concentrations of TTR in lumbar or ventricular CSF)
Sample size and volume	Limited sample volume CSF also used for other assesments
Required/desired frequency of sampling	Chronic study, infrequent dosing If effect would be present, non-acute changes would be expected
Stability of the biomarker	Not known
'Inherent' biomarker variation	Increase of CSF TTR levels, not serum TTR levels with age

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Case Study

Method availability

Parameter	Check
Method performance - sensitivity	Little information from cynomolgus monkey Plasma: 200-300 µg/mL, human CSF: 10-20 µg/mL, human
Method performance - dynamic range	Increase in CSF TTR levels if increased production Decrease in CSF if CP damage and affected homeostasis Neurological disorders: increase up to 100%, decrease up to -75%
Calibrator material	Cynomolgus monkey has 93% sequence homology with human

One single analytical platform for both plasma as CSF TTR levels

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Case study

Transthyretin – Clinical Chemistry Analyser

- Immunoturbidimetric method
- Applicable to most autoanalysers
- Available for serum/plasma
- Liquid ready-to-use reagents
- **Measuring range 26 – 650 µg/mL**

Not sensitive enough for CSF and/or possible decreases

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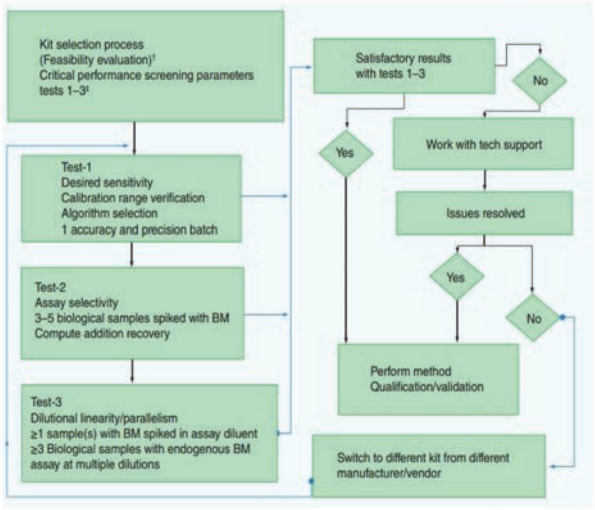
Case Study

Immuno-assay screening



ELISA kits	Calibrator
n = 5	n = 3

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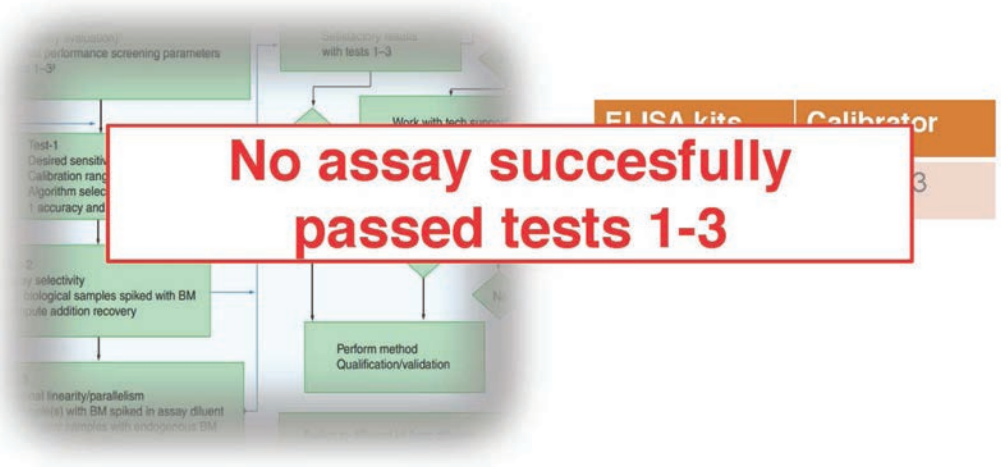
Case Study

Immuno-assay screening



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Case study

LC MS/MS

- Trypsin digestion of samples
- Recombinant cynomolgus monkey TTR as calibrator material

Parameter	Result
Sensitivity	2,5 µg/mL
Precision (CV)	< 10.0%
Bias (|RE| %)	< 6.0%

Parameter	Result
Between subject variation (human, literature)	< 15.0%
Within subject variation (assumed)	< 15.0%

✓ Minimal $CV_A < 0.75*CV_I$

✓ Minimal $B_A < 0.375*(CV_I^2+CV_G^2)^{1/2}$

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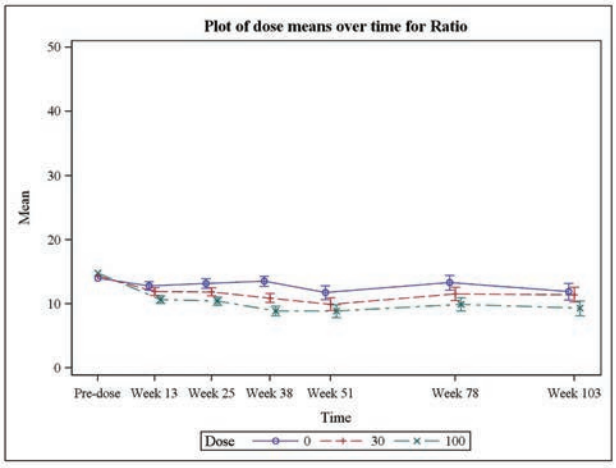
TTR findings in CSF and plasma

Small magnitude changes detected in plasma/CSF ratio



- Small magnitude changes in plasma/CSF ratio throughout the study
  - Control group: 12.9 ± 1.9
  - 30 mg/kg group: 11.7 ± 1.7
  - 100 mg/kg: 10.4 ± 1.9
- Changes are considered significant regarding dose and time
- CSF/plasma levels in line with reported levels within healthy humans

Spector R, Johanson CE. Sustained choroid plexus function in human elderly and Alzheimer's disease patients. Fluids Barriers CNS. 2013 Sep 24;10(1):28. doi: 10.1186/2045-8118-10-28.
Weisner B, Roethig HJ. The concentration of prealbumin in cerebrospinal fluid (CSF), indicator of CSF circulation disorders. Eur Neurol. 1983;22(2):96-105.
Maetzler W et al. Serum and cerebrospinal fluid levels of transthyretin in Lewy body disorders with and without dementia. PLoS One. 2012;7(10):e48042. doi: 10.1371/journal.pone.0048042. Epub 2012 Oct 25.

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Conclusion

A wide variety of Biomarker classification systems exist, each based on a different point of view

- purpose-based lexicon

Selection of biomarkers in (non)-clinical development plan should be initially based on the objectives of the drug development plan after which feasibility and method availability is verified

- Don't measure simply because you can
- Don't stop exploring because there is currently no method available
- Pre-analytical considerations need to be taken into account for rational platform selection

Questions?

Thanks & Acknowledgements!

Bioanalytical Sciences

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Laurent Wautelet
John Smeraglia

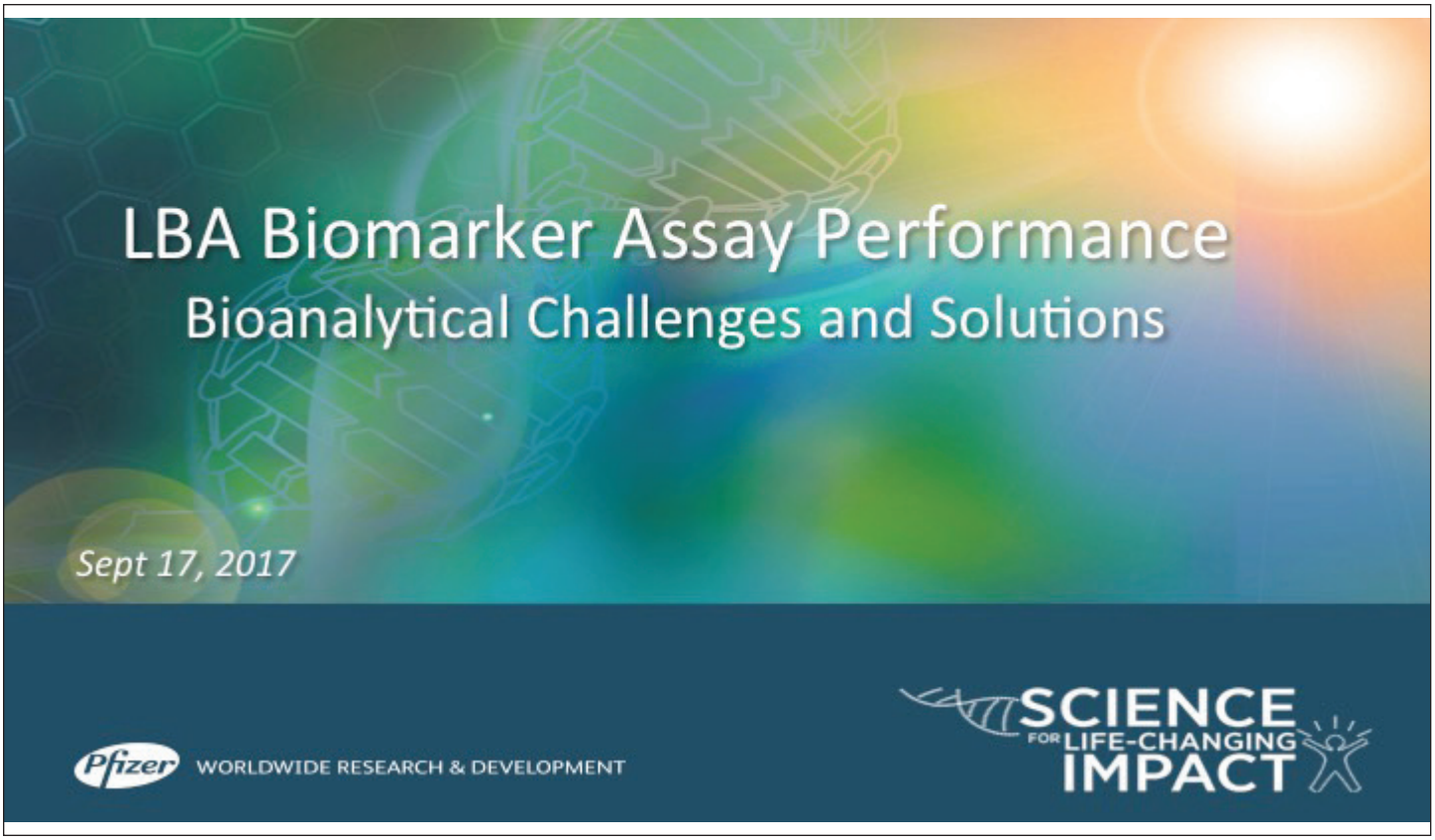
Non-Clinical Safety

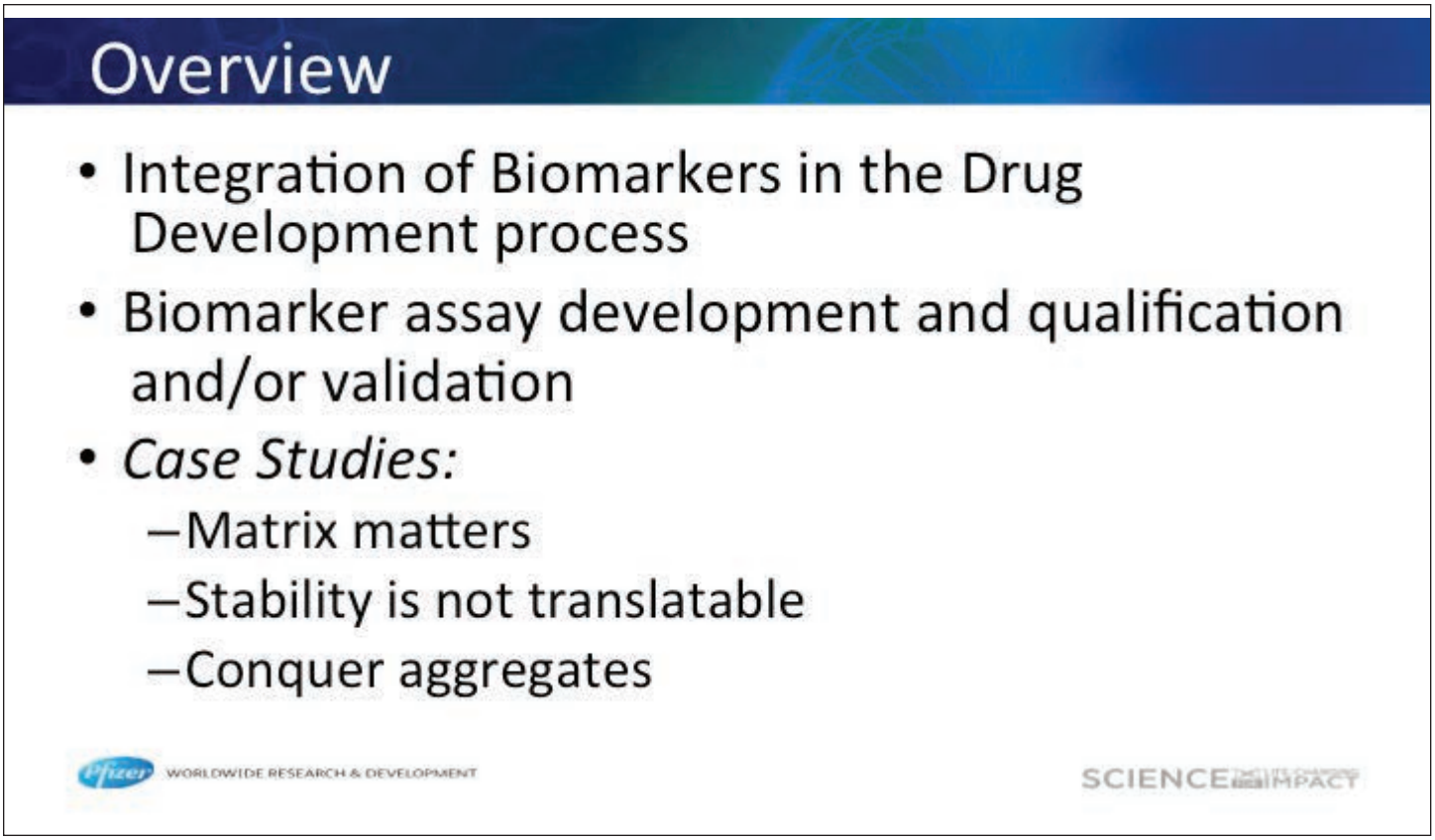
Olympe Depelchin

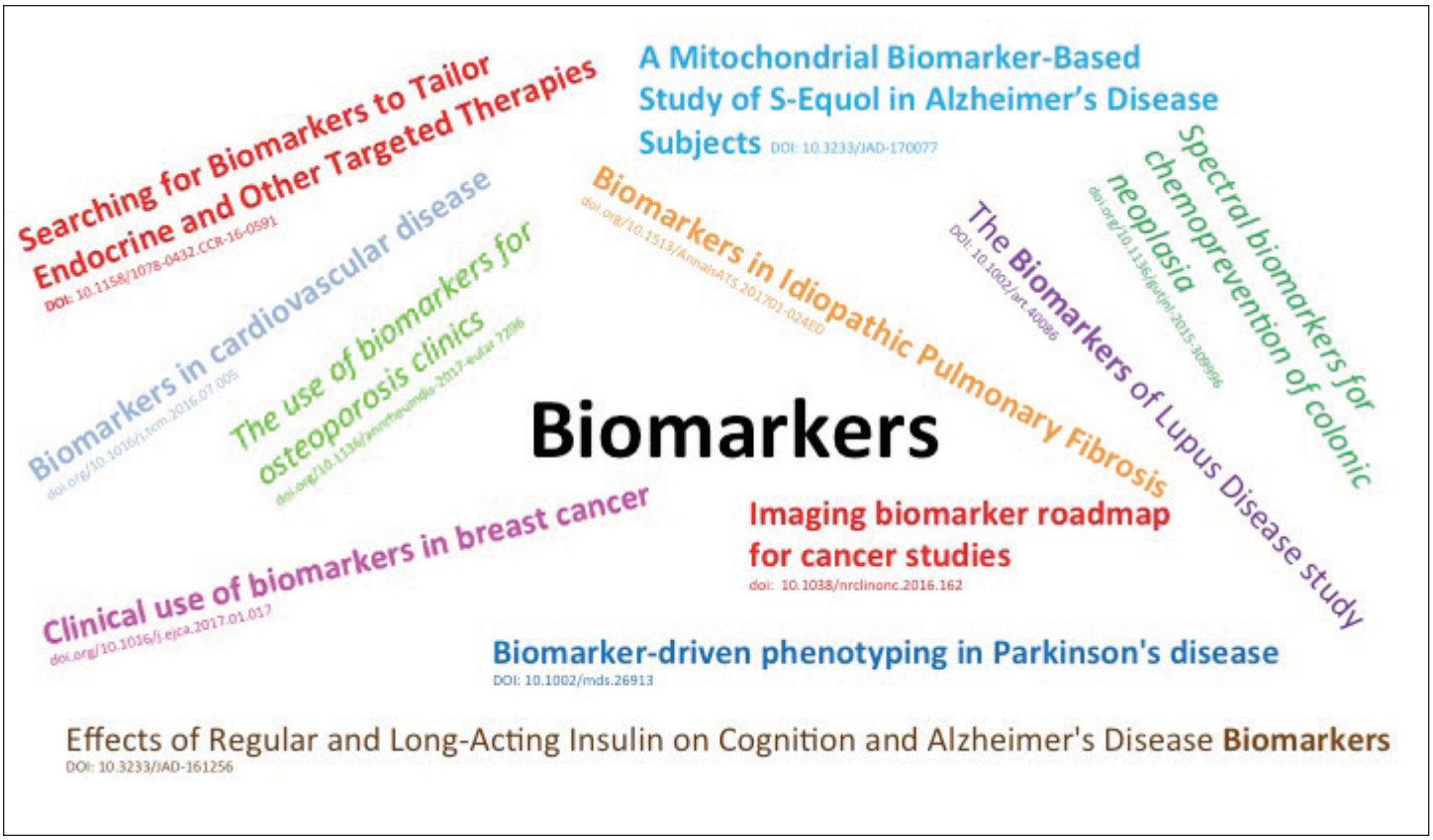
Experimental Medicine and Diagnostics

Andre Da Costa

Stephanie Fraser, Pfizer







Google Scholar Title Search

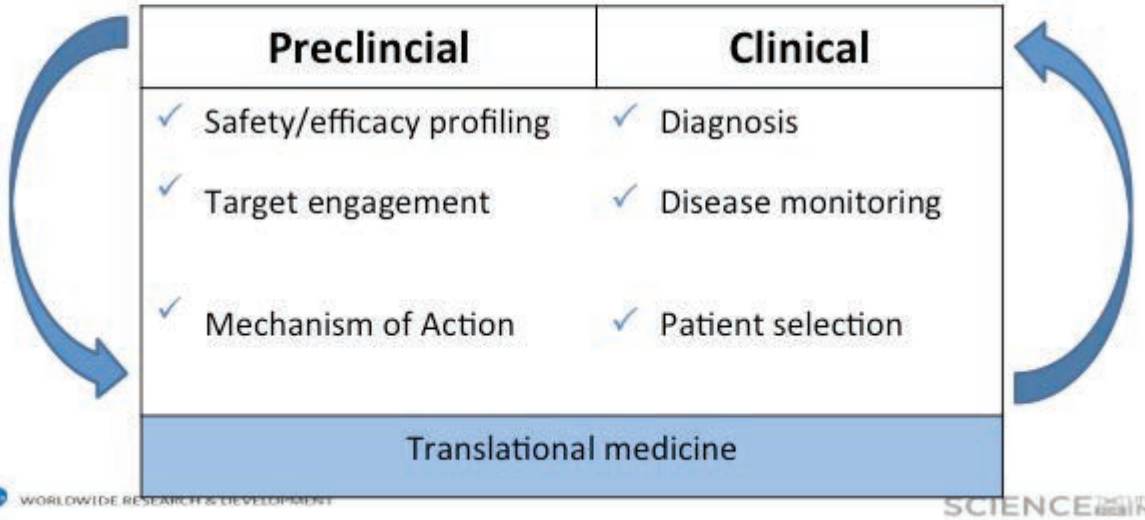
Search	Publications as of 30 Aug 2017				
Biomarker	0	0	0	0	0
Biomarker Assay			0	0	0

Pfizer WORLDWIDE RESEARCH & DEVELOPMENT

SCIENCE THAT IS CHANGING IMPACT

Biomarker Roles

- Biomarkers play many important roles throughout drug development



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Biomarker qualification or validation

- Assay development validation level depends on intended use of the biomarker data

Category I	Category 2	*In Vitro* Diagnostic
Exploratory endpoints for internal decision making	Definitive 1° or 2° endpoint that serves as surrogate of efficacy, safety or disease progression	Distinguish disease from healthy individuals
PD, MOA or hypothesis generation	Supports labeling claim	FDA approved, follows CLIA and CLSI guidelines

WORLDWIDE RESEARCH & DEVELOPMENT

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Biomarker "G and g"uidance

Parameter	FDA Draft Guidance	Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement	Changes to consider
Precision Accuracy (relative)	5 replicates/concentration, 3 concentrations, recovery and CV tolerance are 20% or 25% at LLOQ, total error <30%	2-6 replicates per sample, 3-6 runs; *a priori* working criteria of CV and RE at 20%/25% for LLOQ and TE of 30%	Use patient samples to establish Precision.
Selectivity	Demonstrate lack of cross-reactivity, non-specific binding	Demonstrate lack of interfering substances (endogenous, disease state, con-meds)	Use parallelism across multiple individuals – eliminates reliance on reference materials[1]
Range	Non-linear, 6 non-zero points, covers LLOQ and ULOQ	6 non-zero points, covers LLOQ and ULOQ	Modified analytical measurement range: LLOQ at MRD – ULOQ at maximum dilution
Sensitivity	Lowest QC concentration measured with acceptable accuracy/precision	Lowest QC concentration measured with acceptable accuracy/precision	Estimate using parallelism for endogenous analytes[2]
Stability	Use spiked QCs: Bench top, freeze thaw and storage	Use spiked QCs: Freeze/thaw, storage, benchtop	Use endogenous samples not reference material spikes[3]
Parallelism	Evaluate to detect matrix effects	Use incurred samples: evaluate when calibrators are prepared in a surrogate matrix	Use samples with high endogenous concentrations for validation[2]

Summary

- Biomarkers are clearly integrated into the drug development cycle
- Extent of biomarker development and validation depends on its context-of-use
- The landscape defining validation is evolving
- Biomarker validation is not ☑

Case Studies

WORLDWIDE RESEARCH & DEVELOPMENT

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Matrix Matters

- IL-17 A assay
  - Ultra-sensitive measurements are often necessary for humoral cytokine measurements
  - Cytokine concentration profiles may be altered in disease tissues
- Development of a novel biomarker
  - Variability of a soluble biomarker across multiple anticoagulants

WORLDWIDE RESEARCH & DEVELOPMENT

SCIENCE THAT IMPACT

IL-17A concentration data

Donor	PASI Score	Serum (pg/mL) IL17A	Skin Biopsy (pg/mL) IL17A Non-lesional	IL17A Lesion
101	26.0	0.210	nd	179
102	21.6	0.114	nd	3.70
103	23.9	0.267	nd	nd
104	24.3	0.266	nd	3.50
105	23.9	1.93	nd	59.7

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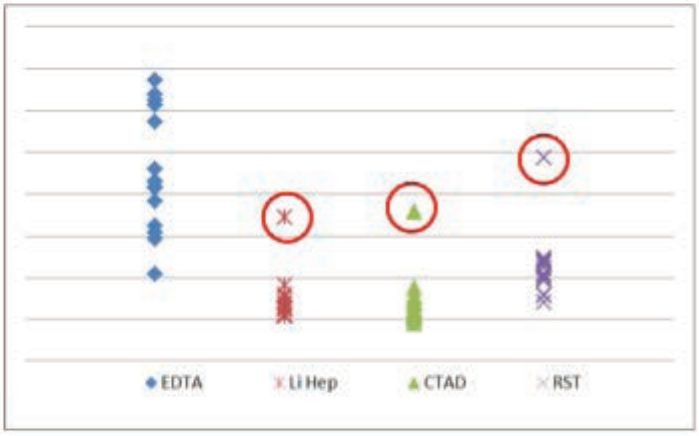
- Serum analysis requires ultrasensitive assays
  - Il-17A LLOQ: 0.05 pg/mL
- Skin biopsies could be evaluated using one of many commercially available assays (MSD, R&D Systems, *etc.*)

Soluble biomarker X

- We have been working to develop a novel biomarker in support of an ongoing project
- Initially assay development was conducted in K_2EDTA plasma
- Recurrent anomalous results led us to investigate the impact of anticoagulant on signal

Soluble biomarker X

- 15 healthy volunteers
- Variable signal
  - K_2EDTA is always highest
- High signal is titratable
- K_2EDTA might be masking true response
- Natural history study to assess impact of matrix on signal from disease state samples



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Stability is not translatable

- Reference material spikes are often used to assess biomarker stability
- This may not be indicative of endogenous stability
- TGF-β1 stability
  - Spiked TGF- β1 into buffer and pooled urine; also evaluated fresh frozen diabetic urine samples
- IL-13 stability
  - established using incurred samples from an ongoing clincal study

WORLDWIDE RESEARCH & DEVELOPMENT

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TGF-β1 stability evaluation

Stability Samples	Sample Concentrations					
	Sample 1		Sample 2		Sample 3	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Purified TGF-β1 spikes in Buffer						
Pre-freeze/thaw	108	108	251	340	981	937
Post-freeze/thaw	119	94	257	296	966	1021
%Recovery	110	87	102	87	98	109
Purified TGF-β1 spikes in Urine						
Pre-freeze/thaw	133	119	124	1260	1280	1250
Post-freeze/thaw	93	78	76	921*	871*	892*
%Recovery	70	66	61	73	68	71
Diabetic Urine Samples						
Pre-freeze/thaw	151	172	196	147	51	282**
Post-freeze/thaw	8	70	7	141	19	15
%Recovery	5	41	4	96	37	5

% Recovery = (Post-freeze thaw /Pre-freeze thaw) x 100
*Standard Deviation was 25%
**Standard Deviation was 34%

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IL-13 QC stability

- IL-13 sample stability was established using serum spiked with reference material

Table 3. IL-13 Spiked Sample Stability		
QC Concentration (pg/mL)	% Recovery Month 4	% Recovery Month 5
20.3	112	57
1.1	94	379

%Recovery = (Concentration at Time/Initial concentration)*100

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IL-13 incurred sample stability

- Some samples were stored beyond established stability
- Incurred samples were analyzed to confirm QC sample stability

Sample	Initial Conc. pg/mL	Reassay Conc. pg/mL	% Difference from Original Conc.*	Months in Storage
1	7.5	0.3	95	9
2	0.3	0.3	-2 ✓	8
3	0.5	0.4	24 ✓	14
4	5.8	0.4	93	9
5	0.4	0.4	21 ✓	14
6	0.8	0.8	1 ✓	15
7	0.9	0.7	25 ✓	8
8	0.9	0.9	-2 ✓	15

*% Difference = |1-(Reassay/Initial)|x100

- 6 of 8 incurred samples met the acceptance criteria of ± 30%
- Stability was extended to 15 months

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Conquer Aggregates

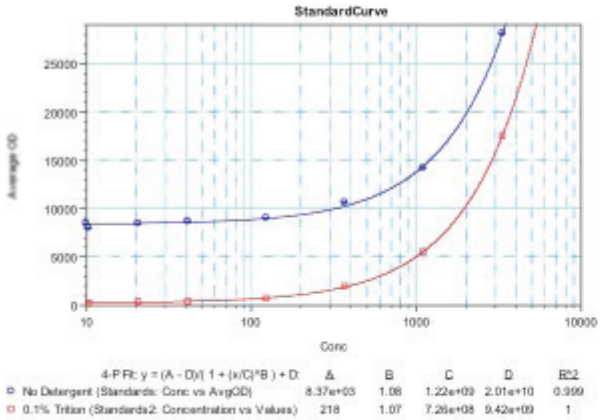
- We have worked with several assays that perform fine until - suddenly they don't
- Often these assays have one or more reagents stored at high concentrations
- We have found that making intermediate dilutions in an assay amenable detergent often restores assay performance

WORLDWIDE RESEARCH & DEVELOPMENT

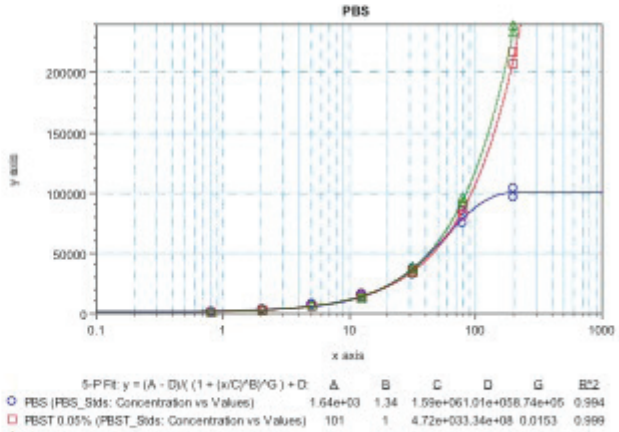
SCIENCE IMPACT

Restoration of assay performance

- Intermittent high background obscured true sample concentrations
- Adding 0.1% Triton to reference material recovered the assay



- Intermittent curve failure at high concentrations
- Adding 0.05% TWEEN to capture antibody dilution recovered the assay



WORLDWIDE RESEARCH & DEVELOPMENT

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References

[1]Valentin MA, Ma S, Zhao A, Legay F, Avrameas A. Validation of immunoassay for protein biomarkers: 1232 bioanalytical study plan implementation to support pre-clinical and clinical studies. J. Pharm. 1233 Biomed. Anal. 55(5), 869–877 (2011).

[2] Stephenson LF, Purushothama S. Parallelism: considerations for the development , validation and implementation of PK and biomarker ligand-binding assays. Bioanalysis. 6(2), 185-198 (2014).

[3]Fraser SA, Fleener C, Ogborne K, Soderstrom C. When close is not close enough: a comparison of endogenous and recombinant biomarker stability samples. Bioanalysis. 7(11), 1355-1360 (2015).

[4]Soderstrom C, Berstein G, Zhang W, Valdez H, Fitz L, Kuhn M, Fraser S. Ultra-sensitive measurement of IL-17A and IL-17A/F in psoriasis patient serum and skin. AAPS Journal. 19(4), 1218-1222 (2017).

WORLDWIDE RESEARCH & DEVELOPMENT

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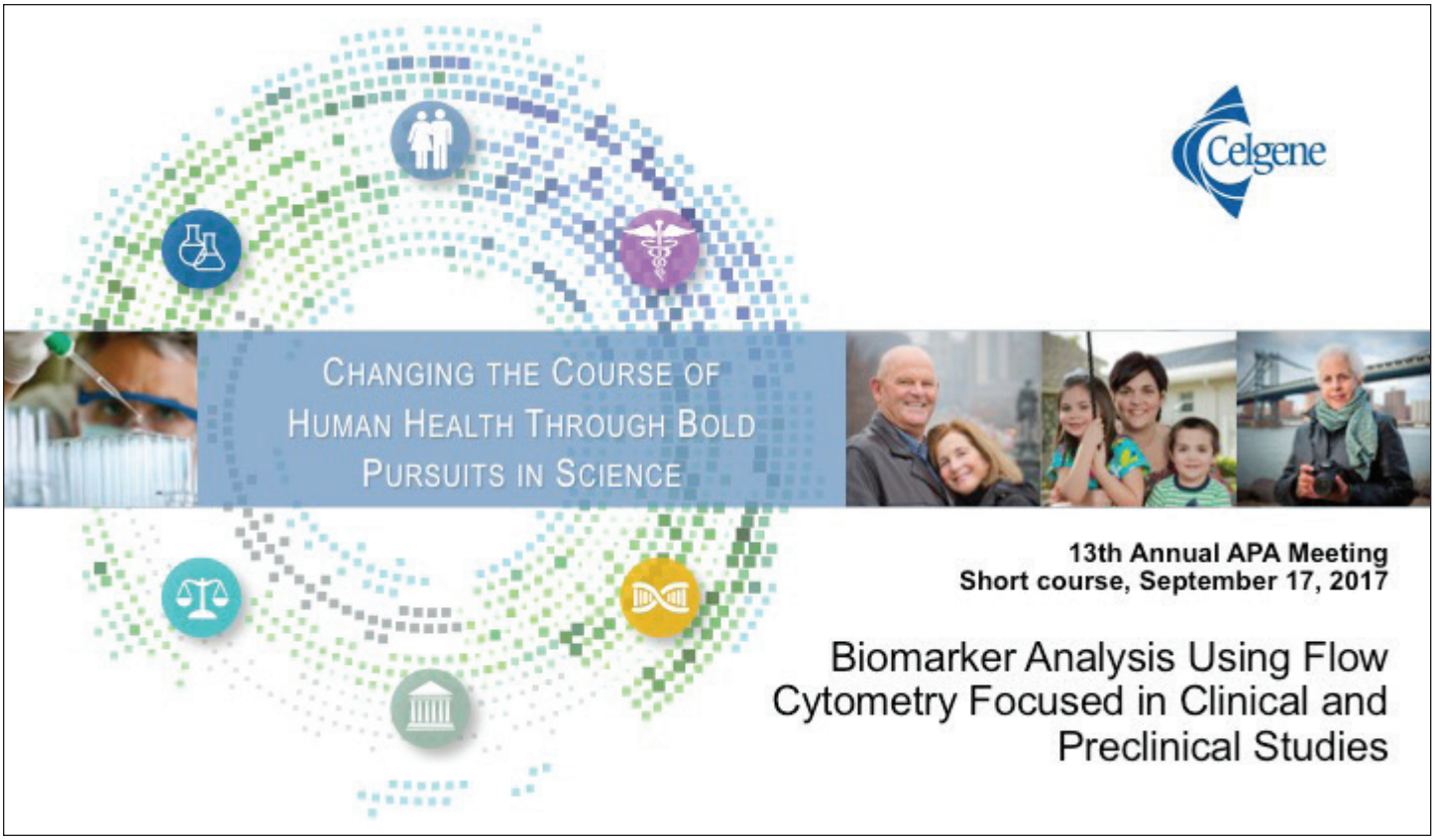
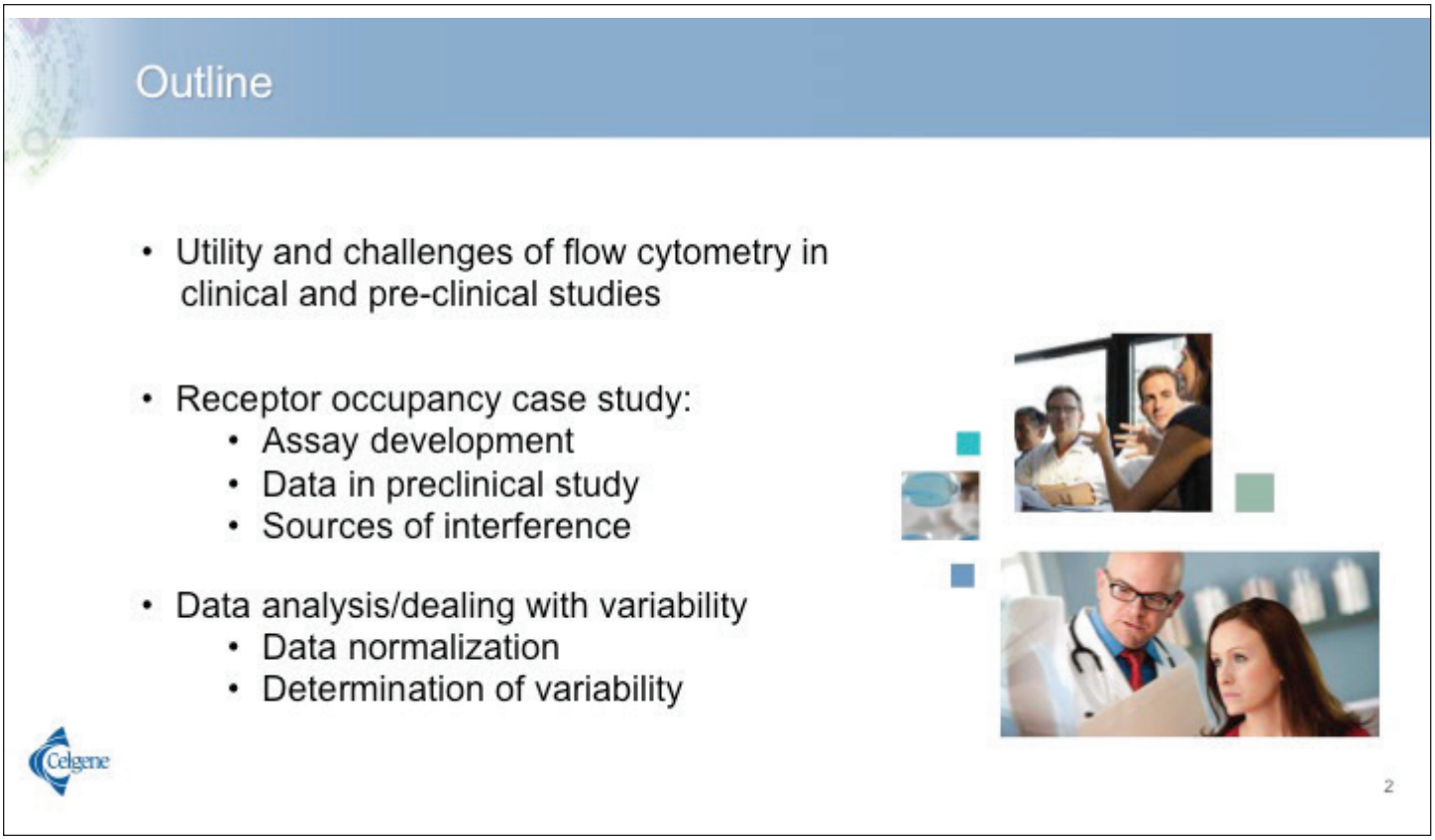
Martin Schwickart, Celgene





Utility of flow cytometry in clinical and pre-clinical studies

Pre-clinical and clinical studies	Additional in Clinical studies

▪ Toxicology markers (e.g. cytopenias)
▪ Markers of proposed MOA
  ▪ Increase or decrease of cell populations (immunophenotyping)
  ▪ Change in expression of cell surface markers (up-or downregulation)
  ▪ Change in intracellular markers (e.g. Ki67 to monitor proliferation)
  ▪ Binding of drug to its target (receptor occupancy)
  ▪ Functional assays – requiring ex-vivo stimulation
	▪ Safety markers (e.g. cytopenias)
	▪ Identification of predictive markers
	▪ Monitoring of markers that correlate with disease or are pathological cells of a disease (e.g. hematological diseases; minimal residual disease)

Celgene

3

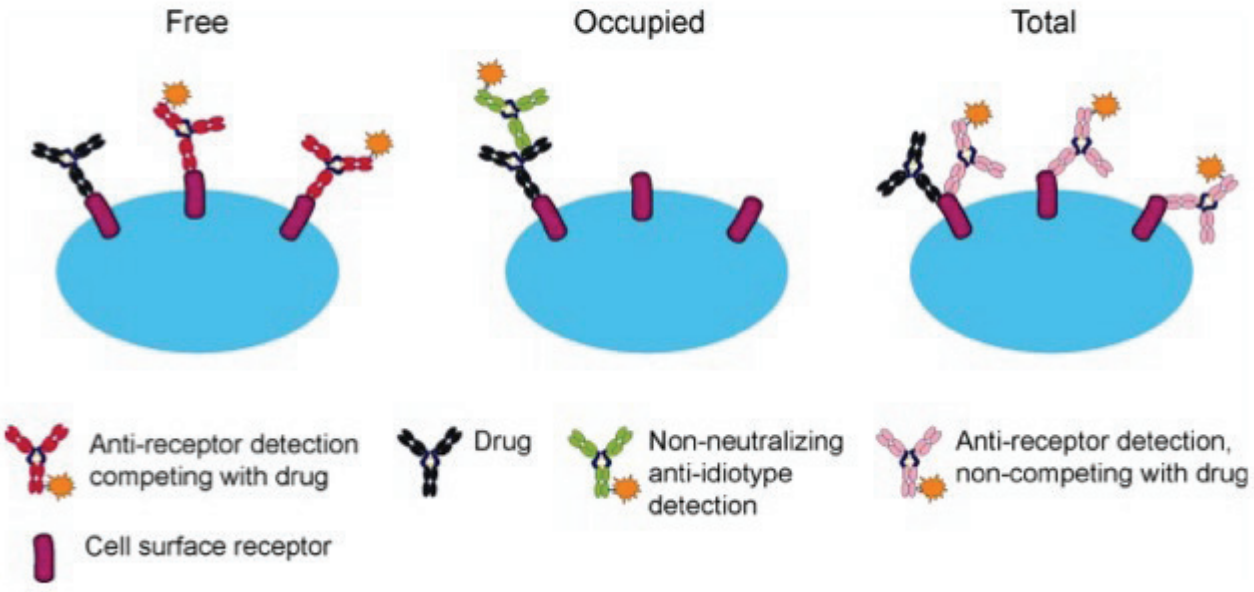
Where to I get my assay from and how difficult is it to develop/ perform?

Assay	Source	Difficulty
▪ Immunophenotyping	▪ Many standard assay available at clinical and non-clinical CRO	▪ Low
▪ Intracellular markers	▪ Clinical and non-clinical CRO	▪ Medium
▪ Receptor occupancy and surface expression ▪ Functional assays	▪ Typically developed in-house and can be outsourced to a competent CRO	▪ High

Celgene

4

Receptor occupancy formats

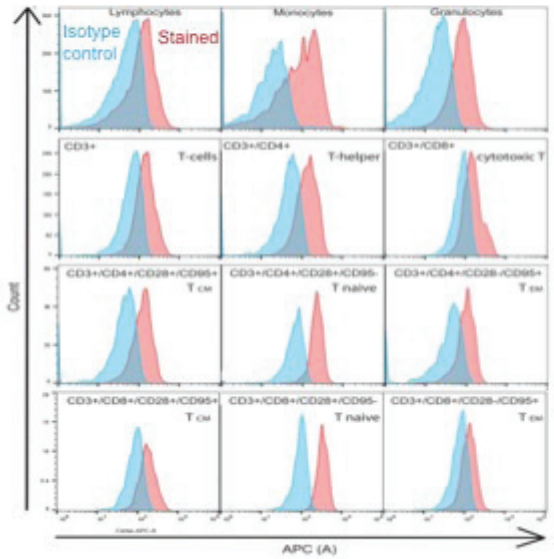


Adapted from Liang et al. 2016

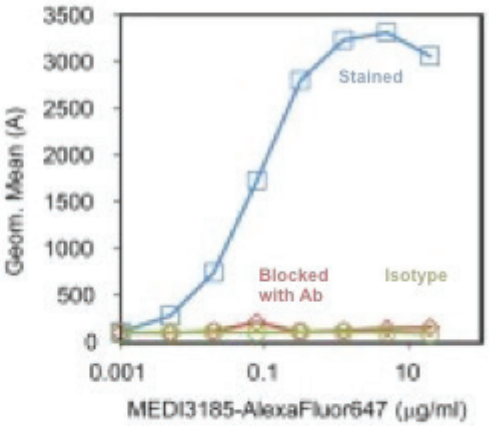
5

Free receptor occupancy assay development

Step 1: Identification of relevant cell population



Step 2: Titration of antibody to maximize signal-to-noise ratio



Advice: Aim to select a biologically relevant population with a large signal-to-noise ratio

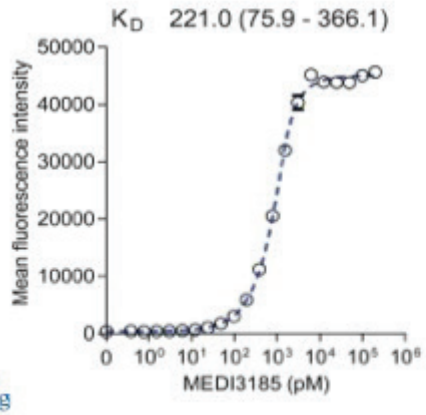
Adapted from Schwickart et al. 2016

6

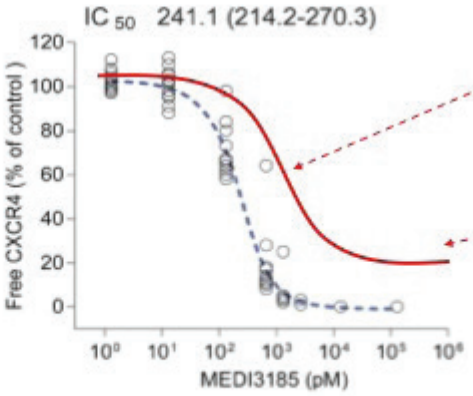
Free receptor occupancy assay development

Step 2: Verification of accuracy of assay

On-cell affinity determination



Free RO Assay characterization by titration of drug into individual blood samples



Common assay problems that will lead to underestimation of occupancy:

Shift in IC50

Incomplete occupancy

Both problems can be due to:

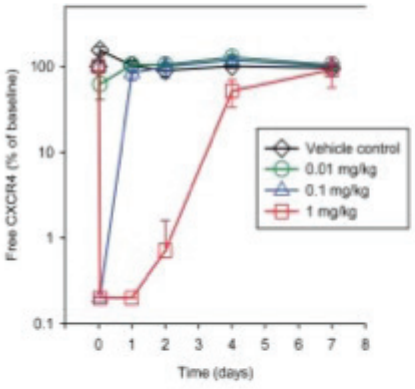
- Long incubation times or high concentration of detection antibody
- Fast off rate of drug
- Background staining

Adapted from Schwickart et al. 2016

7

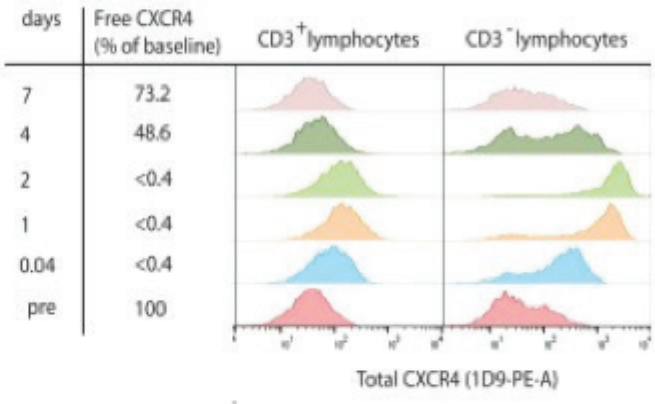
Study Result: The therapeutic antibody occupies all receptors (>99.6%) and leads to increase of surface receptor

Free receptor



Duration of occupancy is dose dependent

Total receptor (free and occupied) at single dose 1mg/kg

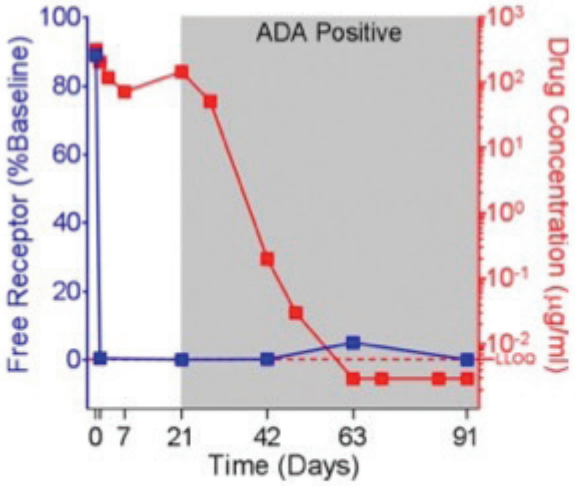


Total receptor accumulates after dosing

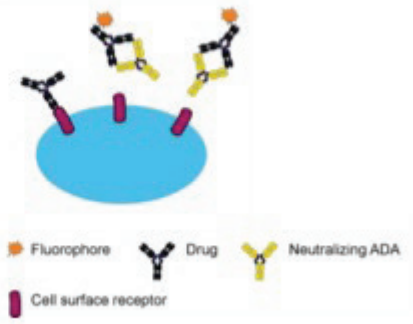
Adapted from Schwickart et al. 2016

8

Risk of using the labeled drug as detection reagent for free assay: Anti-Drug antibodies (ADA) assay interference



Paradoxical result in presence of ADA:
The receptor appears fully occupied (low free receptor), however, drug is not present. This is most likely due to ADA blocking the detection antibody in the assay, which is the labeled drug. Results after appearance of ADA are not reliable if the detection antibody is the labeled drug!



Adapted from Liang et al. 2016

9

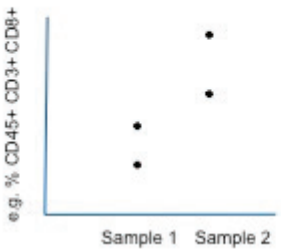
Data analysis considerations: Variability

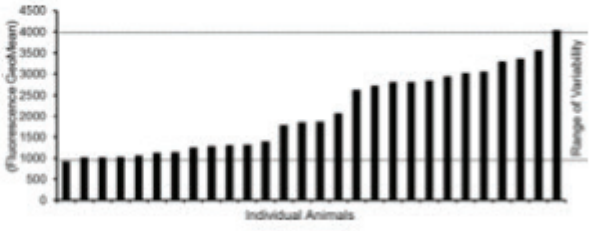
Technical variability

- AKA assay variability
- Can be characterized by the method validation
- Inter-assay variability (%CV) is most informative
- Rare cell populations are more variable than abundant populations

Biological variability

- Difference between two patients is usually greater than between two animals (disease, age, sex, etc.)
- Longitudinal variability – changes over time

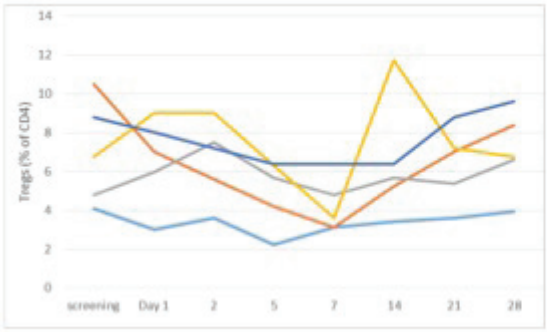




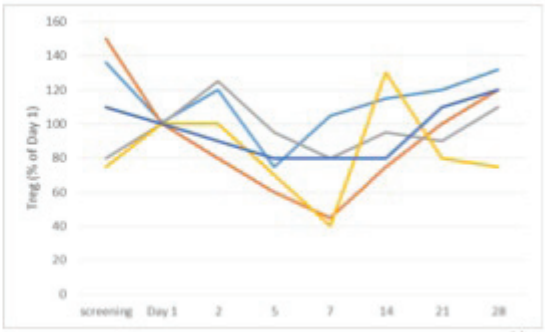
10

Solutions to variability: Normalization

- Identify sources of variability and design the best possible assay
  - Sample stability: Identify sample stability and design study accordingly (if possible)
  - Variability in rare populations can be partially mitigated by acquiring more events
- Data normalization
  - Normalization to % change of baseline helps to visualize trends



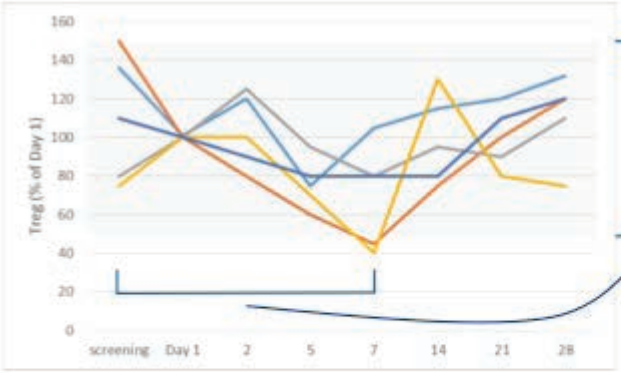
Normalization



11

Solutions to variability: Interpretation of datasets

- Identify the relevant variability in study
  - The variability between two baseline samples of each animal/patient defines the relevant combined variability and only changes greater than that are reliable
  - Define the % change between two baseline samples
  - Compare baseline sample(s) to time point with suspected change by e.g. paired t-test



(1) 95th percentile of difference between screening and day1: 47%

(2) Compare Day7 to screening
Paired 1-tailed t-test: : 0.047 - significant
non-parametric test: 0.450 - not significant

Day7 to Day1 pre-dose:
Paired 1-tailed t-test: 0.037 - significant
non-parametric test: 0.250 - not significant

Decrease in T-regs at Day 7 borders on statistical significance. Going forward, more patient data should help to gain confidence in the trend.

12

Summary and recommendations

- Data analysis
  - Know the overall variability of the data to identify meaningful changes
  - Normalization to baseline helps to comprehend the data better
  - Tell it how it is. Initial trends can disappear later when more patients data becomes available

- Assay development
  - Validation helps to determine whether an assay has a chance to provide meaningful data (variability, stability, etc.)
  - Intracellular markers tend to be more variable
  - Free receptor occupancy assays are prone to interference originating from the detection reagent itself and from anti-drug antibodies if the detection reagent is identical with the biotherapeutic.

Celgene

13

gsk do more feel better live longer

Practical Approaches to Protein Biomarker Quantification by LC-MS: What Are We Actually Measuring?

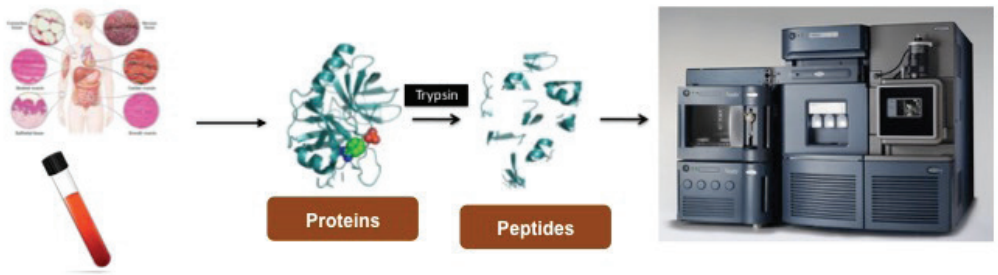
Tim Sikorski
GlaxoSmithKline
September 17, 2017

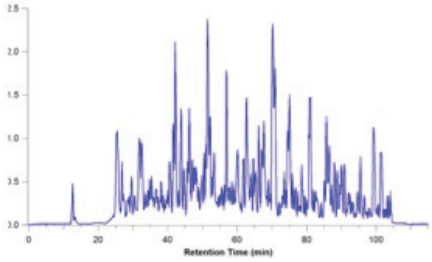
Exploratory Biomarkers at GSK

Supporting Hypothesis-Driven Biomarker Characterization and/or Quantitation to Enable Early and Informed Clinical Decision Making

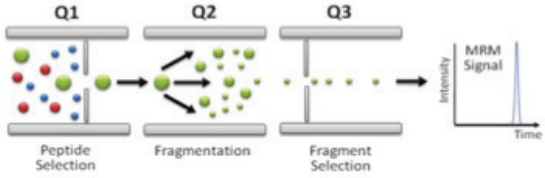
1. *Collection of complimentary techniques and expertise in: flow cytometry, histology, immunohistochemistry, mRNA in-situ hybridization, LC-MS/MS , ligand binding, and qPCR*
2. *Seeks to enable studies to demonstrate target engagement and downstream pharmacology for novel MOA and safety endpoints by development of robust assays*
3. *Strengthen biomarker community and build relationships with the GSK Clinical Unit Cambridge (CUC) to facilitate "Near to Patient Biomarker Testing"*

Typical LC-MS/MS Workflow for Protein Bioanalysis





Liquid Chromatography



Mass Selection using
Triple Quad Mass Spectrometry

Adapted from SRM Atlas

The "Surrogate Peptide" Approach

- An enzymatically derived peptide used to represent a region of a protein or the entire protein
- Ideally we want to do intact analysis but large proteins (>10kDa) are multiply charged and difficult to analyze by ESI-MS.
  - Difficult to resolve large number of proteoforms of endogenous proteins
- Peptide digestion creates a more complex mixture than intact protein, but better sample workflows and chromatography methods are available
- Should start with at least 2 peptides per protein and 2 product ions per peptide
- For quantification, all samples are normalized to a stable isotope labeled ("heavy") internal standard that is spiked into all samples

The not so hard and fast peptide selection rules

- Ideally peptide should be unique sequence at least in your matrix (BLAST searching)
- MS friendly peptide length = 8-18 amino acids
- Avoid peptides containing Cysteine and Methionine Residues
- Try and make peptide selection translatable from preclinical species to human
- **Peptide selection should ultimately be *driven by the biology***
  - Are there isoforms that are important to quantitate? Post translational modifications?

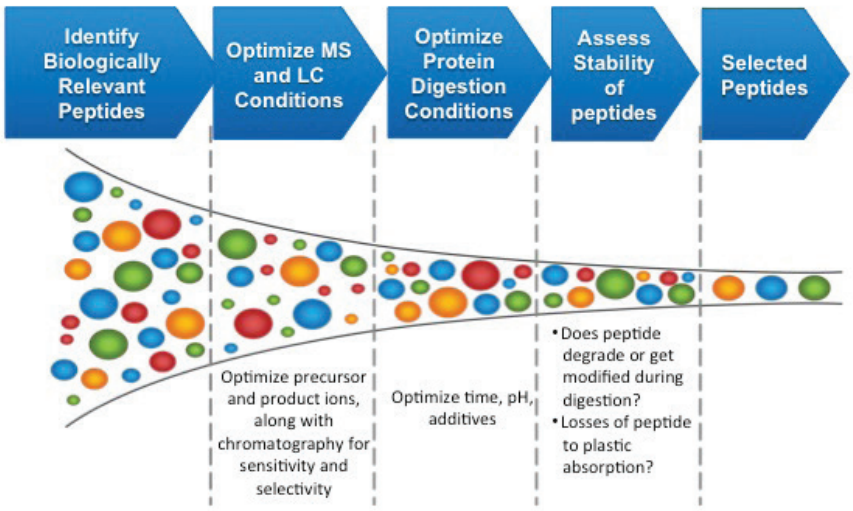
```
GF1    1 DFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGECEFVFLQKYPHTHL
GF2    1 NLGLDCDEHSSESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCSGQCEYMFMQKYPHTHL
          ******** ********************************** **  * ********

GF1   61 VHQANPRGSAGPCCTPTKMSPINMLYFNGKEQIIYGKIPAMVVDRCGCS
GF2   61 VQQANPRGSAGPCCTPTKMSPINMLYFNDKQQIIYGKIPGMVVDRCGCS
         * ************************* * ******** *********
```

Protein Peptide Selection Criteria: Case Study

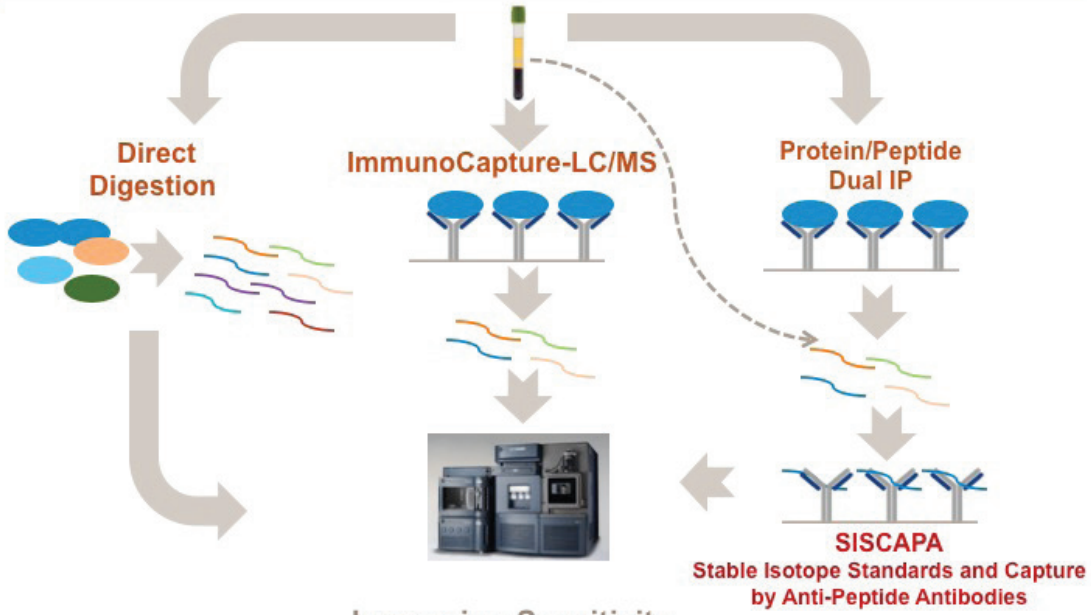
- Protein Biomarker for Inflammatory Disease
- Perform Blast search using UniProt program for selectivity
- Exclude peptides in the C-terminally truncated isoforms region (Splice variants)
- Avoid possible in vivo modifications ,i.e. Glycation at glutamic acid (GLU, E)residues using UniProt
- Avoid Cysteine (Cys, C)residues (di-sulfide bonds) and Methionine (Met, M) residues (oxidation)
- Chose peptide on domain portion of protein for specificity
- Considered peptide length (8 to 18 amino acids)

Selecting the Best Surrogate Peptides



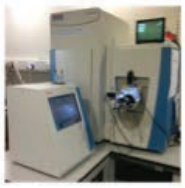
Adapted from Chris Shuford, Lab Corp

Targeted Protein Analysis Workflows: Choosing The Right Fit



Targeted Protein Analysis: Increasing Sensitivity Without Complex Workflows

Low Flow Regimes in Conjunction with High Resolution Mass Spectrometry



Flow Regime	Sensitivity	Analysis Time
Conventional	LOQ ~ 1 ng/mL	3-5 min per sample
MicroFlow (IonKey)	LOQ ~ 50-100 pg/mL	12-15 min per sample
NanoFlow	LOQ ~ 5-10 pg/mL	~30 min per sample

	Hormone 1	Hormone 2	Hormone 3	Hormone 4	Hormone 5	Hormone 6	Hormone 7	Hormone 8
Conventional Flow-Low Res LLOQ	500 pg/mL	500 pg/mL	1 ng/mL	1 ng/mL	10 ng/mL	500 pg/mL	1 ng/mL	1 ng/mL
MicroFlow-High Res LLOQ	10x	10x	2x	20x	10x	10x	10x	10x

Zhuo Chen

Differences Between Protein Biomarker and Biotherapeutic Method Development

Parameter	Biotherapeutic Assay	Biomarker Assay
Assay Method	Absolute Quantification	Absolute or Relative Quantification
Nature of Analyte	Exogenous	Endogenous
Specificity	Drugs are not present in sample matrix	Biomarkers present in sample matrix
QC	Certified standard and blank patient sample matrix available	Certified standard and blank patient sample matrix usually not available
Sensitivity/Range	Higher LLOQ Greater Dynamic Range	Lower LLOQ Less Dynamic Range

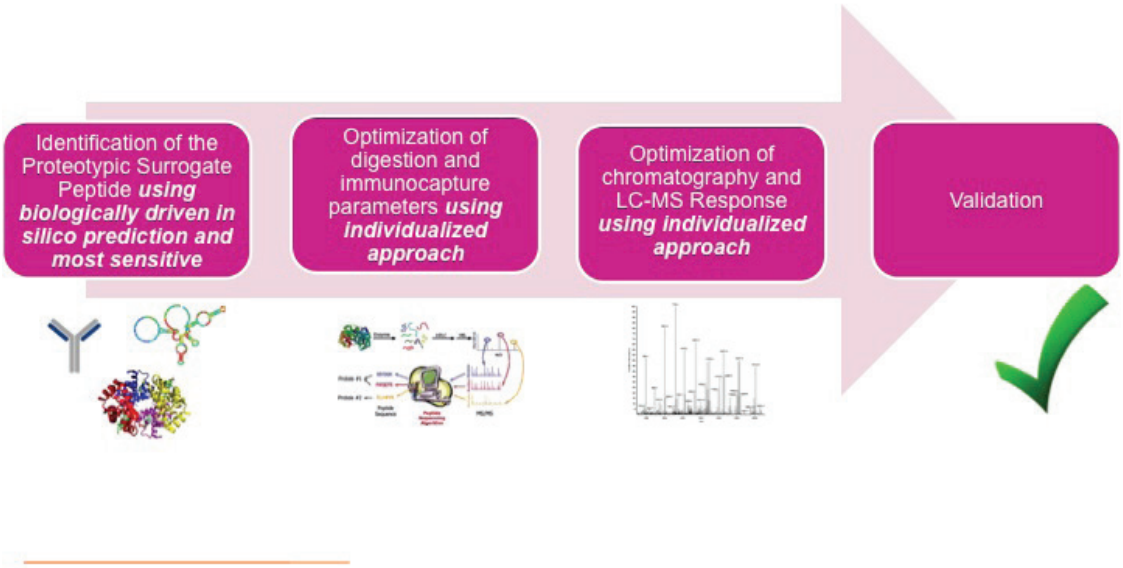
Surrogate Analytes and Surrogate Matrices For Protein Biomarker Analysis

- Accurate quantification of endogenous biomarkers often utilizes a surrogate analyte spiked into a surrogate matrix
  - Typically a recombinant protein spiked into assay buffer containing Albumin
  - All samples normalized to an Internal Standard, typically a heavy version of the surrogate peptide spiked in prior to endoproteinase digestion

Increasing Cost and Assay Development Time

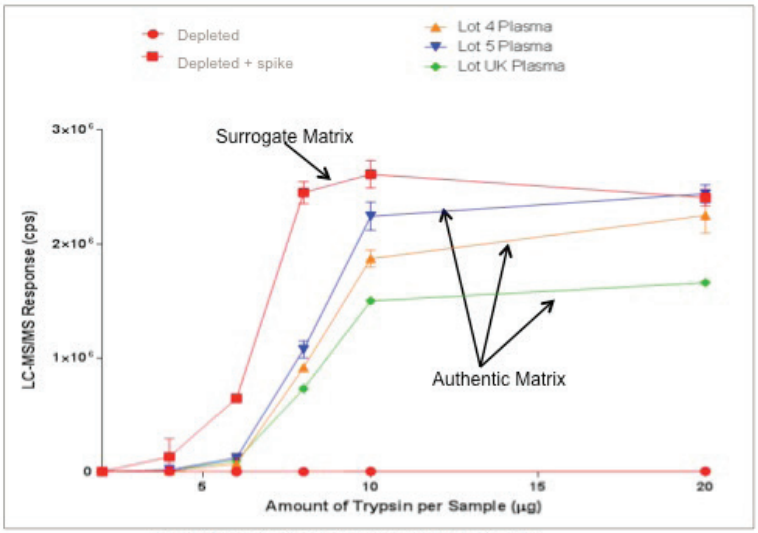
Surrogate Analytes	Surrogate Matrices	Internal Standards
Recombinant Protein	Assay Buffer	Heavy Labeled Surrogate Peptides
"Native" Protein IP'ed from matrix	Depleted Matrix	Heavy Labeled Surrogate Peptide with Overhang Sequence
		SiluPrest Heavy Domain Standard
		Heavy Labeled Surrogate Analyte

Current Method Development/Validation Practices



Digestion Efficiency Differences of Surrogate and Authentic Analytes and Matrices

Standard Curve: Native protein purified from plasma spiked into "depleted" plasma

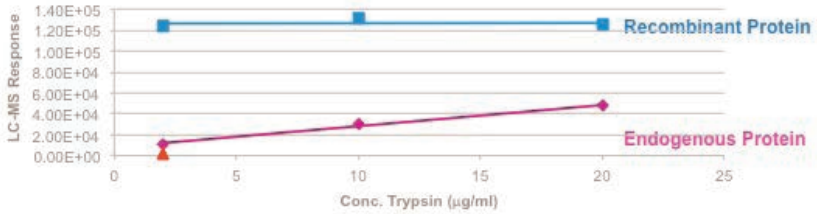


Protein more resistant to digestion in endogenous matrix than in surrogate matrix

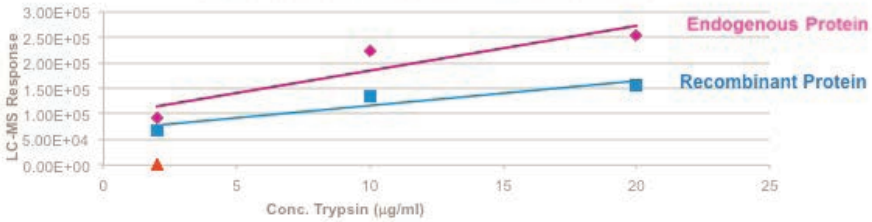
Digestion Efficiency Differences of Surrogate and Authentic Analytes and Matrices

Standard Curve: Recombinant mouse protein purified from E. Coli spiked into human plasma

100mM Sodium Bicarbonate



80/20 Sodium Bicarbonate/Acetonitrile

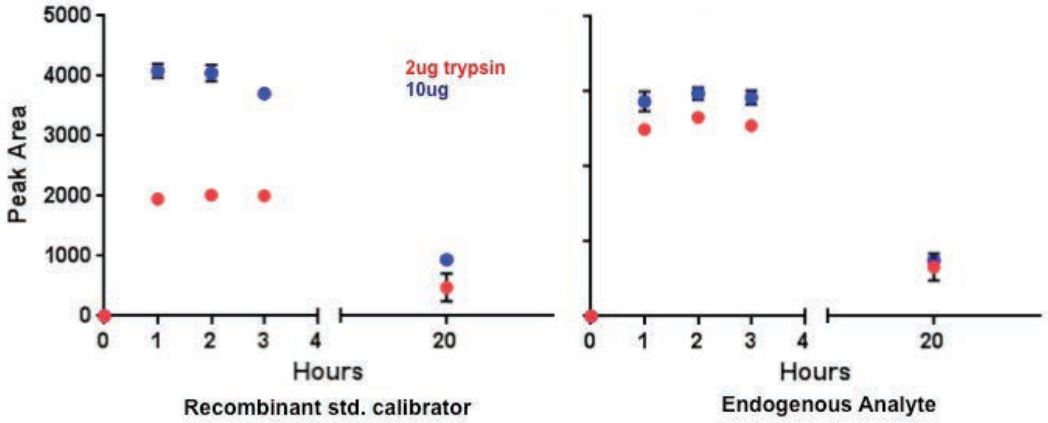


Endogenous protein more resistant to trypsin digestion

Emily Pillet

Digestion Efficiency Differences of Surrogate and Authentic Analytes and Matrices

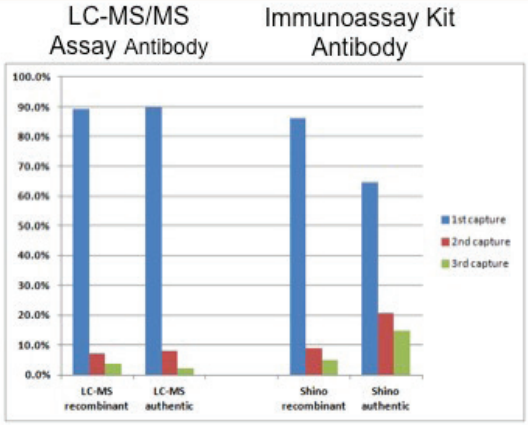
Standard Curve: Recombinant protein expressed and purified from insect cells spiked into Assay Buffer + BSA



- Recombinant standard more resistant to trypsin digestion
- Peptide susceptible to overnight deamidation

Dean McNulty

Immunocapture Differences of Surrogate and Authentic Analytes

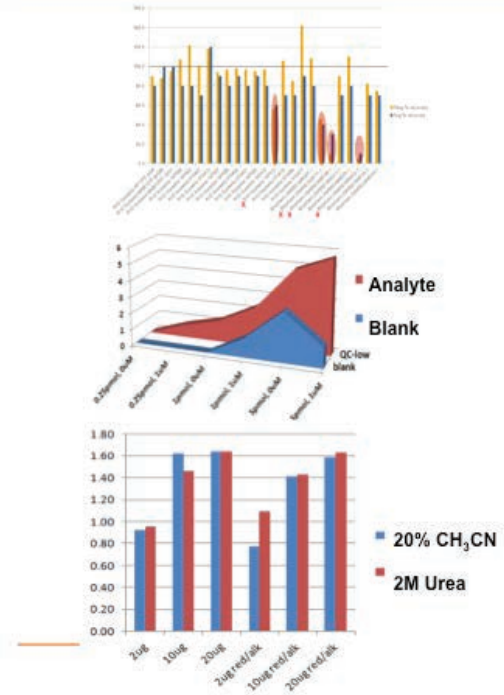


- LC-MS/MS and Kit Based Immunoassay used different capture antibodies
- Sequential immunocapture reveals ELISA kit suffers incomplete capture of authentic samples

Dean McNulty

Progress towards defining best practices

Towards a Systematic Optimization of Digestion and Immunocapture For Protein Biomarkers



Antibody Immunocapture Screen:

- 25 commercial and in-house tool antibodies screened for biomarker capture efficiency in single LC-MS/MS run.

Aptamer Immunocapture Screen:

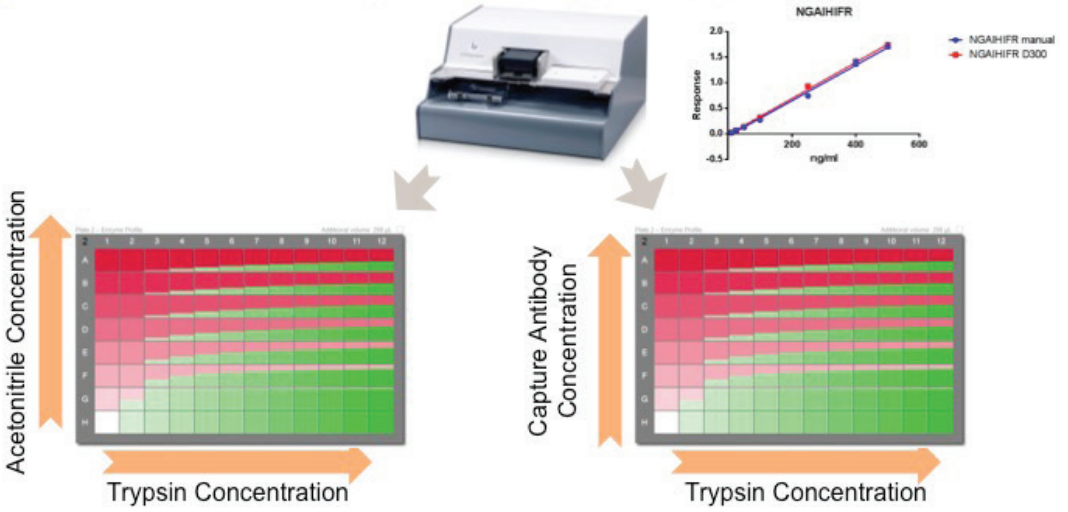
- Immobilized ligand density
- +/- Polyanionic competitor

Enzymatic Digest Screen:

- Endoproteinase
- Digestion Buffer / Denaturants
- Reducing/Alkylating Reagents
- Concentration / Time

Dean McNulty

Using a Digital Dispenser To Quickly Optimize Immunocapture and Trypsin Digestion



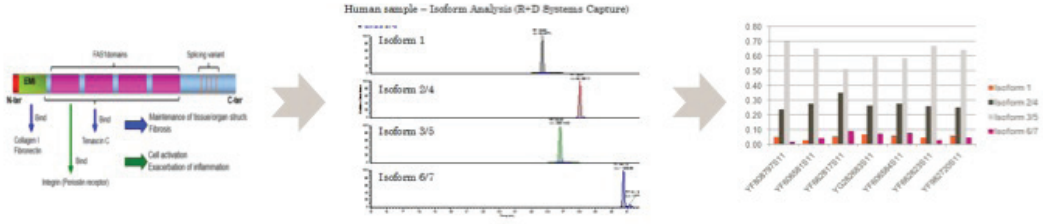
Optimize Digestion in Surrogate and Endogenous Matrix

Optimize Immunocapture in Surrogate and Endogenous Matrix

Characterization of what's being measured: Platform for the Discovery of Novel Proteoforms to Support Experimental Medicine

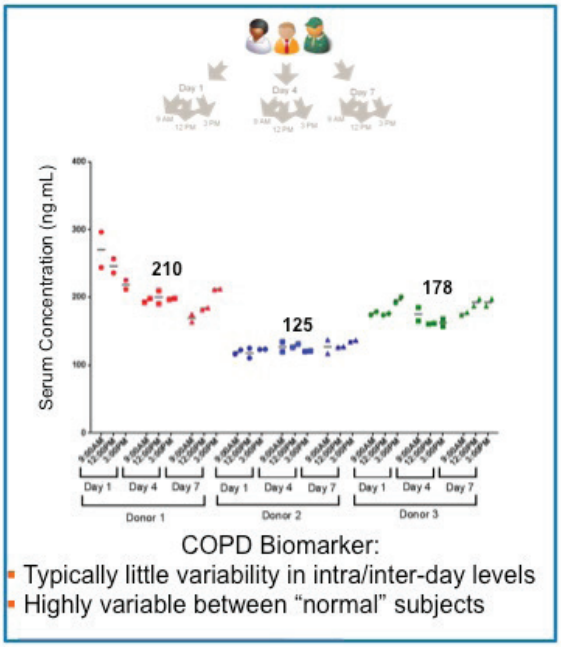
Unbiased, Data Dependent Peptide Identification

- What protein/proteins are being immunocaptured?
- What post-translational modifications can be found on the protein or peptides?
- How accurate are commercial kit based assays? What are they actually measuring?
- Can be applied to LC-MS or ELISA Protein Assays



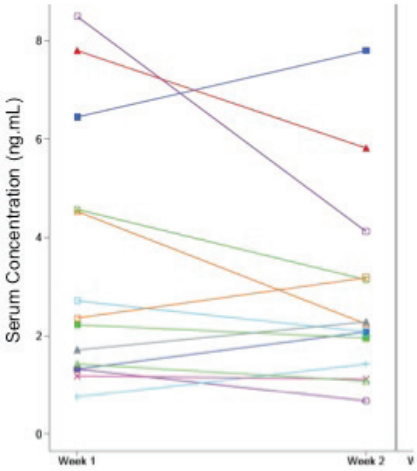
Dean McNulty

What are the assay requirements (CV and Bias): Understanding Endogenous Fluctuations in Biomarkers Over Time



COPD Biomarker:

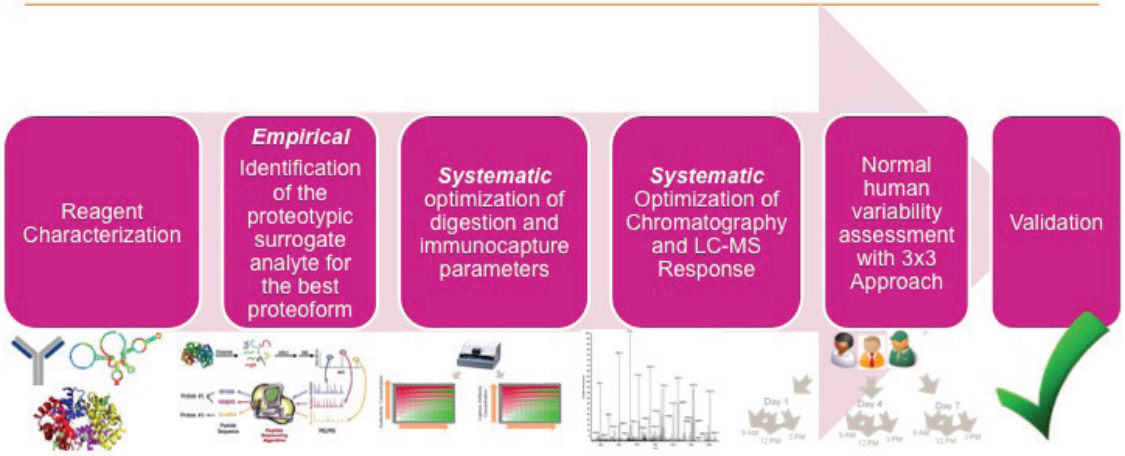
- Typically little variability in intra/inter-day levels
- Highly variable between "normal" subjects



Inflammation Biomarker:

- Highly variable between subjects and across time points

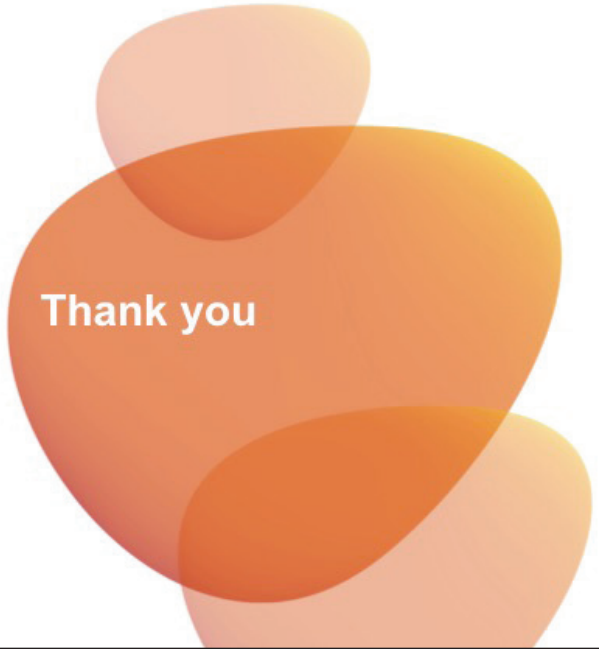
Ensuring the Right Biomarker For Experimental Medicine

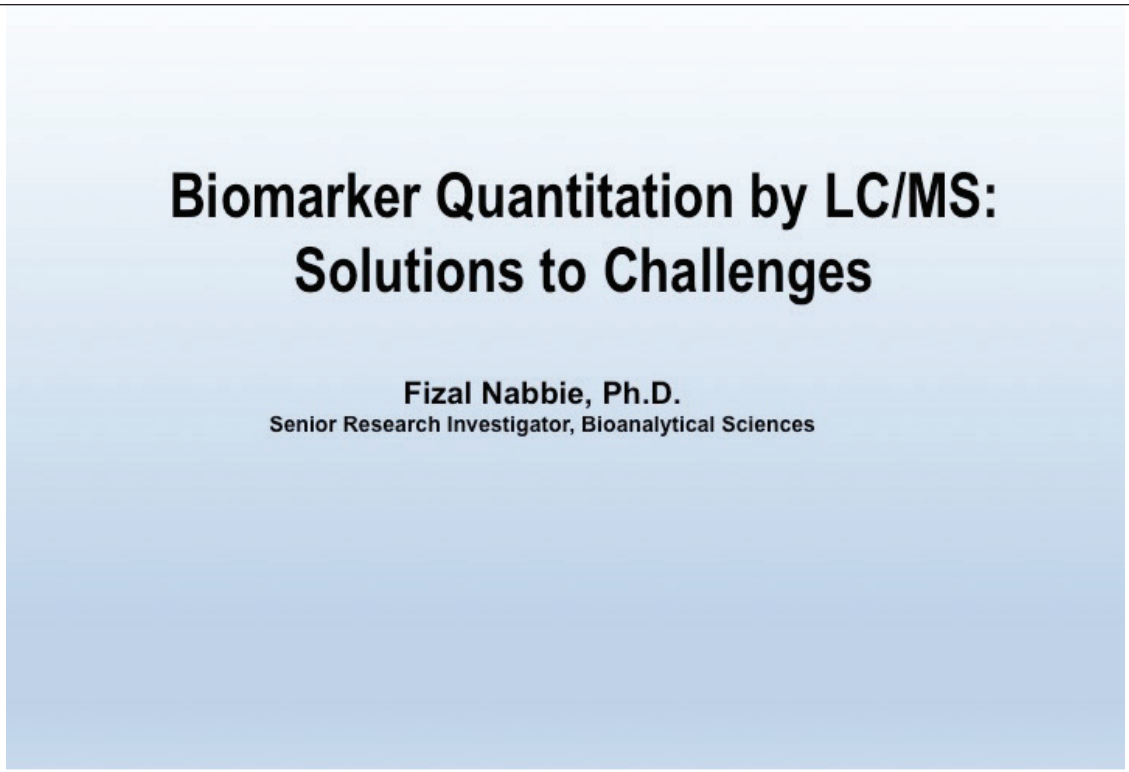


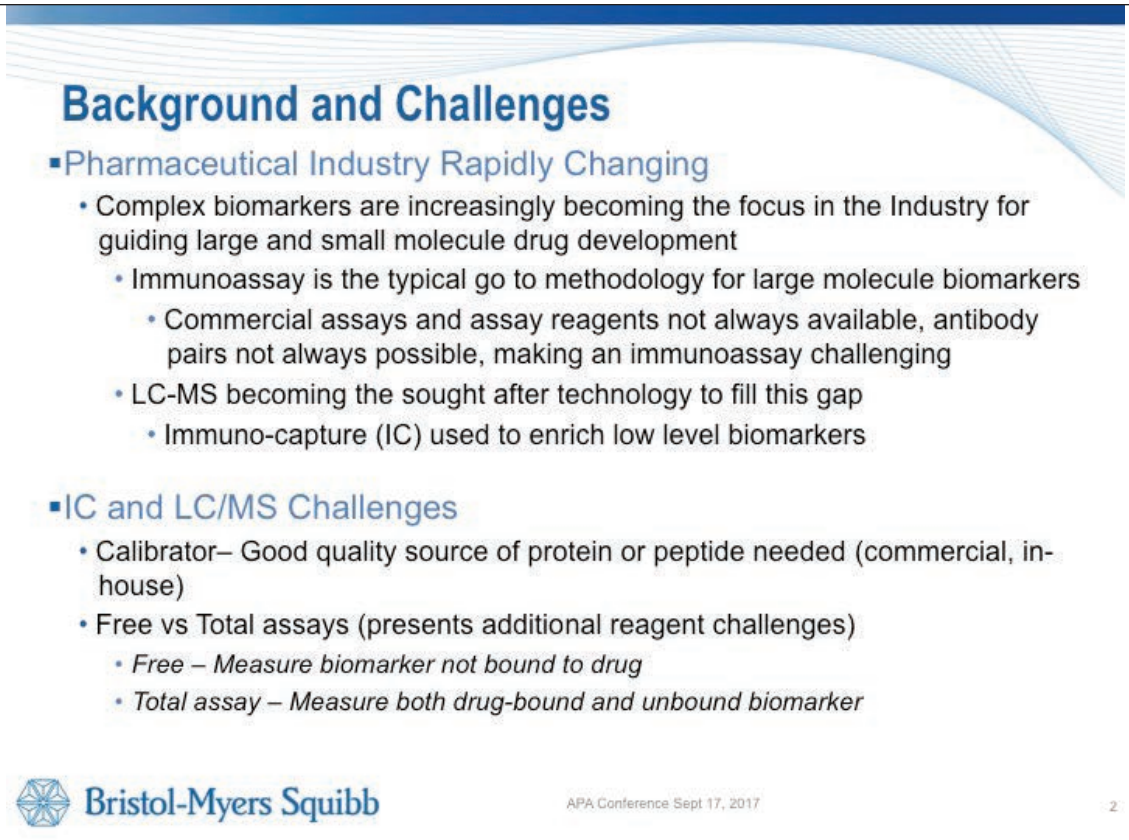
- Understanding the Authentic Analyte in its disease relevant setting
  - Isoform/PTM Specific vs Total Assay?
  - Neo-epitopes
- Confidence in Quantitation
  - Characterized Assay to ensure our assays are specific, quantitative, and robust for the desired analyte
  - Minimize unforeseen obstacles during study support

All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents




Biomarker Quantitation by LC/MS:
Solutions to Challenges
Fizal Nabbie, Ph.D.
Senior Research Investigator, Bioanalytical Sciences
Bristol-Myers Squibb
APA Conference Sept 17, 2017


Background and Challenges
Pharmaceutical Industry Rapidly Changing
Complex biomarkers are increasingly becoming the focus in the Industry for guiding large and small molecule drug development
Immunoassay is the typical go to methodology for large molecule biomarkers
Commercial assays and assay reagents not always available, antibody pairs not always possible, making an immunoassay challenging
LC-MS becoming the sought after technology to fill this gap
Immuno-capture (IC) used to enrich low level biomarkers
IC and LC/MS Challenges
Calibrator– Good quality source of protein or peptide needed (commercial, in-house)
Free vs Total assays (presents additional reagent challenges)
Free – Measure biomarker not bound to drug
Total assay – Measure both drug-bound and unbound biomarker
Bristol-Myers Squibb
APA Conference Sept 17, 2017
2

Ligand Binding Assay vs Hybrid IC-LC/MS

LBA	IC-LC-MS
Sandwich ELISA capture and detect with protein specific antibodies	LBA capture with LC/MS detection methodology
High throughput	Lower throughput
Easy to run, easy to transfer to CROs for global studies	Expensive specialized equipment – requires highly skilled personnel; may require larger quantities of reagents
High sensitivity technology	Sensitivity improving over time
Adequate specificity	High specificity; can differentiate different isoforms, glycosylated vs. non-glycosylated; can work at peptide level
Dynamic range – adequate or large depending on platform	Large dynamic range
Requires multiple binding sites, up to three for total (drug-bound) assays	Requires one binding partner and identification of signature peptide
All dependent on quality reagents which is a long and expensive process	

Bristol-Myers Squibb

IC-LC/MS: As a Solution to LBA Challenges

- Advantages
  - Can work with only one specific antibody (depending on needs)
  - Biomarker enrichment improves specificity and lowers background
  - Can develop quickly compared to sandwich immunoassay
  - Protein digestion offers multiple peptides for quantitation
- Disadvantages
  - Instruments expensive to purchase and maintain (expertise needed to run)
  - Quality source of calibrator protein or peptides needed (this can add to development time to obtain)
  - Enrichment only as good as the capture antibody

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Case Study 1 (Issue)

- Free LBA (kit) to measure soluble target protein used at CRO – over time became "partially-free"
  - It was later determined to be a combination of changes in PAb the kit over time and competition of the kit capture antibody for the protein bound to drug
    - Specificity and sensitivity of kits polyclonal antibody change over time

    Reagent Time Constraints
    - Acquired program, no internal antibodies readily available
    - No time for antibody generation
    - Team agreed to hybrid IC-LC/MS approach, to support ongoing study the kit over time and competition of the kit capture antibody for the protein bound to drug

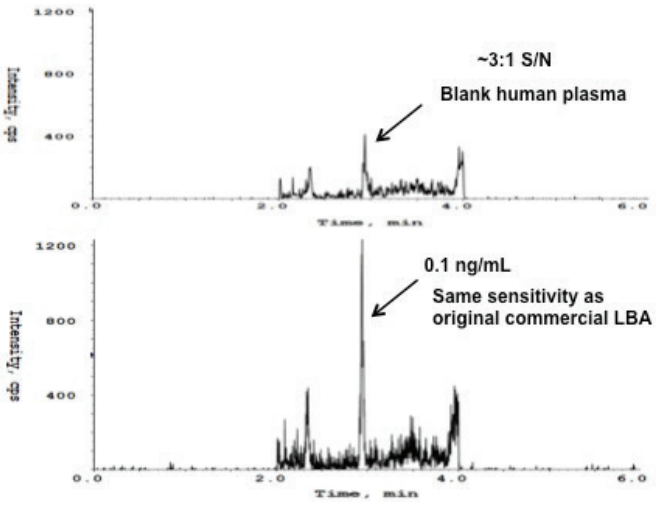
- Reagent Time Constraints
  - Acquired program, no internal antibodies readily available
  - No time for antibody generation
  - Team agreed to hybrid IC-LC/MS approach, to support ongoing study

Case Study 1 (Capture Reagent Screening)

- Commercial monoclonal antibodies screening and selection
  - 7 commercial antibodies identified and purchased
  - Performed physiochemical characterization
    - Binding to target, drug interference, antibody kinetics and purity testing
    - 3 did not bind to protein at all
    - 4 bound to same epitope (same as drug)
      - 3 out of the 4 had non-specific binding/noise issues
      - 1 suitable antibody for binding to protein identified, however it had higher affinity than the drug and thus outcompetes the drug for protein binding
  - Fall back position was using the drug for immuno-capture

Case Study 1: (Solution, Drug as Immuno-capture Reagent in the LC/MS Assay)

	Capture mAb	
STD (ng/mL)	Measured conc.	%Dev
0.1	0.098	-2.0
0.2	0.204	2.0
0.5	0.533	6.6
1.0	0.997	-0.3
2.0	2.026	1.3
10	10.235	2.4
50	46.818	-6.4
100	103.458	3.5
200	186.679	-6.7



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Case Study 2 (Issue)

- Immuno-assay to measure target-drug complex
- Drug binds to target as well as endogenous homologue
  - Homologous protein shares 90% sequence identity with target protein
  - Endogenous levels of homologous protein are 10% of the target endogenous levels
  - Drug binds to both proteins
  - % drug that binds to homologous protein is drug concentration dependent
- LBA challenges
  - Unable to identify an antibody that capture just the protein
    - Cannot differentiate target protein from homologous protein
    - Insufficient sensitivity, 10 ng/mL
  - Diseased patient population has lower concentrations of both proteins than healthy volunteers
    - Need more sensitive assay

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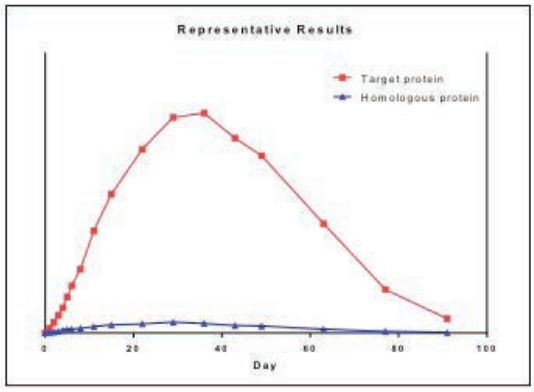
Case Study 2 – (IC-LC/MS Approach)

- Biotinylated drug anti-idiotype was used to capture drug bound to both target protein and homologue protein
  - Complex captured on beads coated with the anti-idiotype antibody
  - Proteins were eluted, digested and unique peptides were measured by LC/MS

Protein	Signature Peptide
Target	xxyxxxxx
Homologue	xxzxxxxx

Case Study 2: (Solution, Hybrid IC-LC/MS)



Day	Target:Homologue
1	6.6
2	9.5
3	9.5
4	8.4
5	9.7
6	12.3
8	13.1
11	16.3
15	16.9
22	20.4
29	19.9
36	23.1
43	26.1
49	25.4
63	29.2
77	27.1
91	27.7

- Assay range
  - 1.1 – 1000 ng/mL Target protein
  - 0.4 – 200 ng/mL homologous protein

Case Study 3 (Issue)

- Measure a specific truncated form of a native protein in the presence of multiple forms of this protein in clinical samples
  - Attempts at generating a specific antibody to bind only to the specific truncated form of the native protein of interest was successful
  - A pair of antibodies suitable for sandwich LBA assay was not achieved.
- Proposed IC-LC/MS Approach
  - Screen commercial and in-house antibodies
  - Use best binding antibody to immuno-capture the specific truncated form of the native protein in the complex sample matrix
  - Elute / digest / and measure unique peptide for the truncated protein by LC/MS
    - Peptides from 4 different domains of the native full length protein was also monitored in the MS to confirm that only the specific protein of interest was captured.

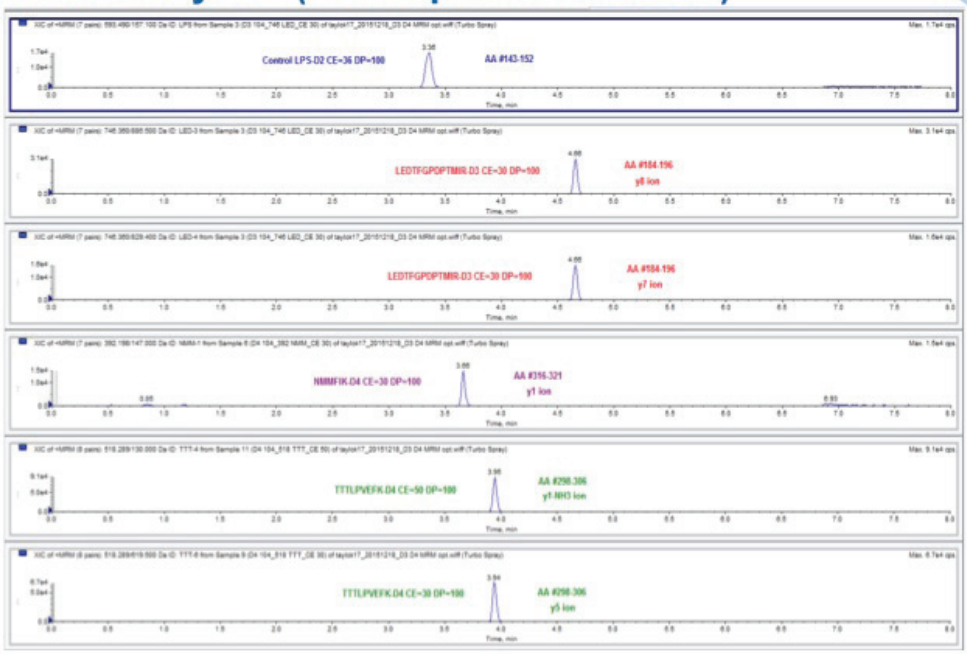
Case Study 3 (Reagent Screening)

- Internal and Commercial Reagents Screened
  - 30 commercial and In-house antibodies screened, only two Abs were identified and the in-house antibody was the stronger binder to the specific native protein of interest.

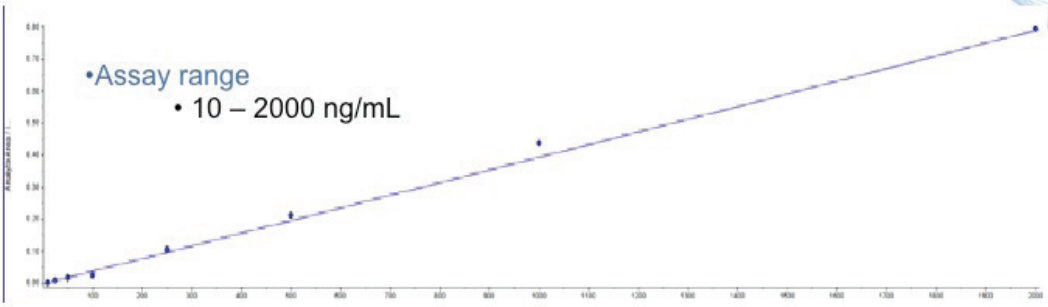
Antibody	Different Forms of the Protein of Interest				
	Protein A	Protein B	Protein C	Protein D	In-House Protein E
Vendor #1	+/-	+		+/-	
Vendor #2	+++		+	++++	
Vendor #3		++			
Vendor #4			+/--		
Vendor #5	+	+/--		+	+/-
Vendor #6	+/-			+	
Vendor #7		++			
Vendor #8					
In House Ab					+/-

+/-- (weakest response), + (good response), ++++ (strongest response)

Case Study 3 – (MS Peptide Selection)

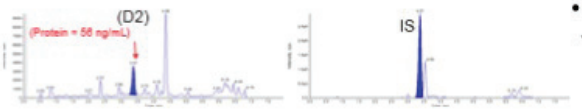


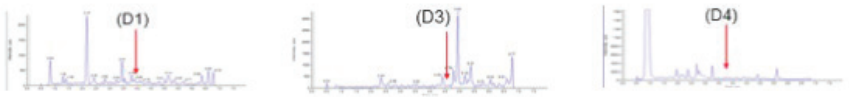
Case Study 3 – (Solution, Hybrid IC-LC/MS)



- Assay range
  - 10 – 2000 ng/mL

- Native protein in a plasma sample
  - D2 MS channel protein of interest
  - No Signal observed in MS channels for domains 1, 3 and 4





Case Study Summary

- Case Study 1
  - On-going trial – no time to generate antibodies (capture and detection)
    - Immuno-capture with drug (free assay) – LC/MS detection
- Case Study 2
  - Drug bound to target protein and homologous protein
    - LBA could not distinguish between the two
    - IC-LC/MS allowed for measurement of both enabling determination of drug-bound target concentration as well as the ratio of drug-bound protein to drug bound homologous protein
- Case Study 3
  - Unable to generate antibody pair for the truncated form of a native protein of interest
    - Immuno-capture with specific antibody, distinguished truncated protein from the other forms by IC-LC/MS using unique peptide

Summary

- Strategy for measurement of biomarker
  1) If quality reagents are available – LBA
     -Selectivity, sensitivity and specificity, particularly for free
     -Two non-competing, non-drug competing for total
  2) If reagents are limited or target protein is of particular difficulty IC-LC/MS is good path forward
     -Use quality capture reagent for best performance
     -Use good quality calibration protein or peptide
- Combining LBA and LC/MS in an orthogonal approach was complimentary and in some cases the best path forward
- IC-LC/MS is a powerful tool for biomarker analysis in drug development
- Employing the capabilities of both technologies resulted in solutions to real world challenging issues in biomarker analysis

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A Regulatory Perspective on Biomarkers for Pivotal Studies: Method Validation, Sample Analysis and Inspections

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Collaboration, Risk Evaluation & Surveillance Team (CREST)
Office of Study Integrity and Surveillance, CDER, U.S. FDA

September 17, 2017
13th Annual APA Meeting – Providence, RI

FDA

Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

2

Background: Guidance Documents

- 2001 – Guidance for Industry: Bioanalytical Method Validation
  - No direct comment on "biomarkers"
- 2013 – Revised Draft Guidance for Industry: Bioanalytical Method Validation
  - Inclusion of biomarker assays
  - Method validation "should address the same questions" as those for PK assays

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Background – Publications

- Workshop Report: 2013 Crystal City V (Booth et al., 2015)
- Workshop Report: 2015 Crystal City VI (Arnold et al., 2016; Lowes and Ackerman, 2016)
- Recommendations on biomarker method validation (Hougton et al., 2012)
- European Bioanalysis Forum recommendation (Timmerman et al., 2012)
- Fit-for-Purpose validation for biomarker measurement (Lee et al., 2006)

4

Types of Biomarkers

- **Protein**
- **Metabolites**
- Nucleic acids
- Cells
- Tissue

Safety biomarkers

Pharmacodynamic biomarkers

Diagnostic biomarkers

Predictive biomarkers

Surrogate endpoint biomarkers

Prognostic biomarkers

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What is the Purpose of the Biomarker Assay?

- Assay for internal decision making, with no impact on label claims ("Category 1")*
- Assay supporting regulatory action for pivotal determinations of effectiveness/ dosage labeling ("Category 2")*
  - Pharmacodynamic interpretation for efficacy and labeling claims
  - For this category, data integrity is critical (full validation)

* *Booth et al. AAPS Journal, 2015*

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Types of Biomarker Assays

- **Qualitative**
- **Relative Quantitative**
- **Definitive Quantitative***

Ligand Binding Assays
Mass spectrometry
Flow cytometry
Molecular pathology
Genomics*

* *Booth et al. AAPS Journal, 2015*

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Essential Parameters for Validation

- Accuracy
- Precision
- Quantification range (Calibration curve)
  - Assay sensitivity
- Selectivity
- Specificity
- Parallelism
- Stability

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Considerations for QC Samples

- QC source and concentration
  - Pooled from patient and/or healthy subject samples?
  - Low QC samples by diluting an endogenous pool (higher concentration) with a surrogate matrix?
  - Spiking reference standard into pools of endogenous matrix? If so, is the spiked reference material identical to the endogenous form?
  - QC pool concentrations cover range of expected study sample concentrations? How many concentration levels and replicates?

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Considerations for Accuracy

- Criteria rely on absolute accuracy or relative accuracy?
- Was the 4-6-X acceptance criteria (i.e. PK) used?
- What is the context of the biomarker's intended use?
  - This may dictate the assay's accuracy criteria
- Is it a small-molecule or peptide biomarker?
- Is there a stable label analogue reference standard?

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Considerations for Precision

- Level of precision may be based on biomarker's biology and amount of change expected
- Will this biomarker have significant differences between
  - Healthy/disease patients?
  - Therapy vs. no treatment?
- Were spiked calibrators used to assess precision?
- Were endogenous samples from target population measured repeatedly to determine precision?
- Criteria? Was total allowable error (TAE) used?

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Considerations for Calibration Curve

- Obtaining appropriate biomarker reference standard or blank matrix not always possible
- Reference standard may not be identical to endogenous biomarker
- Was the disease pool used as the ULOQ?
- Was the healthy pool used as the LLOQ?
- Were endogenous pools mixed in different ratios to develop the calibration curve?
- How many concentration points are on the curve?

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Considerations for Calibration Curve

- What was the *a priori* acceptance criteria used?
- What is the sensitivity of the assay (and criteria used)?
  - LLOQ from parallelism experiments with endogenous biomarker samples?
  - LLOQ from further dilution of healthy pool with analyte-free surrogate matrix?
  - LLOD used?
  - Spiked QC samples used to validate the LLOQ?

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Considerations for Calibration Curve

- Was reference standard spiked into a surrogate matrix (same matrix from different species; PBS)?
  - Were potential differences in the assay's ability to measure analyte in surrogate matrix vs. patient matrix assessed?
  - Did the performance of the calibration curve in matrix used behave similarly to the endogenous biomarker in native matrix? (Parallelism)

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Selectivity/Specificity

- Was extraction used (e.g. for small-molecule and peptide biomarkers) to provide suitable LC-MS assay samples?
- Was an immunocapture step used?
- Were proteins with similar sequences, cleaved portion of pro-proteins, pro-proteins cleaved to the active form, and catabolites evaluated during assay specificity (if applicable)?
- Was matrix from patients (disease-state) evaluated for interference?

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Selectivity/Specificity

- Was recovery of reference standard from matrix of normal and patient (disease-state) populations tested?
- Was interference from additional molecules present in the matrix evaluated?
- What were the critical assay reagents (e.g., ELISA, ECL)?
- Any concerns surrounding hemolyzed or lipemic matrix?

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Parallelism

- Was the validity of the surrogate matrix determined?
  - Demonstration that the surrogate used for the calibration curve correlates with the endogenous matrix or analyte
- How many individual lots of matrix (w/ endogenous concentration) used for assessment?
- Were multiple samples with different concentrations measured?
- How many serial dilutions tested? What was the resulting MRD for the LBA?

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Parallelism

- Is there immunological similarity between the calibrators and the endogenous form of the biomarker (immunoassays)?
- Was parallelism used to assess interference?
- Was accuracy (bias) and precision determined to assure parallelism?

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Stability

- Validation samples – Single pool, multiple sources or samples spiked over endogenous levels?
  - Were healthy and disease pools used as endogenous QC stability samples?
- Was stability evaluated by preparing and analyzing validation clinical samples that mimic the way samples will be collected, processed, shipped, stored, prepared and analyzed?

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Stability

- Was the heterogeneity/metabolism/catabolism/ biotransformation considered in stability evaluation?
- How many replicates at each concentration level were used for stability assessment (3 to 5)?
- Were storage, freeze-thaw and in-process stability evaluated?
- Was stability evaluated for stock solutions with biomarker analytes?

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General Questions for Your Consideration

- What was the purpose of the assay?
- Did you measure what you intended to measure?
- How much variability was in the measurement?
- What are the limits to the measurement?
- How did handling conditions affect the measurement?*

** Arnold et al. AAPS Journal, 2016*

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General Questions for Our Consideration

- If an assay parameter does not meet the criteria of a PK assay, will this pose data quality issues for the biomarker's intended use?
- Multi-analyte analysis:
  - Was the method validated for all analytes simultaneously/ individually?
  - During study sample analysis, if one analyte failed acceptance criteria, how were the passing analytes treated?
- How were assay plates set up (calibrators, QCs, blanks)?

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General Questions for Our Consideration

- Was a commercial diagnostic (Dx) kit used for the biomarker assay?
  - Was it a closed platform (i.e. couldn't be further optimized with additional standards)?
  - If few calibration standards, were additional validation experiments conducted to evaluate range/curve?
  - Were other parameters (stability, selectivity/specificity) validated with additional verification experiments?
  - Was the Dx assay demonstrated to be suitable for the intended use?

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General Questions for Our Consideration

- Were all method validation runs reported (passing and failed)?
- Was there individual plate acceptance criteria?
- Documentation, documentation, documentation

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Conclusion

- Bioanalysis of endogenous molecules present challenges
- Method validation and study sample analysis following the "fit-for-purpose" concept in drug development
- Understanding that all biomarker assays used to support pivotal trials may not be consistent with fully validated PK methods
- Inspectional considerations take into account the nature of the biomarker, the type of assay, and the objective of the method

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26


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THANK YOU
27

APA SHORT COURSE BIOGRAPHIES

Stephanie Fraser, PhD, Pfizer: Dr. Stephanie Fraser is an Associate Research Fellow in the Early Clinical Development organization at Pfizer in Groton, Connecticut. Since 2010 she has led a small but ambitious group of scientists that provide immunoassay based support to clinical biomarker programs across multiple therapeutic areas. Prior to joining Pfizer, Stephanie spent five years in preclinical toxicology at Charles River Laboratories where she managed a flow cytometry laboratory. She received her PhD in Cellular and Molecular Biology from the University of Nevada, Reno in 1999 and has since focused on biomarker development and fit-for-purpose bioanalytical assays.

Darshana Jani, Pfizer: Darshana Jani is Scientific Associate Director at Pfizer, Cambridge, MA, USA, where her role is to serve as scientific and technical lead for both CROs and internal teams for development, validation, and application of bioanalytical assays to comply with scientific and regulatory requirements. Darshana has over 25years of experience in supporting preclinical to clinical studies holding positions with Sanoffi, MedImmune, Biogen and Pfizer. Darshana has risen from the ranks, at the outset developing and applying boianalytical methods, while assuming numerous responsibilities germane to regulatory compliance of the validation and application of group procedure. She has guided bioanalytical lab groups, held responsible for overseeing any and all phases of drug recovery and development; a task which incorporated product characterization, potency determination, immunogenicity testing, as well as surrogate biomarker assay development, validation and sample testing. Darshana has published several recommendation white papers with industry, academic and regulatory peers in the area of biomarkers as well as immunogenicity.

John A. Kadavil, PhD, FDA: Dr. Kadavil received his Bachelor's degree in Biochemistry from the University of Maryland Baltimore County (UMBC). He then received his Ph.D. in Molecular Pharmacology and Experimental Therapeutics from the University of Maryland Baltimore, School of Medicine. Following his Ph.D., he joined the U.S. Food and Drug Administration as a pharmacologist. Dr. Kadavil first worked in the Office of Scientific Investigations (OSI) - Division of Bioequivalence & Good Laboratory Practice under the Office of Compliance at the Center for Drug Evaluation and Research (CDER). During his 8 years at OSI, he conducted both foreign and domestic bioanalytical and clinical inspections. His inspections covered bioavailability/bioequivalence, pharmacokinetic, and GLP studies. In 2011, he joined the Division of Human Food Safety (DHFS) at the Center for Veterinary Medicine (CVM) as a pharmacologist, where he conducted reviews of residue chemistry studies and bioanalytical methods, as well as directed method trials for the implementation of official methods to determine and confirm drug residues. In 2014, Dr. Kadavil returned to CDER, and is currently the Team Lead for the Collaboration, Risk Evaluation and Surveillance Team (CREST) under the Office of Study Integrity and Surveillance (OSIS), which is in the Office of Translational Sciences. CREST supports the implementation and advancement of OSIS' surveillance inspection program of study sites that conduct bioequivalence/bioavailability studies.

Fizal Nabbie, PhD, BMS: Fizal has over 30 years in the Pharmaceutical industry, in different disciplines and he successfully supported development of many pharmaceutical compounds during his career. Fizal has had a long career at Bristol-Myers Squibb and currently holds the position of Senior Research Investigator. In 2006 and again in 2015 Fizal won the "Excellence in Ligand Binding Assays" awards at AAPS conferences. Fizal received his Ph.D. in cell and molecular biology from The University of The Sciences, Philadelphia.

Paul Rhyne, PhD, Biologics Development Services: Dr. Paul Rhyne is a Vice president at Biologics Development Services (BDS). He obtained his Ph.D. in Cellular Immunology from the University of Tennessee at Memphis and gained post-doctoral experience in Virology at St. Jude Children's Research Hospital. Dr. Rhyne began his career in industry

working in a biotechnology company focused on early cancer detection technologies and in the commercial antibody industry where he developed Luminex based biomarker assays for the measurement of phosphorylated proteins. He joined Bristol-Myers Squibb pharmaceutical company overseeing a clinical biomarker assay group that developed and validated assays for BMS clinical trials. Dr. Rhyne continued to expand his career in the contract research organization industry as a Scientific Director at Tandem Laboratories and at Q2 Solutions (Quintiles) overseeing method development and validation of PK and Immunogenicity assays for biologics and biosimilars. Dr. Rhyne is currently a vice president at BDS responsible for all bioanalytical analysis and operations.

Martin Schwickart, PhD, Celgene: Dr. Martin Schwickart is currently Principal Scientist in Translational Development at Celgene. Martin aims to solve translational questions in late stage development with the help of clinical biomarkers, and models of human disease. Martin led a GLP lab, and oversaw assay development/validation of immunoassays, cell-based assays, and flow cytometry assays. Previously, Martin worked at MedImmune and Genentech. He performed his doctoral studies at the Max-Planck Institute for Molecular Cell Biology and Genetics in Dresden, Germany. Martin has published a number of research articles, many in high impact journals describing seminal work.

Timothy Sikorski, PhD, GSK: After graduating from the University of Pennsylvania in 2004, Tim completed his PhD at Harvard University, where he developed proteomic methods to study the dynamics of protein complexes during transcription. Tim joined GSK in October of 2011 as a member of the Biological Mass Spectrometry group in Molecular Discovery Research. There, he developed mass spectrometry-based methods to map post-translational modifications, such as acetylation and phosphorylation, on a proteome-wide scale for mechanism-of-action studies and to identify potential biomarkers. In January 2016, Tim transitioned to the Exploratory Biomarkers Group, where he has been working on developing novel methods for measuring endogenous protein and metabolite biomarkers in systemic matrices to support early Experimental Medicine clinical trials. These assays are serving as important pharmacodynamic endpoints in proving target engagement and mechanisms of action of GSK medicines.

Hans Ulrichts, PhD, UCB Pharma: Hans Ulrichts is a Bioanalytical Scientific Manager at UCB Pharma, Belgium. He obtained his PhD at the Laboratory for Thrombosis Research at the University of Leuven, Belgium, in the field of haematology and thrombosis. After a post-doctoral position at the Royal College of Surgeons in Ireland, Dublin, he joined Ablynx in 2006, where he held several positions with increasing responsibilities. Prior to joining UCB Pharma in 2017, Dr. Ulrichts was the head of the pharmacology department at Ablynx, leading a team of 60 scientists, responsible for the (pre)clinical pharmacology testing of Nanobodies and with a specific focus on bioanalysis. He published extensively, mainly in the field of haematology and thrombosis.

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