BioAgilytix

Biomarker Assay – how to apply Context-of-use to your assay validation

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Key Takeaways

- What is the **context-of-use**?
  - You must revisit this question for each biomarker you analyse

- Learn the critical points of what you need to know to properly apply fit-for-purpose assay validation principles to your biomarker context-of-use.

- What are the key assay validation parameters to produce a reliable bioanalytical assay generating data for your decision making.

Why the Need for Biomarkers?

A biomarker-driven approach can be used to develop **targeted therapies** and **patient selection** strategies, and has the potential to increase success in the drug development process, decrease costs, and ultimately improve patient outcomes.

Research → Non-Clinical → Phase 1 → Phase 2 → Phase 3 → Phase 4

Biomarkers are at the core of the concept of 'patient-centered medicine' or 'personalized medicine'. The goal of this approach is to deliver the right medication to the right patient as a pathway to better treatment.

What is

Context of Use

Fit for Purpose

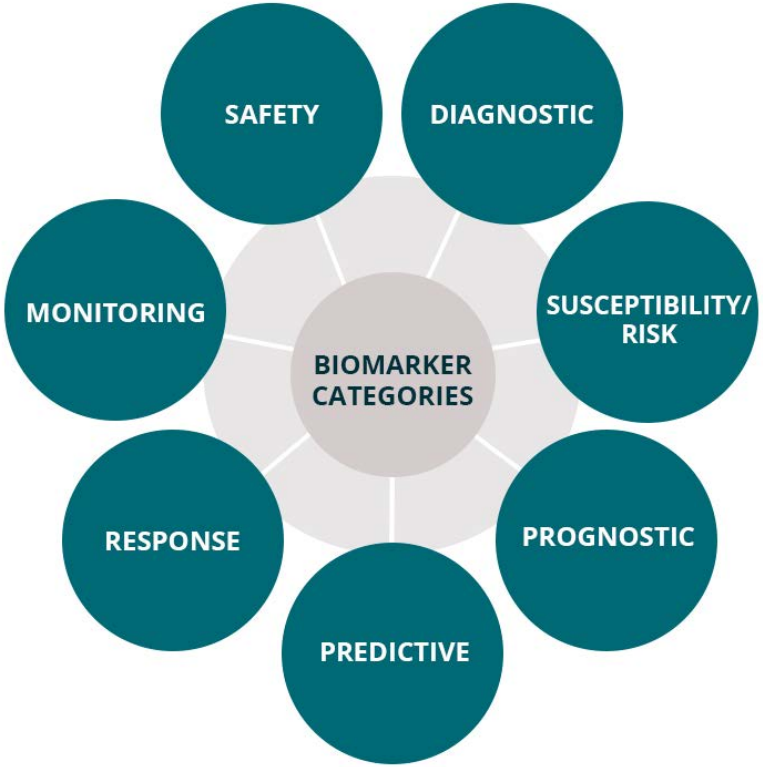
BEST Definition of Biomarker (2015):

evolved as a result of the FDA-NIH Biomarker Working Group

A defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.

Molecular, histologic, radiographic, or physiologic characteristics are types of biomarkers. A biomarker is not an assessment of how a patient feels, functions, or survives.

Categories of Biomarkers



https://www.ncbi.nlm.nih.gov/books/NBK326791/

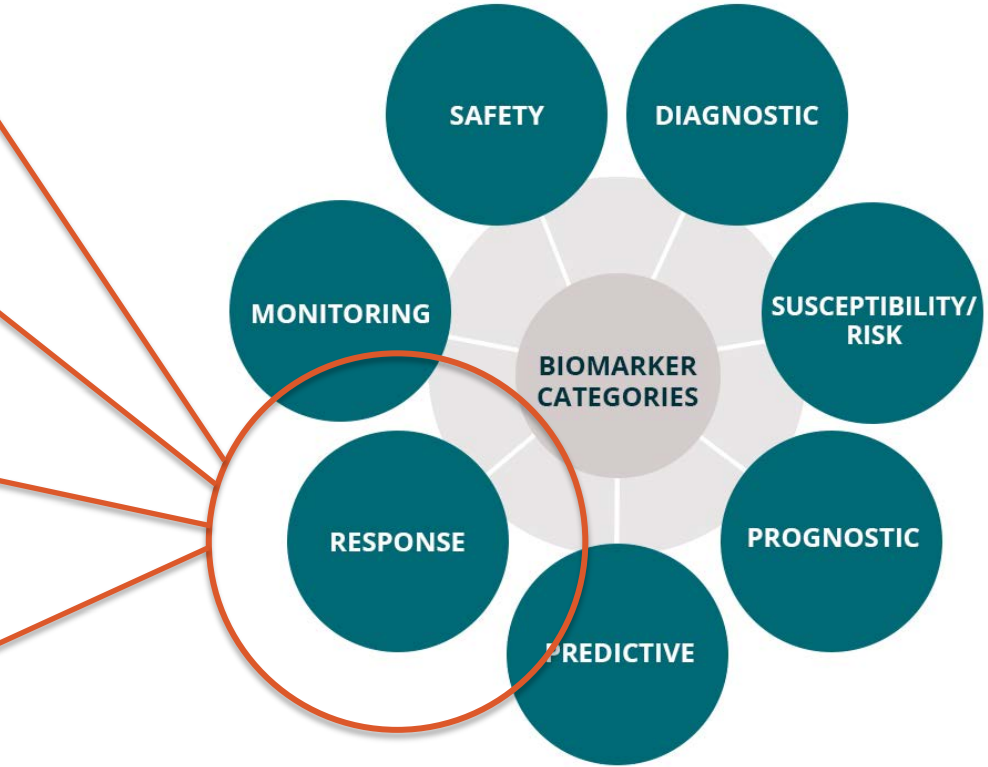
Pharmacodynamic Biomarkers

Target Engagement (TE)
Demonstrates that the drug binds to the intended target

Proof of Mechanism (PoM)
Demonstrates that the drug has the expected (immediate) pharmacological effect

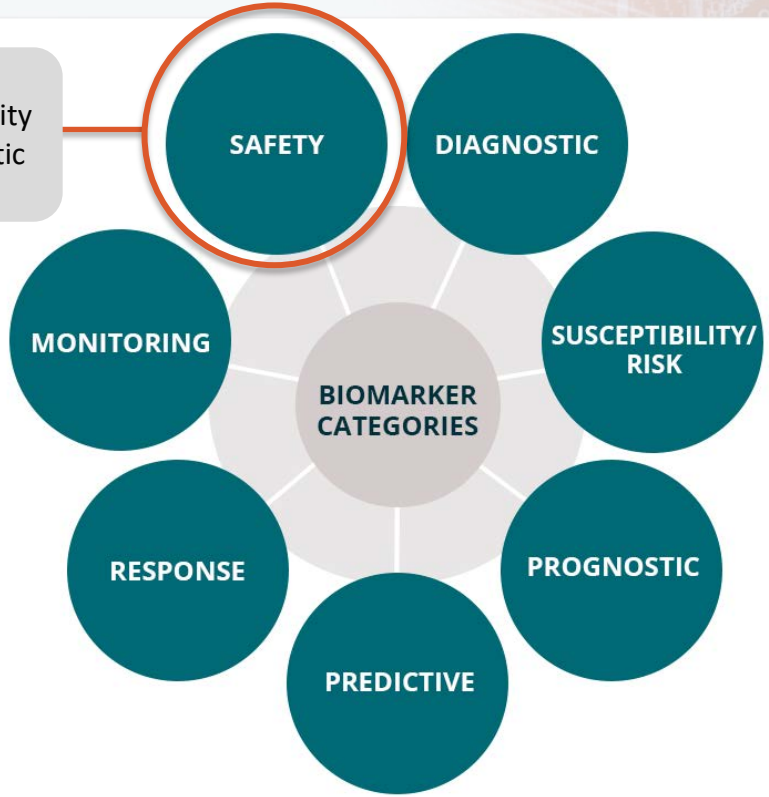
Proof of Principal (PoP)
Proves that the drug has a pharmacological impact on disease pathophysiology

Proof of Concept (PoC)
Proves that the drug produces a meaningful clinical change on an accepted disease endpoint



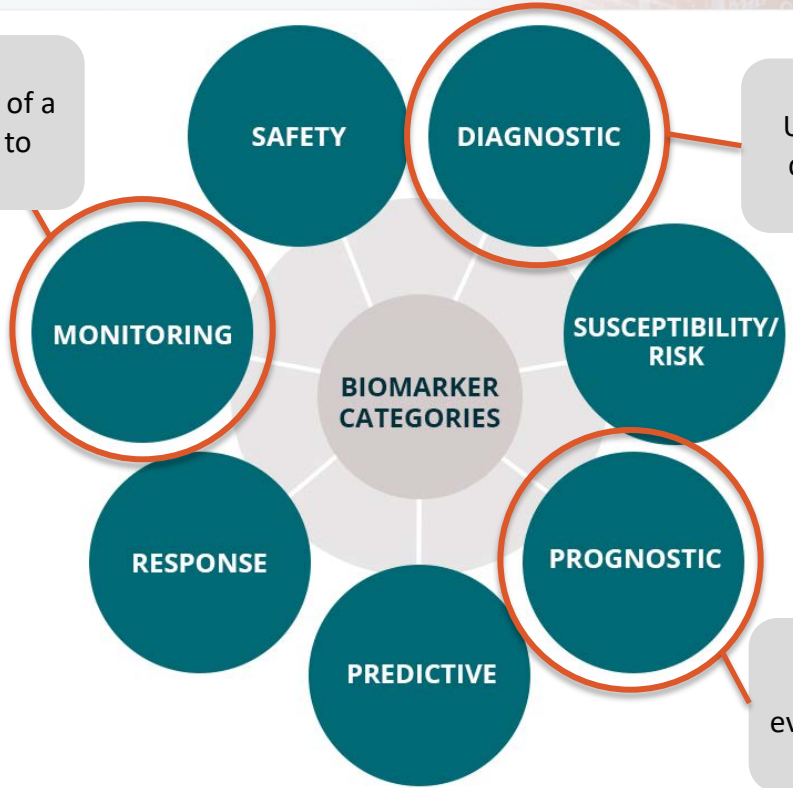
Safety Biomarkers

Safety Biomarker
Indicate the presence or extent of toxicity related to an intervention or therapeutic drug exposure



Precision Medicine Biomarkers

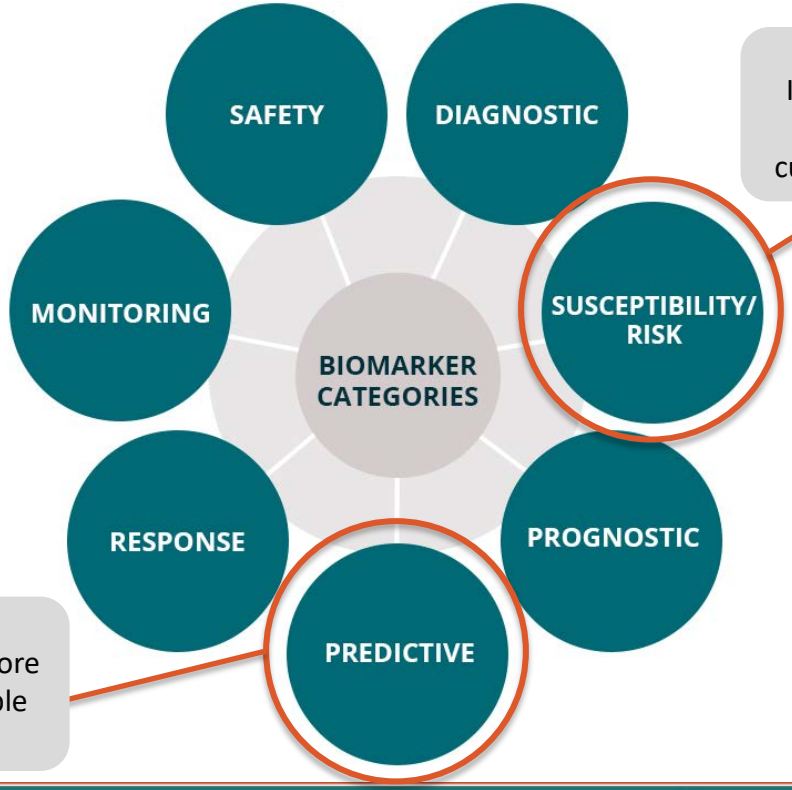
Monitoring Biomarker
Measured serially for assessing status of a disease or for evidence of exposure to (or effect of) a therapeutic drug



Diagnostic Biomarker
Used to detect or confirm presence of a disease or to identify individuals with a subtype of the disease

Prognostic Biomarker
Used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease

Precision Medicine Biomarkers



Susceptibility/Risk
Indicates the potential for developing a disease in an individual who does not currently have clinically apparent disease

Predictive Biomarker
Used to identify individuals who are more likely to have a favorable or unfavorable effect from a therapeutic drug

What is Context-of-Use?

The Context-of-Use (COU) is a precise description of the biomarker´s specific use in the drug development process.

Consists of:

- The BEST biomarker category
- The biomarker´s intended use in drug development
- Context-of-use is the ‘purpose’ in fit-for-purpose
- Each biomarker qualification effort should identify a single COU

What is Fit-for-Purpose?

- A misperception in industry that fit-for-purpose is not good quality
  - There is a misunderstanding that everything is allowed for biomarkers
    - Increase acceptance criteria
    - Deliver data outside range
  - Fit-for-purpose is Good Quality!

- The fit-for-purpose status of a biomarker method is deemed acceptable if the assay is capable of discriminating changes that are **statistically significant** from the subject variation associated with the biomarker.
    - What degree of change is expected to be measured?
    - What amount of inter- and intra-subject variability is normal for the population of interest?
    - What amount of precision is expected or tolerated for the instrument platform being considered?

Clinical Validation and Analytical Validation are Two Distinct Aspects of Biomarker Validation

Clinical validation ensures the biomarker reflects (identifies, measures, and predicts) the outcome of interest and are acceptable for the proposed Context-of-Use

Analytical validation ensures that the performance characteristics of the biomarker assay are acceptable for the proposed Context-of-Use

Biomarker Assay Life Cycle Management

Target Identification → Target Validation → Lead Candidate Identification & Selection → Pre-Clinical Safety → Phase I → Phase II → Phase III

- Critical Reagents selection (commercial or in-house generated)
- Define pre-analytical conditions
- Set analytical performance requirements for assay

- Set-up Assay Validation parameters
- Establish assay validation acceptance criteria
- Define documention of assay performance

- Determine Biomarker levels and inter-individual variability
- Biomarker levels correlation to disease stage or progression
- Change in Biomarker levels correlation to clinical meaningful endpoint(s)

Ask Questions!

- Biomarker assays follow a fit-for-purpose driven assay validation approach which lead by the biomarker´s context-of-use (COU).
  - The level of assay characterization is determined by the end-use of the biomarker data

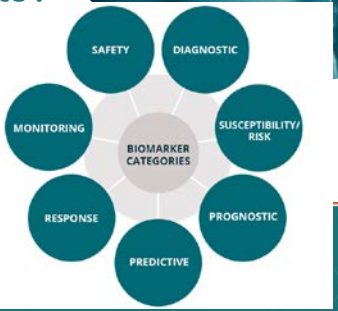
- Initial data-set generated for exploratory use may be of such subtantial interest that it changes its COU, and now becomes a decision making biomarker.

Data sometimes ends up getting used in ways you did not originally expect!

Define Context-of-Use

Key questions to ask when evaluating a biomarker:

- Is it related to a **biological** response of disease?
- Can enrichment studies increase the **success** of the drug?
- Can the patients be **selected** for treatment?
- Will the measurement of biomarker levels help speed up the **drug development process**?
- Will the **stratification** help delivering drug faster to the patients?



Primary and Secondary endpoints are not COU

For an Assay Validation - You Need to Ask Questions

- Will the study involve **healthy subjects or patients?** What is the expected levels in the population
- Will the therapeutic drug cause **interference?**
- Will the **biomarker** be up or down regulated? What is the magnitude of the change
- Which **sensitivity (LLOQ)** is needed?
- How do **sample handling** conditions affect the measurement?
- What is the analytical **precision** compared to the biological variation?
- And is this variability suitable to **detect** the drug effect?
- Which **matrix** to collect in & will the matrix affect the **assay performance?**

What is the intended use of data?

What to Consider

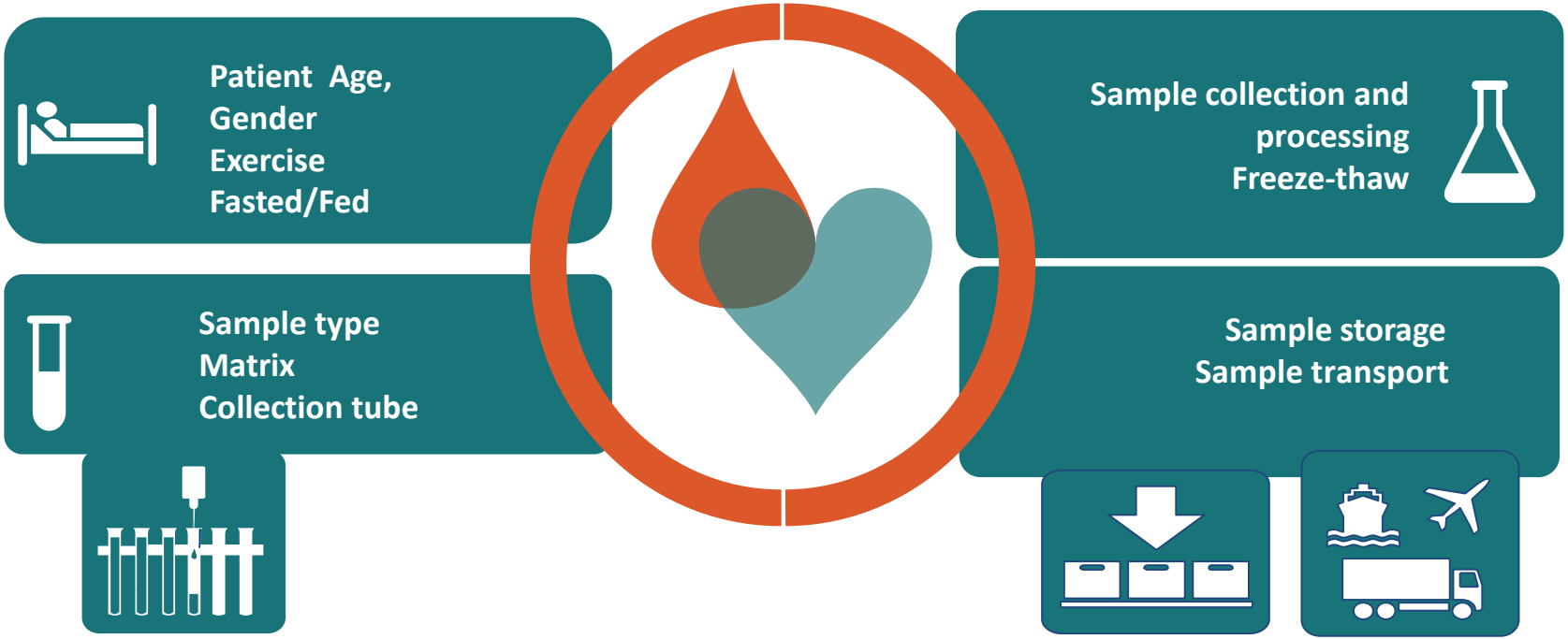
Reagents

- Commercial Kit
  - Convenient – speed up process
  - Antibody pairs already chosen
  - Reference Standard is available

- Realize that not all kits perform or measure what you would expect
  - Calibration material
  - Assay performance not meeting expectations; ‘what are we measuring?!’

- Kit Supply
  - Lot-to-lot variation
  - Delay in kit production
  - Termination of kit supply

Pre-Analytical Considerations



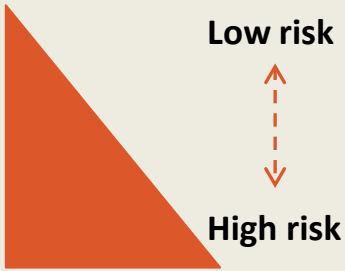
How are the data used?

Diagnostic

Disease confirmation

Patient Selection

Prognostic



Predictive

Drug Selection

Pharmacodynamic

Dose Selection

Biomarkers can guide patient selection for clinical trials

Biomarkers can guide dose setting in clinical trials

Biomarker Assays ≠ PK Assays

The difference between **Biomarker assays** and **PK assays**?

- Recombinant standard calibrator material ≠ endogenous analyte
- Relative Accuracy
  Accurate recovery of spiked recombinant material ≠ endogenous analyte accuracy (1+1≠ 2)
- Parallelism ≠ Dilution linearity
- Matrix contain endogenous analyte => surrogate matrix
- Recombinant material stability ≠ endogenous analyte stability;
  - Include Stability assessment of endogenous biomarker
- Pre-setting of Acceptance criteria

Understand the Biology

What to do in Assay Development and Validation

- During **Assay Development** you characterize the assay
  - Specificity
  - Measure the biomarker in matrix
  - Define the Minimum required dilution (MRD)
  - Parallelism
  - Estimate LLOQ
  - Precision both analytical variation and biological variation
  - Stability
  - Pre-analytical assessment

- During **Assay Validation** you **confirm** the data from the assay development

Case studies

What to include of assay characterization parameters

1. Exploratory Biomarer
2. Pharmacodynamic Biomarker
3. Predictive Biomarker

Exploratory Biomarkers

- Bridge the results of animal models to clinical expectation
- Are used to understand the Pharmacodynamic or Mechanism of Action of the drug
- Used for Hypothesis generation
- Can be used to fill the gaps of uncertainty about disease targets or variability in drug response
- Data are not suited to judge safety and efficacy of the drug
- Data are for internal decisions

Exploratory Biomarker

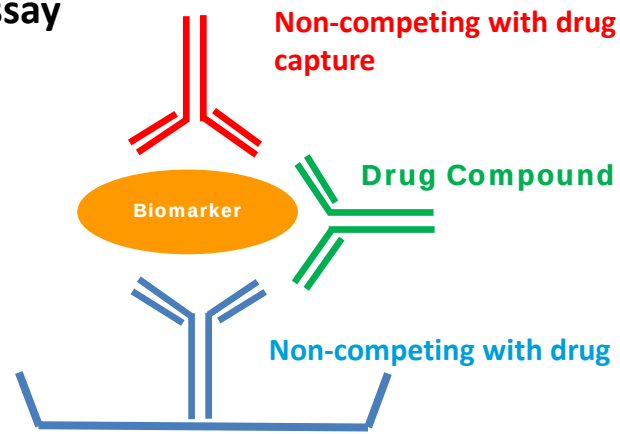
Assay Characterization parameters

- Calibrators and Assay range
  - Spike recombinant protein in Buffer (surrogate matrix)
- QCs
  - Spike recombinant protein in Buffer; Relative accuracy as recombinant protein is not always the same as the endogenous protein
  - Use the QCs for assay acceptance
- Precision & Relative Accuracy
- Specificity
  - Is the assay measuring what it is intended to?
- Consider to use
  - Stability (but have in mind that Stability of recombinant spike protein is not the same of the endogenous)

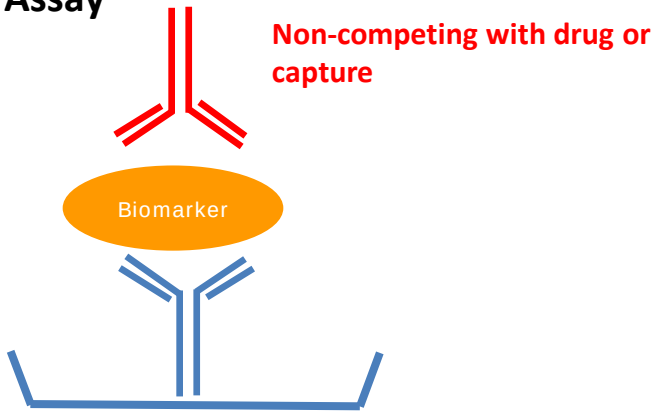
Pharmacodynamic Biomarker

- Use for defining the mechanism of action of the Drug – Did the drug hits it's target?
- Do the drug have its intended pharmacological effect?
- Consider if there is a need for Total and Free Biomarker measurement
  - Total – when biomarker is bound to drug compound
  - Free – when biomarker is not bound to drug compound

Total Assay



Free Assay



Pharmacodynamic Biomarker

Assay Characterization parameters

- Calibrators and Assay range
  - Spike recombinant protein in Buffer (surrogate matrix)
- QCs
  - Use Buffer QCs and at least one matrix QC level
    - Matrix QC level can be measured several times and the establish value is used as the nominal value
    - Use the QCs for assay acceptance
- Precision & Relative accuracy
- Specificity
  - Test Total or Free assay by preparing QC with different spiked levels of Drug and Biomarker
  - Free assay: Prepare an inhibition curve by spiking different levels of drug to a fixed level of biomarker
- Consider to use
  - Parallelism if you can hold of ‘real matrix sample’
  - Selectivity
  - Stability (but have in mind that Stability of recombinant spike protein is not the same of the endogenous)

Predictive Biomarker

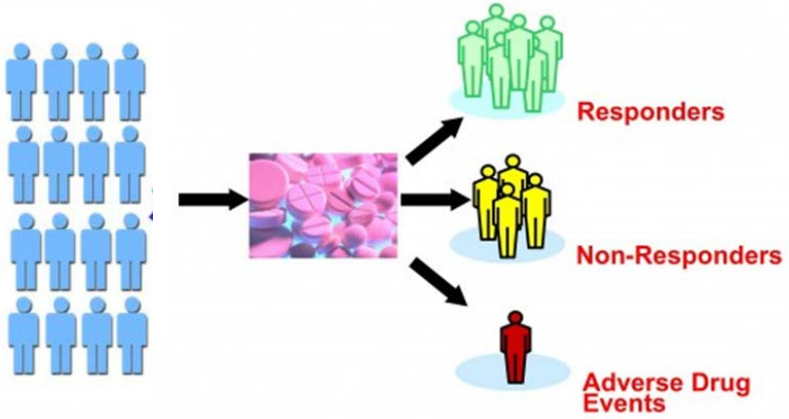
- Used to identify individuals who are more likely to have a favorable or unfavorable effect from a therapeutic drug

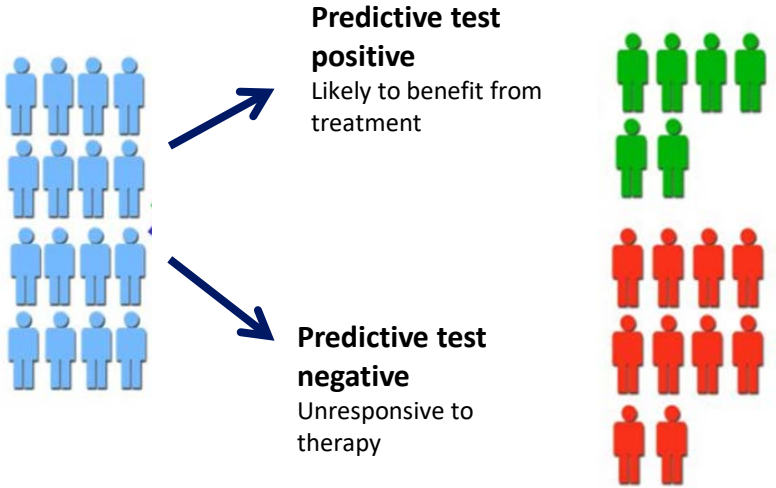
- Most often an exploratory biomarker in Phase I and II
- Data are used retrospective – and used internal – to define if the biomarker can be used as a potential Precision Medicine decision making

- Often samples from previous trials are used to characterize the assay (ensure the informed consent)
  - These samples are used for Parallelism, Selectivity and Stability assessment

Precision Medicine

Shifting the Paradigm by use of biomarkers





Predictive Biomarker

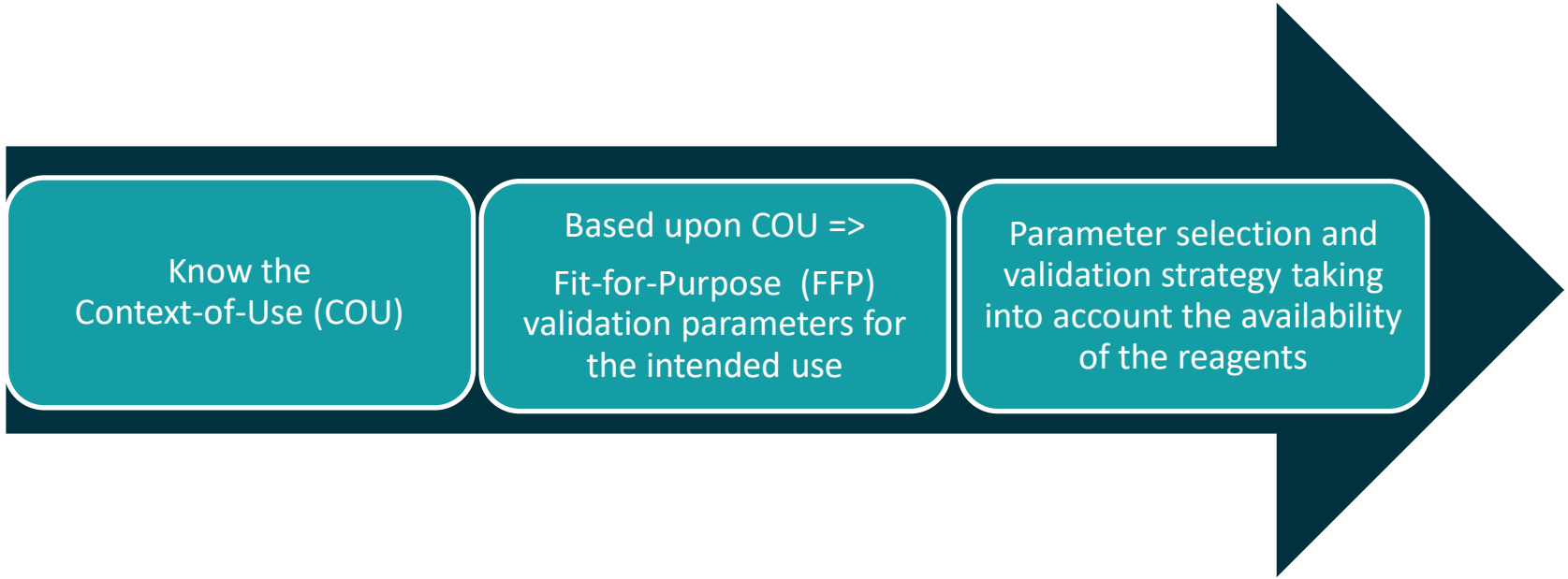
Assay Characterization parameters

- Calibrators and Assay range
  - Spike recombinant protein in Buffer (surrogate matrix). The Reference material needs to be characterized.
- QCs
  - Use Matrix QCs
    - Matrix QC level can be measured several times and the establish value is used as the nominal value
    - Use the QCs for assay acceptance
- Precision and Relative Accuracy
- Specificity
- Parallelism
  - Dilute a real sample to verifify that it dilute in parallel to the recombinant protein calibrator
- Selectivity
- Stability (use endogenous level sample)

Biomarker assay are not PK assays

- Validation parameters are **NOT to be used** as check list where all listed bullet points must be evaluated
- The point is that **YOU** pick the analytical elements which are directly relevant to the biomarker of interest, so it matches the Context of Use (COU) in **YOUR** drug development process.
- Based on these considerations, the analytical elements should be experimentally evaluated.
- The level of assay validation is determined by the intended use of biomarker data

Biomarker Validation Philosophy at BioAgilytix



Context-of-Use

The level of assay validation is determined by the end-use of biomarker data

Always consider:

1. Know the Context-of-Use
2. Defining pre-analytical conditions
3. Setting analytical characterization requirements for the assay
4. Characterizing and Documenting assay performance
5. Establishing assay validation acceptance criteria

Consider key analytical characterization parameters when preparing the validation plan:

1. Accuracy (Relative)
2. Analytical Measurement Range
3. Parallelism
4. Precision
5. Selectivity
6. Specificity
7. Stability (endogenous)

Final Remarks

- What is the **Context-of-Use**?
  - You must revisit this question for each study and biomarker you analyse
- When does **exploratory** become **confirmatory**?
- The assay validation is an **iterative process** that takes into account how and at which stage the data are used

The assay must be reliable in order to be confident in the decisions taken from the biomarker data.

Question & Answer

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Thank You!

Merci

Tak

Danke

Grazie

Obrigado

Спасибо

Gracias

Efharisto

Tack

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