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Influence of Context of Use (COU) on Biomarker Method Development and Validation

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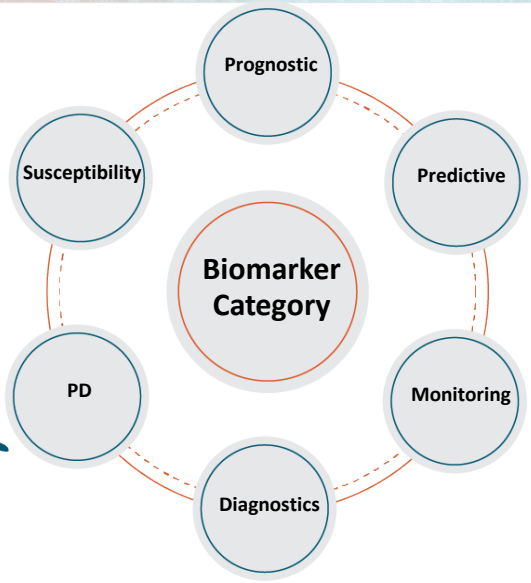
Biomarker Applications and COU

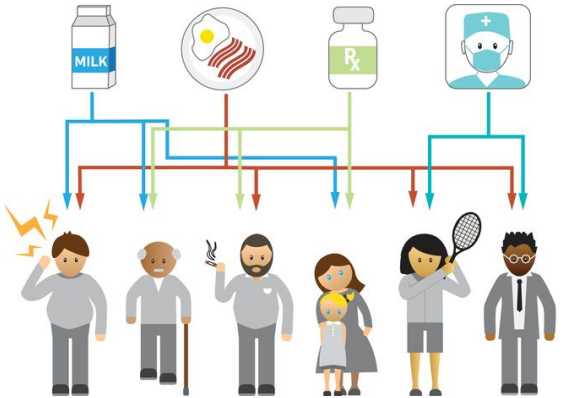
Method Development

- COU
- Patient population
- Sample type
- Biological variation
- Sample collection
- Interferents
- Platforms and Technologies
- Quantitative, Qualitative, or Relative Quantitative
- Available reagents
- Performance criteria



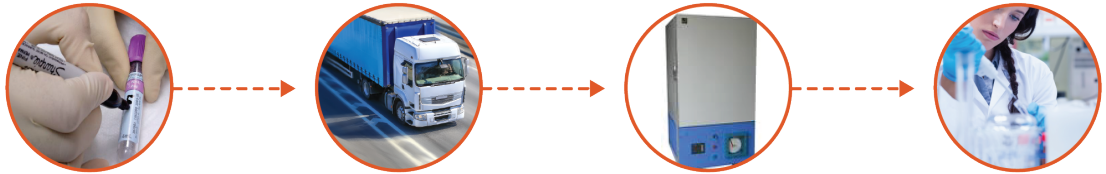
Context of Use

- A biomarker's COU should be understood during development of the bioanalytical method designed to measure it.
- COU influences technology selection and assay design.
- COU will be further defined by the study design, which will have practical implications for design of the assay.
  - Test population (healthy, disease)
  - Population comorbidities
  - Potential interferents
  - Sample collection
  - Testing environment



Influence of COU – Assay Development

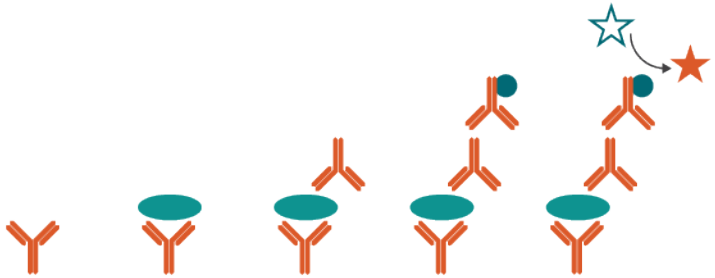
- Platform/technology selection
  - Qualitative or Quantitative
  - Sensitivity requirements
  - Throughput expected
  - Validation requirement/status of instrument and software
- Reagent availability
  - Specificity/selectivity requirements
- Preanalytical factors



  - Sample type
  - Sample collection, processing, transportation, storage – short term/long term

Influence of COU – Assay Design and Performance

- Assay Design
  - Format
  - Specificity/selectivity/interference
  - Reagents



- Parallelism
  - Assess as early as possible during development
  - Commercial sources of samples available
  - Assess during in-study validation in the study population

Influence of COU – Assay Performance

- Assay Performance Requirements
- The COU will help to determine the performance requirements for the assay.
  - As discussed by Lee et al. (2006), a biomarker method is deemed fit-for-purpose if the assay is capable of discriminating changes that are statistically significant from the intra- and inter-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 imprecision is expected or tolerated for the platform being considered?

Can the Assay Discriminate Changes?

- An assay suitable for detecting a statistically meaningful effect in a population with a low degree of biological variability may not be suitable for a different study population that has much greater physiological variability.
- The acceptable amount of total error associated with a method must consider the degree of biological variability among the population of interest and the magnitude of the anticipated response.

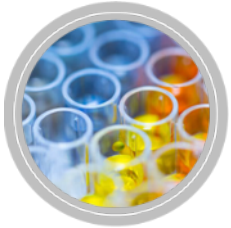
Can you spot the difference?

Example – COU 1

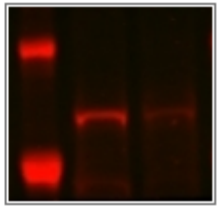
- Early phase clinical PD marker for POC
  - Phosphoprotein
  - Phosphorylation present or absent
- Few subjects (< 10)
- Pre- and post-treatment collections per subject
- Sample type: tissue biopsy
- Control samples (cells, tissues) are available for development
- Specific detection reagents are available

Platform Selection: COU 1

- ELISA (commercial kit available for total protein)
  - Medium – high throughput
  - Qualitative or relative-quantitative



- Western Blot
  - Low throughput
  - Qualitative or relative-quantitative



- IHC
  - Low throughput
  - Qualitative

Other Factors Influencing Platform Selection

- Availability of platforms
- Sample type
  - Collection
  - Fixation
  - Storage
- Reagents
  - Characterization
  - Specificity

Analytical Validation for COU 1

- Intra-assay precision
- Inter-assay precision
- Sample processing variability
  - Within operator
  - Between operators
- Stability of endogenous analyte
  - Bench top/4 °C storage
  - Freeze/thaw
  - Frozen storage to cover required storage period

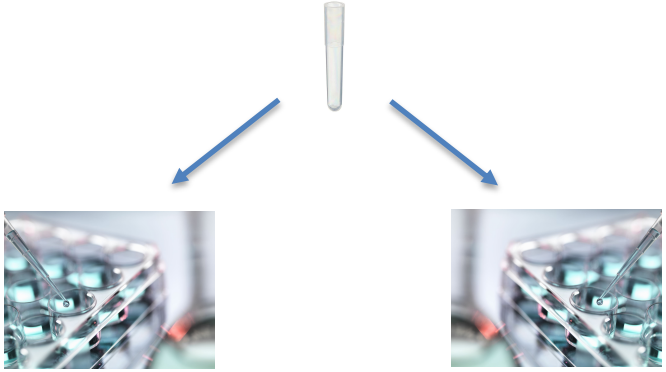
Example – COU 2

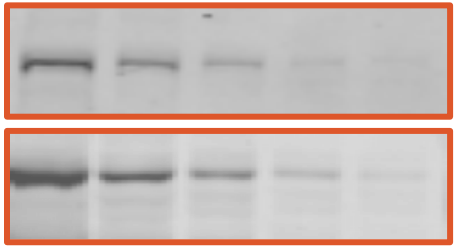
- Clinical PD biomarker for possible treatment decisions
  - Dose-dependent inhibition
- Study includes multiple cohorts and multiple subjects per cohort
- Sample type: PBMC
- Several collections per subject over time for monitoring
- Control samples available for development
- Specific detection reagents available

Platform Options for COU 2

- ELISA (commercial kit available for total protein)
  - Separate assays for phospho/total
  - Relative quantitative (surrogate standard)
  - Requires reagents to be highly specific
- Multi-plex platforms
- Western Blot
  - May be used if detection reagents are not specific
  - Inter-gel variability can be high

A surrogate standard will be required, and parallelism will need to be assessed.





Analytical Validation for COU 2

- Calibration range
- Intra-assay precision and relative accuracy
- Inter-assay precision and relative accuracy
- Sample processing variability
- Parallelism in normal and/or disease state samples
- Sensitivity/selectivity/specificity
- Stability of endogenous analyte
  - Bench top/4 °C storage
  - Freeze/thaw
  - Frozen storage to cover required storage period

Summary

- Context of use influences all facets of biomarker method development, from platform selection through validation.
- Context of use influences the evolution of a method as a drug progresses through discovery and development.
- Platform selection is dependent upon the availability of reagents appropriate to meet the context of use of the biomarker.
- The attributes and validation requirements of an analytical method will likely have to evolve to ensure that the method is appropriate to measure the biomarker for its intended purpose at various stages of drug development.

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Question & Answer

감사합니다

Thank You!

Danke

Merci

Tak

Grazie

obrigado

ありがとう

Спасибо

Gracias

Tack

Efharisto