Making Haste, Slowly, in Bioanalysis of Biomarkers

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# Overview

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In the bioanalytical laboratory, we often receive requests for biomarker assays that come with little to no context as to what questions the biomarker data are expected to answer.

In its simplest form, the request comes as:
• ‘Do you have a validated assay for biomarker X?’

When faced with this situation, how should we proceed?
Festina lente

‘Make haste, slowly’
- Classical adage and oxymoron dating back to Roman times
- Activities should be performed with a proper balance of urgency and diligence
- If tasks are too rushed then mistakes are made and good long-term results are not achieved
Making haste, slowly, in bioanalysis of biomarkers

- Make time to understand the background to biomarker assay requests
  - Take the correct path for assay establishment and validation
  - Gives us confidence that the biomarker data our laboratories produce are fit for their intended purpose
How will the biomarker data be used?

Understand the biomarker biology & Mechanism of action (MoA) of the drug

Bioanalysis (2012); 4(15): 1883–94
Case Study 1: Biomarker of target engagement
Case Study 1: Enquiry

- We would like to measure the levels of free biomarker X. The endogenous level are very variable so maybe the use of a surrogate will be necessary. We have already established a parallelism between the recombinant biomarker and the endogenous biomarker
  - How will you validate this assay?
Workflow for analysis of a biomarker using a new assay

- Understand biology of BM
- Translate BM biology and science into Bioanalysis
- Qualify assumptions
  - Agree on final assay requirements
  - Set up the assay
  - Analyze samples

Success

Bioanalysis (2012); 4(15): 1883–94
Workflow for analysis of a biomarker using a new assay

Focus for this case study
• Biomarker biology
• MoA of drug
• Usage of data

Analysis of BM using a new assay.

Bioanalysis (2012); 4(15): 1883–94
Case Study 1: The biology of the biomarker

The biomarker is a membrane-bound extracellular protein involved in modulation of immune function

- In some cancers, the protein is overexpressed in the tumor microenvironment inhibiting anti-tumor immunity
- High levels of soluble protein biomarker are associated with poor overall survival and progression-free survival

What concentration levels do we expect in the study population?
- How much intra- and inter-individual variability do we expect?
Case Study 1: The MoA of the drug

- Drug is a monoclonal antibody (mAb)

- The biomarker is the **target** of the drug
  - Drug inhibits activity of the protein biomarker to restore a pro-inflammatory microenvironment and help activate an anti-tumor immune response

- What changes do we expect to see in biomarker levels upon drug administration?
  - What assay sensitivity do we require?
  - Should we measure free or total biomarker?
Case Study 1: Expected changes during treatment

- **Biomarker Concentration**
- **Time**
- **Drug administration**

**Tumour reduction/killing**

**Total biomarker**

**Free biomarker**

Examples:
- Davis et al. (1999) Drug Delivery 6(3), 171-9 [https://doi.org/10.1080/1071754992669222](https://doi.org/10.1080/1071754992669222)
- Neubert et al. (2013) Anal. Chem. 85, 1719–26 [https://doi.org/10.1021/ac303031q](https://doi.org/10.1021/ac303031q)
Case Study 1: Usage of the biomarker data

- Supporting early clinical trial (phase I/II) evaluating safety, efficacy and optimal dosing regime of the drug

- Biomarker data will be used to evaluate pharmacodynamic (PD) response to the drug
  - Want to achieve target engagement >95%
  - Analysis of PD data at interim and end of study
  - Biomarker data will not support dose escalation
Case Study 1: Biomarker assay establishment

A number of assay options:

- Free biomarker assay on standard immunoassay platform
  - Unlikely to detect levels after drug administration
  - Assay results <LOQ may indicate appropriate level of target engagement

- Free biomarker assay on ultra-sensitive platform
  - Additional investment, but may be capable of measuring free target levels
  - Measurement may be impacted by change in drug:biomarker binding kinetics during sample processing

- Total biomarker assay
  - Need to identify reagents that are non-competitive with the drug
  - Free biomarker levels can be modelled based on drug concentration measurement (PK) and drug binding kinetics
Case Study 1: Biomarker assay establishment – path taken

► A number of assay options:
  • Free biomarker assay on standard immunoassay platform
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Case Study 2: Changing use of biomarker data
Case Study 2: Enquiry

- We would like to measure the levels of biomarker X in our clinical study as part of our patient enrollment
  - Can we move ahead with the current assay you have already validated?
Workflow for analysis of a biomarker using an existing assay

- Existing BM platform
- Overlay BM assay performance on BM request
- BM assay performance and BM request fits
- Agree on final assay requirements
- Set up assay and analyze samples
- Analyze samples

Flowchart: “New Biomarker”

Analysis of BM using an existing assay.

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Focus for this case study

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Overlay BM assay performance on BM request

BM assay performance and BM request fits

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Set up assay and analyze samples

Analyze samples

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Case Study 2: Biology of the biomarker & MoA of the drug

- The biomarker is a small cytokine important in recruitment of immune cells to sites of infection
  - Production of this cytokine is induced by the target of the drug, and serves as a surrogate for activity of the drug target

- The drug is a monoclonal antibody that binds to and neutralizes the activity of its target by preventing it binding to its receptor
  - The target of the drug plays a role in a number of different inflammatory diseases
Case Study 2: Usage of the biomarker data

- Previous clinical studies
  - The biomarker data were used as an pharmacodynamic indicator that drug target was neutralized
  - In patients, biomarker levels were typically high (several thousand pg/mL) at initiation of treatment, returning to ‘normal’ levels (a few hundred pg/mL) when the drug maintained neutralization of its target

Simulated data for illustrative purposes
Case Study 2: Usage of the biomarker data

► New clinical study
  • Biomarker data will similarly be as an indicator of drug target activity
  • However, patients expected to have low levels of biomarker initially, with an increase at the onset of an ‘inflammatory episode’
  • If biomarker levels move above a ‘threshold’, it will indicate active disease and patients will be randomized to receive drug or placebo
Case Study 2: Does the assay fit the biomarker request?

► Do we have data that can be used to set the threshold?
  • Collected data on inter-individual variation during assay development and validation
    – Approx. 50 healthy individuals
    – However healthy individual ≠ subject without disease
  • Data on within individual variation with this method
    – Longitudinal within subject data was collected within previous clinical studies
    – However previous study subject ≠ current study subject

► Initiated collection of samples from this indication to better define subjects with and without active disease
Case Study 2: Does the assay fit the biomarker request?

- Can the current assay support the new context of use?
  - What level of precision is needed for the end use of the data?
Case Study 2: Does the assay fit the biomarker request?

- The assay is under control for current data usage
  - But level of variation could lead to misclassification of results in new context

Performance chart for endogenous QC samples

Nominal concentration = 432 pg/mL
(36 measurements: 6 replicates x 6 runs)

Inter-assay precision = 7.6%
Case Study 2: Changes to the assay to meet the new CoU

► Assay moved from manual process to automated platform
  • Improved precision to <5%

► Additional endogenous QC samples included in each run
  • Allowed normalization/correction of study sample data against a reference value
4 Case Study 3: Assessing parallelism and what it tells us
Case study 3: Assessing parallelism and what it tells us

► Assay for an inflammatory cytokine was developed and validated:
  • Precision
    – 6 runs with endogenous QC samples
    – Inter-assay precision of 8.0%
  • Stability
    – Stable through 5 freeze-thaw cycles
    – Stable stored at -80°C for 3 months (assessed with freshly collected samples)
  • Parallelism
    – 5 individuals assessed at multiple dilutions
    – Target parallelism results within 3SD (3x inter-assay precision)
    – No pass/fail criteria – assay characterization
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Case study 3: Parallelism assessment in validation

- 5 individual analyzed and multiple dilutions across the assay range

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<th>Dilution Factor</th>
<th>Log Dilution Factor</th>
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<tbody>
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<td>2</td>
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Simulated data for illustrative purposes
Case study 3: Parallelism assessment in validation

- Dilution-adjusted concentration calculated

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Case study 3: Parallelism assessment in validation

- Dilution-adjusted relative error calculated (vs MRD result)

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Simulated data for illustrative purposes
Case study 3: Parallelism assessment in validation

- All 5 individuals have dilutions within 3SD
  - But potential concerns…?

![Data Graph]

Positive bias at higher dilutions…?

Interference…?
Case study 3: Parallelism assessment in-study

- 3 samples assessed for parallelism from each subject
  - Selected samples from low, mid and high part of observed range
Case study 3: Parallelism assessment in-study

Subject 1
Parallel

Subject 2
Parallel

Subject 3
Non-parallel

Subject 4
Parallel

Simulated data for illustrative purposes
Case study 3: Parallelism assessment in-study

- No indication of positive bias from higher sample dilutions
- A small number of subjects had negative bias at dilutions after MRD
  - Parallelism present at dilutions ≥5
  - Reporting for these subjects amended – reported results from a dilution factor ≥5
5. Summary & Conclusions
Establishing a biomarker assay requires an understanding of the biomarker biology, the mechanism of action of the drug, the intended use of the data and the capabilities/limitations of the bioanalytical methods

- Dialog with stakeholders is essential to understand the biomarker CoU
- Enables us to adopt a suitable bioanalytical strategy where the appropriate level of assay performance is understood
- Give us confidence that the data our laboratories produce are fit for their intended purpose
Parallelism is a key assessment in understanding biomarker assay performance both in validation and in the study population.

• Determines whether recombinant analyte and/or surrogate matrix are representative of endogenous analyte

Parallelism is not a straightforward pass/fail assessment.

• We have to evaluated what the data tell us…
  - whether the assay has the appropriate level of performance for the intended use
  - whether we have to put mitigations in place for analysis and/or reporting
References

► European Bioanalysis Forum (EBF) recommendation on method establishment and bioanalysis of biomarkers in support of drug development

► Update to the European Bioanalysis Forum (EBF) Recommendation on Biomarkers Assays; Bringing Context of Use into Practice

► EBF Topic Team 61: Non-parallelism in biomarker assays
Acknowledgments

- European Bioanalysis Forum (EBF) community and Topic Team 61
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Questions

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