Do you know what you are measuring?

Developing biomarkers that have clinical impact

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EBF 2019, Autumn Focus Workshop
Overview

• Strategies and Challenges of Building COU-based Biomarker Assays
• Communication is a required part of the Biomarker Solution
• Case study: Discovery to Clinical Implementation
• Parallelism and Endogenous Controls are Foundational for Developing Biomarker Assays
Biomarkers in 3 easy steps...

- **Context of Use**: Ask the right questions
- **Knowledge Map**: Generate the right data
- **Analytical Tools**: With the right (FFP) tool

Path to biomarker utility: Iterate, iterate, iterate…
Strategic Approach to COU-based Biomarker Assay Development

Goal: Understand “what is being measured”

• Understand the behavior of the endogenous analyte in the context of the assay
  • Recombinant reference material ≠ endogenous analyte
  • ALL BIOMARKER ASSAY DEVELOPMENT REQUIRES A TRUE POSITIVE CONTROL
  • Utility of the assay for the endogenous analyte is driven by both assay reagents and preanalytical factors

• Understand the behavior of the biomarker in the context of the study
  • Is the biomarker variability (analytical + biological) appropriate for the clinical hypothesis to be tested?
Roadmap to Initiating COU-based Biomarker Assay Development

• Start with **Why**
  - Why does the team think this biomarker will help the program?
  - What data-driven decisions need to be made and how will the biomarker data be interpreted?

• Then determine **How**:
  - Build the necessary biomarker knowledge map:
    - The biology
    - The assay
    - The sample (pre-analytical factors)
    - The study

**CLEAR COMMUNICATION**

**SOLID SCIENCE**
Transition from research into clinical development

Establish the COU as early as possible:
- What are the goals and needs of the study/program?
- How is the biomarker expected to behave in the study?
- What decisions will be made with this data?
- WHAT IF this biomarker is not measurable in the clinic?
Case Study: Aligning COU and Biomarker (Assay) Development

*R2D transition: Clinical BM assay request & stakeholders sub team formed*

**NEEDS/ EXPECTATIONS/DECISIONS:**

- Need a relative-quantitative PD assay to establish a FIH PK/PD model for the therapeutic agent.
- Treatment is expected to cause a decline in the biomarker, targeting 50-90% inhibition
- Program will Go/No Go based on levels of BM change and select therapeutic doses
Iteration #1: Discovery Biomarker Assay Design

Biomarker Hypothesis

-Drug

Sample Pre-analytics

Sodium Heparin Whole Blood (+ Drug response curve)

Lysis buffer + protease and phosphatase inhibitors

60 min @ 4°C shaking

Immediately add to MSD plate

+Drug

Assay Design

Target (Lysed WB)

Primary antibody (anti-target)

Precoated MSD plate

Secondary antibody (Biotin-anti-target)

Detection antibody (SA-SULFO-TAG)

Data Readout

- Dose-response curve with IC\textsubscript{50} reported
- No calibration curve
- Limited range of signal detection
Planning for Success

KNOWN UNKNOWNS:
• Translating from discovery-based murine assay into human studies.
• Require significant sensitivity to quantify nadir of response & broad dynamic range to capture dose-dependent differences in BM levels.
• Clinical implementation challenges using a complex matrix, including sample processing and sample stability.
• Strategy for long-term clinical implementation of BM as program expands globally.

UNKNOWN UNKNOWNS:
• Cross-functional communication maintains our ability to react and problem-solve
No such thing as a “good” donor….

- Discovery colleagues leveraged the murine assay to evaluate healthy human donors.
- Preclinical evaluation of healthy volunteers indicated only “selected” donors had a “good” biomarker response.
- Performed all IND-enabling studies with only “good” donors (<8 of 50 donors).

- Random selection of healthy donors show poor dynamic range between Maximum (untreated) and Minimum (drug-treated) response.
- 50% of individuals ≤ 2-fold dynamic range
- 20-30% of individuals no response was detected.
And then there is practicality….

- Discovery only evaluated freshly lysed whole blood samples in assay.
- In clinical setting, a minimum of one freeze-thaw to ship samples to testing lab would be expected.

- Freeze-thaw results in signal response below detection limit.
- Curious behavior of “high” biomarker donors.
Differential Response of Recombinant Standard vs. Endogenous Analyte

☑ Titration of recombinant STD curve results in a broad dynamic range.

☒ Poor detection of endogenous analyte.
Parallelism: Cornerstone of Biomarker Assays

Why Parallelism?
- Validates the use of surrogate matrix & calibrator material
- Identifies appropriate MRD for endogenous analyte
- Estimates sensitivity of endogenous analyte
- Assessment of parallelism in multiple individuals = Selectivity
- Investigational tool for anomalous results

Parallelism requires samples with the endogenous analyte
- The positive control of your experiment!
- No a priori acceptance criteria for parallelism, it depends on the COU
- Use it as a tool to characterize the limits of the biomarker assay
- Use it early and repeat as needed!

Stevenson L & Purushothama S, Bioanalysis 2014
Parallelism Investigation: Case of the curious donor

- Most individuals have signal responses at the limits of detection
- High signal responses appear to represent non-specific matrix interference

<table>
<thead>
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<th>Sample Dilution</th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
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<td>Neat</td>
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<td>1435</td>
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ECL Units (LOD~600)
Build the Assay using the Endogenous Analyte

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<tr>
<th>Coat</th>
<th>ECL Signal</th>
<th>Detection Ab</th>
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<tr>
<td></td>
<td>2ug/mL</td>
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- Recombinant calibrators also perform well with new assay format

Graph showing Max/Min signal ratios with data points for different conditions.
90% of individuals:
• MRD 1:4
• Dilution acceptable to 1:54 (%CV: <20%, Bias: <25%)
• Endogenous sensitivity ~ 5.5 pg/mL
Assay is suitable for team goal of detecting 50-90% change in BM

In study, use both recombinant and endogenous QCs to monitor assay performance:
- Recombinant QCs inform standard curve performance (run acceptance)
- Monitoring EQCs informs integrity of sample measurements (identify drift or systemic biases)
Knowing the limits of Clinical Implementation

- Establish limits of preanalytical sample processing

- Informed stakeholders that BM analysis had to be limited to an experienced Ph1 unit, could not be deployed in multi-site study
- Supplied and trained clinical site, including qualification of staff through a feasibility study
Knowing the limits of Clinical Implementation

- Establish biomarker stability to support the COU

Samples can be batch tested every 3 weeks (< 10% change from baseline)
Knowing the limits of Biology

- Establish longitudinal variability of biomarker

For expected study duration (2 weeks), the biomarker is expressed at consistent levels in untreated subjects.

- Maximum: %CFB<30%, %CV<20%.

Communicated to stakeholders:

- Based observed analytical and longitudinal variability, changes in BM >30% are likely to be treatment-related.
- Changes <30% will be difficult to distinguish from BM variability.
Study Results: Dose-dependent Inhibition of Biomarker

- Placebo group:
  - Consistent with validation results of ± 30% biomarker variability
  - Changes in dosed groups <30% were indistinguishable from placebo group

- Dosed groups:
  - Dose-dependent increase and maintenance of inhibition of biomarker observed over the required 50-90% target thresholds for program to advance.
Summary

- Biomarker assays take an iterative path as the COU, knowledge, and assays evolve
- Continuity of communication with your stakeholders is critical to success
- All biomarker assays should start with the endogenous analyte
- What if you don’t have the endogenous analyte? Do what you can but don’t assume you’ve crossed the finish line, map out a plan....