DETECTION AND DOSAGE OF SOLUBLE BIOMARKERS IN CLINICAL SAMPLES

WHAT DO ALL CANDIDATE IMMUNOTHERAPIES HAVE IN COMMON?

Whether in the preclinical or clinical stage, all immunotherapies need robust and reliable monitoring tools to characterize and track the immune response. ABL’s expertise lies in the monitoring of the immune response within a regulatory compliant setting. We have supported hundreds of preclinical and clinical studies evaluating the safety and efficacy of candidate Monoclonal Antibodies (MAbs), Antibody Drug Conjugates (ADCs), therapeutic vaccines, Chimeric Antigen Receptor T cells (CAR-T cells) and several other novel immunotherapies.

INTRODUCTION

In the past two decades, multiple studies have shown the critical role of cytokines, chemokines and other soluble mediators as biomarkers in various indications, including cancer, infectious diseases, neurodegenerative disorders, etc. The availability of suitable methods to measure such biological markers with high sensitivity and specificity is essential for many reasons such as: early diagnosis, patient stratification, drug effect monitoring, drug safety assessment.

Enzyme-Linked Immuno-Sorbant Assay (ELISA), is a robust and widely used method for biomarker detection. However, classical ELISA is limited by its ability to measure only one analyte per test, its demand on sample volume and its sensitivity. Innovative strategies, including multiplexing combined with ultra-sensitive technologies, such as the SIMOA® platform from Quanterix. SIMOA® offers deeper characterization of the complexity and dynamics of circulating biomarkers in a limited sample volume. As the range of immunotherapeutic drugs in development continues to grow, the importance of cytokines and other soluble mediators in a variety of biological fluids (serum, plasma, cerebrospinal fluid, synovial fluid, urine...) becomes more evident and provides major information on the host's immune response to the drug, including potential toxic effects.

SOLUBLE BIOMARKERS

As an expert in immunoassay development and validation, ABL provides its customers with:
- Advice on the most relevant mediators and methods to detect them.
- Assay development and fit-for-purpose analytical validation.
- Sample testing in the context of preclinical and clinical studies.
- Assay optimization and prototyping for future diagnostic tests.

Leveraging our qualified ELISA, Luminex, SIMOA®, Erenna®, Aushon Circascan and Mesoscale Discovery platforms, ABL offers state-of-the-art assays for the measurement of soluble biomarkers in biological specimens.
In the context of a phase II clinical trial, the sponsor was interested in characterizing the host immunological response during the administration of a therapeutic vaccine.

Because the number of analytes of interest were limited in this study, ABL was requested to optimize and validate a specific ELISA based methods for each biomarker. The measurement of type-I IFN-beta is described below.

After selecting an appropriate commercially available kit, the first step was to optimize it, looking for the minimal required dilution to avoid a matrix effect.

In this study, a 5-fold dilution of the serum was needed to remove matrix interference. Once optimized, a fit-for-purpose validation of the method was implemented according to international guidelines (Figure 1).

Figure 1: Parameters, strategy and acceptance criteria for the method validation

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>DESCRIPTION</th>
<th>ACCEPTANCE CRITERIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td></td>
<td>No cross reactivity between cytokine</td>
</tr>
<tr>
<td>Calibration curve</td>
<td></td>
<td>75% of back-calculated calibration points with 75-125% recovery, except for lower and upper standard points (70-130%).</td>
</tr>
<tr>
<td>Trueness</td>
<td></td>
<td>Except for LLOQ, trueness on other points between 75-125% recovery. Trueness for the LLOQ/UULOQ between 70-130% recovery.</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
<td>Except for LLOQ, %RSD (%CV) for all points +/-20% %RSD for the LLOQ/UULOQ +/- 25%</td>
</tr>
<tr>
<td>Accuracy / Total error</td>
<td></td>
<td>Tolerance interval ≤35%, except for LOQ (≤ 45%) Probability : 95%</td>
</tr>
<tr>
<td>Dilution Linearity / Hook effect</td>
<td></td>
<td>Testing of samples from patients with unknown levels of type I IFN evaluation of the concentration. If necessary, serial dilutions of corresponding spiked sample. Back calculated concentration for each dilution of spiked samples within 25% of the nominal value after correction for dilution. Precision of the final concentrations across all dilutions should not exceed 25%.</td>
</tr>
<tr>
<td>Limit of quantification / Dynamic Range</td>
<td></td>
<td>Lower and upper validation sample concentration values measured with recovery ranging from 70-130% and intermediate precision +/- 25% and accuracy ≤ 45%</td>
</tr>
<tr>
<td>Stability</td>
<td></td>
<td>Trueness +/- 25%</td>
</tr>
</tbody>
</table>

Overall performance of the method is illustrated by the following accuracy profile.

Figure 2: Accuracy profile of the method

LLOQ* is 50 IU/mL, i.e. 10 IU/mL x 5 (dilution factor) The used ULOQ in this method is 1000 IU/mL, i.e. 200 IU/mL x 5 (dilution factor) (*) LLOQ : Lower Limit Of Quantification / ULOQ : Upper Limit Of Quantification

Using this well validated method, ABL tested several hundreds of serum samples collected during a longitudinal clinical study. An example of the reported results is shown in Figure 3.

Figure 3: Type 1 IFN-beta assessment in clinical trial samples

ABL’s step-wise approach is performed systematically and can be adapted to our partners’ needs. The use of state-of-the-art technology and fit-for-purpose validated bio-analytical methods strengthens the reliability of the results which can therefore be exploited with a high level of confidence to document regulatory applications and drive development decisions.