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Single-analyte biomarker assays for pharmacodynamic or safety assessment during biotherapeutic development

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Contents

Technology Digest: Single-analyte biomarker assays for pharmacodynamic or safety assessment during biotherapeutic development

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Biomarkers and biotherapeutic development

In a US FDA-supported effort to further the success of new therapeutics in clinical studies, the use of biomarkers in biotherapeutic development has been steadily increasing in recent years. Biotherapeutics are often designed to disturb specific steps in biochemical pathways; the therapeutic target molecule – or molecules involved in subsequent steps in the pathway – can serve as biomarkers, with changes in their concentration being indicative of pharmacodynamic (PD) effects of the biotherapeutic [1]. Biomarkers used in biotherapeutic development also monitor drug safety and efficacy by helping to guide the dosing regimen, i.e., the frequency and dose at which a drug is to be administered to maintain optimum drug exposure to maximize efficacy and minimize side effects [2]. In this Tech Digest, we review multiplex and singleplex assays for biotherapeutic development and provide an overview of the Gyrolab® microfluidic immunoassay platform for biomarker assays.

Multiplex *vs* singleplex

Measurement of clinically relevant analytes such as biomarkers is often achieved through ligand binding assays (LBAs), which were initially designed to detect a single analyte in a biological matrix. These assays, referred to from here onwards as singleplex assays, typically capture and detect analytes using antibodies as critical reagents and can utilize enzymes, chemiluminescence, radioactive isotopes, fluorescence and electrochemiluminescence [3].

When used to make decisions concerning regulatory submissions and drug development, it is essential that biomarker assays deliver high performance and reliability. To meet the demands of biomarker discovery, assay technologies to measure biomarker proteins in blood, other biological fluids and tissues have evolved to have significantly higher sensitivity, as well as the ability to measure increasing numbers of analytes concurrently in a single sample via multiplex assays. This has resulted in the development of a substantial number of premade, commercially available multiplex assay kits, helping to reduce sample volumes and time spent in the laboratory. However, researchers can find themselves employing assay kits that are not specifically designed for their intended study. This requires adaptation of the kit before the assay can be validated, as the kits are usually intended for drug discovery or clinical diagnostics rather than drug development [3]. Performance and feasibility testing should therefore be conducted to ensure the assay is fit for the intended application and researchers should be aware that adaptations may be required for use with a different sample matrix or to meet regulatory requirements [4].

Other challenges with quantitative ranges, optimal sample dilution, cross-talk, sensitivity, specificity and lot-to-lot variability restrict multiplex kits and create additional hurdles for researchers [4]. Therefore, there is an unmet need for well-defined commercial kits that are suitable for biotherapeutic development [3]. Although some commercial kit manufacturers have begun classifying their kits according to higher levels of characterization, there has not been an industry-wide effort to improve the overall quality of these kits for use in drug development. Standardized qualification criteria is also lacking, as manufacturers use their own specific requirements for kit characterization and documentation.

Singleplex assays carry their own unique challenges. In addition to limitations in the number of analytes that can be measured at one time, singleplex assays often require higher sample volumes due to the increased number of measurements required. Obtaining large sample volumes can be difficult, particularly when concerning the sampling of infants or critically ill patients. Singleplex assays are also often less cost-effective than multiplex assays. However, when used for validation of regulated studies, singleplex assays are preferred. Acceptance criteria is likely to be more flexible to accommodate for multiplex assays, or an analyte may have to be removed from the panel if these criteria aren't met [5]. Whereas, for single-analyte assays, the acceptance criteria are clearer and easier to achieve. Whether validation is for exploratory purposes or to inform critical decisions, fit-for-purpose validation of these assays is critical [5].

During biomarker discovery, multiplex platforms are favored as large numbers of potential biomarker candidates can be screened simultaneously. Once these biomarkers have been identified and qualified, the need to screen a high volume of analytes at once diminishes and the ability to screen fewer analytes with greater robustness becomes critical. As development progresses through preclinical and clinical phases, the value of high-quality, well-characterized single-analyte assays with suitably high sensitivity, reproducibility and broad dynamic range becomes essential to the utility of these assays as surrogate endpoints, efficacy markers or safety biomarkers and for validation of regulated studies. Therefore, single-analyte, well-characterized assays are typically developed for use and favored in later stages of development.

Cytokines as biomarkers for biotherapeutic development

Cytokines – encompassing interleukins, interferons, growth factors and chemokines – are a diverse group of soluble proteins and are key modulators of immunity, regulating responses to proximal events of inflammation, immune response and repair [6]. Cytokines are secreted by immune cells, including monocytes, macrophages, T-cells, B-cells and natural killer cells, as well as some nonimmune cells. Cytokine concentration changes regulate the tumor microenvironment, change the proliferation and differentiation of immune cells and even influence the metastasis of cancer cells. These, among other factors, make cytokines apt candidates for therapeutic biomarkers, enabling the establishment of safe yet maximized starting doses for biotherapeutics [7].

In recent years, there has been an increase in the use of single-analyte biomarker assays for PD or safety assessment during biotherapeutic development and cytokines have been one of the many biomarker categories helping contribute to this increase [7].

Numerous platforms now utilize cytokine kits for biomarker quantification, including the Gyrolab microfluidic immunoassay platform. The latest Gyrolab kits utilize five single-analyte biomarker reagents to facilitate the quantification of human inflammatory cytokines IL-4, IL-6, IL-10, IFN-gamma and TNF-alpha in human and cynomolgus monkey serum [101]. Optimized for use on all Gyrolab assay platforms, these reagents demonstrate high sensitivity, matrix tolerance and wide dynamic range to cover the entire spectrum of cytokine levels observed in disease states or PD studies. To meet the requirements of regulated environments and maximize assay reproducibility, the Gyrolab human cytokine assays provide rapid and entirely automated biomarker assays and the use of low sample volumes makes the platform well adapted for biotherapeutic development [101]. The minimal matrix effect experienced in the Gyrolab assays facilitates the shift from traditional serum screening to targeted tissue screening and the introduction of the Gyrolab Bioaffy™ 4000 CD to the Gyrolab platform has improved the overall sensitivity of these immunoassays, extending quantification levels to low pg/mL or high fg/mL levels [102].

Biomarker assay platforms: Gyrolab® Technology

The features of the Gyrolab platform address the key existing challenges within biomarker discovery and analysis. As explained by Ourania Tzara, Research Scientist for Biotherapeutic Development, H. Lundbeck A/S (Valby, Denmark), biomarker assay development can be simplified with the appropriate automated tools:

> *"Gyrolab technology serves as a unique system for rapid development of robust and sensitive biomarker assays for use in drug development programs. As a fully automated immunoassay platform, it enables smooth transfer between analysts and laboratories and the low sample volume requirements allow it to be effectively implemented in the preclinical phase."*

There is a strong need, particularly during preclinical stages of research, for biomarker platforms to be optimized for flexible assay development to adjust to the user's immunoassay design and reagents. This need is highlighted by Kenneth Munroe, Principal Scientist I, In Vitro Pharmacology, Charles River Laboratories (MA, USA);

> "Developing biomarker assays for drug discovery requires an assay platform that is robust and versatile to meet changing demands of early research. The Gyrolab technology not only can be applied in preclinical assays where samples are frequently of low volume and tend to be split during collection, but it also allows for assay formats being adapted to research where several biomarkers need to be evaluated while testing drug effects."

The general movement towards plug-and-play kits has minimized pipetting errors as well as the time needed for assay development, to deliver high-performance immunoassays. Aruni Karunanayake M, Scientist II, Charles River Laboratories, explained:

> "Biomarker assay development is challenged by utilizing the appropriate analytical tool to scientifically justify the choice of a relevant biomarker and its measurement. The Gyrolab technology supports customizing sensitive, robust, and reliable immunoassays for biomarker readouts. The easy-to-use platform allows for minimal sample volume and automated protocol execution, while ensuring precision and accuracy of an analytical method."

As several platforms may be suitable for a particular biomarker application, it is critical that the user selects a platform that is optimized for the intended application, able to meet the demands of preclinical research through clinical studies and delivers sensitive and selective biomarker immunoassays.

Concluding remarks

Although biomarker discovery benefits from the ability of multiplex assays to screen multiple potential biomarkers simultaneously, the accuracy, sensitivity and reproducibility of well-characterized single-analyte biomarker assays is essential for quantification during the later stages of biomarker development. Researchers have a range of tools at their disposal to provide fit-for-purpose methods for quantification of biomarkers, which is essential for the use of these assays to guide efficacy and safety of biotherapeutics.

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