The breadth of biomarkers and their assays: part 2

“...it is biomarkers that are opening up new vistas and forcing the expansion of existing technology solutions and the exploration of alternatives.”

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Once again, we are excited to serve as Guest Editors for this second issue of the Special Focus on Bioanalysis of Biomarkers, in *Bioanalysis*. In this edition, we are pleased to introduce new content that lends additional insights into the central themes explored in the first issue, where we highlighted the range of challenges encountered in the measurement of biomarkers and the breadth of technological solutions being applied to meet those challenges. In the following pages, we present a variety of content that includes commentary, research and review articles that expand upon some common challenges, leverage experience with tried and true technologies, and introduce us to new methods, new technologies and an array of different analytes, which together speak to the complexity of biomarker bioanalysis.

Two topics at the center of many biomarker discussions are accuracy and parallelism [1–4]. It is now widely acknowledged that demonstration of true accuracy is a rarity in the biomarker space. In a Commentary provided here, Rand Jenkins discusses some of the complexities associated with accuracy, the realities of relative accuracy, and provides perspective on the commonalities in this regard between biomarkers and therapeutics with endogenous counterparts [5]. On the parallelism front, Lindsay King's Commentary summarizes and contextualizes key concepts and focuses on the application of parallelism to demonstrate immunological similarity between the calibrator and the endogenous analyte and how this differs from assessment of matrix interference [6]. Amaravadi also provides an editorial

article on the general perspective on biomarker measurements, the associated challenges and where we are heading [7].

The use of biomarkers as surrogates for drug–drug interaction studies is an area of growth as pharmaceutical companies search for ways to identify potential side effect risks to clinical study subjects and accelerate the development process. Two papers are presented in this special issue using the familiar UHPLC-MS/MS platform. Using a surrogate matrix, Yuan *et al.* provide a case example where 7 α -hydroxy-cholesten-3-one (C4) was measured as a biomarker by UHPLC-MS/MS to assess CYP7A1 enzyme activity as it relates to changes in bile acid synthesis [8]. Similarly, Zheng *et al.* developed a UHPLC-MS/MS assay to quantify the total unconjugated forms of urinary dehydroepiandrosterone (DHEA), 7 β -hydroxy-DHEA, cortisone and 6 β -hydroxycortisone as potential biomarkers of CYP3A inhibition and induction [9].

The increasing need for improved analysis of protein biomarkers, in particular the quantification of cytokines in biological fluids, has prompted the development of more efficient and more sensitive ligand-binding assay platforms [10]. In this issue, Gupta and colleagues describe the sensitivity, accuracy, precision and other analytical characteristics of multiplexed cytokine assays in plasma using the Simple Plex™ Ella microfluidic platform, ProteinSimple, CA, USA. Their demonstration of analytical performance, and comparison with traditional ELISA, pave the way for assay implementation in

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exploratory clinical biomarker studies, such as those that investigate pharmacodynamic changes resulting from immune checkpoint inhibitor treatment [11].

While extensive activity is seen in the pages of *Bioanalysis* and other journals on protein and small-molecule biomarker analytes, other chemotypes are being explored as part of mechanistic studies. da Costa *et al.* provide a review of the potential use of such an alternative analyte, miRNAs, in a clinical setting as circulating biomarkers in Parkinson's disease [12]. miRNAs have the potential for being an early diagnostic of the disease which is normally diagnosed through later stage neuromotor symptoms. In addition to discussing the various technologies employed, the authors note the regulatory authorities' interest and consortia focused on validating the relevance of specific miRNAs as diagnostic biomarkers.

Finally, the measurement of cellular biomarkers by flow cytometry for characterization of the disease state or evaluation of response to treatment is an area of increasing emphasis in drug development. To this end, Sun and colleagues describe a case study where a quantitative flow cytometry assay was developed for the detection of intracellular expression of Ki67, a cell proliferation marker, in cellular subsets of human blood [13]. Assay validation demonstrates robustness of the method to monitor Ki67 expression in clinical samples from immunotherapy studies.

The issue also includes the parts two and three of the White Papers to be published following the Workshop on Recent Issues in Bioanalysis annual meeting held in April this year, which focus on biomarker assay validation [14,15].

While the variety of new chemo- and proteo-type drug constructs continue to provide challenges to the bioanalytical scientist, it is biomarkers that are opening up new vistas and forcing the expansion of existing technology solutions and the exploration of alternatives. As part of the scientific community working in this space, the Guest Editors and hopefully the readers, have found the content of both Special Focus Issues on biomarkers inspiring and eliciting a further commitment to being active participants in the development of new therapies for patients with serious diseases.

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