Rapid Assay Development for Protein Biomarkers

The bottleneck in biomarker development has been the ability to develop assays in a timeframe acceptable to move viable candidates forward in line with the therapeutic compound. NextGen Sciences' platform is a solution that greatly shortens the assay development time relative to more conventional platforms such as immunoassays.

The pharmaceutical and biotechnology industry is under pressure to reduce the attrition rate of compounds through the development process. The industry is looking towards biomarkers and better target selection as the best means of improving the attrition rate. Industry and its regulators see these as key solutions to the drug development problem especially as related to efficacy and safety. So much so, that the use of molecular biomarkers is becoming mandatory for drug development programs. The role of biomarkers spans all aspects of drug discovery and development. It has been recognized that integration of biomarkers through the different phases of drug development can yield safer drugs with enhanced therapeutic efficacy in a cost-effective manner. Biomarkers also provide the critical link in translational medicine (bench to bedside) and are essential for the realization of personalized medicine.

The ability to discover putative biomarkers increases every year with improved discovery platforms in multiple disciplines. Proteomic technologies have been used successfully for biomarker discovery projects, producing many lists of candidate or putative protein biomarkers. However, further verification work is typically limited by the small number of proteins for which there are commercially available assays (~500 human proteins), by the assays inability to be used in multiple biofluids and by the timescales and costs involved in the development and validation of immunoassays to novel targets. The lack of assays for protein biomarkers has created a bottleneck in biomarker development. That is the ability to develop assays in an acceptable timeframe in order to begin to validate putative biomarkers and to move the viable candidates forward.

NextGen Sciences has developed a workflow for rapid development of robust, precise and accurate protein biomarker assays, thus overcoming the bottleneck. The workflow is described below and outlined in Figure 1. NextGen Sciences' platform greatly shortens the assay development time relative to more conventional platforms such as immunoassays.

NextGen Sciences' platform for biomarker discovery is a powerful method that enables the detection and quantitation of a large number of protein species in the sample type of interest. Typically the number of proteins detected in a discovery analysis can be from 500 proteins in plasma to 3,000 from a tissue sample. The proteins are also from a broad range of classes and activities. Frequently entire pathways critical to the therapeutic area of interest can be mapped. An example would be the insulin signaling pathway in pancreatic islets. The end product of this in depth analysis includes an index of proteins identified with quantitative data for each protein in the sample and The data not only provides the quantitation needed to select putative biomarkers but also provides the empirical information required for assay development.

The objective of stage 1 assay development is to develop an assay that will be used to confirm putative biomarkers. The platform used in this workflow is a mass spectrometry-based assay. Measurements are made on unique peptides that are used as surrogates specific to the target proteins, this approach brings very high specificity for each protein in the assay. The assays do not require antibodies for detection and can be designed to monitor a specific protein, single or multiple isoforms of a protein, and even a single amino acid mutation within the protein(s) being measured. Having the necessary data on the candidate proteins captured during biomarker discovery allows for rapid assay development.

The assay is then used to monitor the level of the target proteins in a sample set, often three to five times the size of the sample set used at the discovery stage. The assay is relative to a reference sample which is either a selected sample or a pool of selected samples. The level of each protein is calculated based on the response in the mass spectrometer of the corresponding surrogate peptides relative to the level in the reference sample. Statistical analysis (p-value and false discovery rate) is applied to provide an assessment of the data this helps determine the significance of each biomarker. Ideally, many of the biomarkers would be confirmed after testing the larger sample set. However, this is where a number of the putative markers may fall out of the analysis as they are found not to be specific to the physiological changes. This ensures that assay development is as robust and cost effective as it can be since only those proteins which are confirmed in stage 1 testing as following the desired trend will move forward for further assay development. This is an empirical selection/confirmation based on actual results and information.

While determination of the relative protein levels is sufficient for biomarker confirmation, protein concentration levels are required for biomarker validation. In order to achieve this, the relative quantitation assay from stage 1 biomarker testing is further developed to provide concentration values for each protein – often called absolute quantitation. This is accomplished in stage 2 assay development. Isotopically labeled peptide standards (typically ^{13}C or ^{15}N labeled amino acids) are used to convert mass spectrometry data (analyte peak areas) to protein concentrations. The assay can also be validated on a fit-for-purpose basis with a timeline for this stage being typically two to six months. Validation can range from being suitable for internal decision making all the way through to suitable for regulatory filing.

The objective of stage 2 testing is to validate the protein biomarkers. Data are reported as the concentration of each protein in each sample. This step of biomarker development typically involves hundreds of samples that are tested in batches in order to empirically validate the proteins as biomarkers.

Biomarker projects are supported by NextGen Sciences' proprietary **biomarker**library™ which contains catalogues of proteins from a variety of biological fluids, tissues and cell lines from pre-clinical models to human. This knowledge accelerates the development of robust, accurate and precise multiplexed assays suitable during all phases of clinical development.

Proteomics is the high throughput comparative, quantitative analysis of proteins. Proteomic analysis offers a unique means to identify protein changes related to a physiological response. These changes identify physiologically relevant proteins and as such are putative targets for therapeutic intervention (pharmaceutical targets), members of signaling pathways (functional analysis of proteins) and proteins involved in the molecular effects of known compounds (functional analysis of proteins and off target effects). The platform and methods allow rapid insight to target validation studies

NextGen Sciences' high quality, comprehensive protein profiling services, that include the identification and characterization of clinically relevant proteins utilized in biomarker discovery, also facilitates better pharmaceutical target selection and validation with the aim of reducing compound attrition rates.

NextGen Sciences' protein characterization services include advanced analytical methods leading to a better understanding of a therapeutic protein's characteristics and in addition the assay development methods for protein biomarkers confers the ability to monitor therapeutic proteins and these characteristics through processing, scale up and in preclinical and clinical samples. Therapeutic proteins and antibodies are characterized in depth including (re)-sequencing, glycan mapping and native molecular weight information.

NextGen Science's biomarker and target validation services offer a way to improve drug development for all therapeutic areas. NextGen Sciences is currently using the workflow described in this article to develop protein biomarker assays for pharmaceutical and biotechnology companies. This has helped relieve the bottleneck of assay development. NextGen Sciences has demonstrated that the platform can be used to develop multiplex assays in the timelines presented in this workflow.

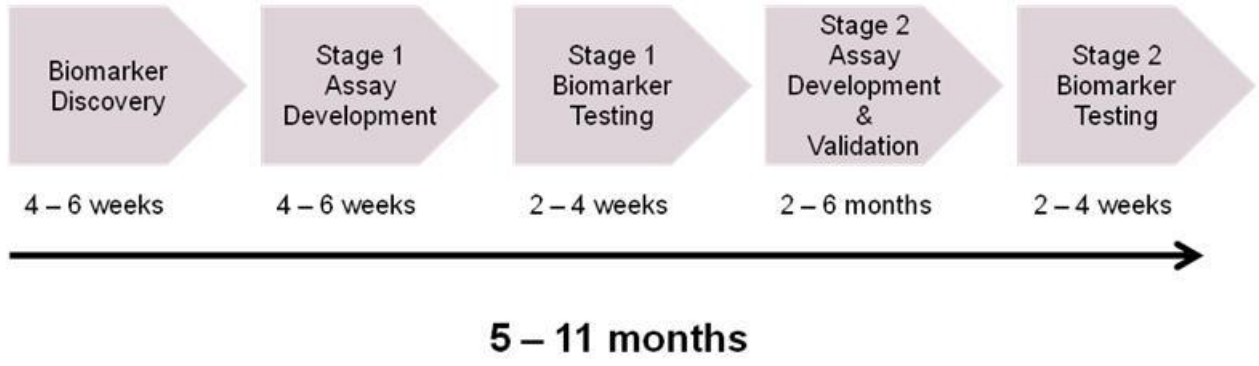
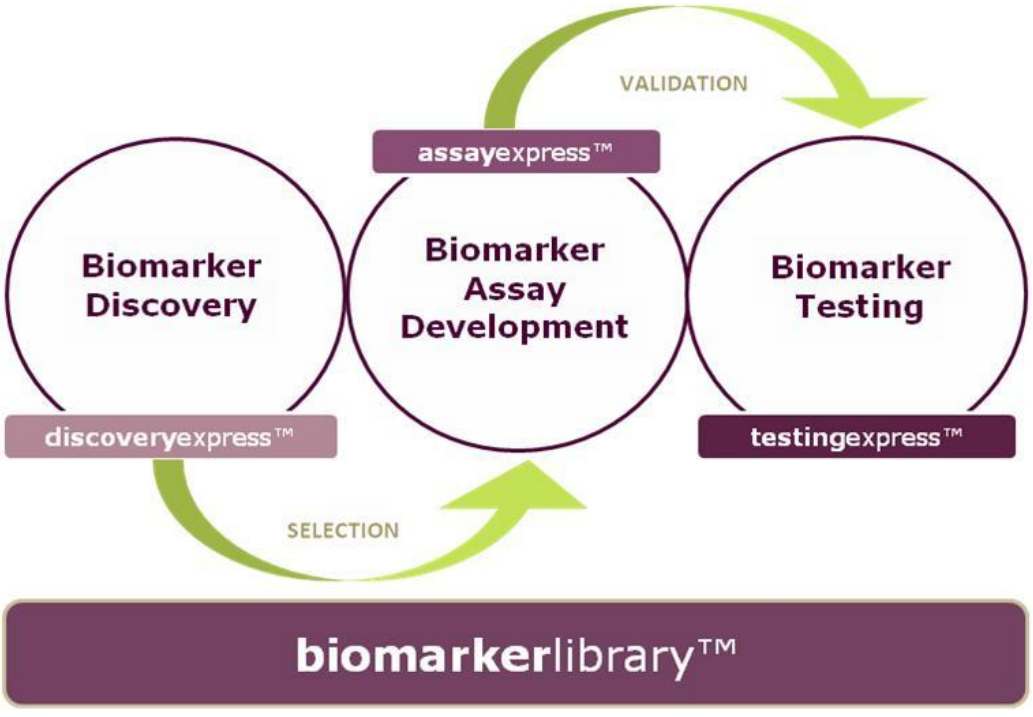


Figure 1. Workflow for Biomarker Assay Development. The data generated in the biomarker discovery step identifies protein changes and, in addition, the preliminary information for stage 1 and stage 2 assay development. Both stages of biomarker testing provide high throughput multiplexed assays.



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